Official Title: A Single-Center, Multiple-Dose, Randomized, Double-Blind, Placebo-Controlled, Positive-Controlled, Crossover Study to Investigate the Effect of Balovapton on the Qtc Interval in Healthy Subjects

NCT Number: NCT03808298

Document Date: Protocol Version 3: 23-April-2019

PROTOCOL

TITLE:	**A SINGLE-CENTER, MULTIPLE-DOSE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, POSITIVE-CONTROLLED, CROSS-OVER STUDY TO INVESTIGATE THE EFFECT OF BALOVAPTAN ON THE QTC INTERVAL IN HEALTHY SUBJECTS**
PROTOCOL NUMBER:	WP40734
VERSION NUMBER:	V3.0
EUDRACT NUMBER:	NA
IND NUMBER:	116483
TEST PRODUCT:	Balovaptan (RO5285119)
MEDICAL MONITOR:	[redacted], MD
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	See electronic date stamp below

FINAL PROTOCOL APPROVAL

Approver's Name	Title	Date and Time (UTC)
[redacted]	Company Signatory	23-Apr-2019 06:19:00

Document	Date of Issue	Approver(s)	Summary of Changes
Original Protocol	Oct-23-2018		• Not Applicable
Protocol v2.0 Amendment 1 and Administrative letter 1, Nov-13-2018	See date stamp on the cover page.		• Changes in wording regarding intensive PK sampling for balovaptan and moxifloxacin outlined in Administrative letter from Nov-13-2018 incorporated. • Reference to balovaptan sampling on Day 16 have been removed. • The 1.25 h time point for PK sampling and ECG readings has been changed to 1.0 h post-dose. This information has been updated in Appendices and protocol text. • Section 4.4 has been edited to clarify that concomitant therapy should be captured from 30 days prior to initiation of study drug. • The text in Section 4.4.2 regarding prohibited therapies has been edited for clarity. • Text in Section 4.4.4 has been edited to remove the statement "Subjects should have fasted for at least 8 hours prior to the screening visit, each admission to the CRU, and the Follow up visit" as it redundant with the collection description in Section 4.5.5.1. • Text has been added to Section 4.4 to clarify that the clinical site is not required to track subject's fluid intake. • In Section 4.5.3 Physical Examinations the phrase "Any abnormality identified" has been updated to read "Any clinically significant abnormalities identified" • Instructions for collection and handling of PK samples in Section 4.5.5.2 has been updated. • Text reading safety ECG readings in Section 4.5.6.1 has been edited for clarity. • Section 4.6.1 has been edited for clarity regarding assessments for subjects who withdraw or are terminated from the study early. • Text regarding ECG extraction on consecutive days has been edited throughout the document to clarify that when a reading is taken on consecutive days (Day 2 and Day 15) the 24 h time point can be taken from the 3 pre-dose Holter extractions. • Text regarding analysis of samples for cardiac biomarkers has been edited to indicate that samples will be taken but only analyzed for specific subjects on request. • Text in Section 5.3.1 has been modified to make it consistent with Section 5.4.2.1 and Appendix 2 clarifying that only SAE's will be recorded prior to study drug administration. • Section 6.7.2 has been edited to make text consistent with ECG extraction time points throughout protocol. • TQT exclusion criteria #3 and #7 have been edited to clarify that criteria should be assessed at both Screening

			and at Day -2 of Treatment Period 1. • TQT exclusion criteria #4 has been edited for clarity. • lactate dehydrogenase (LDH) has been added to the list of blood chemistry parameters to be assayed for based on FDA feedback. • Troponin 1 has been changed to Troponin I throughout the document • Troponin T has been replaced in the text with cardiac troponin (cTnT) to add specificity • Minor editorial and administrative changes have been made to multiple sections of the text for clarity.
Protocol v3.0 Amendment 2	See date stamp on the cover page.		• An optional blood sample for DNA extraction and Clinical Genotyping has been added. [redacted] or if genetic variants of cytochrome P450s (e.g., CYP3A4), transporters, or receptors may affect the metabolism, pharmacokinetics of balovaptan, or whether any potential effect of balovaptan on ECG parameters may be associated with any genetic variants in genes. • Changes to SOA regarding cardiac biomarkers sampling outlined in Administrative letter from Feb-08-2019 incorporated

