CLINICAL STUDY PROTOCOL

Comprehensive *in vitro* Proarrhythmia Assay (CiPA) Clinical Phase 1 ECG Biomarker Validation Study (CiPA Phase 1 ECG Biomarker Study)

PROTOCOL NO. SCR-004

Sponsor:	U.S. Food and Drug Administration White Oak Building #64, Room 2072 10903 New Hampshire Avenue Silver Spring, MD 20993
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Version of Protocol:	9.1
Date of Protocol:	24 March 2017

CONFIDENTIAL

Sponsor Signature Page

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- International Council for Harmonisation (ICH) harmonised tripartite guideline E6 (R1): Good Clinical Practice; and
- All applicable laws and regulations, including without limitation, data privacy laws and compliance with appropriate regulations, including human subject research requirements set forth by the Research Involving Human Subjects Committee (RIHSC), the Institutional Review Board (IRB) of the U.S. Food and Drug Administration (FDA).

Digitally signed by David Strauss -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=David Strauss -S, 0.9.2342.19200300.100.1.1=2000507494
Date: 2017.03.25 16:02:16 -04'00'

David Strauss, MD, PhD
Director, Division of Applied Regulatory Science
U.S. Food and Drug Administration

Date

Investigator Signature Page

I confirm that I have read and that I understand this protocol, the investigator brochure, and other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki;
- ICH harmonised tripartite guideline E6 (R1): Good Clinical Practice;
- All applicable laws and regulations, including without limitation data privacy laws and regulations;
- Human subject research requirements set forth by the RIHSC, the IRB of the FDA;
- Regulatory requirements for reporting of serious adverse events (SAEs) defined in Section 4.7.3.1 of this protocol; and
- Terms outlined in the Clinical Study Site Agreement.

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Section 6 of this protocol.

Carlos R. Sanabria MD

Carlos Sanabria, MD
Principal Investigator

27 MAR 2017

Date

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Protocol Synopsis

Protocol Number:	SCR-004
Title:	Comprehensive *in vitro* Proarrhythmia Assay (CiPA) Clinical Phase 1 ECG Biomarker Validation Study (CiPA Phase 1 ECG Biomarker Study)
Investigators:	Principal Investigator: Carlos Sanabria, MD Subinvestigator: Paul Rice, MD
Study Phase:	1
Study Period:	The duration of study participation will be up to 12 days (excluding the screening period).
Study Site:	Spaulding Clinical Research LLC, 525 South Silverbrook Drive, West Bend, WI 53095
Background and Motivation:	At clinical concentrations, drugs that predominantly block the potassium channel encoded by the human ether-à-go-go-related gene (hERG) without late sodium or L-type calcium current block ("predominant hERG" drugs) cause heart rate-corrected QT interval (QTc) prolongation and intermediate or high risk of torsade de pointes (TdP). However, at clinical concentrations, drugs that block hERG with approximately equipotent late sodium and/or calcium block ("balanced ion channel" drugs) can cause QTc prolongation, but have a low risk of TdP. Thus, not all hERG block and QTc prolongation is associated with TdP. The Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative is developing a preclinical cardiac safety evaluation of new drugs that provides a more accurate and comprehensive mechanistic-based assessment of proarrhythmic potential. The CiPA assay will involve assessment of drug block on multiple cardiac ion currents (including hERG, calcium, and late sodium) that will be integrated in a computational in silico model to classify drugs as low, intermediate, or high risk of TdP. CiPA proposes to utilize an electrocardiogram (ECG) assessment in early clinical Phase 1 studies (single ascending dose [SAD] or multiple ascending dose [MAD] studies) to determine if there are unexpected ion channel effects, such as due to a human-specific metabolite or protein binding, compared to the preclinical ion channel data. Through analysis of prior clinical studies, the CiPA Phase 1 ECG working group identified the heart rate-corrected early repolarization (J-T_{peak}) interval (J-T_{peakC}) as the best biomarker to differentiate "balanced ion channel" drugs from "predominant hERG" drugs. Drugs that exhibit "balanced ion channel" effects often have QTc prolongation, but do not exhibit J-T_{peakC} prolongation. While this has been shown for multiple drugs and drug combinations blocking

either hERG + late sodium or hERG + late sodium + calcium (analysis of thorough QT [TQT] studies and results from previous prospective clinical trials SCR-002 and SCR-003), it is still not clear if drugs that only block hERG + calcium (without late sodium block) exhibit this phenotype.

This study will assess whether exposure-response analysis of the electrocardiographic QTc and J-T_{peakC} intervals in Phase 1 clinical pharmacology studies can be used to confirm that "balanced ion channel" drugs do not cause J-T_{peakC} prolongation and that "predominant hERG" drugs cause QTc prolongation.

This clinical study consists of 2 parts: a 50-subject parallel part (Part 1) and a 10-subject crossover part (Part 2). These parts are included in the same protocol and study due to the similarity of the inclusion and exclusion criteria, similar procedures, and similar primary goals. In addition, the 10-subject placebo group from the parallel part of the study can potentially be used interchangeably with the crossover part in exploratory analysis. Up to 74 healthy subjects will be enrolled (including 14 potential replacement subjects).

Part 1: Drugs with Well Characterized Ion Channel Effects

Resampling of the data from prior clinical studies demonstrated that, with 9 subjects on active drug and 6 subjects on placebo, the use of exposure-response modeling could confirm the absence of J-T_{peakC} prolongation (upper bound of confidence interval [CI] <10 milliseconds [msec]) approximately 94% of the time for a "balanced ion channel" drug. This is nearly identical to the ability of QTc exposure-response analysis to confirm the absence of QTc prolongation in small sample sizes. Thus, Part 1 of the current study was designed with a sample size and structure similar to the "IQ-CSRC Prospective Study" (Darpo et al, 2014 and Darpo et al, 2015) that had similar power as a SAD study to exclude small QTc effects and supported the replacement of TQT studies with exposure-response modeling in early clinical studies, except the current study will focus on J-T_{peakC} for "balanced ion channel" drugs. Due to the long half-life of some drugs that are included in this study and the desire to more closely mimic SAD/MAD studies, Part 1 of the current clinical study will use an entirely parallel study design with 10 subjects in each arm.

Part 2: Combination of hERG and Calcium Block

Part 2 of this study will assess whether calcium block can reduce J-T_{peakC} prolongation from hERG block. Resampling and further simulation of the data from our prior clinical study (SCR-003) demonstrated that, with 8 subjects, the use of exposure-response modeling could detect the effect of inward current block in the QTc

and J-T_{peakC} slopes associated with hERG block approximately 99% and 99% of the time using a crossover design for QTc and J-T_{peakC}, respectively (i.e., the late sodium blocker significantly reduced prolongation from the hERG blocker). For Part 2, we anticipate the selected dose for the calcium blocker will result in similar or larger QTc and J-T_{peakC} effects than those observed in SCR-003 because the calcium current is larger than the late sodium current. Therefore, and to account for potential dropouts between periods, Part 2 of the study will use a crossover design with 10 subjects.

Summary and Objectives:

The primary objectives of this study are:

1. To confirm that exposure-response analysis of the electrocardiographic QTc and J-T_{peakC} intervals in Phase 1 clinical pharmacology studies can be used to confirm that "balanced ion channel" drugs do not cause J-T_{peakC} prolongation and "predominant hERG" drugs cause QTc prolongation.
2. To test the hypothesis that calcium channel block (diltiazem) can reduce the QTc prolongation from hERG block by shortening J-T_{peakC}.

Primary Objective 1 (Part 1)

Part 1 of the study will assess primary objective 1. Part 1 will include 4 drugs with well-characterized ion channel effects, QTc effects, and TdP risk. Three (3) drugs will be "balanced ion channel" blockers (approximately equipotent hERG and late sodium and/or calcium block) with low TdP risk (ranolazine, verapamil and lopinavir + ritonavir) and 1 drug will be a "predominant hERG" blocker with TdP risk (chloroquine). Fifty (50) healthy subjects will be enrolled. Multiple doses of each drug will be given to 40 subjects on 3 consecutive days to achieve low and high exposures on Days 1 and 3, respectively, and 10 subjects will receive placebo. Data will be analyzed using linear mixed-effects exposure-response models.

The criterion for the "balanced ion channel" drugs will be an upper bound of the 2–sided 90% CI that is <10 msec for the projected J-T_{peakC} effect at the peak plasma level on Day 3.

The criterion for the "predominant hERG" drug will be an upper bound of the 2–sided 90% CI that is ≥10 msec for the projected QTc effect at the peak plasma level on Day 1.

Primary Objective 2 (Part 2)

Part 2 of the study will assess primary objective 2. Part 2 will include 2 oral drugs (dofetilide and diltiazem) with well-characterized individual ion channel effects and TdP risk. Dofetilide is a "predominant hERG" blocker while diltiazem is a calcium channel blocker. Ten (10) healthy subjects will be enrolled. Multiple doses of

each drug will be given either separately or together on 3 consecutive days in the first period and again on 3 consecutive days in the second period, depending on the randomization, to achieve low and high exposures on Days 1 and 3, respectively. There will be no placebo arm. Data will be analyzed using linear mixed-effects exposure-response models.

The criterion for calcium block (diltiazem) effects on the QTc prolongation from hERG block (dofetilide) will be whether the projected QT_C effect of dofetilide alone is significantly greater (i.e., $p<0.05$) than the projected QT_C effect of the combination of dofetilide + diltiazem. This will be assessed at the dofetilide peak plasma level on Day 3 (computed from the combination of dofetilide + diltiazem) on the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem data using a linear mixed-effects model. Subsequently, and if the test is significant for QTc, the same test will be performed to assess calcium block (diltiazem) effects on J-T_{peakC}.

Study Design:

Part 1

Part 1 will be a double-blind, randomized, placebo-controlled, 1-period parallel design to assess the effect of 4 marketed drugs and 1 placebo on the QTc and J-T_{peakC} intervals in 50 healthy subjects. A parallel design similar to a SAD or MAD Phase 1 study will be used that will result in each study drug being administered to 10 subjects, and placebo to 10 subjects, in 1 period of 3 consecutive days as follows:

Subjects (n)	Treatment Group	Ion Channel Effects
10	A	balanced
10	B	balanced
10	C	balanced
10	D	predominant hERG
10	E	N/A (reference)

Abbreviations: hERG: human ether-à-go-go-related gene; N/A, not applicable.
A: ranolazine; B: verapamil; C: lopinavir + ritonavir; D: chloroquine; E: placebo

Part 2

Part 2 will be a double-blind, randomized, 2-period crossover design to assess the effect of hERG block (dofetilide) versus calcium block (diltiazem) on the QTc and J-T_{peakC} intervals in 10 healthy subjects. On Days 1, 2, and 3 (Period 1) and Days 8, 9, and 10 (Period 2), subjects will receive their assigned treatment according to the randomization schedule. The project biostatistician will prepare the randomization schedule, and subjects will be randomly assigned to 1 of 2 different treatment sequences during which each subject will receive each of the following treatments by the end of the study: 1)

dofetilide and 2) diltiazem alone and dofetilide + diltiazem.

The treatment sequences are as follows:

Treatment Sequence	Subjects (n)	Period 1 (Days 1, 2, 3)	Period 2 (Days 8, 9, 10)
1	5	F	G
2	5	G	F

F: dofetilide alone; G: diltiazem alone on Days 1 and 2 and dofetilide + diltiazem on Day 3

Parts 1 and 2

Subjects will be screened for study eligibility from Days –28 to –1. During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent form will be reviewed with the subject by a member of the study team and the subject will be encouraged to ask questions to ensure he or she has a good understanding of the study. If the subject is eligible and agrees to participate, the subject will be asked to sign the informed consent form before any study-specific procedure is performed, including randomization.

After the consent process is complete, demographic data, medical history, and concomitant medications will be recorded. A physical examination will be performed by a study team member. Clinical laboratory tests (hematology, serum chemistry, and urinalysis) will be performed. Female subjects must have a negative pregnancy test result. Screening tests will be performed within 28 days of and no later than 1 day before Day 1.

Results of all screening tests will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm subject eligibility. At each visit, eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented, vital sign measurements and a 12-lead ECG will be performed, clinical laboratory, drug and alcohol, and pregnancy tests (for females) will be performed, an intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed), study drug will be administered, and blood samples will be collected per protocol.

U.S. Food and Drug Administration (FDA) will prepare the randomization schedule. Subjects will enter the study clinic for check-in procedures the day before study drug administration (Day 0). At check-in, continuous/Holter 12-lead ECGs will be recorded from early afternoon until the subjects go to sleep. During this period, subjects will perform a sequence of postural maneuvers (e.g., 10 minutes supine, 10 minutes sitting, 10 minutes standing,

	10 minutes sitting, 10 minutes supine) to characterize ECG biomarkers and heart rate relationship. At each treatment period, continuous/Holter 12-lead ECGs will be recorded from 1 hour before the first oral dose on Day 1 until 73 hours after the first dose. All study drugs will be administered orally. At each treatment period, there will be multiple doses over a period of 3 consecutive days. Subjects will be discharged from the study after completion of all study procedures. If a subject discontinues from the study prematurely, all procedures scheduled for the end of the study will be performed. Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days.
Subject Population:	Approximately 60 healthy subjects are planned for enrollment, of which 50 will be assigned to Part 1 and 10 will be assigned to Part 2 at randomization. Up to 14 subjects may be qualified as replacements. Thus, a maximum of 74 subjects will be exposed to study drugs and procedures during the study. Every effort will be made to maintain an approximate 50:50 male to female gender distribution. Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the local Institutional Review Board (IRB) and FDA Research Involving Human Subjects Committee (RIHSC) before telephone screening. Subjects will be offered payment for Screening and participation in the study, but no special incentives are offered.
Study Drugs, Dosage, and Route of Administration:	For all drugs, standard drug doses or doses used in prior studies in healthy subjects will be used in this research study. Multiple doses of the assigned study drug will be administered to each subject during the treatment period. All study drugs will be administered orally. The dofetilide + diltiazem combination dose (dofetilide 0.25 mg and diltiazem 120 mg) was selected below the maximum doses in the label (dofetilide 0.5 mg twice daily [BID] and diltiazem 480 mg daily) and below dofetilide doses studied in a previous drug-drug interaction study with dofetilide and verapamil. An increase in dofetilide exposure of 40% to 50% may occur from diltiazem pharmacokinetic (PK) interaction, similar to what has been previously reported in interaction studies with higher doses of dofetilide + verapamil (dofetilide 0.5 mg BID + verapamil 80 mg three times daily) and dofetilide + ketoconazole (dofetilide 0.5 mg BID + ketoconazole 400 mg daily). A single oral dose of dofetilide + diltiazem will be given on Day 3 or Day 10 to subjects assigned to Part 2 of the study.

Part 1 Study Drugs

- Ranolazine 1500 mg BID for 2.5 days
- Verapamil 120 mg immediate release (IR) morning and afternoon doses on Days 1 and 2, 240 mg extended release (ER) evening dose on Days 1 and 2, and 120 mg IR morning dose on Day 3
- Lopinavir + ritonavir 800 mg/200 mg BID for 2.5 days
- Chloroquine 1000 mg on Day 1, 500 mg on Day 2, 1000 mg on Day 3

Part 2 Study Drugs

- Dofetilide 0.125 mg on Day 1 and 0.375 mg on Day 3 (dofetilide alone period) or 0.25 mg (dofetilide + diltiazem period) on Day 3
- Diltiazem 120 mg IR morning dose on Day 1, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR (when coadministered with dofetilide) on Day 3

Reference Drug, Dosage, and Route of Administration:

Part 1 Reference Drug

- Placebo

Part 2 Reference Drug

- There is no placebo. Subjects will be randomized to 2 different treatment sequences.

Inclusion Criteria:

Subjects who meet all of the following inclusion criteria will be eligible to participate in the study:

1. Subject signs an IRB-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] authorization) before any study-related procedures are performed.
2. Subject is a healthy man or woman, 18 to 50 years of age, inclusive, who weighs at least 50 kg (110 pounds) and has a body mass index of 18 to 30 kg/m^2, inclusive, at Screening.
3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 12-lead ECG results, and physical examination findings at Screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee).
4. Female subjects will be at least 2 years postmenopausal, surgically sterile, or practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique).
5. Female subjects must not be pregnant or lactating before enrollment in the study.

6. Male or female subjects must agree to practice 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from Screening until 30 days after the last dose of study drug.
7. Subject is highly likely (as determined by the investigator) to comply with the protocol-defined procedures and to complete the study.

Exclusion Criteria:

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subject has a safety 12-lead ECG result at Screening or Check-in with evidence of any of the following abnormalities:
   - QTc using Fridericia correction (QTcF) >430 msec
   - PR interval >220 msec or <120 msec
   - QRS duration >110 msec
   - Second- or third-degree atrioventricular block
   - Complete left or right bundle branch block or incomplete right bundle branch block
   - Heart rate <50 or >90 beats per minute
   - Pathological Q-waves (defined as Q-wave >40 msec)
   - Ventricular pre-excitation
2. Subject has more than 12 ectopic beats during the 3-hour Holter ECG at Screening.
3. Subject has a history of unexplained syncope, structural heart disease, long QT syndrome, heart failure, myocardial infarction, angina, unexplained cardiac arrhythmia, TdP, ventricular tachycardia, or placement of a pacemaker or implantable defibrillator. Subjects will also be excluded if there is a family history of long QT syndrome (genetically proven or suggested by sudden death of a close relative due to cardiac causes at a young age) or Brugada syndrome.
4. Subject has a history or current evidence of any clinically significant (as determined by the investigator) cardiovascular, dermatologic, endocrine, gastrointestinal, hematologic, hepatic, immunologic, metabolic, neurologic, psychiatric, pulmonary, renal, urologic, and/or other major disease or malignancy (excluding nonmelanoma skin cancer). The investigator may allow exceptions to these criteria (e.g., cholecystectomy, childhood asthma) following discussion with the medical monitor.
5. Subject has a history of thoracic surgery.