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PROTOCOL ACCEPTANCE FORM

TITLE:	**A SINGLE-CENTER, MULTIPLE-DOSE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, POSITIVE-CONTROLLED, CROSS-OVER STUDY TO INVESTIGATE THE EFFECT OF BALOVAPTAN ON THE QTC INTERVAL IN HEALTHY SUBJECTS**
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I agree to conduct the study in accordance with the current protocol.

_____

Principal Investigator's Name (print)

_____ _____

Principal Investigator's Signature Date

Please retain the signed original of this form for your study files. Please return a copy of the signed form by your local study monitor to the contact provided below.

[redacted]
Roche Products Limited
6 Falcon Way, Shire Park
Welwyn Garden City,
AL7 1TW, United Kingdom

PROTOCOL SYNOPSIS

TITLE:	**A SINGLE-CENTER, MULTIPLE-DOSE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, POSITIVE-CONTROLLED, CROSS-OVER STUDY TO INVESTIGATE THE EFFECT OF BALOVAPTAN ON THE QTC INTERVAL IN HEALTHY SUBJECTS**
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IND NUMBER:	116483
TEST PRODUCT:	Balovaptan (RO5285119)
PHASE:	Phase 1
INDICATION:	Not applicable
SPONSOR:	F. Hoffmann-La Roche Ltd

Objectives and Endpoints

This study will investigate the effect of balovaptan (RO5285119) on cardiac repolarization by measuring ECGs in healthy subjects following multiple doses of the targeted therapeutic and supra-therapeutic doses.

Primary Objective	Corresponding Endpoint
1. To investigate the effect of balovaptan 50 mg QD on the QTcF interval after 14 days of dosing.	1. Placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 14
Secondary Objectives	**Corresponding Endpoints**
1. To investigate the effect of a single dose of balovaptan 50 mg on the QTcF interval at Day 1.	1. Placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1.
2. To investigate the effect of balovaptan 10 mg QD on the QTcF interval at Day 1 and Day 14.	2. Placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1 and Day 14.

3. To investigate the effect of balovaptan on the following ECG parameters: PR, RR, QRS, QT, T-wave and U-wave morphology.	3. Placebo-corrected, change-from-baseline heart rate (HR), PR and QRS interval ($\Delta\Delta$HR, $\Delta\Delta$PR and $\Delta\Delta$QRS) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1 and Day 14. • Categorical outliers for QTcF, HR, PR, and QRS. • Frequency of treatment emergent changes of T-wave morphology and U-wave presence.
4. To investigate the pharmacokinetics of balovaptan, M2 (as appropriate) and M3.	4. Pharmacokinetic parameters estimation such as Tmax, Cmax, and AUC0-24h for plasma concentrations of balovaptan, M2 (as appropriate) and M3. Other pharmacokinetic parameters may also be added.
5. To investigate the pharmacokinetics of moxifloxacin.	5. Pharmacokinetic parameters estimation such as Tmax, Cmax, and AUC0-24h for plasma concentrations of moxifloxacin. Other pharmacokinetic parameters may also be added.
6. To investigate the relationship between plasma concentrations of balovaptan, M2 (as appropriate), M3 and QTcF interval length.	6. Pharmacokinetic-pharmacodynamic model of balovaptan, M2 (as appropriate) and M3 concentrations vs. QTcF changes.
7. To investigate the relationship between plasma concentrations of moxifloxacin and QTcF interval length (if warranted).	7. Pharmacokinetic-pharmacodynamic model of moxifloxacin concentrations vs. QTcF changes (if warranted).
8. To investigate the ECG assay sensitivity to detect a change in the QTcF interval, using moxifloxacin 400 mg as an active control	8. Placebo-corrected, change-from-baseline QTcF ($\Delta\Delta$QTcF) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 2 and Day 15.
9. To investigate the safety and tolerability of balovaptan and moxifloxacin at the dosing regimens scheduled.	9. Safety assessments: adverse events, physical examination, vital signs, laboratory tests, C-SSRS and HR monitoring.
Exploratory Objectives	**Corresponding Endpoints**
1. To investigate any effect of balovaptan at 50 mg QD and 10 mg QD doses on pulmonary blood pressure as assessed by the use of TT-doppler echocardiography.	1. TT-Doppler echocardiography: pulmonary artery acceleration time (PAAT), right ventricular outflow tract - velocity time integral (RVOT-VTI) and derived mean pulmonary arterial pressure as change to baseline relative to placebo treatment.