6. Subject has any condition possibly affecting study drug absorption (e.g., gastrectomy, Crohn's disease, irritable bowel syndrome).
7. Subject has a skin condition likely to compromise ECG electrode placement.
8. Subject is a female with breast implants.
9. Subject's laboratory test results at Screening or Check-in are outside the reference ranges provided by the clinical laboratory and considered clinically significant (as determined and documented by the investigator or designee).
10. Subject's laboratory test results at Screening or Check-in indicate hypokalemia, hypocalcemia, or hypomagnesemia according to lower limits of the reference ranges provided by the clinical laboratory.
11. Subject's laboratory test results at Screening or Check-in are >2 × the upper limit of normal (ULN) for alanine aminotransferase or aspartate aminotransferase, >1.5 × ULN for bilirubin, or >1.5 × ULN for creatinine.
12. Subject has a positive test result at Screening for human immunodeficiency virus I or II antibody, hepatitis C virus antibodies, or hepatitis B surface antigen.
13. Subject has a mean systolic blood pressure <100 or >140 mmHg or a mean diastolic blood pressure <50 or >90 mmHg at either Screening or Check-in. Blood pressure will be measured in triplicate after the subject has been resting in a supine position for a minimum of 5 minutes.
14. Subject has a known hypersensitivity to any of the study drugs or related compounds.
15. Subject has consumed alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice within 48 hours before dosing or anticipates an inability to abstain from these products throughout the duration of the study.
16. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks before Screening (self-reported).
17. Subject is unable to tolerate a controlled, quiet, study conduct environment, including avoidance of music, television, movies, games, and activities that may cause excitement, emotional tension, or arousal during the prespecified time points (e.g., before and during ECG extraction windows).
18. Subject is unwilling to comply with study rules, including the study-specific diet, attempting to void at specified times

(e.g., before ECG extraction windows), remaining quiet, awake, undistracted, motionless, and supine during specified times, and avoiding vigorous exercise as directed.

19. Subject has a history of consuming more than 14 units of alcoholic beverages per week within 6 months before Screening, has a history of alcoholism or drug/chemical/substance abuse within 2 years before Screening (Note: 1 unit = 12 ounces of beer, 4 ounces of wine, or 1 ounce of spirits/hard liquor), or has a positive test result for alcohol or drugs of abuse at Screening or Check-in.
20. Subject has used any prescription or nonprescription drugs (including aspirin or nonsteroidal anti-inflammatory drugs [NSAIDs] and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer), or complementary and alternative medicines within 28 days before the first dose of study drug.
21. Subject is currently participating in another clinical study of an investigational drug or has been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.
22. Subject has had any significant blood loss, donated 1 unit (450 mL) of blood or more, or received a transfusion of any blood or blood products within 60 days, or donated plasma within 7 days before Check-in.
23. Subject has any other condition that precludes his or her participation in the study (as determined by the investigator).
24. Subject is unwilling to have genetic analysis performed or a blood sample collected for isolating peripheral blood mononuclear cells (PBMCs).

ECG Assessments:

At Screening, a 3-hour Holter ECG will be performed in order to exclude subjects who have more than 12 ectopic beats during this collection period. Subjects will be supine during the Holter monitoring period.

At check-in, continuous/Holter 12-lead ECGs will be recorded from early afternoon until the subjects go to sleep. During this time, subjects will perform a sequence of postural maneuvers (e.g., 10 minutes supine, 10 minutes sitting, 10 minutes standing, 10 minutes sitting, 10 minutes supine) to characterize ECG biomarkers and heart rate relationship. On the treatment days within each of the periods, continuous 12-lead ECGs will be recorded using the telemetry system for a total of approximately 74 hours (starting 1 hour before first dosing of the treatment period and continuing until 73 hours after time 0 or first oral dose on Day 1).

Ten-second 12-lead ECG tracings with the most stable heart rate and highest signal-to-noise ratio will be extracted in at least 3 replicates at 28 ECG extraction time points during each treatment period (i.e., total of 28 and 56 ECG extractions per subject for Part 1 and Part 2, respectively).

During all ECG extraction periods, the subjects will be in a supine position. Three (3) ECG extractions will be performed for a baseline measurement before the first oral dose on Day 1 of each treatment period. The other 25 ECG extractions/period time-matched to the PK samples will be obtained before the PK sample collection time. Subjects will be isolated (not able to see each other) in a quiet environment that is free from external stimuli.

The position of the electrodes must be marked using a nonirritant marker to ensure that the position of the electrodes on the treatment days is the same. On all days, the ECGs will be recorded using the same model of ECG recorder at a sampling frequency of at least 500 Hz with an amplitude resolution of at least 2.5 μV.

Pharmacokinetic Assessments:

Pharmacokinetic blood samples will be collected on Days 1, 2, 3, and 4 of each period (i.e., on Days 1, 2, 3, and 4 for Part 1 and Days 1, 2, 3, 4, 8, 9, 10 and 11 for Part 2). Blood samples will be collected by direct venipuncture or by inserting an IV catheter. There will be a total of 25 and 50 PK samples per subject for Study Part 1 subjects and Study Part 2 subjects, respectively (10 samples on Day 1, 3 samples on Day 2, 11 samples on Day 3 and 1 sample on Day 4 for subjects in Parts 1 and 2. Part 2 subjects will have that repeated for the second period. Plasma concentrations will be determined with a validated liquid chromatography with tandem mass spectroscopy method for all PK samples.

Maximum observed plasma concentration (C_{max}) along with the time of C_{max} (T_{max}) and area under the plasma concentration-time curve (AUC) will be computed for each drug for Days 1 and 3 of the treatment period. The PK samples will also be used for exposure-response analysis of ECG biomarkers.

Safety Assessments:

Safety will be evaluated in terms of adverse events (AEs), clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG results, and physical examination findings.

Other Assessments:

At Check-in, blood samples will be required for genetic analysis and PBMC isolation. Genetic variations may be examined as potential predictors of PK or pharmacodynamic (PD) response. The PBMCs will be stored for potential generation of induced pluripotent stem

cells (iPSCs) to be differentiated into terminal cells (e.g., cardiomyocytes, hepatocytes) for subject-specific drug testing.

Sample Size and Threshold Determination:

The sample size (10 subjects per arm) was selected based on analysis by the sponsor of ECG biomarkers in Studies SCR-002 and SCR-003 and by resampling of data from previously conducted TQT clinical studies, similar to the methodology of Ferber et al (2015).

Part 1

The results of resampling analysis suggest that 10 subjects on active study drug and 10 subjects on placebo will be sufficient to detect QTc prolongation for the "predominant hERG" drug and exclude J-T_{peakC} prolongation for the "balanced ion channel" drugs.

Part 2

The results of resampling analysis demonstrated that inward current block effects on the QTc and J-T_{peakC} slopes could be detected with 8 subjects using a crossover design (i.e., the inward current blocker significantly reduced prolongation from the hERG blocker).

Statistical Methods:

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics. The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized. Demographic and baseline characteristics will be summarized overall and by treatment for all subjects.

Exposure-response population: The exposure-response population will include all subjects who receive at least 1 dose of any of the study drugs and have digital ECG data (QTc and J-T_{peakC}) for the treatment period collected before dosing and at 1 or more time points after dosing as well as plasma concentration data from the same time points after dosing.

Additional analysis details will be specified in the Statistical Analysis Plan.

Primary Analysis

Part 1

The primary variable for the exposure-response analysis will be the change-from-baseline in QTc (ΔQTc) for the "predominant hERG" drug and change-from-baseline in J-T_{peakC} (ΔJ-T_{peakC}) for the

"balanced ion channel" drugs, where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline. The concentration of the drug will be used as a covariate. Exposure-response analysis will be done following most recent best practices in concentration-QTc modeling.

Part 2

The primary variable for the exposure-response analysis will be the change-from-baseline in QTc (ΔQTc) for the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem, where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline. The concentration of dofetilide and diltiazem will be used as covariates.

Parts 1 and 2

Investigation of hysteresis: Prior to model selection for the exposure-response analysis, the presence of hysteresis will be assessed for QTc and J-T_{peakC}. To detect hysteresis, individual change-from-baseline J-T_{peakC} (ΔJ-T_{peakC}) will be computed. For each day, the time of the largest mean ΔJ-T_{peakC} (U_{max}) will be determined. If the largest mean ΔJ-T_{peakC} exceeds 5 msec at ≥3 time points, the time difference between U_{max} and the T_{max} of the drug level exceeds 1 hour, and the 1-sided, 1-sample Wilcoxon test for the difference between ΔJ-T_{peakC} at T_{max} and at U_{max} is formally significant at the 1% level, it will be concluded that hysteresis existed. In such a case, a PK model with an additional effect compartment will replace the model described below. The same steps will be followed for QTc as part of the primary endpoint.

Model selection: To assess the appropriateness of a linear model, normal QQ-plots for the residuals and plots of weighted residuals versus concentration and versus fitted values will be produced. A model with a quadratic term in concentration will be fitted and the quadratic term will be tested on the 2-sided 5% alpha level. In case of a significant quadratic term, nonlinear models, such as a log-linear model and an E_{max} model, will be investigated and the primary model will be selected based on the Akaike Information Criterion and plausibility arguments.

Exposure-response analysis: In the absence of hysteresis and unless the prespecified test procedure for linearity indicates otherwise, the primary analysis will be based on a linear mixed-effects model implemented in SAS® or R software, with ΔJ-T_{peakC} as the dependent variable, drug plasma concentration and baseline J-T_{peakC} as continuous covariates, treatment and time point as categorical factors, and subject-specific random effects for the intercept and slope. All postdose data will be used. The degrees of freedom for the model estimates will be determined by the Kenward-Rogers method. From

the model, the slope (i.e., the regression parameter for the concentration) and the treatment effect will be estimated together with 2-sided 90% CIs.

The predicted mean placebo-adjusted change-from-baseline J-T_{peakC} ($\Delta\Delta$J-T_{peakC}) at the observed geometric mean C_{max} (i.e., the product with the slope estimate + treatment effect [ΔJ-$T_{peakCactive}$ – ΔJ-$T_{peakCplacebo}$]) and the 2-sided 90% CI of the estimate will be calculated. The same steps will be followed for QTc as part of the primary endpoint.

Part 1

Criteria for primary QTc and J-T_{peakC} assessment:

Criteria for the 3 "balanced ion channel" drugs (ranolazine, verapamil, lopinavir + ritonavir) will be based on the predicted J-T_{peakC} effect on the third day of dosing. To demonstrate a lack of J-T_{peakC} prolongation for each of the 3 drugs:

- The upper bound of the 2-sided 90% CI of the predicted mean $\Delta\Delta$J-T_{peakC} must be <10 msec at the observed geometric mean C_{max} on Day 3.

Criteria for the "predominant hERG" drug (chloroquine) will be based on the predicted QTc effect on the first day of dosing. To demonstrate the presence of QTc prolongation for chloroquine:

- The upper bound of the 2-sided 90% CI of the predicted mean placebo-adjusted change-from-baseline QTc ($\Delta\Delta$QTc) must be $\geq$10 msec at the observed geometric mean C_{max} on Day 1.

Part 2

Criteria for primary J-T_{peakC} assessment:

To demonstrate that calcium channel block (diltiazem) reduces the QTc prolongation from hERG block (dofetilide) by shortening J-T_{peakC}:

- We will assess whether the projected QT_C effect of dofetilide alone will be significantly greater (i.e., $p<0.05$) than the projected QT_C effect of the combination of dofetilide + diltiazem. This will be assessed at the dofetilide peak plasma level on Day 3 (computed from the combination of dofetilide + diltiazem) on the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem data using a linear mixed-effects model.

- If the previous test is statistically significant for QTc, the same test will be performed for J-T_{peakC}.

Additional ECG Analyses

Exposure-response analysis of secondary ECG biomarkers: For Part 1, exposure-response analysis similar to that described for QTc and J-T_{peakC} will be applied to PR, QRS, QTc, J-T_{peakC}, and T_{peak}-T_{end} for Day 1 and Day 3. This will include analysis of change from baseline for dofetilide alone, diltiazem alone, and dofetilide + diltiazem combination from Part 2 of the study. Additional details will be specified in the Statistical Analysis Plan.

ΔΔECG measurements by time point: For each time point, an analysis of variance model will be fitted with ΔJ-T_{peakC} as the dependent variable, treatment (active or placebo) as factor, and baseline J-T_{peakC} as a covariate. From this model, the difference (ΔJ-$T_{peakCactive}$ – ΔJ-$T_{peakCplacebo}$) will be estimated with a 2-sided 90% CI. Separate models will be fitted for each treatment, all of them using the same placebo data. The same steps will be followed for QTc. Change from Baseline in heart rate, PR, QRS, and T_{peak}-T_{end} will be calculated using descriptive summary statistics.

Pharmacokinetics: The PK population will include all subjects who receive study drug and have at least 1 estimable PK parameter after dosing. The PK parameters C_{max}, T_{max}, and AUC will be summarized using descriptive statistics (number of subjects, mean, standard deviation [SD], coefficient of variation [CV], median, minimum, and maximum) for Day 1 and Day 3 for each active drug and period (i.e., Days 1 and 3 of Part 1 and Days 1, 3, 8 and 10 of Part 2). The PK parameters will be analyzed using noncompartmental methods based on actual sampling times. All parameters will be calculated using SAS or R software. Mean and individual concentration-time profiles will be presented in graphs.

Safety: The safety population will include all subjects who receive at least 1 dose of any of the study drugs. Descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum) will be used to summarize all safety and clinical laboratory data. All AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities. The incidence of treatment-emergent adverse events (TEAEs), organized by system organ class and frequency, will be summarized by seriousness, severity, relationship to treatment, and by treatment at onset of the TEAE. A detailed listing of serious AEs and TEAEs leading to withdrawal will also be provided. Vital sign measurements, safety 12-lead ECG results, and changes from Baseline for these parameters will be summarized by treatment and time point. Physical examination findings will be presented in a data listing, and abnormal physical examination findings will be recorded as AEs. All concomitant medication usage and medications that changed in daily dose, frequency, or both since the subject provided

informed consent will be summarized for each subject.

Additional Analyses: Blood samples will be collected for genetic analysis. PBMCs will be isolated for potential generation of iPSCs.

Date of Protocol: 24 March 2017

1. List of Abbreviations

Abbreviation	Definition
AE	adverse event
Ag/Ab	antigen/antibody
AUC	area under the plasma concentration-time curve
BID	twice daily
CFR	Code of Federal Regulations
CI	confidence interval
CiPA	Comprehensive *in vitro* Proarrhythmia Assay
C_{max}	maximum observed plasma concentration
CPT	cell preparation tube
CV	coefficient of variation
CYP	cytochrome P450
ECG	electrocardiogram
eCRF	electronic case report form
ER	extended release
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HCl	hydrochloride
HCV	hepatitis C virus
hERG	human ether-à-go-go-related gene
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICH	International Council for Harmonisation
iPSC	induced pluripotent stem cell
IR	immediate release
IRB	Institutional Review Board
IV	intravenous
J-T_{peak}	early repolarization interval
J-T_{peakC}	heart rate-corrected J-T_{peak} interval
ΔJ-T_{peakC}	change-from-baseline in J-T_{peakC}
$\Delta\Delta$J-T_{peakC}	placebo-adjusted change-from-baseline J-T_{peakC}
MAD	multiple ascending dose
msec	millisecond(s)
NSAID	nonsteroidal anti-inflammatory drug
PBMC	peripheral blood mononuclear cell

PD	pharmacodynamic
PK	pharmacokinetic
QA	quality assurance
QTc	heart rate-corrected QT interval
ΔQTc	change-from-baseline in QTc
ΔΔQTc	placebo-adjusted change-from-baseline QTc
QTcF	heart rate-corrected QT interval using the Fridericia correction
RIHSC	Research Involving Human Subjects Committee
SAD	single ascending dose
SAE	serious adverse event
SD	standard deviation
TdP	torsade de pointes
TEAE	treatment-emergent adverse event
T_{max}	time of C_{max}
TQT	thorough QT
ULN	upper limit of normal
U_{max}	time of the largest mean $\Delta J\text{-}T_{peakC}$

2. Introduction

At clinical concentrations, drugs that predominantly block the potassium channel encoded by the human ether-à-go-go-related gene (hERG) without late sodium or L-type calcium current block ("predominant hERG" drugs) cause heart rate-corrected QT interval (QTc) prolongation and intermediate or high risk of torsade de pointes (TdP) (Johannesen et al, 2014; Crumb et al, 2016). However, at clinical concentrations, drugs that block hERG with approximately equipotent late sodium and/or calcium block ("balanced ion channel" drugs) can cause QTc prolongation, but have a low risk of TdP. Thus, not all hERG block and QTc prolongation is associated with TdP.

The Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative is developing a preclinical cardiac safety evaluation of new drugs that provides a more accurate and comprehensive mechanistic-based assessment of proarrhythmic potential (Colatsky et al, 2016). The CiPA assay will involve assessment of drug block on multiple cardiac ion currents (including hERG, calcium, and late sodium) that will be integrated in a computational in silico model to classify drugs as low, intermediate, or high risk of TdP.

CiPA proposes to utilize an electrocardiogram (ECG) assessment in early clinical Phase 1 studies (single ascending dose [SAD] or multiple ascending dose [MAD] studies) to determine if there are unexpected ion channel effects, such as due to a human-specific metabolite or protein binding, compared to the preclinical ion channel data (Colatsky et al, 2016). Through analysis of prior clinical studies, the CiPA Phase 1 ECG working group identified the heart rate-corrected early repolarization ($J\text{-}T_{peak}$) interval ($J\text{-}T_{peakC}$) as the best biomarker to differentiate "balanced ion channel" drugs from "predominant hERG" drugs (Vicente et al, 2016). Drugs that exhibit "balanced ion channel" effects often have QTc prolongation, but do not exhibit $J\text{-}T_{peakC}$ prolongation (Johannesen et al, 2014; Johannesen et al, 2016). While these ECG signatures have been shown for multiple drugs and drug combinations blocking either hERG + late sodium or hERG + late sodium + calcium, it is still not clear whether QTc prolongation caused by hERG + calcium block (with no late sodium block) exhibits $J\text{-}T_{peakC}$ prolongation (Johannesen et al, 2014; Johannesen et al, 2016).

This clinical study consists of 2 parts: a 50-subject parallel part (Part 1) and a 10-subject crossover part (Part 2). These parts are included in the same protocol and study due to the similarity of the inclusion and exclusion criteria, similar procedures, and similar primary goals. In addition, the 10-subject placebo group from the parallel part of the study can potentially be used interchangeably with the crossover part in exploratory analysis. Up to 74 healthy subjects will be enrolled (including 14 potential replacement subjects).