2. To investigate any effect of 50 mg qd and 10 mg qd on right and left heart as assessed by TT-Doppler echo.	2. TT-Doppler-echocardiography: LVEF and additional secondary TT-Doppler-echocardiography parameters of left and right heart, as described in Section 4.5.7 (also for objective 1)
3. To investigate the effect 10 mg and 50 mg QD balovaptan on blood pressure and heart rate as measured by the use of Holter-ECG and ABPM.	3. Ambulatory blood pressure monitoring (ABPM): mean diastolic and systolic blood pressure (daytime and nighttime mean). • Heart rate will also be analyzed as derived from Holter ECG. – Heart rate as derived from ABPM and/or Holter ECG may be explored against fluctuations in blood pressure measured by ABPM (if warranted).
4. To investigate whether genetic variants in genes are associated with natural or potential drug induced neutropenia; with differences in pharmacokinetics of balovaptan, M2 or M3, or with any potential effect of balovaptan on ECG parameters.	5. The genetic variants in genes that are associated with natural or potential drug induced neutropenia; genetic variants in genes that are associated with pharmacogenetics of metabolizing enzymes, transferases, transporters, etc, (eg,CYP3A4 and P-glycoprotein); genetic variants in genes that may be associated with a potential effect of balovaptan on ECG parameters.

Study Design

This will be a single-center, multiple-dose, randomized, double-blind, placebo-controlled, positive-controlled, twelve sequence, 3-period cross-over study to investigate the effect of balovaptan on the QTc interval in healthy subjects.

Screening

Screening will be conducted up to 28 and 3 days (inclusive) before each subject's first dosing day (Day 1 of Treatment Period 1).

In-Clinic Confinement

Day -2

Subjects will be confined to the CRU starting on Day -2 until the morning of Day 16 during each Treatment Period. Subjects are entered into CRU on Day -2 so that subjects are rested and prepared for the baseline ABPM and baseline TT-Doppler echocardiogram on Day -1. Eligibility assessments will be performed on Day -2 as outlined in the Schedule of Activities (SoA), Appendix 1.

Day -1

On Day -1 of each Treatment Period, ABPM and TT-Doppler echocardiogram assessments will be performed. These results will serve as the baseline for the subjects. On days when TT-Doppler echocardiogram and ABPM assessments are performed subjects will be given low salt standardized meals.

Randomization (Day -1)

At the start of the study in Treatment Period 1, at the end of the day of Day -1, subjects will be randomized to one of the 12 treatment sequences comprised of the 4 treatments described:

Treatments

Treatment A (balovaptan therapeutic dose)

Days 1-14: A single once daily oral dose of 10 mg (1 × 10 mg tablet) of balovaptan and 4 tablets of matching placebo for balovaptan for 14 days.

Day 2 and 15: A single oral dose of a matching placebo capsule for moxifloxacin.

Treatment B (balovaptan supra-therapeutic dose)

Days 1-14: A single once daily oral dose of 50 mg (5 x 10 mg tablets) of balovaptan for 14 days.