2.1. Part 1: Drugs with Well Characterized Ion Channel Effects

Resampling of the data from prior clinical studies demonstrated that, with 9 subjects on active drug and 6 subjects on placebo, the use of exposure-response modeling could confirm the absence of J-T_{peakC} prolongation (upper bound of confidence interval [CI] <10 milliseconds [msec]) approximately 94% of the time for a "balanced ion channel" drug. This is nearly identical to the ability of QTc exposure-response analysis to confirm the absence of QTc prolongation in small sample sizes. Thus, Part 1 of the current study was designed with a sample size and structure similar to the "IQ-CSRC Prospective Study" (Darpo et al, 2014 and Darpo et al, 2015) that had similar power as a SAD study to exclude small QTc effects and supported the replacement of thorough QT (TQT) studies with exposure response modeling in early clinical studies, except the current study will focus on J-T_{peakC} for "balanced ion channel" drugs. Due to the long half-life of some drugs that are included in this study and the desire to more closely mimic SAD/MAD studies, Part 1 of the current clinical study will use an entirely parallel study design with 10 subjects in each arm.

Part 1 of the study will include 4 drugs with well-characterized ion channel effects, QTc effects, and TdP risk. Three (3) drugs will be "balanced ion channel" blockers (approximately equipotent hERG and late sodium and/or calcium block) with low TdP risk (ranolazine, verapamil and lopinavir + ritonavir), and 1 drug will be a "predominant hERG" blocker with TdP risk (chloroquine) (Crumb et al, 2016).

Approximately 50 healthy subjects are planned for enrollment. Multiple doses of each drug will be given to 40 subjects on 3 consecutive days to achieve low and high exposures on Days 1 and 3, respectively, and 10 subjects will receive placebo. Data will be analyzed using linear mixed-effects exposure-response models with the following primary endpoints:

- The criterion for the "balanced ion channel" drugs (ranolazine, verapamil, lopinavir + ritonavir) will be an upper bound of the 2–sided 90% CI that is <10 msec for the projected J-T_{peakC} effect at the peak plasma level on Day 3.
- The criterion for the "predominant hERG" drug (chloroquine) will be an upper bound of the 2–sided 90% CI that is ≥10 msec for the projected QTc effect at the peak plasma level on Day 1.

Of note, based on prior studies, ranolazine, lopinavir + ritonavir, and chloroquine are all expected to cause mean QTc prolongations of approximately 10 msec on Day 1 and 20 msec on Day 3.

2.2. Part 2: Combination of hERG and Calcium Block

Resampling and further simulation of the data from our prior clinical study (SCR-003) demonstrated that, with 8 subjects, the use of exposure-response modeling could detect

the effect of inward current block in the QTc and J-T_{peakC} slopes associated with hERG block approximately 99% and 99% of the time using a crossover design for QTc and J-T_{peakC}, respectively (i.e., the late sodium blocker significantly reduced prolongation from the hERG blocker). For Part 2, we anticipate the selected dose for the calcium blocker will result in similar or larger QTc and J-T_{peakC} effects than those observed in SCR-003 because the calcium current is larger than the late sodium current. Therefore, and to account for potential dropouts, Part 2 of the study will use a crossover design with 10 subjects.

Part 2 of the study will include 2 oral drugs (dofetilide and diltiazem) with well-characterized individual ion channel effects and TdP risk. Dofetilide is a "predominant hERG" blocker while diltiazem is a calcium channel blocker.

Approximately 10 healthy subjects are planned for enrollment. Multiple doses of each drug will be given either separately or together to 5 subjects in each treatment sequence on 3 consecutive days in each of the 2 periods to achieve high diltiazem concentration and similar dofetilide exposures (dofetilide alone versus dofetilide + diltiazem) on Day 3. Data will be analyzed using linear mixed-effects exposure-response models with the following primary endpoints:

- We will assess whether the projected QT_C effect of dofetilide alone will be significantly greater (i.e., $p<0.05$) than the projected QT_C effect of the combination of dofetilide + diltiazem. This will be assessed at the dofetilide peak plasma level on Day 3 (computed from the combination of dofetilide + diltiazem) on the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem data using a linear mixed-effects model.
- If the previous test is statistically significant for QTc, the same test will be performed for J-T_{peakC} .

Of note, based on prior studies, dofetilide alone is expected to cause mean QTc prolongation of approximately 10 msec on Day 1 and 30 msec on Day 3.

While not part of the primary objective, as in the prior FDA-Spaulding studies (Johannesen et al, 2014 and 2016), genetic variations may be examined as potential predictors of pharmacokinetic (PK) and pharmacodynamic (PD) response in an exploratory analysis. A weighted score of more than 60 common genetic variants associated with baseline QTc has been observed to correlate with individual subject PD response. In addition, as in the prior FDA-Spaulding study (SCR-003), a blood sample will be collected to isolate peripheral blood mononuclear cells (PBMCs) to enable potential reprogramming to induced pluripotent stem cells (iPSCs). The iPSCs can then be differentiated to other cell types (e.g., iPSC-cardiomyocytes, iPSC-hepatocytes). While all subjects will provide informed consent for this analysis, it is anticipated that iPSCs may only be generated for subjects with extreme PK or PD response to enable further study of mechanisms for individual subject variation.

3. Study Objectives

3.1. Primary Objectives

The primary objectives of this study are:

1. To confirm that exposure-response analysis of the electrocardiographic QTc and J-T_{peakC} intervals in Phase 1 clinical pharmacology studies can be used to confirm that "balanced ion channel" drugs do not cause J-T_{peakC} prolongation and "predominant hERG" drugs cause QTc prolongation.
2. To test the hypothesis that calcium channel block (diltiazem) can reduce the QTc prolongation from hERG block (dofetilide) by shortening J-T_{peakC}.

4. Investigational Plan

4.1. Study Design

4.1.1 Part 1

Part 1 of the study will assess primary objective 1. Part 1 will be a double-blind, randomized, placebo-controlled, 1-period parallel design to assess the effect of 4 marketed drugs and 1 placebo on the QTc and J-T_{peakC} intervals in 50 healthy subjects. Part 1 will include 4 drugs with well-characterized ion channel effects, QTc effects, and TdP risk. Three (3) drugs will be "balanced ion channel" blockers (approximately equipotent hERG and late sodium and/or calcium block) with low TdP risk (ranolazine, verapamil, and lopinavir + ritonavir) and 1 drug will be a "predominant hERG" blocker with TdP risk (chloroquine). A parallel design similar to a SAD or MAD Phase 1 study will be used that will result in each study drug being administered to 10 subjects, and placebo to 10 subjects, in 1 period of 3 consecutive days.

4.1.2 Part 2

Part 2 of the study will assess primary objective 2. Part 2 will be a double-blind, randomized, 2-period crossover design to assess the effect of hERG block (dofetilide) versus calcium block (diltiazem) on the QTc and J-T_{peakC} intervals in 10 healthy subjects. Part 2 will include 2 oral drugs (dofetilide and diltiazem) with well-characterized individual ion channel effects and TdP risk. Dofetilide is a "predominant hERG" blocker while diltiazem is a calcium channel blocker. A crossover design similar to a drug-drug interaction Phase 1 study will be used that will result in each study drug or a combination of both being administered to 10 subjects in 2 periods of 3 consecutive dosing days each.

4.1.3 Common Procedures

To maintain the study blind, subjects will be blindfolded during study drug administration. The ECG analysis will be blinded to treatment, time, and study day/subject identifiers.

At the study clinic (Spaulding Clinical Research unit in West Bend, Wisconsin), subjects will be screened for study eligibility from Day –28 to Day –1. On Day 0, subjects will enter the study clinic for check-in procedures the day before study drug administration. During the screening visit, the inclusion and exclusion criteria will be reviewed to ensure the subject is appropriate for the study. The informed consent form will be reviewed with the subject by a member of the study team and the subject will be encouraged to ask questions to ensure the subject has a good understanding of the study. If the subject is eligible and agrees to participate, the subject will be asked to sign the informed consent form before any study-specific procedure is performed, including randomization.

After the consent process is complete, demographic data, medical history, and concomitant medications (including over-the-counter and complimentary/alternative supplements) will be recorded. A physical examination will be performed by a study team member. Clinical laboratory tests (hematology, serum chemistry, and urinalysis) will be performed. Female subjects must have a negative pregnancy test result. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant or the subject has a positive pregnancy test result, the subject will be instructed to follow-up with his or her personal physician. Screening tests will be performed within 28 days of and no later than 1 day before Day 1.

Screening procedures will be performed by clinic staff, and all screening results will be evaluated by the study clinician/investigator against the inclusion/exclusion criteria to confirm subject eligibility. At check-in, eligibility criteria will be reviewed, any changes in medical history (including concomitant medications) will be documented, vital sign measurements and a 12-lead ECG will be performed, clinical laboratory, drug and alcohol, and pregnancy tests (for females) will be performed, an intravenous (IV) catheter may be inserted into the subject's forearm region for blood collection (if needed), study drug will be administered, and blood samples will be collected per protocol.

The FDA project biostatistician will prepare the randomization schedule, and subjects will be randomly assigned to 1 of 2 different study parts. Subsequently:

- Subjects assigned to Part 1 will be randomly assigned to 1 of 5 different treatments, thus each subject will receive one of the following treatments by the end of the study: 1) ranolazine, 2) verapamil, 3) lopinavir + ritonavir, 4) chloroquine, or 5) placebo.
- Subjects assigned to Part 2 will be randomly assigned to 1 of 2 different treatment sequences, during which each subject will receive each of the following treatments by the end of the study: 1) dofetilide and 2) diltiazem alone and dofetilide + diltiazem.

Subjects will enter the study clinic for check-in procedures the day before study drug administration of the first period (Day 0). At check-in, continuous/Holter 12-lead ECGs will be recorded from early afternoon until the subjects go to sleep. During this period, subjects will perform a sequence of postural maneuvers (e.g., 10 minutes supine, 10 minutes sitting, 10 minutes standing, 10 minutes sitting, 10 minutes supine) to characterize ECG biomarkers and heart rate relationship. Continuous/Holter 12-lead ECGs will be recorded from 1 hour before the first oral dose on Day 1 until 73 hours after the first dose at each treatment period. All study drugs will be administered orally. On Days 1, 2, and 3 of each period subjects will receive their assigned treatment according to the randomization schedule (i.e., on Days 1, 2 and 3 for subjects in Part 1, and on Days 1, 2, 3, 8, 9 and 10 for subjects in Part 2).

In the study clinic, study drugs will be administered throughout 3 consecutive days per period as described in the dosing schedule below, and continuous 12-lead ECGs will be recorded using the telemetry system for approximately 74 hours. In Part 1, the first oral dose on Days 1, 2, and 3 (chloroquine or placebo) will be administered after subjects have fasted for at least 8 hours and 1 hour before breakfast. The morning and evening oral doses on Days 1, 2, and 3 (ranolazine, verapamil, lopinavir + ritonavir, or placebo) will be administered together with food (e.g., with breakfast at 8:30 am on Days 1, 2, and 3 and with a snack at 8:30 pm on Days 1 and 2). The afternoon doses (verapamil or placebo) will be given with a snack at 2 hours after lunch (e.g., approximately 2:30 pm). In Part 2, the oral doses of dofetilide or diltiazem or diltiazem + dofetilide or placebo, depending on treatment sequence, on Days 1, 2, and 3 of each period will be administered together with food (e.g., with breakfast at 8:30 am on Days 1, 2, and 3 and with a snack at 8:30 pm on Days 1 and 2 of each period). Subjects will stay in the study clinic overnight during each period, and blood samples for PK analysis will be collected at a set of pre-specified time points on Days 1, 2, 3, and 4 for Part 1 and Part 2 and on Days 8, 9, 10, and 11 for Part 2. While in the study clinic, subjects will receive standardized meals at consistent times relative to dosing.

Collection of the PK samples will be time-matched to the ECG extractions; therefore, the subjects will be supine for approximately 10 minutes before the defined time points and for approximately 5 minutes after for ECG extraction/PK sample collection. The PK sample will be collected after the ECG extraction to avoid changes in autonomic tone associated with the psychological aspects of blood collection and the reduction in blood volume subsequent to blood collection. A 5-minute window for PK sample collection is allowed after the ECG extraction window (see Table 8-2).

4.1.4 Dosing Schedule

Part 1:

- Treatment A: Ranolazine (1500 mg twice daily [BID] for 2.5 days) and placebo.

  - On Days 1, 2, and 3, an oral dose of placebo will be administered 1 hour before breakfast and a single 1500 mg oral dose of ranolazine will be administered with breakfast.
  - On Days 1 and 2, an oral dose of placebo will be administered in the afternoon approximately 2.5 hours after lunch and a single 1500 mg oral dose of ranolazine will be administered in the evening with a snack.
- Treatment B: Verapamil (120 mg IR morning and afternoon doses on Days 1 and 2, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR morning dose on Day 3) and placebo.
  - On Days 1, 2, and 3, an oral dose of placebo will be administered 1 hour before breakfast and a single 120 mg oral dose of verapamil immediate release (IR) will be administered with breakfast.
  - On Days 1 and 2, a single 120 mg oral dose of verapamil IR will be administered in the afternoon approximately 2 hours after lunch.
  - On Days 1 and 2, a single 240 mg oral dose of verapamil extended release (ER) will be administered in the evening with a snack.
- Treatment C: Lopinavir + ritonavir (800 mg/200 mg BID for 2.5 days) and placebo
  - On Days 1, 2, and 3, an oral dose of placebo will be administered 1 hour before breakfast and a single 800 mg/200 mg oral dose of lopinavir + ritonavir will be administered with breakfast.
  - On Days 1 and 2, an oral dose of placebo will be administered in the afternoon approximately 2.5 hours after lunch and a single 800 mg/200 mg oral dose of lopinavir + ritonavir will be administered in the evening with a snack.
- Treatment D: Chloroquine (1000 mg on Days 1 and 3, 500 mg on Day 2) and placebo
  - On Days 1 and 3, a single 1000 mg oral dose of chloroquine will be administered 1 hour before breakfast and an oral dose of placebo will be administered with breakfast.
  - On Day 2, a single 500 mg oral dose of chloroquine will be administered 1 hour before breakfast and an oral dose of placebo will be administered with breakfast and in the afternoon approximately 2.5 hours after lunch.
  - On Days 1 and 2, an oral dose of placebo will be administered in the afternoon approximately 2.5 hours after lunch and in the evening with a snack.
- Treatment E: Placebo

- On Days 1 and 2, an oral dose of placebo will be administered 1 hour before breakfast, with breakfast, in the afternoon approximately 2.5 hours after lunch and in the evening with a snack.
- On Day 3, an oral dose of placebo will be administered 1 hour before breakfast and with breakfast.

Part 2: Note that the subjects in Part 2 will receive both Treatment F and Treatment G in a randomized crossover fashion with Day 1 of each treatment period separated by 1 week.

- Treatment F: Dofetilide (0.125 mg on Day 1, placebo on Day 2, 0.375 mg on Day 3), and placebo
  - On Day 1, an oral dose of placebo will be administered 1 hour before breakfast, a single 0.125 mg oral dose of dofetilide will be administered with breakfast, an oral dose of placebo will be administered in the afternoon approximately 2.5 hours after lunch, and an oral dose of placebo will be administered in the evening with a snack.
  - On Day 2, an oral dose of placebo will be administered 1 hour before breakfast, with breakfast, in the afternoon approximately 2.5 hours after lunch, and in the evening with a snack.
  - On Day 3, an oral dose of placebo will be administered 1 hour before breakfast, and a single 0.375 mg oral dose of dofetilide will be administered with breakfast.
- Treatment G: Diltiazem (IR and ER) 120 mg IR morning dose on Day 1, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR (when coadministered with dofetilide) on Day 3 and placebo.
  - On Day 1, an oral dose of placebo will be administered 1 hour before breakfast, a single 120 mg oral dose of diltiazem IR will be administered with breakfast, an oral dose of placebo will be administered in the afternoon approximately 2.5 hours after lunch, and a single 240 mg oral dose of diltiazem ER will be administered in the evening with a snack.
  - On Day 2, an oral dose of placebo will be administered 1 hour before breakfast, with breakfast, and in the afternoon approximately 2.5 hours after lunch, and a single 240 mg oral dose of diltiazem ER will be administered in the evening with a snack.
  - On Day 3, an oral dose of placebo will be administered 1 hour before breakfast, and a single 120 mg oral dose of diltiazem IR in combination with a single 0.25 mg oral dose of dofetilide will be administered with breakfast.

Scheduled safety ECGs will be performed at the approximate time of maximum observed plasma concentration (C_{max}) (T_{max}) of drugs (10:30 am) following the morning dose on

Days 1, 2 and 3 for Part 1 and Days 1, 2, 3, 8, 9 and 10 for Part 2 (see Table 8-2). The 2 drugs associated with TdP (dofetilide and chloroquine) will only be given in the morning. At all other times, subjects will be monitored via telemetry from their 12-lead Holter ECGs. On the safety ECGs, if the absolute QTc using the Fridericia correction (QTcF) is greater than 500 msec (as defined by automatically measured intervals) and the change in QTcF from the Baseline ECG is greater than 60 msec, which is confirmed on a second safety ECG, then the oral dose(s) will not be administered for that treatment day. An adverse event (AE) will be reported and the medical monitor will be notified as quickly as possible (see Section 4.7.3.4). Additionally, if a subject has an on-treatment 12-lead ECG with third degree heart block except if brief (no more than 2 consecutive dropped beats per episode) during sleep, or second degree heart block occurring at night with persistent severe bradycardia (heart rate <40 bpm), or second degree heart block during the day that is symptomatic or progressive, the finding should be recorded as an AE and reported immediately to the medical monitor. Conditions for whether a subject may receive subsequent doses or be discontinued are described in Section 4.7.3.4.

Subjects assigned to Part 1 will be discharged from the study on Day 4 and subjects assigned to Part 2 will be discharged from the study on Day 11 after completion of all study procedures and removal of IV catheter (if applicable). If a subject discontinues from the study prematurely, all procedures scheduled for discharge Day (i.e., Day 4 for Part 1 and Day 11 for Part 2) will be performed. Meal timing and components, activity levels, and general conditions in the study clinic will be as similar as possible on the treatment days.