Day 2 and 15: A single oral dose of a matching placebo capsule for moxifloxacin.

Treatment C (active control [400 mg moxifloxacin] on Day 2)

Days 1-14: A single once daily oral dose of 5 tablets of matching placebo for balovaptan for 14 days.

Day 2: A single oral dose of 400 mg moxifloxacin capsule

Day 15: A single oral dose of a matching placebo capsule for moxifloxacin

Treatment D (active control [400 mg moxifloxacin] on Day 15)

Days 1-14: A single once daily oral dose of 5 tablets of matching placebo for balovaptan for 14 days.

Day 2: A single oral dose of a matching placebo capsule for moxifloxacin

Day 15: A single oral dose of 400 mg moxifloxacin capsule.

Treatment Sequences

1. A, B, C
2. A, C, B
3. B, A, C
4. B, C, A
5. C, A, B
6. C, B, A
7. A, B, D
8. A, D, B
9. B, A, D
10. B, D, A
11. D, A, B
12. D, B, A

Dosing (Days 1-15)

Each Treatment Period will involve 15 days of dosing (Day 1 – Day 15). Subjects must fast for at least 8 hours prior to and 4 hours after dosing on study days when 24-hour Holter ECG assessments will be performed (study Days 1, 2, 14 and 15). Dosing on study days with 24-hour Holter ECG assessment should be at approximately the same time of day ± 1h.

Prior to dosing on Day 1 subjects will undergo pre-dose assessments as outlined in the SoA (Appendix 1). Following pre-dose assessments subjects will receive a single dose of study medication preceded by a ≥ 8 hour fast the night before. Study drugs will be taken with 240 mL of water and be followed by a 4-hour post-dose fast. If subjects require more liquid to take all study drugs, additional water may be provided in aliquots of 50 mL. If additional

water is provided the amount should be captured. Water provided for study drug administration will be identical on all dosing days. Identical fasting procedures from Day 1 will be followed on study Days 2, 14 and 15. No fasting will be required on other dosing days. Subjects will receive standardized low salt meals on study days when 24-hour Holter ECGs (Days 1, 2, 14 and 15), ABPM (Days -1 and 12) or TT-Doppler echocardiogram (Days -1 and 13) assessments will be performed. Meals will be given at approximately the same time on each of these study days.

Days 1 and 2: 24-hour Holter ECG Assessments
On study Days 1 and 2 of each Treatment Period, 24-hour ECG assessments will be performed. Subjects will be administered study drug following a ≥ 8 hour fast and continue to be fasted for 4 hours post-dose on these days. Subjects will be provided with standardized low salt meals on these days. Subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Day 12
On study Day 12 of each Treatment Period, ABPM assessments will be performed for each subject. On study days when ABPM assessments are performed subjects will be provided with standardized low salt meals. Subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Day 13
On study Day 13 of each Treatment Period, at approximately the same time of day as on Day -1, TT-Doppler echocardiogram will be performed to assess any effects of steady state balovaptan levels on cardiac parameters. Subjects will receive standardized low salt meals on this study day. In addition to TT-Doppler echocardiogram subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Days 14 and 15: 24-hour Holter ECG Assessments
On study Days 14 and 15 of each study Treatment Period, 24-hour ECG assessments will be performed. Subjects will be administered study drug following a ≥ 8 hour fast and continue to be fasted for 4 hours post-dose. Subjects will be provided with standardized low salt meals on these days. Subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Day 16
Subjects will leave the clinical research unit (CRU) on the morning of Day 16 after final assessments as described in the SoA (Appendix 1).

Washout Period:
Following each Treatment Period subjects will undergo a washout period lasting a minimum of 13 and a maximum of 20 days from the last dose of study drug.

Follow-up:
Subjects will return to the unit for a follow-up visit between 14 and 21 days after the final dose of study drug. This will include a general physical examination, 12-lead-ECG, vital signs, C-SSRS, adverse event review, body weight and laboratory safety tests.