Safety will be evaluated in terms of AEs, clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG results, and physical examination findings (see Section 8.1).

4.1.5 Risk/Benefit

Subjects will be informed that participation in a human PK-PD study like the present one cannot be of benefit to healthy volunteers. Nevertheless, the information from the physical examination, vital sign measurements, and ECG results may be shared with the subject's personal physician if this is the subject's choice. Subjects will be informed that it is also their choice to inform their personal physician that they are participating in this research study.

Subjects will be informed that their contribution to the study is of major importance to agencies like the U.S. FDA for helping this agency better evaluate the effects of different medications on the ECG. However, since this is a study involving healthy volunteers, subjects will be informed that they have the alternative not to participate.

Subjects will be informed that they may be exposed to risks associated with the pharmacological properties of the investigational product and the study procedures. The following summary of potential AEs for the study drugs will be provided to and discussed with the subjects:

1. Ranolazine 1500 mg BID for 2.5 days: QT interval prolongation, dizziness, nausea, asthenia, constipation, headache, bradycardia, palpitations, tinnitus, vertigo, abdominal pain, dry mouth, vomiting, peripheral edema, dyspnea, hypotension, orthostatic hypotension
2. Verapamil hydrochloride (HCl) 120 mg IR morning and afternoon doses on Days 1 and 2, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR morning dose on Day 3: constipation, dizziness, nausea, hypotension, headache, edema, congestive heart failure, pulmonary edema, fatigue, elevated liver enzymes, dyspnea, bradycardia, atrioventricular block, rash, flushing
3. Lopinavir + ritonavir 800 mg/200 mg BID for 2.5 days: QT and PR interval prolongation, hyperglycemia, hepatic transaminase elevations, pancreatitis, diarrhea, nausea, vomiting, hypertriglyceridemia, hypercholesterolemia
4. Chloroquine 1000 mg on Day 1, 500 mg on Day 2, 1000 mg on Day 3: Blurred vision, nausea, vomiting, abdominal cramps, headache, diarrhea
5. Dofetilide 0.125 mg on Day 1, 0.25 mg or 0.375 mg on Day 3: QT interval prolongation, dizziness, fainting, tachycardia, headache, chest pain, diarrhea, unusual sweating, vomiting, anorexia, polydipsia.
6. Diltiazem 120 mg IR morning dose on Day 1, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR on Day 3: hypotension (asymptomatic and symptomatic), injection site reactions; vasodilation (flushing), and arrhythmia (junctional rhythm or isorhythmic dissociation).

All of the selected doses (Section 4.8.1) have been evaluated previously for all drugs, except for the coadministration of dofetilide and diltiazem. An increase in dofetilide exposure of 40% to 50% is expected when dosed in combination with diltiazem (dofetilide 0.25 mg and diltiazem 120 mg) because of cytochrome P450 (CYP) 3A4 inhibition by diltiazem. This is expected to result in a C_{max} of 2.25 ng/mL, which corresponds with the levels expected from a single oral dose of 0.375 mg of dofetilide, which is below the maximum dose in the label and previous interaction studies with other CYP3A4 inhibitors that assessed interaction with dofetilide 0.5 mg BID and observed an increase in dofetilide exposure of 40% to 50% (dofetilide + verapamil and dofetilide + ketoconazole). In addition, dofetilide-induced QTc prolongation is expected to be lower with dofetilide + diltiazem versus dofetilide alone due to QTc shortening caused by calcium current block associated with diltiazem. In summary, and from a risk standpoint, it is important to note that: 1) this will be a single oral dose expected to result

in dofetilide exposure below dofetilide levels associated with recommended dose in the label (0.5 mg BID), 2) that the associated QTc prolongation is expected to be less than QTc prolongation caused by a dofetilide 0.375 mg single dose, and 3) that subjects will be continuously monitored. Section 4.8 contains details of dose selection rational for the dofetilide + diltiazem combination.

The study drugs will not be administered to anyone who is pregnant. All women must take a pregnancy test before receiving any study drug in this study. All woman of childbearing potential enrolled on this study will be informed that they must use effective birth control methods (abstinence, intrauterine device, and contraceptive foam and a condom [i.e., double-barrier method]) during treatment. Subjects will be informed that they must notify the investigator if they or their female partners become pregnant during the course of the study.

Subjects will be informed that insertion of an IV catheter may be required for blood sample collection and, during insertion of the catheter, soreness, bruising, or infection at the insertion site are possible but unlikely. Subjects will also be informed that dizziness and lightheadedness may occur during direct venipuncture, insertion of the IV catheter, or during blood collection.

Subjects will be informed that they may eat only meals and snacks that are provided during periods of their stay in the study clinic, and that they must consume all of each meal that is served at a reasonable pace (within 25 minutes).

Subjects will be informed that blood samples will be collected for genetic testing to explore how a person's genes affect the way the body and drug interact.

Subjects will be informed that blood samples will be collected for generation of subject-specific iPSCs, which can enable studying how the individual's cells respond to the drug in the laboratory.

Subjects will be informed that the confidentiality of their data will be respected at all times according to state law, and the study personnel handling their study data are bound by confidentiality agreements.

Subjects will be informed that the study drug and all tests, procedures, and visits required by the study are provided at no cost to them. If subjects become ill or physically injured because of participation in this study, they will be informed that costs of treatment will not be covered by the sponsor.

If a subject becomes pregnant, she will be informed that neither Spaulding Clinical Research nor the sponsor will be responsible for the cost of any obstetric or related care, or for the child's care.

4.2. Selection of Study Population

Subjects will be screened and the data collected will be reviewed by the principal investigator. Only those subjects who meet all of the eligibility criteria will be enrolled. Approximately 60 healthy subjects are planned for enrollment, of which 50 will be assigned to Part 1 and 10 will be assigned to Part 2 at randomization. Up to 14 subjects may be qualified as replacements as described in Section 4.5.2. Thus, a maximum of 74 subjects will be exposed to study drugs and procedures during the study. Every effort will be made to maintain an approximate 50:50 male to female gender distribution.

4.2.1 Inclusion Criteria

Subjects who meet all of the following inclusion criteria will be eligible to participate in the study:

1. Subject signs an Institutional Review Board (IRB)-approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] authorization) before any study-related procedures are performed.
2. Subject is a healthy man or woman, 18 to 50 years of age, inclusive, who weighs at least 50 kg (110 pounds) and has a body mass index of 18 to 30 kg/m^2, inclusive, at Screening.
3. Subject has normal medical history findings, clinical laboratory results, vital sign measurements, 12-lead ECG results, and physical examination findings at Screening or, if abnormal, the abnormality is not considered clinically significant (as determined and documented by the investigator or designee).
4. Female subjects must be either at least 2 years postmenopausal, surgically sterile, or practicing 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique).
5. Female subjects must not be pregnant or lactating before enrollment in the study.
6. Male or female subjects must agree to practice 2 highly effective methods of birth control (as determined by the investigator or designee; one of the methods must be a barrier technique) from Screening until 30 days after the last dose of study drug.
7. Subject is highly likely (as determined by the investigator) to comply with the protocol-defined procedures and to complete the study.

4.2.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Subject has a safety 12-lead ECG result at Screening or Check-in with evidence of any of the following abnormalities:
   - QTcF >430 msec
   - PR interval >220 msec or <120 msec
   - QRS duration >110 msec
   - Second- or third-degree atrioventricular block
   - Complete left or right bundle branch block or incomplete right bundle branch block
   - Heart rate <50 or >90 beats per minute
   - Pathological Q-waves (defined as Q-wave >40 msec)
   - Ventricular pre-excitation
2. Subject has more than 12 ectopic beats during the 3-hour Holter ECG at Screening.
3. Subject has a history of unexplained syncope, structural heart disease, long QT syndrome, heart failure, myocardial infarction, angina, unexplained cardiac arrhythmia, TdP, ventricular tachycardia, or placement of a pacemaker or implantable defibrillator. Subjects will also be excluded if there is a family history of long QT syndrome (genetically proven or suggested by sudden death of a close relative due to cardiac causes at a young age) or Brugada syndrome.
4. Subject has a history or current evidence of any clinically significant (as determined by the investigator) cardiovascular, dermatologic, endocrine, gastrointestinal, hematologic, hepatic, immunologic, metabolic, neurologic, psychiatric, pulmonary, renal, urologic, and/or other major disease or malignancy (excluding nonmelanoma skin cancer). The investigator may allow exceptions to these criteria (e.g., cholecystectomy, childhood asthma) following discussion with the medical monitor.
5. Subject has a history of thoracic surgery.
6. Subject has any condition possibly affecting study drug absorption (e.g., gastrectomy, Crohn's disease, irritable bowel syndrome).
7. Subject has a skin condition likely to compromise ECG electrode placement.
8. Subject is a female with breast implants.
9. Subject's laboratory test results at Screening or Check-in are outside the reference ranges provided by the clinical laboratory and considered clinically significant (as determined and documented by the investigator or designee).

10. Subject's laboratory test results at Screening or Check-in indicate hypokalemia, hypocalcemia, or hypomagnesemia according to lower limits of the reference ranges provided by the clinical laboratory.
11. Subject's laboratory test results at Screening or Check-in are >2 × the upper limit of normal (ULN) for alanine aminotransferase or aspartate aminotransferase, >1.5 × ULN for bilirubin, or >1.5 × ULN for creatinine.
12. Subject has a positive test result at Screening for human immunodeficiency virus (HIV) I or II antibody, hepatitis C virus (HCV) antibodies, or hepatitis B surface antigen (HBsAg).
13. Subject has a mean systolic blood pressure <100 or >140 mmHg or a mean diastolic blood pressure <50 or >90 mmHg at either Screening or Check-in. Blood pressure will be measured in triplicate after the subject has been resting in a supine position for a minimum of 5 minutes.
14. Subject has a known hypersensitivity to any of the study drugs or related compounds.
15. Subject has consumed alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice within 48 hours before dosing or anticipates an inability to abstain from these products throughout the duration of the study.
16. Subject has used nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks before Screening (self-reported).
17. Subject is unable to tolerate a controlled, quiet, study conduct environment, including avoidance of music, television, movies, games, and activities that may cause excitement, emotional tension, or arousal during the prespecified time points (e.g., before and during ECG extraction windows).
18. Subject is unwilling to comply with study rules, including the study-specific diet, attempting to void at specified times (e.g., before ECG extraction windows), remaining quiet, awake, undistracted, motionless, and supine during specified times, and avoiding vigorous exercise as directed.
19. Subject has a history of consuming more than 14 units of alcoholic beverages per week within 6 months before Screening, has a history of alcoholism or drug/chemical/substance abuse within 2 years before Screening (Note: 1 unit = 12 ounces of beer, 4 ounces of wine or 1 ounce of spirits/hard liquor), or has a positive test result for alcohol or drugs of abuse at Screening or Check-in.
20. Subject has used any prescription or nonprescription drugs (including aspirin or nonsteroidal anti-inflammatory drugs [NSAIDs] and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer), or

complementary and alternative medicines within 28 days before the first dose of study drug.

21. Subject is currently participating in another clinical study of an investigational drug or has been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.
22. Subject has had any significant blood loss, donated 1 unit (450 mL) of blood or more, or received a transfusion of any blood or blood products within 60 days, or donated plasma within 7 days before Check-in.
23. Subject has any other condition that precludes his or her participation in the study (as determined by the investigator).
24. Subject is unwilling to have genetic analysis performed or a blood sample collected for isolating PBMCs.

4.3. Screening Failures

Subjects who sign and date the informed consent form but who fail to meet the inclusion and exclusion criteria are defined as screening failures. A screening log, which documents the subject initials and reason(s) for screening failure, will be maintained by the investigator for all screening failures. A copy of the log should be retained in the investigator's study files.

If a subject fails the screening process because of an abnormal laboratory result, they can receive a copy of the results upon request. The investigator will determine if follow-up for the abnormal laboratory result is needed, and will encourage the subject to follow-up with his or her personal physician as appropriate. All subjects will be informed as to the reason(s) they are excluded from study participation, even if follow-up is not required. If a subject fails the screening process because of a positive test result for human immunodeficiency virus or hepatitis, the positive result will be reported to local health authorities as required by law.

4.4. Termination of Study or Investigational Site

4.4.1 Criteria for Termination of the Study

The study will be completed as planned unless one of the following criteria is satisfied that requires early termination of the study.

- New information regarding the safety or efficacy of the study drug(s) that indicates a change in the known risk profile for the study drug(s), such that the risk is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objective or compromises subject safety.

4.4.2 Criteria for Termination of Investigational Site

The study site may be terminated if the site (including the investigator) is found in significant violation of GCP, the protocol, the contractual agreement, or is unable to ensure adequate performance of the study.

In the event that the sponsor elects to terminate the study or the investigational site, a study-specific procedure for early termination will be provided by the sponsor; the procedure will be followed by the applicable investigational site during the course of termination.

4.5. Criteria for Subject Withdrawal

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn by the investigator without the approval of the subject based on the investigator's clinical judgment. A subject is not required to provide a written request to withdraw from the study; however, a written request is required if a subject withdraws consent for his or her personal data to be used for study-related purposes.

A subject may be discontinued for any of the following reasons:

- AE: The subject has experienced an AE that, in the opinion of the investigator, requires early termination. The appropriate electronic case report form (eCRF) must be completed for each AE. If a subject is discontinued from the study due to an AE, the investigator is required to follow-up with the subject until the event resolves or becomes stable. If a subject dies during the study, the cause of death must be reported as a serious AE (SAE), with an outcome of death noted in the eCRF.

  Additionally, if a subject has an on-treatment safety 12-lead ECG with a QTcF interval ≥500 msec (as defined by automatically measured intervals) and an increase in QTcF interval >60 msec over Baseline at any time point where a safety 12-lead ECG is recorded, which is confirmed by a second safety 12-lead ECG performed within 1 hour, the ECG change should be recorded as an AE and reported immediately to the medical monitor. Conditions for whether a subject may receive subsequent doses or be discontinued are described in Section 4.7.3.4.

  Additionally, if a subject has an on-treatment 12-lead ECG with third degree heart block except if brief (no more than 2 consecutive dropped beats per episode) during sleep, or second degree heart block occurring at night with persistent severe bradycardia (heart rate <40 bpm), or second degree heart block during the day that is symptomatic or progressive, the finding should be recorded as an AE and reported immediately to the medical monitor. Conditions for whether a subject may receive subsequent doses or be discontinued are described in Section 4.7.3.4.

- Protocol Violation: The subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unnecessary risk to the subject's health.
- Withdrawal by Subject: The subject (or other responsible individual [e.g., caregiver]) wishes to withdraw from the study in the absence of a medical need.

  NOTE: Withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category.
- Study Terminated by Sponsor: The sponsor, IRB, FDA, or other regulatory agency terminates the study.
- Pregnancy: The subject is found to be pregnant.

  NOTE: If the subject is found to be pregnant, the subject must be withdrawn immediately. The pregnancy will be followed-up to term, and the outcome, including any premature termination will be recorded. All live births must be followed for a minimum of 30 days or until the first well-baby visit.
- Other.

  NOTE: This category records withdrawals caused by an accidental or a medical emergency, unblinding, and other rare cases. The specific reason should be recorded in the comment space of the eCRF.

4.5.1 Handling of Withdrawals

The investigator may terminate a subject's study participation at any time during the study when the subject meets the criteria described in Section 4.5. In addition, a subject may discontinue his or her participation without giving a reason at any time during the study. Subjects will be informed that their participation in the study is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Should a subject's participation be discontinued, the primary reason for termination must be recorded. In addition, efforts should be made to perform all procedures scheduled for the early termination visit. Any data and samples collected before subject withdrawal will become the property of the sponsor.

4.5.2 Replacement Subjects

Approximately 60 healthy subjects are planned for enrollment, of which 50 will be assigned to Part 1 and 10 will be assigned to Part 2 at randomization. Up to 14 subjects may be qualified as replacements. Thus, a maximum of 74 subjects will be exposed to study drugs and procedures during the study.

4.5.2.1 Part 1

It is anticipated that Part 1 of this study will be completed in 3 cohorts, with 20 subjects in Cohort 1, 20 subjects in Cohort 2, and 10 subjects in Cohort 3 if no replacement subjects are needed. After consultation with the sponsor, subjects may be replaced in a later cohort (e.g., Cohort 3) if it is estimated that fewer than 8 subjects will complete the study in a study arm of Part 1. A replacement algorithm will be pre-specified in the subject replacement plan to guide the unblinded Spaulding pharmacist to determine this while the rest of the staff and sponsor remain blinded. A maximum of 14 replacement subjects may be enrolled in the study, and replacement subjects (if needed) must complete the treatment period. A replacement subject will receive the same treatment as the subject being replaced. During Part 1 of the study, a maximum of 64 subjects will be exposed to study drugs and procedures.

If a subject vomits after dosing, a PK blood sample will be collected and, if the subject vomits immediately after dosing, the sponsor will be consulted regarding a decision to continue the subject in the study. Under no circumstances will a dose of any of the study drugs be repeated.

4.5.2.2 Part 2

It is anticipated that Part 2 of this study will be completed in 1 cohort with 10 subjects with a crossover design. As described in Section 4.9.1, this cohort is preloaded with 2 replacements, and the study should be statistically powered with just 8 completers. Similar to Part 1, subjects may be replaced in a later cohort if it is estimated that fewer than 8 subjects will complete Part 2. A maximum of 14 replacement subjects may be enrolled in the study, and replacement subjects (if needed) must complete the treatment period. A replacement subject will receive the same treatment as the subject being replaced. During Part 2 of the study, a maximum of 24 subjects will be exposed to study drugs and procedures.

4.6. Study Visits

4.6.1 Recruitment

Recruitment materials (e.g., internet, radio, and print advertisements, social media posts) will be approved by the local IRB and FDA Research Involving Human Subjects Committee (RIHSC) before telephone screening. The sponsor is responsible for registration of the study on clinicaltrials.gov; however, this may not occur until RIHSC has approved the final study protocol.

4.6.1.1 Compensation

Subjects will be offered payment for Screening; however, if the results of their alcohol and drug screening tests are positive, they will not be compensated. Subjects who complete the entire study (Day 0 to Day 4 [Part 1] or Day 11 [Part 2]) will receive payment according to the schedule provided in the informed consent form. No special incentives are offered. Final payment will not be released until all follow-up procedures have been completed and accepted by the investigator.

If a subject chooses to withdraw from the study prematurely, he or she will only be compensated for completed days. If subjects are withdrawn for medical reasons or if the study is halted temporarily or permanently, the subjects will receive compensation proportional to the time spent in the study. No compensation will be provided if a subject is dismissed from the study for noncompliance (e.g., improper conduct, ingesting alcohol and/or drugs [including recreational drugs], tampering with the study drug, consuming any prohibited foods or beverages).

If subjects are required to stay in the clinic for a longer period for safety reasons, they will be compensated at a rate proportional to the entire compensation for the study. If a subject becomes ill or physically injured because of participation in this study, the subject will be referred for treatment.

4.6.2 Screening

The following procedures and assessments will be performed at Screening (Day –28 to Day –1):

- Obtain informed consent/HIPAA authorization (The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding.)

After informed consent is obtained:

- Review inclusion/exclusion criteria to confirm subject eligibility
- Record demographic information
- Measure height, weight, and calculate body mass index
- Perform serology screening (HIV antigen/antibody [Ag/Ab] Combo 1/2, HCV antibody, HBsAg)
- Record medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)

- Perform a serum pregnancy test (female subjects only)
- Record prior medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a 3-hour Holter ECG
- Perform a complete physical examination

4.6.3 Study Periods

Part 1 of this study has a parallel design with only 1 treatment period. Part 2 of this has a crossover design with 2 treatment periods; however, the subjects will be kept in the study clinic between the 2 treatment periods.

4.6.3.1 Check-in

The following procedures and assessments will be performed at Check-in (Day 0):

- Review inclusion/exclusion criteria to confirm subject eligibility
- Review medical history
- Perform alcohol and drug screening (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone)
- Perform a serum pregnancy test (female subjects only)
- Collect blood sample for genetic testing and isolating PBMCs. If it has been more than 14 days (prior to check-in) since the subject was screened for HIV Ag/Ab Combo 1/2, HCV antibody, and HBsAg, the subject will be re-screened for HIV Ag/Ab Combo 1/2, HCV antibody, and HBsAg
- Admit subject to the study clinic
- Randomization (after completion of check-in procedures on Day 0 or just before dosing on Day 1)
- Record concomitant medications
- Monitor for AEs

- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Perform a safety 12-lead ECG
- Perform a continuous Holter ECG from early afternoon until the subjects go to sleep; during this time, subjects will perform a sequence of postural maneuvers (e.g., 10 minutes supine, 10 minutes sitting) to characterize ECG biomarkers and heart rate relationship
- Perform a complete physical examination

4.6.3.2 Treatment

The following procedures and assessments will be performed during the treatment period according to the Schedules of Events (Section 8.1):

- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests at time points indicated in Table 8-1
- Begin the continuous 12-lead ECG recording using the telemetry system at least 1 hour before first dosing, verify that the ECG electrodes are connected appropriately, and verify that the time on the ECG recorder is synchronized to the study clock. At each ECG sampling time point, lead connections must be checked prior to the nominal time point.
- Administer study drug according to the randomization schedule
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature) at the time points specified in Table 8-2. If vital signs are scheduled at the same time as another event, vital signs will be measured after the ECG, but before blood sample collection.
- Perform a safety 12-lead ECG at the time points specified in Table 8-2. If scheduled for the same time, safety 12-lead ECGs will always be performed before vital sign measurement and blood sample collection.
- Collect PK blood samples (5 mL) at the time points specified in Table 8-2. The PK blood samples will always be collected after the ECG extraction, safety 12-lead ECGs, and vital sign measurement, if applicable at the specific time point.

4.6.3.3 Washout

There are no washout periods for Part 1 of this study. For Part 2, there will be a 5-day washout period between the last dose of Period 1 (Day 3 morning) and first dose of Period 2 (Day 8 morning).

4.6.4 Discharge (or Early Termination)

The following procedures and assessments will be performed before the subject is discharged from the study (Day 4 and/or Day 11) or at early termination:

- Perform a serum pregnancy test (female subjects only)
- Record concomitant medications
- Monitor for AEs
- Perform clinical laboratory tests (hematology, serum chemistry, and urinalysis)
- Measure vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature)
- Perform a safety 12-lead ECG (if QTcF is ≥500 msec or QTcF prolongation from baseline is >60 msec, the subject will continue to be monitored until discharge)
- Perform a complete physical examination
- End the continuous 12-lead ECG recording using the telemetry system at least 73 hours after time 0 for the treatment period
- Remove IV catheter (if applicable)
- Collect a single PK blood sample (5 mL). The PK blood samples will always be collected after the ECG extraction, safety 12-lead ECGs, and vital sign measurement (Table 8-2).
- Discharge subject from the study clinic after completion of all study procedures

4.7. Study Procedures

4.7.1 Electrocardiogram Assessments

4.7.1.1 Continuous 12-lead Electrocardiogram Acquisition

At Screening, a 3-hour Holter ECG will be performed in order to exclude any subjects who have more than 12 ectopic beats during this collection period. Subjects will be supine during the 3-hour Holter monitoring period, but will be provided with breaks as needed to use the restroom. At check-in, continuous/Holter 12-lead ECGs will be recorded from early afternoon until the subjects go to sleep. During this period, subjects

will perform a sequence of postural maneuvers (e.g., 10 minutes supine, 10 minutes sitting, 10 minutes standing, 10 minutes sitting, 10 minutes supine) to characterize ECG biomarkers and heart rate relationship.

On the treatment days, continuous 12-lead ECGs will be recorded using the telemetry system for a total of approximately 74 hours (starting 1 hour before first dosing of the treatment period and continuing until 73 hours after time 0 or first oral dose on Day 1). Ten-second 12-lead ECG tracings with the most stable heart rate and highest signal-to-noise ratio will be extracted in at least 3 replicates at 28 ECG extraction time points during the treatment period as follows: -1 hour, -30 minutes, 0 (immediately before first oral dose on Day 1), 0.5, 2, 2.5, 3.5, 4.5, 5, 6, 8.5, 14, 24, 27.5, 32.5, 38, 48, 50, 50.5, 51.5, 52.5, 53, 54, 54.5, 55, 59, 61, and 73 hours after time 0 or the first oral dose on Day 1 or Day 8. The ECG extractions at -1 hour, -30 minutes, and time 0 (immediately before first oral dose on Day 1) will be used for the baseline (see Table 8-2).

For all time points, the subjects will be in a supine position for approximately 10 minutes before and approximately 5 minutes after the time point. The subjects will also be in a supine position during the baseline and 73-hour time point.

All ECG extractions time-matched to the PK samples will be obtained before the PK sample collection time. At all times during ECG extractions (from supine rest until after the blood sample is collected), subjects will be isolated (not able to see each other) in a quiet environment that is free from external stimuli. A 10-minute ECG extraction window that either precedes or straddles the extraction time point will be utilized to capture ECGs of adequate quality.

The ECGs will be over-read in a blinded manner by a central ECG laboratory.

Continuous 12-lead ECGs will be obtained digitally using a Mortara telemetry recorder and read centrally (Section 4.7.1.2). The continuous ECG recording will contain simultaneously recorded 12 leads sampled at a frequency of at least 500 Hz with an amplitude resolution of at least 2.5 μV. All continuous ECG recordings will be performed using recorders of the same model, produced by the same manufacturer, and configured to store the raw ECG without any filtering. Each subject will have his or her own ECG recorder for use throughout the study. Subjects will be instructed not to interfere with the recording equipment or cables, and any necessary procedures will be implemented to monitor the quality of the ECG recordings, such as detection of disconnected cables or electrodes. In addition, the clock on the ECG recorder will be confirmed to be consistent with the study clock at the clinical site; this will be confirmed on each treatment day.

The position of the electrodes must be marked using a nonirritant marker to ensure that the position of the electrodes on the treatment days is the same. Prior to placing the

electrodes on the body, the skin will be properly prepared, including surface abrasion and hair shaving, if necessary.

For all time points that require an ECG, PK blood sample collection, and vital signs measurement, the ECG will be matched closest to the time point, the PK blood samples will be collected after completion of the supine rest period, and vital signs will be measured before the PK blood sample.

4.7.1.2 Twelve-lead Electrocardiogram Core Laboratory Analysis

The ECGs will be over-read in a blinded manner by a central ECG laboratory. Fiducial points necessary for the primary endpoint will be measured using previously developed semi-automatic reading methodology. Prior to locating fiducial points, the ECGs will be preprocessed to remove baseline wander, powerline interference, and other noise artifacts, and a median beat will be constructed. The lead chosen for the measurement will be the vector magnitude (computed using the Guldenring transformation). This will allow for a global assessment of ECG effects.

Two (2) independent reviewers blinded to treatment and time will review the adjudications (P onset, QRS onset, QRS offset, T_{peak}, and T_{end}) of all ECGs. If the 2 independent readers disagree more than 5 msec or about the measurability of the ECG, they will reassess the ECG blinded to their previous measurement. If they still disagree, the difference will be resolved by an ECG interpretation expert. Detailed ECG analysis methods and workflow will be described in the ECG analysis plan, which will be completed before the study starts.

4.7.2 Pharmacokinetic Assessments

4.7.2.1 Pharmacokinetic Sample Collection

Pharmacokinetic blood samples will be collected on Days 1, 2, 3, and 4 of each period (i.e., on Days 1, 2, 3, and 4 for Part 1 and Days 1, 2, 3, 4, 8, 9, 10, and 11 for Part 2). Blood samples will be collected by direct venipuncture or by inserting an IV catheter into the subject's forearm region. A single PK blood sample (5 mL) will be collected at the following time points within each period: 0.5, 2, 2.5, 3.5, 4.5, 5, 6, 8.5, 14, 24, 27.5, 32.5, 38, 48, 50, 50.5, 51.5, 52.5, 53, 54, 54.5, 55, 59, 61, and 73 hours after time 0 or first oral dose on Day 1 or Day 8 (Table 8-2). Each blood sample will be labeled with subject number, study number, study day, time point, event, and a barcode that matches that belonging to the subject.

Note: there is no PK blood sample collection at the predose time point on Day 1.

Collection of the PK samples will be time-matched to the ECG extractions; therefore, the subjects will be supine for approximately 10 minutes before the defined time points and for approximately 5 minutes after for ECG extraction/PK sample collection. The PK

sample will be collected after the ECG extraction to avoid changes in autonomic tone associated with the psychological aspects of blood collection and the reduction in blood volume subsequent to blood collection. A 5-minute window for PK sample collection is allowed after the ECG extraction window.

4.7.2.2 Pharmacokinetic Specimen Handling

The PK blood samples (5 mL each) will be collected into tubes containing K_2EDTA, inverted several times to mix the blood with the anticoagulant, and placed in an ice bath. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes, at 3000 revolutions per minute, at 4°C, by a study team member.

The plasma will be separated using a disposable plastic pipette and approximately half of the plasma will be transferred into duplicate cryotube vials labeled as Aliquot A (primary) and Aliquot B (backup). The plasma samples will be appropriately labeled and stored frozen at –70°C or below until shipment. Temperature monitoring logs should be maintained and accessible for review by the study monitor.

The Aliquot A samples (primary) will be shipped first, on dry ice, to the bioanalytical laboratory at FDA for processing when requested by the sponsor. The Aliquot B samples (backup) will be held for a second shipment at a time after the completion of all Aliquot A sample shipment(s) and the timing of the Aliquot B shipment communicated by the sponsor. None of the PK blood samples will be stored for future use.

Plasma concentrations will be determined with a validated liquid chromatography with tandem mass spectrometry method for all PK samples.

4.7.2.3 Pharmacokinetic Parameters

Due to the nature of this study, only C_{max} along with the T_{max} and area under the plasma concentration-time curve (AUC) will be computed for each drug on Days 1 and 3 of the treatment period. Pharmacokinetic samples will also be used for concentration-dependent analysis of ECG biomarkers.

4.7.3 Safety Assessments

Safety will be evaluated in terms of AEs, clinical laboratory results (hematology, serum chemistry, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral body temperature), safety 12-lead ECG results, and physical examination findings.

4.7.3.1 Adverse Events

4.7.3.1.1 Adverse Event Definitions

An AE is defined as any untoward and/or unintended sign, including an abnormal clinical laboratory finding, symptom, or disease temporally associated with the use of a study drug, whether or not considered related to the study drug. Events or conditions that increase in frequency or severity during or as a consequence of use of a drug in human clinical trials will also be considered AEs.

A treatment-emergent adverse event (TEAE) is defined as an AE that begins after study drug administration.

An unexpected AE is any AE having a specificity or severity not consistent with the current investigator's brochure for the study drug(s).

An SAE is defined as any AE occurring at any dose that meets the following criteria:

- Results in death,
- Is life threatening,
- Requires hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability or incapacity,
- Is a congenital anomaly/birth defect, or
- Is an important medical event that may not meet the previous criteria but, based upon appropriate medical judgment, jeopardizes the subject or requires medical or surgical intervention to prevent one of the outcomes listed previously.

4.7.3.1.2 Adverse Event Reporting

The recording of AEs will begin after the subject signs the informed consent form and will continue until discharge (or early termination). All AEs, whether serious or nonserious and whether or not related to the study drug, must be recorded in the eCRF. Study subjects will be instructed to warn study staff if he or she has any unexpected symptoms. In addition, all subjects will receive a reminder telephone call approximately 24 hours before Check-in.

Any SAE (whether expected or unexpected) must be entered into the eCRF system and reported by facsimile to the medical monitor or designee using the SAE Reporting Form within 24 hours of the investigator or study clinic staff becoming aware of the event. It is the responsibility of the investigator to report all SAEs to the medical monitor, to provide the most complete report possible, and to assess each SAE for its relationship to the study drug. The investigator is responsible for obtaining follow-up information on all SAEs and submitting follow-up SAE data. Any unexpected SAEs must be reported promptly to the investigator's IRB as per the IRB's requirements.

In the event of a fatal or life-threatening SAE, the sponsor will notify the appropriate FDA authorities by telephone or facsimile within 7 calendar days of receipt of the report.

The sponsor will follow all 7-day alert reports with a written report within 10 working days of receipt of the case. Serious AE cases that concern nonfatal, nonlife-threatening events that are unexpected and at least possibly related to the study drug will be submitted in writing to the FDA within 10 working days of receipt.

Furthermore, any AEs that are not expected, occur at a higher frequency, or would require modification of the study protocol and/or informed consent must be reported to the FDA within 10 working days.

Adverse events that are assessed by the investigator as possibly or probably related to the study drug will be followed until they resolve or stabilize. All SAEs will be followed until resolution.

4.7.3.1.3 Assessment of Severity

The investigator will assess the severity of each AE using the following scale:

- Mild: The subject is aware of the AE but is still able to perform all activities; minimal or no medical intervention or therapy is required.
- Moderate: The subject has to discontinue some activities due to the AE; minimal or no medical intervention or therapy is required.
- Severe: The subject is incapacitated by the AE and is unable to perform normal activities; significant medical intervention or therapy is required and hospitalization is possible.

4.7.3.1.4 Assessment of Causality

The investigator will assess the causal relationship/relatedness of each AE to the study drug using the following scale:

- Not Related: Onset of the AE has no reasonable temporal relationship to administration of the study drug, a causal relationship to administration of the study drug is biologically implausible, or the event is attributed to an alternative etiology.
- Unlikely Related: Onset of the AE has a reasonable temporal relationship to study drug administration and although a causal relationship is unlikely, it is biologically plausible.
- Possibly Related: Onset of the AE has a strong temporal relationship to administration of the study drug, cannot be explained by the subject's clinical state or other factors, and a causal relationship is biologically plausible.
- Probably Related: Onset of the AE shows a distinct temporal relationship to administration of the study drug that cannot be explained by the subject's clinical

state or other factors, the AE is a known reaction to the product or chemical group, or can be predicted by the product's pharmacology.

4.7.3.1.5 Pregnancy

A serum pregnancy test will be performed for female subjects at the time points presented in the Schedules of Events (Section 8.1). If a subject becomes pregnant while on the study, this should be reported immediately to the investigator, the subject will be withdrawn from the study and the medical monitor and the subject will be instructed to follow-up with his or her personal physician. All pregnancies are to be reported as an AE and followed for outcome.

4.7.3.2 Clinical Laboratory Tests

Clinical laboratory and diagnostic screening tests will be performed at the time points presented in the Schedules of Events (Section 8.1) and will be collected in accordance with acceptable laboratory procedures. Clinical laboratory testing will be performed by Spaulding Clinical Laboratory, West Bend, Wisconsin, and Laboratory Corporation of America (LabCorp), Dublin, Ohio. The clinical laboratory tests that will be performed are presented in Table 4-1. Unused clinical laboratory test samples will not be stored for future use.

Table 4-1 Clinical Laboratory Tests and Diagnostic Screening Tests

Hematology	Serum Chemistry	Urinalysis
Hematocrit Hemoglobin Platelet count Red blood cell count White blood cell count (with automated differential)	Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Bicarbonate Bilirubin (total, direct, and indirect) Blood urea nitrogen Calcium Chloride Creatinine (including calculated creatinine clearance) Glucose Lactate dehydrogenase Magnesium Phosphorus Potassium Sodium Total protein Uric acid	Appearance Bilirubin Blood Color Glucose Ketones Leukocyte esterase Microscopic examination: red blood cells, white blood cells, epithelial cells, bacteria, crystals, and casts (if present) Nitrite pH Protein Specific gravity Urobilinogen

Diagnostic Screening Tests:	
Serum	**Urine**
Serology (human immunodeficiency virus Ag/Ab Combo 1/2, hepatitis C virus antibody, and hepatitis B surface antigen) **Female Subjects Only** Human chorionic gonadotropin (for pregnancy)	Drug screen including: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, alcohol, opiates, phencyclidine, propoxyphene, and methadone

Clinical laboratory results will be reviewed by the investigator or designee together with data in the eCRF. Any values outside the reference range will be evaluated for clinical significance. If a value is determined to be clinically significant, the subject will be instructed to follow-up with his or her personal physician. The investigator or designee may repeat the clinical laboratory tests if deemed appropriate. The investigator will maintain a copy of the laboratory accreditation and the reference ranges for the laboratory used.