Number of Subjects

56 subjects will be enrolled with the goal of obtaining 48 evaluable subjects.

Target Population

The target population will be healthy male or female volunteers aged 18 to 60 years inclusive. An attempt will be made to have each Treatment population contain at least 1/3 male subjects by ensuring that a minimum of 40% of enrolled population are male.

Inclusion Criteria

Subjects must meet the following criteria for study entry:

1. Healthy male or female volunteers aged 18 to 60 years inclusive, at screening. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECG, hematology, blood chemistry, urinalysis, and serology.
2. Body Mass Index (BMI) between 18 and 32 kg/m^2 inclusive, at Screening.
3. For women of childbearing potential: agreement to use at least 1 acceptable form of contraception during the entire study and for 90 days following last dose of study drug.
    a. A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (> 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).
    b. The following are acceptable contraceptive methods: abstinence from heterosexual activity; bilateral tubal ligation; sterilization of male partner; hormonal contraceptives; hormone-releasing intrauterine devices; copper intrauterine devices; Essure.
    c. Barrier methods alone are not considered an acceptable form of contraception for this study and if utilized must be supplemented by one of the acceptable methods described above. For this study, barrier contraception methods are defined as: male or female condom with or without spermicide (male and female condom must not be used simultaneously); and cap, diaphragm, or sponge with spermicide.
4. For men: Vasectomized, agreement to remain abstinent (refrain from heterosexual intercourse) or use of a condom during intercourse. Must also agree to refrain from donating sperm, as defined below:
    a. With female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during the entire study and for 90 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.
5. Able to participate, and willing to give written informed consent and to comply with the study restrictions.
6. Fluent in English.

Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from study entry:

Past Medical History

1. History of any clinically significant gastrointestinal, renal, hepatic, broncho-pulmonary, neurological, psychiatric, cardiovascular, endocrinological, hematological, lymphatic, musculoskeletal, genitourinary, immunological, dermatological or connective tissue or allergic disease, metabolic disorder, or cancer.
2. Signs and symptoms potentially indicative of peripheral neuropathy.
3. Unexplained orthostatic reaction with syncope within last 6 months.
4. A history of clinically significant hypersensitivity (e.g., to drugs, including moxifloxacin or any quinolone antibiotics and balovaptan, and excipients) or allergic reactions.
5. History of alcohol and/or drug abuse or addiction within the last 2 years before study enrollment.

6. Subjects on hormone replacement therapy if post-menopausal status cannot be documented from medical history.
7. Previous treatments with iron for iron deficiency anemia within three months of screening.
8. History of coagulopathies, bleeding disorders, or blood dyscrasias.
9. History of porphyria, active liver disease or permanent or unexplained elevation of serum transaminases.
10. In the opinion of the Investigator or designee, any major illness within one month before the screening examination or any febrile illness within one week prior to Screening and up to first study drug administration.

Current Status

1. Clinically relevant deviation from normal in the physical examination including vital signs.

2. Positive result on human immunodeficiency virus (HIV) 1, HIV 2, hepatitis C virus (HCV) antibody, or hepatitis B virus surface antigen (HBsAg).

3. Clinically significant abnormalities in laboratory test results (including complete blood count, chemistry panel, and urinalysis). In the case of uncertain or questionable results, tests performed during Screening may be repeated on Day -1 or Day -2 to confirm eligibility. Repeat of abnormal test results are allowed during Screening and eligibility assessment at the discretion of the Investigator or designee provided results will be available prior to Randomization on Day -1.

4. If female, a positive pregnancy test at screening or prior to Day 1 of any Treatment Period.

5. Lactating women.

6. Any condition or disease detected during the medical interview / physical examination that would render the subject unsuitable for the study, place the subject at undue risk or interfere with the ability of the subject to complete the study in the opinion of the Investigator or designee.