4.7.3.3 Vital Sign Measurements

Vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature) will be measured using an automated device at the time points presented in the Schedules of Events (Section 8.1). The subject should be in a supine position, if possible, for a minimum of 5 minutes before vital signs are measured.

4.7.3.4 Safety 12-lead Electrocardiograms

Subjects will be placed on continuous 12-lead ECG Holter monitoring throughout the entire 3 dosing days. In addition, safety 12-lead ECGs will be performed at the time points presented in the Schedules of Events (Section 8.1). The subject should be in a supine position, if possible, for approximately 10 minutes before safety 12-lead ECGs are measured. While collecting the safety 12-lead ECG, there should be minimal to no interruption of the continuous 12-lead ECG collection (Section 4.7.1.1).

The safety 12-lead ECGs will be reviewed by the investigator at the study clinic to detect any immediate safety concerns. If a subject has an on-treatment safety 12-lead ECG with a QTcF interval ≥500 msec (as defined by automatically measured intervals) and an increase in QTcF interval >60 msec over Baseline at any time point where a safety 12-lead ECG is recorded, which is confirmed by a second safety 12-lead ECG performed within 1 hour, the subject will not receive any more study drug doses, the subject will be instructed to follow-up with his or her personal physician, an AE will be recorded, and the event should be reported immediately to the medical monitor. The subject can continue to be monitored and undergo PK sampling and ECG measurements per protocol until the next dose would have been administered or until Discharge on Day 4 if this occurs after the last dose on Day 3.

Additionally, if a subject has an on-treatment 12-lead ECG with third degree heart block except if brief (no more than 2 consecutive dropped beats per episode) during sleep, or second degree heart block occurring at night with persistent severe bradycardia (heart rate <40 bpm), or second degree heart block during the day that is symptomatic or progressive, the subject will not receive any more study drug doses, the finding should be recorded as an AE and reported immediately to the medical monitor. The subject will be instructed to follow-up with his or her personal physician. The subject can continue to be monitored and undergo PK sampling and ECG measurements per protocol until the next dose would have been administered or until Discharge on Day 4 if this occurs after the last dose on Day 3.

At discharge from the study, if a subject's 12-lead ECG has a QTcF interval ≥500 msec (as defined by automatically measured intervals) or an increase in QTcF interval >60 msec over Baseline, the subject will be advised to remain in the clinic until the QTcF is below those thresholds.

4.7.3.5 Physical Examinations

A complete physical examination will be performed at the time points presented in the Schedules of Events (Section 8.1).

The complete physical examination will include, but not be limited to, assessments of the head, eyes, ears, nose, throat, skin, thyroid, nervous system, respiratory system, cardiovascular system, abdomen (liver and spleen), lymph nodes, and extremities. Height, weight (without shoes and wearing the lightest possible clothing), and calculation of body mass index will be performed at Screening only.

If an abnormality is observed upon physical examination, the subject will be instructed to follow-up with his or her personal physician.

4.7.4 Demographics and Medical History

Demographic data (date of birth, gender, race, and ethnicity) will be collected at Screening.

Each subject will provide a complete medical history at Screening that will be reviewed at Check-in. Specific information relating to any prior or existing medical conditions/surgical procedures will be recorded in the subject's eCRF.

4.7.5 Blood Sample Collection and Handling for Genetic Testing

Whole blood samples (two 5-mL EDTA tubes) for isolation of DNA and genetic testing will be collected by a research team member at the time point presented in the Schedules of Events (Section 8.1) and will only be used for the purposes of the current study. Each sample will be labeled with the subject number and a barcode that matches those

belonging to the subject. After collection, the samples will be gently inverted and then frozen immediately at -70°C. Samples will be shipped to FDA, Silver Spring, MD. As performed in the prior FDA-sponsored clinical trials SCR-002 and SCR-003 (Strauss et al. 2016), samples will be used for analysis of genetic variants or potential genome sequencing if indicated by individual subject PK or PD response. These analyses will be considered hypothesis-generating and exploratory.

4.7.6 Blood Collection for PBMC Isolation to Enable iPSC Generation

Vacutainer tubes will be stored at room temperature until ready to perform blood sample collection. A trained phlebotomist will collect blood samples by venipuncture using 4 Vacutainer cell preparation tubes (CPTs) (8 mL/tube) for PBMC isolation from each subject. Tubes will be immediately inverted gently 4 to 6 times to ensure even mixing. CPT tubes will be incubated at room temperature for a minimum of 30 minutes and a maximum of 2 hours. CPT tubes will then be centrifuged at room temperature at 1500 to 1800 × *g* for 20 minutes for swinging bucket rotors or 10 minutes for fixed angle rotors. Tubes will be inspected to ensure proper blood separation. Tubes will be maintained on ice until ready for shipment. Samples will be maintained on dry ice during shipment. Samples will be shipped to FDA, Silver Spring, MD where PBMC isolation will be performed. Based on the individual-subject PK and PD response, iPSCs may be generated for subjects in the study and differentiated to terminal cell types (e.g., cardiomyocytes, hepatocytes) to enable subject-specific drug testing as performed in the prior FDA-sponsored clinical trial SCR-003 (Blinova et al. 2016).

After material transfer agreements are in place, FDA may biobank the iPSCs at a biobank such as the Coriell Institute for Medical Research (www.coriell.org), WiCell (www.wicell.org) or another biobank. This will enable additional researchers to study the iPSCs, as differentiation techniques are expected to evolve and improve over the next years.

4.8. Study Treatments

For each of the study drugs, doses were selected based on the doses studied in prior PK studies (verapamil and diltiazem) or TQT studies (lopinavir + ritonavir) or the lowest dose that causes 10 msec mean increase in ΔΔQTc on Day 1 and 20 to 30 msec ΔΔQTc prolongation on Day 3 (ranolazine, chloroquine and dofetilide) that is also within previously studied doses.

The dofetilide + diltiazem combination dose (dofetilide 0.25 mg and diltiazem 120 mg) was selected below the maximum doses in the label (dofetilide 0.5 mg BID and diltiazem 480 mg daily) and below dofetilide doses studied in a previous drug-drug interaction study with dofetilide and verapamil (Johnson et al, 2001). An increase in dofetilide exposure of 40% to 50% may occur from diltiazem PK interaction, similar to what has

been previously reported in interaction studies with higher doses of dofetilide + verapamil (dofetilide 0.5 mg BID + verapamil 80 mg three times daily) and dofetilide + ketoconazole (dofetilide 0.5 mg BID + ketoconazole 400 mg daily [dofetilide label). A single oral dose of dofetilide + diltiazem will be given on Day 3 or Day 10 to subjects assigned to Part 2 of the study.

An increase in dofetilide exposure of 40% to 50% is expected when dosed in combination with diltiazem (dofetilide 0.25 mg and diltiazem 120 mg) because of CYP3A4 inhibition by diltiazem. This is expected to result in a C_{max} of 2.25 ng/mL, which corresponds with the levels expected from a single oral dose of 0.375 mg of dofetilide, which is below the maximum dose in the label and previous interaction studies with other CYP3A4 inhibitors that assessed interaction with dofetilide 0.5 mg BID and observed an increase in dofetilide exposure of 40% to 50% (dofetilide + verapamil and dofetilide + ketoconazole). The increase in exposure is expected to result in similar concentrations to the dofetilide 0.375 mg dose in the dofetilide alone arm. However, dofetilide-induced QTc prolongation is expected to be lower due to QTc shortening caused by calcium current block associated with diltiazem. From a risk standpoint, it is important to note that: 1) this will be a single oral dose expected to result in dofetilide exposure below dofetilide levels associated with recommended dose in the label (0.5 mg BID), 2) that the associated QTc prolongation is expected to be less than QTc prolongation caused by a dofetilide 0.375 mg single dose, and 3) that subjects will be continuously monitored.

4.8.1 Treatments Administered

On Days 1, 2, 3, 8, 9, and 10 subjects will receive 1 of the following 2 treatments according to the randomization schedule:

Part 1:

- Treatment A: Ranolazine 1500 mg BID for 2.5 days
- Treatment B: Verapamil 120 mg IR morning and afternoon doses on Days 1 and 2, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR morning dose on Day 3
- Treatment C: Lopinavir + ritonavir 800 mg/200 mg BID for 2.5 days
- Treatment D: Chloroquine 1000 mg on Day 1, 500 mg on Day 2, 1000 mg on Day 3
- Treatment E: Placebo

Part 2:

- Treatment F: Dofetilide 0.125 mg on Day 1, 0.375 mg on Day 3

- Treatment G: Diltiazem 120 mg IR morning dose on Day 1, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR on Day 3 with coadministration of 0.25 mg dofetilide on Day 3.

Study drugs will be administered by a clinical research nurse on the study clinic floor at the subject's bedside. The pharmacist and investigator will be available if needed during study drug administration. Study drugs will be administered with 240 mL of room-temperature water.

First morning oral dose (placebo or chloroquine) will be administered in the morning with 240 mL of room-temperature water after subjects have fasted for at least 8 hours and 1 hour before breakfast. Second morning oral dose (placebo, ranolazine, verapamil or lopinavir + ritonavir, dofetilide, diltiazem IR or dofetilide + diltiazem IR) will be administered with breakfast. Afternoon oral dose (placebo or verapamil) will be administered approximately 2 hours after lunch. Evening oral dosing (placebo, ranolazine, verapamil, lopinavir + ritonavir, dofetilide or diltiazem ER) will be administered together with a snack (e.g., doses at approximately 7 am, 8:30 am, 2:30 pm, and 8:30 pm). Meals will be administered 30 minutes before oral dosing. Subjects will be instructed to consume all of contents of the meals at a reasonable pace (within 25 minutes) to allow time to return to their room for dosing.

4.8.2 Dose Selection

4.8.2.1 Ranolazine

The dose selected for ranolazine is 1500 mg BID for 2.5 days. In the prior FDA-Spaulding clinical study (Johannesen et al, 2014), a single dose of 1500 mg caused a QTc prolongation of approximately 10 msec. Ranolazine is a drug with "balanced ion channel" effects that prolongs QTc but has a low risk of TdP (Crumb et al, 2016). As the primary endpoint of this study is to test whether J-T_{peakC} is not prolonged at high exposure levels for "balanced ion channel" drugs, 1500 mg BID for 2.5 days will be administered. This dose has been safely administered to 14 healthy subjects for up to 5 days (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021526_s000_Ranexa_Medr.pdf) and 191 patients for 1 week (Chaitman et al, 2004).

4.8.2.2 Verapamil

The dose selected for verapamil is 120 mg IR morning and afternoon doses on Days 1 and 2, 240 mg ER evening dose on Days 1 and 2, and 120 mg IR morning dose on Day 3. This is within the maximum label dose (480 mg daily) that has also been administered to healthy subjects (Hartter et al, 2012).

4.8.2.3 Lopinavir + Ritonavir

The dose selected for lopinavir + ritonavir is 800 mg/200 mg BID for 2.5 days. This dose was selected to be equivalent to that previously administered in a TQT study in 39 healthy subjects (http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021251s053,021906s047lbl.pdf).

4.8.2.4 Chloroquine

The dose selected for chloroquine is 1000 mg on Day 1, 500 mg on Day 2, and 1000 mg on Day 3. This dose is consistent with the labeled dose of 2.5-g chloroquine phosphate in 3 days (http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/006002s043lbl.pdf) and has been administered to 40 healthy subjects (Miller et al, 2013).

4.8.2.5 Dofetilide

The dose selected for dofetilide is 0.125 mg on Day 1, placebo on Day 2, and 0.375 mg on Day 3. This dose is less than the labeled dose (0.5 mg BID) and less than the dose of 0.5 mg previously administered to healthy subjects (Johannesen et al, 2014).

4.8.2.6 Diltiazem

The dose selected for diltiazem is 120 mg IR in the morning on Day 1, 240 mg ER in the evening on Day 1 and 2, and 120 mg IR on Day 3. This dose is less than the maximum label dose of 480 mg daily for IR and 540 mg daily for ER.

4.8.3 Method of Assigning Subjects to Treatment Sequence

4.8.3.1 Randomization Process

The project biostatistician will create the specifications that will be used to generate the randomization schedule. The specifications will be based on the protocol requirements and appropriate statistical programming with consideration for study design, number of treatments, number of subjects planned for enrollment, stratification, and blocking.

Based on these specifications, the project biostatistician (or designee) will generate a dummy randomization schedule. The schedule is generated by a SAS® program, which produces output file(s) and/or SAS dataset(s).

The project biostatistician (or designee) distributes the 'dummy' randomization schedule to specified personnel for review. Any change (e.g., change in block size, change in stratification levels) that requires an update to the specifications will reset this process. Minor changes (e.g., display formatting) will not require a change to the specifications.

After the approval of the 'dummy' randomization schedule, the project biostatistician (or designee) transfers the program used to generate the 'dummy' schedule to the

randomization biostatistician (unblinded), who is an independent party and will not be participating in any programming or statistical decisions for the study before breaking the blind. No transfer is necessary if the unblinded randomization biostatistician also created the 'dummy' randomization.

The randomization biostatistician is responsible for generating the final randomization schedule. The output is sent only to designated unblinded recipients.

Archival of the programs and output is accomplished by the creation of an encrypted, password-protected ZIP file containing the program, .log and .lst files, output file(s) and/or SAS dataset(s). This ZIP file is sent to the Spaulding Clinical Quality Assurance (QA) department, without the password, via a CD-ROM. The ZIP file is copied to a CD-ROM and placed in a secure location in the Spaulding Clinical QA department. The password to the ZIP file is sent in a tamper-evident envelope to the Spaulding Clinical QA department.

Randomization will occur after informed consent is obtained, either after completion of check-in procedures on Day 0 or just before dosing on Day 1. Approximately 60 healthy male and female subjects are planned for enrollment, of which 50 will be assigned to Part 1 and 10 will be assigned to Part 2 at randomization. Up to 14 subjects may be qualified as replacements as described in Section 4.5.2. Thus, a maximum of 74 subjects will be exposed to study drugs and procedures during the study. Unique subject numbers will be used in sequential order based on each subject's order of qualification.

Enrolled subjects will be randomly assigned to 1 of 2 different parts. Subsequently:

- Subjects assigned to Part 1 will be randomly assigned to 1 of 5 different treatments, thus each subject will receive one of the following treatments by the end of the study: 1) ranolazine, 2) verapamil, 3) lopinavir + ritonavir, 4) chloroquine, or 5) placebo. Replacement subjects (if needed) will be assigned to the treatment group of the subject they are replacing.
- Subjects assigned to Part 2 will be randomly assigned to 1 of 2 different treatment sequences, during which each subject will receive each of the following treatments by the end of the study: 1) dofetilide and 2) diltiazem alone and dofetilide + diltiazem.

The treatment groups for each part are presented in the 2 tables below.

Table 4-2 Study Part 1 Treatment Groups

Subjects (n)	Treatment Group	Ion Channel Effects
10	A	balanced
10	B	balanced
10	C	balanced
10	D	predominant hERG
10	E	N/A (reference)

Abbreviations: hERG: human ether-à-go-go-related gene; N/A, not applicable.

A: ranolazine; B: verapamil; C: lopinavir + ritonavir; D: chloroquine; E: placebo

Table 4-3 Study Part 2 Treatment Groups

Treatment Sequence	Subjects (n)	Period 1 (Days 1, 2, 3)	Period 2 (Days 8, 9, 10)
1	5	F	G
2	5	G	F

F: dofetilide alone; G: diltiazem alone on Days 1 and 2 and dofetilide + diltiazem on Day 3

All randomization information will be secured and housed in a locked storage area, accessible only by the randomization personnel and the assigned pharmacist and his or her verifier.

4.8.4 Identity of Study Drugs

Ranolazine is indicated for the treatment of chronic angina. The tablets are designed for extended release of the drug in the gastrointestinal tract. Ranolazine has a molecular weight of 427.54 and the molecular formula $C_{24}H_{33}N_3O_4$. The physical form is a white to off-white solid that is soluble in dichloromethane and methanol; sparingly soluble in tetrahydrofuran, ethanol, acetonitrile, and acetone; slightly soluble in ethyl acetate, isopropanol, toluene, and ethyl ether; and very slightly soluble in water (Ranexa® prescribing information, 2016).

Verapamil HCl is available as 120 mg IR (Calan®) and 240 mg ER (Isoptin SR®) formulations. Verapamil HCl is a calcium ion influx inhibitor (slow channel blocker or calcium ion antagonist) with a molecular weight of 491.07 and the molecular formula $C_{27}H_{38}N_2O_4 \bullet HCl$. The physical form is an almost white, crystalline powder, practically free of odor, with a bitter taste that is soluble in water, chloroform, and methanol (Calan package insert, 2015 and Isoptin SR product monograph, 2014).

Lopinavir + ritonavir is indicated for the treatment of HIV infection. Lopinavir has a molecular weight of 628.80 and the molecular formula $C_{37}H_{48}N_4O_5$. The physical form is a white to light tan powder that is freely soluble in methanol and ethanol, and soluble in isopropanol. Ritonavir has a molecular weight of 720.95 and the molecular formula $C_{37}H_{48}N_6O_5S_2$. The capsules are available for oral administration in a strength of 133.3 mg lopinavir and 33.3 mg ritonavir (Kaletra® package insert, 2010).

Chloroquine is indicated for the treatment of malaria infection. Chloroquine has a molecular weight of 515.87 and the molecular formula $C_{18}H_{26}ClN_3 \bullet 2H_3PO_4$. Chloroquine is a white, odorless, bitter tasting, crystalline substance, that is freely soluble in water (Chloroquine package insert, 2009).

Dofetilide is an antiarrhythmic drug with Class III (cardiac action potential duration prolonging) properties, which occur by blocking the hERG potassium channel. Dofetilide has a molecular weight of 441.6 and the molecular formula $C_{19}H_{27}N_3O_5S_2$. The physical

form is a white to off-white powder that is very slightly soluble in water and propan-2-ol and is soluble in 0.1M aqueous sodium hydroxide, acetone, and aqueous 0.1M hydrochloric acid. (Tikosyn® package insert, 2016).

Diltiazem HCl is available as 120 mg IR (Cardizem®) and 240 mg ER (Cardizem CD®) formulations. Diltiazem HCl is a calcium ion flux inhibitor (slow channel blocker or calcium antagonist) indicated for the treatment of hypertension and for the management of chronic stable angina (effort-associated angina) where there is no evidence of vasoplastic or unstable angina. Diltiazem HCl has a molecular weight of 450.98 and the molecular formula $C_{22}H_{26}N_2O_4S \bullet HCl$. The physical form is a white to off-white crystalline powder with a bitter taste that is soluble in water, methanol, and chloroform (Cardizem CD package insert, 2009).

Placebo capsules containing only cornstarch will be used as the placebo control in this study.

4.8.5 Management of Clinical Supplies

4.8.5.1 Study Drug Packaging and Storage

The active study drugs will be obtained from commercial sources. Storage instructions for the active study drugs are as follows:

- Ranolazine should be stored at 25°C (77°F) with excursions permitted from 15°C to 30°C (59°F-86°F) (Ranexa prescribing information, 2016).
- Verapamil IR should be stored from 15°C to 25°C (59°F-77°F) and protected from light and moisture (Calan package insert, 2016). Verapamil ER should be stored at 15°C to 25°C (Isoptin SR product monograph, 2014).
- Lopinavir + ritonavir should be stored from 2°C to 8°C (36°F-46°F) until dispensed. Exposure to excessive heat should be avoided (Kaletra package insert, 2010).
- Chloroquine should be stored at 20°C to 25°C (68°F-77°F) and protected from light (Chloroquine package insert, 2009).
- Dofetilide should be stored at controlled room temperature (15°C to 30°C; 59°F to 86°F) and protected from moisture and humidity (Tikosyn package insert, 2016).
- Diltiazem IR should be stored below 30°C and protected from light and moisture (Cardizem product information, 2014). Diltiazem ER should be stored at 25°C (77°F), with excursions permitted to 15°C to 30°C (59°F to 86°F) and protected from humidity (Cardizem CD package insert, 2009).

Placebo capsules will be supplied by the clinical site, stored at controlled room temperature (15°C to 30°C; 59°F to 86°F), and protected from light and moisture.

4.8.5.2 Study Drug Accountability

Good clinical documentation practices will be employed to record the receipt, storage conditions, accountability, and use or return of the study drug. The study drug will be stored in a secure location with access to the study personnel who will be managing the storage, dispensing, and accountability of the study drug.

Upon completion or termination of the study, final accountability review by the study monitor, and written authorization from the sponsor, all unused and/or partially used study drug should be returned or destroyed at the study clinic. It is the investigator's responsibility to ensure that the sponsor has provided written authorization for study drug disposal, the disposal process follows the study clinic's standard operating procedures, and appropriate records of the disposal are documented and maintained. No unused study drug may be disposed until fully accounted for by the study monitor (or designee). Documentation of unused study drug should include subject number, medication identity (medication #, period #), date, and quantity of study drug used.

4.8.6 Blinding

The study will be double-blind and the blind will be maintained through a randomization schedule held by the dispensing pharmacist. In addition, subjects will be blindfolded during study drug administration and the ECG analysis will be blinded to treatment, time, and study day/subject identifiers. The pharmacist (and designated staff member responsible for confirmation of study drug dose) will be unblinded to subject treatment assignment; however, the pharmacist will not perform any study procedures other than study drug preparation and dispensing.

The clinical research nurse will administer the oral study drug in unit dose containers that are not transparent; the nurse will hand the dose container to the subject to open and consume the dose at the correct time point. Thus, the nurse will not observe the substance in the dose container and will remain blinded. The subjects will be blindfolded during oral dosing so they will be unaware of the study drug they are receiving during the study. All other clinic personnel and the cardiologist at the central ECG laboratory will be blinded to treatment, time, and study day/subject identifiers.

4.8.6.1 Breaking the Blind

The study drug blind will not be broken by the investigator or designee unless information concerning the study drug is necessary for the medical treatment of the subject. For unblinding a subject, the randomization information for unblinding can be obtained by contacting the dispensing pharmacist. The sponsor or medical monitor must be notified immediately if the study drug blind is broken. The date, time, and reason that the blind was broken will be recorded in the source documents. If the blind is broken by the investigator or designee, the study drug must be stopped immediately and the subject

must be withdrawn from the study. Data or specimens already collected from subjects who discontinue prematurely and for whom the blind is broken will be made available for analysis if needed.

4.8.7 Treatment Compliance

At Screening, as part of the inclusion criteria, it will be confirmed that subjects are able to comply with the protocol-defined procedure of ingesting oral study drug. All doses of the study drug will be administered in the study clinic either under direct observation of or administered by clinic personnel, and recorded in the eCRF. Clinic personnel will perform a hand and mouth check to confirm and document that the subject has ingested the entire dose of study drug. If a subject vomits after dosing, the event will be documented as an AE. The decision to replace any subject who vomits after dosing will be made as described in Section 4.5.2.

4.8.8 Prior and Concomitant Medications

Subjects are prohibited from using any prescription or nonprescription drugs (including aspirin or NSAIDs and excluding oral contraceptives and acetaminophen) within 14 days or 5 half-lives (whichever is longer), or complementary and alternative medicines within 28 days before the first dose of study drug.

Subjects are also prohibited from currently participating in another clinical study of an investigational drug and may not have been treated with any investigational drug within 30 days or 5 half-lives (whichever is longer) of the compound.

Subjects must be instructed not to take any medications, including over-the-counter products, without first consulting with the investigator.

4.8.9 Subject Restrictions

Subjects are not allowed to use nicotine-containing products (e.g., cigarettes, cigars, chewing tobacco, snuff) within 6 weeks before Screening. In addition, subjects are not allowed to ingest alcohol, xanthine-containing products (e.g., tea, coffee, chocolate, cola), caffeine, grapefruit, or grapefruit juice for 48 hours before dosing and throughout the study. Subjects are not allowed to use aspirin or NSAIDs within 14 days before the first dose of study drug. Subjects will be asked if they have used any of these substances and their responses will be recorded on the eCRF.

Subjects must be able to tolerate a controlled, quiet study conduct environment, including avoidance of music, television, movies, games, and activities that may cause excitement, emotional tension, or arousal during prespecified times (e.g., before and during ECG extraction windows) throughout the duration of the study.

Subjects must be willing to comply with study rules, including the meal schedule, attempting to void at specified times (e.g., before ECG extraction windows), remaining quiet, awake, undistracted, motionless, and supine during specified times, and avoiding vigorous exercise as directed throughout the duration of the study. Subjects will not be allowed to sleep during any ECG extraction periods.

All subjects will fast overnight for a minimum of 8 hours (no food or fluid except water) before blood collection for clinical laboratory testing. Standardized meals will be served at consistent times relative to dosing, and no food or fluids will be served containing caffeine. Outside of meal times, the subjects will only be allowed to intake water, which will be available ad libitum.

4.9. Statistical Methods

4.9.1 Sample Size

Up to 74 healthy subjects will be enrolled (including 14 potential replacement subjects). The sample size (10 subjects per arm) was selected based on analysis by the sponsor of ECG biomarkers in Studies SCR-002 and SCR-003 and by resampling of data from previously conducted TQT clinical studies, similar to the methodology of Ferber et al (2015). The data sources used in this analysis are appropriate to determine the sample size because (i) the inclusion and exclusion criteria of this study is similar to inclusion and exclusion criteria in SCR-002, SCR-003 and typical TQT studies; (ii) this study will be conducted at the same clinical site than SCR-002 and SCR-003; (iii) the ECG analysis will be performed at FDA using the same methodology used in SCR-002 and SCR-003; and (iv) we observed consistent drug-induced ECG signatures between SCR-002 and SCR-003 (Vicente et al. 2016 Figure 1 and Figure 2).

4.9.1.1 Part 1

For Part 1, the sample size of 10 subjects per arm in a parallel design was selected to mirror the sample size in standard SAD/MAD studies. For example, a waiver for a TQT study was recently granted based on exposure response analysis of a SAD/MAD study (Nelson et al, 2015). This study had 20 subjects on placebo, 10 subjects in the SAD portion, and 10 subjects in the MAD portion.

In addition, resampling of the data from the ranolazine arm from Johannesen et al (2014) using a similar methodology to Ferber et al (2015) suggests that 9 subjects on active study drug and 6 subjects on placebo will be sufficient to exclude J-T_{peakC} prolongation for ranolazine. Of note, this was with a single dose of 1500 mg, while the current study will include ranolazine 1500 mg BID for 2.5 days. Similar analysis of previously conducted TQT studies suggests that 9 subjects on active study drug and 6 subjects on placebo will also be sufficient to exclude J-T_{peakC} prolongation for the "balanced ion channel" drugs in this study. This is also consistent with the method used in the IQ-CSRC

study (Darpo et al, 2014). That study was able to detect QTc prolongation for the "predominant hERG" drugs and to exclude QTc prolongation for the "no ion channel" drug (Darpo et al, 2015).

It is anticipated that this study will be completed in 3 cohorts with approximately equal numbers of subjects per cohort. Cohorts 1 and 2 will have 20 subjects each (4 subjects per arm). Cohort 3 will have 10 subjects (2 subjects per arm). Up to 14 replacement subjects may be added to Cohort 3 if it is estimated that fewer than 8 subjects will complete the study in a study arm. A replacement algorithm will be pre-specified in the subject replacement plan to guide the unblinded Spaulding pharmacist to determine this while the rest of the staff and sponsor remain blinded. Table 4-5 shows an example of this balanced design. Please note that this is an example and is not necessarily the exact number of cohorts and subjects allocated per treatment and cohort in Part 1 of the current study.

Table 4-4 Example of Subjects per Cohort Scheme in Part 1

Subjects (n)	Treatment Group	Ion Channel Effects
10	A	balanced
10	B	balanced
10	C	balanced
10	D	predominant hERG
10	E	N/A (reference)

Abbreviations: hERG: human ether-à-go-go-related gene; N/A, not applicable.
A: ranolazine; B: verapamil; C: lopinavir + ritonavir; D: chloroquine; E: placebo

4.9.1.2 Part 2

For Part 2, the sample size of 10 subjects, with 5 subjects per treatment sequence in a crossover design was selected by resampling and further simulation of the data from our prior clinical study (SCR-003). This analysis demonstrated that , with 8 subjects, the use of exposure-response modeling could detect the effect of inward current block in the QTc and J-T_{peakC} slopes associated with hERG block approximately 99% and 99% of the time using a crossover design for QTc and J-T_{peakC}, respectively (i.e., the late sodium blocker significantly reduced prolongation from the hERG blocker). For Part 2, we anticipate the selected dose for the calcium blocker (diltiazem) will result in similar or larger QTc and J-T_{peakC} effects than those observed in SCR-003 because the calcium current is larger than the late sodium current. Therefore, and to account for potential dropouts, Part 2 of the study will use a crossover design with 10 subjects.

It is anticipated that Part 2 of this study will be completed in 1 cohort with 10 subjects with a crossover design. As described in Section 4.9.1, this cohort is preloaded with 2 replacements, and the study should be statistically powered with just 8 completers. Similar to Part 1, subjects may be replaced in a later cohort if it is estimated that fewer than 8 subjects will complete Part 2. A maximum of 14 replacement subjects may be

enrolled in the study, and replacement subjects (if needed) must complete the treatment period. A replacement subject will receive the same treatment as the subject being replaced. During Part 2 of the study, a maximum of 24 subjects will be exposed to study drugs and procedures. Table 4-5 shows an example of this balanced design.

Table 4-5 Example of Subjects per Treatment Sequence in Part 2

Treatment Sequence	Subjects (n)	Period 1 (Days 1, 2, 3)	Period 2 (Days 8, 9, 10)
1	5	F	G
2	5	G	F

F: dofetilide alone: G: diltiazem alone on Days 1 and 2 and dofetilide + diltiazem on Day 3

4.9.2 Analysis Populations

The exposure-response population will include all subjects who receive at least 1 dose of any of the study drugs and have digital ECG (QTc and J-T_{peakC}) data for the treatment period collected before dosing and at 1 or more time points after dosing as well as plasma concentration data (except for the placebo arm) from the same time points after dosing. Subjects in this population will be used for the exposure response analysis.

The PK population will include all subjects who receive study drug and have at least 1 estimable PK parameter after dosing.

The safety population will include all subjects who receive at least 1 dose of any of the study drugs.

4.9.3 General Statistical Considerations

All data will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings, but not included in the calculation of summary statistics. Additional analysis details will be specified in the Statistical Analysis Plan.

4.9.4 Subject Disposition

The number of subjects who enroll in the study and the number and percentage of subjects who complete each assessment will be presented. The frequency and percentage of subjects who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will be summarized.

4.9.5 Demographics and Baseline Characteristics

Continuous demographic and baseline characteristic variables (date of birth, height, weight, and body mass index) will be summarized overall and by treatment using descriptive statistics (number of subjects, mean, standard deviation [SD], median,

minimum, and maximum). The number and percentage of subjects in each class of categorical demographic and baseline characteristic variables will also be summarized.

4.9.6 Electrocardiogram Analyses

The QT interval will be corrected for heart rate using Fridericia's formula ($QTc=QT/RR^{1/3}$). The J-T_{peak} interval will be corrected for heart rate using the Johannesen et al (2014) formula ($J\text{-}T_{peakC} = J\text{-}T_{peakC}/RR^{0.58}$). Baseline will be the mean of the 3 predose ECG extractions of Day 1.

4.9.6.1 Primary Analysis

Part 1

Part 1 of the study will assess primary objective 1. The primary objective of Part 1 is to confirm that exposure-response analysis of the electrocardiographic QTc and J-T_{peakC} intervals in Phase 1 clinical pharmacology studies can be used to confirm that "balanced ion channel" drugs do not cause J-T_{peakC} prolongation and "predominant hERG" drugs cause QTc prolongation.

The primary variable for the exposure-response analysis will be the change-from-baseline in QTc (ΔQTc) for the "predominant hERG" drug and change-from-baseline in J-T_{peakC} ($\Delta J\text{-}T_{peakC}$) for the "balanced ion channel" drugs, where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline. The concentration of the drug will be used as a covariate. Exposure-response analysis will be done following most recent best practices in concentration-QTc modeling (Darpo et al, 2014, Darpo et al, 2015 and ICH E-14 Q&A 2015).

Part 2

Part 2 of the study will assess primary objective 2. The primary variable for the exposure-response analysis will be the change-from-baseline in QTc (ΔQTc) for the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem, where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline. The concentration of dofetilide and diltiazem will be used as covariates.

4.9.6.1.1 Investigation of Hysteresis

Prior to model selection for the exposure response analysis, the presence of hysteresis will be assessed for QTc and J-T_{peakC}. To detect hysteresis, individual change-from-baseline J-T_{peakC} ($\Delta J\text{-}T_{peakC}$) will be computed. For each day, the time of the largest mean $\Delta J\text{-}T_{peakC}$ (U_{max}) will be determined. If the largest mean $\Delta J\text{-}T_{peakC}$ exceeds 5 msec at $\geq$3 time points, the time difference between U_{max} and the T_{max} of the drug level exceeds 1 hour, and the 1-sided, 1-sample Wilcoxon test for the difference between $\Delta J\text{-}T_{peakC}$ at T_{max} and at U_{max} is formally significant at the 1% level, it will be concluded that hysteresis existed. In such a case, a PK model with an additional effect compartment

will replace the direct effect model described below. Briefly, the delayed effect of the drug will be modeled by using a sequential approach, where a two-compartment model will be fitted to the PK data alone first, and afterwards the PK model will be reapplied to the data with an added effect compartment to compute the PD response (Holford et al 1981, Vicente et al 2015). The same steps will be followed for QTc as part of the primary endpoint.

4.9.6.1.2 Model Selection

To assess the appropriateness of a linear model, normal QQ-plots for the residuals and plots of weighted residuals versus concentration and versus fitted values will be produced. A model with a quadratic term in concentration will be fitted and the quadratic term will be tested on the 2-sided 5% alpha level. In case of a significant quadratic term, nonlinear models, such as a log-linear model and an E_{max} model, will be investigated and the primary model will be selected based on the Akaike Information Criterion and plausibility arguments.

4.9.6.1.3 Exposure-Response Analysis

In the absence of hysteresis and unless the prespecified test procedure for linearity indicates otherwise, the primary analysis will be based on a linear mixed-effects model implemented in SAS or R software, with ΔJ-T_{peakC} as the dependent variable, drug plasma concentration and baseline J-T_{peakC} as continuous covariates, treatment and time point as categorical factors, and subject-specific random effects for the intercept and slope. All postdose data will be used. The degrees of freedom for the model estimates will be determined by the Kenward-Rogers method. From the model, the slope (i.e., the regression parameter for the concentration) and the treatment effect will be estimated together with 2-sided 90% CIs.

The predicted mean placebo-adjusted change-from-baseline J-T_{peakC} ($\Delta\Delta$J-T_{peakC}) at the observed geometric mean C_{max} (i.e., the product with the slope estimate + treatment effect [ΔJ-$T_{peakCactive}$ – ΔJ-$T_{peakCplacebo}$]) and the 2-sided 90% CI of the estimate will be calculated. The same steps will be followed for QTc as part of the primary endpoint.

4.9.6.1.4 Criteria for QTc and J-T_{peakC} Assessment

Part 1:

Criteria for the 3 "balanced ion channel" drugs (ranolazine, verapamil, lopinavir + ritonavir) will be based on the predicted J-T_{peakC} effect on the third day of dosing. To demonstrate a lack of J-T_{peakC} prolongation for each of the 3 drugs:

- The upper bound of the 2-sided 90% CI of the predicted mean $\Delta\Delta$J-T_{peakC} must be <10 msec at the observed geometric mean C_{max} on Day 3.

Criteria for the "predominant hERG" drug (chloroquine) will be based on the predicted QTc effect on the first day of dosing. To demonstrate the presence of QTc prolongation for chloroquine:

- The upper bound of the 2-sided 90% CI of the predicted mean placebo-adjusted change-from-baseline QTc ($\Delta\Delta$QTc) must be $\geq$10 msec at the observed geometric mean C_{max} on Day 1.