7. Subjects with CPK values > 2x upper limit of normal (ULN), confirmed by repeat sample.

8. Positive urine alcohol test or urine drug test at Screening or Day -2 of each Treatment Period (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, methadone, methamphetamines, and 3,4-methylenedioxymethamphetamine [MDMA]).

9. Subject has been a regular smoker (> 5 cigarettes, > 1 pipeful, > 1 cigar per day or equivalent as determined by Investigator or designee) within 6 months of study enrollment.

10. Coffee (or tea) consumption > 5 cups per day or methylxanthine-containing drinks >1.5 liter/day or more than 250 g/day of chocolate within 3 months of first dose of study drug.

11. Alcohol consumption of > 18 units per week for males and females. One unit of alcohol equals 12 ounces of regular beer, 5 ounces of wine or 1.5 ounces of 80 proof spirits within 3 months of first dose of study drug.

12. Subjects who have lost or donated in excess of ~450 mL blood in the 3 months prior to Day 1 of Treatment Period 1.

13. Subjects under judicial supervision, guardianship, or curatorship.

14. Dietary restrictions that would prohibit the consumption of standardized meals.

15. Poor venous access for blood sampling.

Concomitant Medication

1. Taken greater than 2 g/day paracetamol within 48 hours of study drug administration or greater than 4 g/day paracetamol within 7 days of study drug administration.
2. Any nonprescribed systemic or topical medication or herbal remedies are prohibited for 7 days prior to the first study drug administration through Follow-up.
3. Participation in an investigational drug study within 60 days or 5 times the elimination half-life, whichever is longer, prior to first dosing, or within 5 months prior to first dosing in case of a study with a biological, as calculated from the last day of follow-up from the previous study.
4. Participation in an investigational device study within 60 days of first dosing.
5. Subjects who have received any prescribed systemic or topical medication within 14 days of the first dose administration are excluded, unless in the opinion of the Investigator (or designee) the medication will not interfere with the study procedures or compromise safety.
6. Any slow release medicinal formulations considered to still be active within 4 weeks (or within 5 times the elimination half-life of the medication, whichever is longer) prior to the first study drug administration are prohibited, unless in the opinion of the Investigator or designee the medication will not interfere with the study procedures or compromise safety.
7. Taking any inhibitor of CYP3A taken within 4 weeks (or within 5 half-lives, whichever is longer) prior to study drug administration or taking any inducer of CYP3A within 4 weeks prior to the first dosing.
8. Use of any prohibited medications and food before study start and subjects who do not agree to refrain from consuming prohibited medications or food during the study.
9. Subjects who have previously enrolled in, or withdrawn after enrollment, from this study.

TQT-related Exclusion Criteria

1. Personal history of unexplained black-outs or faints.
2. Personal history of reactions or faints at having their blood taken, unexplained black-outs or faints or any condition which could relapse during or immediately after the study which in the opinion of the Investigator or designee makes the subject unsuitable to participate in this study.
3. Subjects experiencing fainting or pre-syncopal events during orthostatic challenge testing, performed according to site SOP, at Screening or Day -2 of Treatment Period 1.
4. Subjects with Screening or Treatment Period 1 (Day -2) baseline mean QT interval corrected using Fridericia's formula (QTcF) > 450 ms or < 300 ms (using the same upper QTcF limit in both males and females) or > 30 ms difference between the highest and the lowest of the triplicate readings.