Part 2:

To demonstrate that calcium channel block (diltiazem) reduces the QTc prolongation from hERG block (dofetilide) by shortening J-T_{peakC}:

- We will assess whether the projected QT_C effect of dofetilide alone will be significantly greater (i.e., $p<0.05$) than the projected QT_C effect of the combination of dofetilide + diltiazem. This will be assessed at the dofetilide peak plasma level on Day 3 (computed from the combination of dofetilide + diltiazem) on the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem data using a linear mixed-effects model.
    - The primary variable for the exposure-response analysis will be the change-from-baseline in QTc (ΔQTc) for the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem, where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline.
- If the previous test is statistically significant for QTc, the same test will be performed for J-T_{peakC}.
    - The primary variable for the exposure-response analysis will be the change-from-baseline in J-T_{peakC} (Δ J-T_{peakC}) for the pooled dofetilide alone, diltiazem alone, and dofetilide + diltiazem, where the mean of the 3 predose ECG readings on Day 1 will be used as the Baseline.

4.9.6.2 Additional ECG Analyses

4.9.6.2.1 Exposure-Response Analysis of Secondary ECG Biomarkers

Exposure-response analysis similar to that described in the primary analysis section for QTc and J-T_{peakC} will be applied for all ECG intervals (i.e., PR, QRS, QTc, J-T_{peakC}, and T_{peak}-T_{end}) for Day 1 and Day 3. This will include analysis of change from baseline for dofetilide alone, diltiazem alone, and dofetilide + diltiazem combination from Part 2 of the study. Additional details will be specified in the Statistical Analysis Plan.

4.9.6.2.2 $\Delta\Delta$ECG Measurements by Time Point

For each time point, an analysis of variance model will be fitted with ΔJ-T_{peakC} as the dependent variable, treatment (active or placebo) as factor, and baseline J-T_{peakC} as a covariate. From this model, the difference (ΔJ-$T_{peakCactive}$ – ΔJ-$T_{peakCplacebo}$) will be

estimated with a 2-sided 90% CI. Separate models will be fitted for each treatment, all of them using the same placebo data. The same steps will be followed for QTc. Change from Baseline in heart rate, PR, QRS, and T_{peak}-T_{end} will be calculated using descriptive summary statistics.

4.9.6.3 Additional Analyses

4.9.6.3.1 Genetic Testing Analyses

Gene (genotype) variances may be explored that may contribute to the PK or PD of the study drugs. These analyses will be considered hypothesis-generating and exploratory.

4.9.6.3.2 Subject-Specific iPSC Analyses

Isolated PBMCs from the study subjects will be stored and may be used to generate iPSCs and differentiate to terminal cell types (e.g., cardiomyocytes or hepatocytes) depending on the PK and PD results of individual subjects.

4.9.7 Pharmacokinetic Analyses

The PK parameters C_{max}, T_{max}, and AUC will be summarized using descriptive statistics (number of subjects, mean, SD, coefficient of variation [CV], median, minimum, and maximum) for Day 1 or 8 and Day 3 or 10 for each active drug. The PK parameters will be analyzed using noncompartmental methods based on actual sampling times. All parameters will be calculated using SAS or R software. Mean and individual concentration-time profiles will be presented in graphs.

4.9.8 Safety Analyses

4.9.8.1 Adverse Events

All AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities. The incidence of TEAEs, organized by system organ class and frequency, will be summarized by seriousness, severity, relationship to treatment, and by treatment at onset of the TEAE. A detailed listing of serious AEs and TEAEs leading to withdrawal will also be provided.

4.9.8.2 Clinical Laboratory Tests

Clinical laboratory results (hematology, serum chemistry, and urinalysis) will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum). Clinical laboratory results will be classified as normal or abnormal, according to the reference ranges of the individual parameter. The number and percentage of subjects with abnormal laboratory results will be provided. No statistical testing will be performed on clinical laboratory data.

4.9.8.3 Vital Sign Measurements

Vital sign measurements and changes from Baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time point.

4.9.8.4 Safety 12-lead Electrocardiograms

Safety 12-lead ECG data and changes from Baseline will be summarized using descriptive statistics (number of subjects, mean, SD, minimum, median, and maximum) by treatment and time point. The extent of change in each of the treatments will also be compared. The incidence of pathological ECG interpretive statements at Baseline and during treatment will be assessed among the treatments.

4.9.8.5 Physical Examinations

Physical examination findings will be presented in a data listing, and abnormal physical examination findings will be recorded as AEs.

4.9.8.6 Other Safety Data

All concomitant medication usage and medications that changed in daily dose, frequency, or both since the subject provided informed consent will be summarized for each subject.

4.9.9 Interim Analyses

No interim analyses are planned.

4.9.10 Missing Data

Missing data will not be imputed. Data that are excluded from the descriptive or inferential analyses will be included in the subject data listings. This will include data from subjects not in the particular analysis population, measurements from unscheduled visits, or extra measurements that may arise from 2 or more analyses of a plasma sample at the same time point.

4.10. Data Quality Assurance

Completed eCRFs are required for each subject randomly assigned to study drug. Electronic data entry will be accomplished through the ClinSpark® remote electronic data capture system, which allows for on-site data entry and data management. This system provides immediate, direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner. Each person involved with the study will have an individual identification code and password that allows for record traceability. Thus, the system, and subsequently any

investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records.

Furthermore, the investigator retains full responsibility for the accuracy and authenticity of all data entered into the electronic data capture system. The completed dataset and their associated files are the sole property of the sponsor and should not be made available in any form to third parties, except for appropriate governmental health or regulatory authorities, without written permission of the sponsor.

5. Ethical Considerations

5.1. Ethical Conduct of the Study

This study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, 1964 and later revisions, as well as, United States Title 45 Code of Federal Regulations (CFR) Part 46 GCP, and International Council for Harmonisation (ICH) guidelines describing technical requirements for registration of pharmaceuticals for human use.

5.2. Institutional Review Board (IRB)

The investigator will provide both the local IRB and FDA's research in human subjects committee (RIHSC) with all required documents, including the study protocol and informed consent form. The study will not be initiated until appropriate IRB approval is obtained from both the local IRB and RIHSC. The investigator will provide the sponsor with copies of the approval documents for the protocol, informed consent form, and all recruiting materials. The sponsor will provide a copy of the RIHSC approval letter to the investigator or designee before the study is initiated. The local IRB and RIHSC will also receive copies of any original or amended information sheets or pamphlets given to the study subject in support of the informed consent process and any advertisements or other recruitment material. Such materials will not be employed in the study before approval by both the local IRB and RIHSC.

Subjects will be informed that they have the right to contact the local IRB or Office for Human Research Protections if they have any questions, concerns, complaints, or believe they have been harmed by the participation in this research study as a result of investigator negligence. Subjects will be given the address and phone number of the local IRB and the RIHSC.

6. Administrative Procedures

6.1. Responsibilities of the Investigator

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes may be reported to the IRB but will not result in protocol amendments.

6.1.1 Form FDA 1572

The investigator will complete and sign the Form FDA 1572.

6.1.2 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with the ICH E6(R1) and all applicable guidelines and regulations.

6.1.3 Reporting Requirements

By participating in this study, the investigator agrees to submit reports of SAEs according to the time line and method outlined in the protocol (Section 4.7.3.1.2). In addition, the investigator agrees to submit reports to the IRB as appropriate. The investigator also agrees to provide the sponsor with an adequate report shortly after completion of the investigator's participation in the study.

6.1.4 Source Documentation

By participating in this study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories.

6.1.5 Retention of Records

The investigator agrees to keep the records stipulated in this protocol and those documents that include (but are not limited to) the study-specific documents, identification log of all participating subjects, medical records, source worksheets, all original signed and dated informed consent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent form), copies of all eCRFs, query responses, and detailed records of drug disposition, to enable evaluations or audits from regulatory authorities, the sponsor, or its designees.

Furthermore, ICH 4.9.5 requires the investigator to retain essential documents specified in ICH E6(R1) (Section 8) until at least 2 years after the last approval of a marketing application for a specified drug indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory

authorities are notified. In addition, ICH 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the clinical study site agreement between the investigator and sponsor.

Refer to the clinical study site agreement for the sponsor's requirements on record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

6.1.6 Financial Disclosure and Obligations

The investigator is required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 45 CFR 45. In addition, the investigator must provide to the sponsor a commitment to update this information promptly if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.

Neither the sponsor nor the study clinic is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process.

6.2. Confidentiality and Disclosure of Data

All subjects will sign a HIPAA-compliant authorization form containing the mandated core elements and requirements before participation in this clinical study. The sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject's source data will only be linked to the sponsor's electronic data capture system database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes such as gender, age or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the sponsor requires that the investigator allow review of the subject's original medical records (source data or documents) by the study monitor, representatives from any regulatory authority (e.g., FDA), the sponsor's designated auditors, and the appropriate IRB. These medical records will include, but will not be limited to, clinical laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process.

Copies of any subject source documents that are provided to the sponsor must have certain personally identifiable information removed (i.e., subject name, address, and other identifier fields not collected in the subject's eCRF).

Data will be maintained and backed up in the electronic data capture system. All access to the data is protected by username and password, and each staff member and all sponsor staff will have separate access that requires a separate username and password. Access is only given to site staff and requested sponsor staff who have completed the appropriate training.

6.3. Subject Consent

Written informed consent in compliance with 45 CFR 46 will be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An informed consent template may be provided by the sponsor to the study clinic. If any institution-specific modifications to study-related procedures are proposed or made by the study clinic, the consent should be reviewed by the sponsor or its designee or both before IRB submission. Once reviewed, the consent will be submitted by the investigator to the IRB for review and approval before the start of the study. If the informed consent form is revised during the course of the study, all active participating subjects must sign the revised form.

Before enrollment, each prospective subject will be given a full explanation of the study and be allowed to read the approved informed consent form. The informed consent process will be performed by a clinical research nurse in a private room. The subject will be given unlimited time to ask questions regarding study participation, and each subject will be questioned to ensure their understanding. Once the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing the informed consent form.

The investigator will provide a copy of the signed informed consent form to the subject. The original form will be maintained in the subject's medical records at the site.

6.4. Data Collection

Full details of procedures for data collection and handling will be documented in the data management plan, which is initiated with the final protocol receipt. The data management plan is a changing document that evolves over the course of the study and is finalized by database lock.

6.5. Publications

No information related to or generated by this study will be released to the public until it has been reviewed by the sponsor. The sponsor shall own intellectual rights for the data and analysis resulting from this study and results cannot be presented or published without written permission from the sponsor. Authorship on publications will be determined by standard journal requirements.

7. Study Management

7.1. Release of Study Drug to the Study Clinic

Before the study drug can be released to the study clinic, the following documents will be collected from the study clinic by the clinical research organization, retained in the trial master file, and a study drug shipment approval form will be completed by the clinical research organization:

- Protocol signature page signed by the investigator
- IRB approval of the protocol and informed consent form and IRB membership list
- Completed Form FDA 1572, curriculum vitae, and medical licenses from each investigator
- Financial disclosure and debarment certification from each investigator
- Executed contract with investigator and study clinic

7.2. Monitoring

The sponsor or its designee will monitor the study to ensure that it is being conducted according to the protocol, GCP standards, and applicable region-specific requirements, and to ensure that study initiation, conduct, and closure are adequate. The investigators and the study clinic staff will be expected to cooperate fully with the study monitors and personnel or agents of the sponsor, and be available during monitoring visits to answer questions sufficiently and to provide any missing information. The investigators and their institutions will permit direct access to source data/documents for study-related monitoring activities, audits, IRB reviews, and regulatory inspections.

During any on-site visits, the study monitor will:

- Check and assess the progress of the study
- Review all informed consent forms
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution
- Verify that the facility remains acceptable
- Conduct study drug accountability

These monitoring activities will be done in order to verify that the:

- Data are authentic, accurate, and complete.
- The safety and rights of the subjects are being protected.

- The study is being conducted in accordance with the currently approved protocol (including any amendments), GCP, and all applicable regulatory requirements.

In addition, the sponsor, designated auditors, and government inspectors must be allowed access to eCRFs, source documents, and other study files that may be required to evaluate the conduct of the study.

7.3. Management of Protocol Amendments and Deviations

7.3.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent immediate hazard to the subject, must be submitted to the sponsor or designee and reviewed and approved by RIHSC before implementation. Amendments to the protocol must be submitted in writing to the investigator's IRB and RIHSC for approval before subjects are enrolled into an amended protocol.

7.3.2 Protocol Violations and Deviations

Any significant protocol deviations that the investigator or study clinic staff believes are of major importance (e.g., incorrect randomizations, subject enrolled but not eligible) should be reported to the sponsor and the investigator's IRB as soon as possible. Significant protocol deviations may include the following:

- Deviations from the inclusion/exclusion criteria that may affect subject safety
- Deviations (omission or delay) of safety monitoring procedures
- Deviations in the administration of the study drug
- Deviations in obtaining informed consent

All subjects who are enrolled and receive the study drug, regardless of whether they have a major protocol violation, must continue to be followed for safety for all follow-up study visits.

8. Appendices

8.1. Appendix A: Schedules of Events

Table 8-1 Overall Schedule of Events

	Screening	Check-in	Treatment Days			Discharge Day (or Early Termination)
Study Day(s)[a]	–28 to –1	0	1 or 8	2 or 9	3 or 10	4 or 11
Informed consent/HIPAA authorization	X					
Eligibility assessment	X	X				
Demographics	X					
Height, weight, body mass index	X					
Serology	X	X[k]				
Holter ECG[b]	X	X				
Medical history	X	X				
Drug and alcohol screening	X	X				
Pregnancy test (female subjects)	X	X				X
Blood sample for genetic testing		X				
Blood sample for PBMC isolation		X				
Admission to study clinic		X				
Randomization[c]		X				
Prior and concomitant medications	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X
Clinical laboratory tests[d]	X	X				X
Vital sign measurements[e]	X	X	X	X	X	X
Safety 12-lead ECG[f]	X	X	X	X	X	X
Physical examination	X	X				X
Study drug administration[g]			X	X	X	
Continuous 12-lead ECG[h]			X	X	X	X
PK blood sample collection[i]			X	X	X	X
Discharge from study clinic[j]						X

Abbreviations: ECG, electrocardiogram; HIPAA, Health Insurance Portability and Accountability Act; PBMC, peripheral blood mononuclear cells; PK, pharmacokinetic.

a For Part 1, subjects will check-in on Day 0 and remain confined in the study clinic until Day 4. For Part 2 (2-period crossover), subjects will check-in on Day 0 (Period 1) and remain confined until Day 11 (end of Period 2). In Part 2, events scheduled on Days 1, 2, and 3 (Period 1) will be repeated on Days 8, 9, and 10 (Period 2).

b At Screening, a 3-hour Holter ECG will be performed in order to exclude subjects who have more than 12 ectopic beats during this collection period. At check-in, continuous/Holter 12-lead ECGs will be recorded from early afternoon until the subjects go to sleep. During this period, subjects will perform a sequence of postural maneuvers (e.g., 10 minutes supine, 10 minutes sitting, 10 minutes standing, 10 minutes sitting, 10 minutes supine) to characterize ECG biomarkers and heart rate relationship.

c Randomization will occur either after completion of check-in procedures on Day 0 or just before dosing on Day 1.

d All subjects will fast overnight for a minimum of 8 hours (no food or fluid except water) before blood collection for clinical laboratory testing.

e Vital signs will be measured at the time points described in Table 8-2. Vital signs will be measured before the PK sample is collected, but after safety 12-lead ECG and continuous ECG extractions. The subject should be in a supine position, if possible, for a minimum of 5 minutes before vital signs are measured.

f Safety 12-lead ECGs will be performed at the time points described in Table 8-2. The subject should be in a supine position, if possible, for approximately 10 minutes before safety 12-lead ECGs are measured. While collecting the safety 12-lead ECG, there should be minimal to no interruption of the continuous 12-lead ECG collection.

g First morning oral dose (placebo or chloroquine) will be administered in the morning with 240 mL of room-temperature water after subjects have fasted for at least 8 hours and 1 hour before breakfast. Second morning oral dose (placebo, ranolazine, verapamil or lopinavir + ritonavir, dofetilide, diltiazem IR or dofetilide + diltiazem IR) will be administered with breakfast. Afternoon oral dose (placebo or verapamil) will be administered approximately 2 hours after lunch. Evening oral dosing (placebo, ranolazine, verapamil, lopinavir + ritonavir, dofetilide or diltiazem ER) will be administered together with a snack (e.g., doses at approximately 7 am, 8:30 am, 2:30 pm, and 8:30 pm). Meals will be administered 30 minutes before oral dosing. Subjects will be instructed to consume all of contents of the meals at a reasonable pace (within 25 minutes) to allow time to return to their room for dosing.

h On the treatment days (Days 1, 2 and 3 within each period), continuous 12-lead ECGs will be recorded using the telemetry system for a total of approximately 74 hours (starting 1 hour before first dosing of the treatment period and continuing until 73 hours after time 0 or first oral dose on Day 1). Ten-second 12-lead ECG tracings with the most stable heart rate and highest signal-to-noise ratio will be extracted in at least 3 replicates at 28 ECG extraction time points during the 2 treatment periods as follows: at 3 time points before the first oral dose (Baseline) and at 25 time points following the first oral dose (Table 8-2). For all time points, the subjects will be in a supine position for approximately 10 minutes before and approximately 5 minutes after the time point. The subjects will also be in a supine position during the baseline and 73-hour time point.

i Pharmacokinetic blood samples will be collected on the treatment days (Days 1, 2, 3, and 4 within each period). Blood samples may be collected by direct venipuncture or by inserting an intravenous catheter into the subject's forearm region. A single PK blood sample (5 mL) will be collected at the following time points within each period: 0.5, 2, 2.5, 3.5, 4.5, 5, 6, 8.5, 14, 24, 27.5, 32.5, 38, 48, 50, 50.5, 51.5, 52.5, 53, 54, 54.5, 55, 59, 61, and 73 hours after time 0 or first oral dose on Day 1 or Day 8 (Table 8-2). Note: there is no PK blood sample collection at the predose time point on Day 1.

j Subjects will be discharged from the study after completion of all study procedures. If a subject discontinues from the study prematurely, all procedures scheduled for the end of the study will be performed.

k If it has been more than 14 days (prior to check-in) since the subject was screened for human immunodeficiency virus (HIV) antigen/antibody (Ag/Ab) Combo 1/2, hepatitis C virus (HCV) antibody, and hepatitis B surface antigen (HBsAg), the subject will be re-screened for HIV Ag/Ab Combo 1/2, HCV antibody, and HBsAg.

Table 8-2 Schedule of Events by Time Point

See attached document that presents a schedule of events by time point.

9. Reference List

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