5. Screening or baseline (Day -2, Treatment Period 1) ECG evidence of atrial fibrillation, atrial flutter, second or third-degree AV block, complete right or left bundle branch block, Wolff-Parkinson-White-syndrome, or cardiac pacemaker.
6. Screening or baseline ECGs (Day -2 of Treatment Period 1) with QRS and/or T-wave judged to be unfavorable for a consistently accurate QT measurement (i.e., neuromuscular artifact that cannot be readily eliminated, arrhythmias, indistinct QRS onset, low amplitude T-wave, merged T- and U-waves, prominent U-waves, etc.).
7. Confirmed (based on the average of ≥ 3 consecutive measurements) systolic blood pressure greater than 139 or less than 90 mmHg, and diastolic blood pressure greater than 89 or less than 45 mmHg at Screening or Day -2 of Treatment Period 1.
8. Confirmed (based on the average of ≥ 3 consecutive measurements) resting HR > 100 bpm or < 40 bpm at Screening or Day -2 of Treatment Period 1.
9. Notable resting bradycardia (HR < 40 bpm) on ECG at Screening or Day -2 of Treatment Period 1. Notable resting tachycardia (mean HR > 100 bpm) on ECG at Screening or Day -2 of Treatment Period 1.
10. Family history of congenital long QT syndrome or sudden death.
11. History or presence of clinically significant ECG abnormalities, as determined by study Investigator or designee, before study drug administration.

End of Study

The end of this study is defined as the date when the last subject, last visit occurs, or safety follow-up is received from the last subject, whichever occurs later.

Length of Study

The total length of the study, from screening of the first subject to the end of the study, is expected to be approximately 14-20 weeks. For each enrolled subject the duration of the study from Screening to the end of Follow-up will be a minimum of 122 days or a maximum of 143 days.

- Screening: up to 28 days
- Dosing periods including washout: 80 – 94 days
    - Treatment Period 1: 18 Days (Day -2 – Day 16)
    - Washout: 13-20 Days
    - Treatment Period 2: 18 Days (Day -2 – Day 16)
    - Washout: 13-20 Days
    - Treatment Period 3: 18 Days (Day -2 – Day 16)
- Follow up: 14 - 21 days after last dosing

Investigational Medicinal Products

Test Product (Investigational Drug)

Balovaptan 10 mg (Therapeutic dose) orally, once-daily for 14 days

Balovaptan 50 mg (Supratherapeutic dose) orally, once-daily for 14 days

Moxifloxacin 400 mg, single, oral dose

Comparator

Placebo controlled

Statistical Methods

For cardiodynamic ECG evaluation, the primary analysis will be based on by-time point analysis to evaluate the effect of balovaptan on the placebo-corrected change-from-baseline QTcF (ΔΔQTcF) at each post-dosing time point using the Intersection Union Test. The effect of balovaptan on placebo-corrected change-from-baseline in HR, PR, and QRS (ΔΔHR, ΔΔPR, and ΔΔQRS) will also be evaluated using the Intersection Union Test. In addition, the relationship between plasma concentrations of balovaptan and its metabolites (M2 [as appropriate] and M3) and ΔΔQTcF will be evaluated using a linear mixed-effects modeling approach. An analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence. Assay sensitivity will also be evaluated using by-time point analysis of the effect on ΔΔQTcF of moxifloxacin using a similar model as for the primary analysis.

Determination of Sample Size:

A sample size of 56 subjects was chosen to obtain 48 evaluable subjects. Subjects are evaluable if ECGs are available for all three periods to allow (1) the derivation of the primary endpoint (i.e., change from baseline on Day 14), and (2) the assessment of the effect of moxifloxacin. Assuming a 1-sided 5% significance level and a within-subject standard deviation (SD) of 8 ms for ΔQTcF and a true mean difference of 3 ms in ΔQTcF between balovaptan and placebo, a sample size of 48 evaluable subjects will provide a power of 96.6% to demonstrate that the upper bound of all the 2-sided 90% CIs will fall below 10 ms for up to 7 post-dose time points on Day 14. Sample Size Determination for Assay Sensitivity: Assuming a 1-sided 5% significance level and a within-subject SD of 8 ms for ΔQTcF, a sample size of 48 evaluable subjects will provide a power of 99% to exclude a mean difference of 5 ms in ΔQTcF between moxifloxacin and placebo groups from the lower bound of the 2-sided 90% CI at least one of the three pre-specified time points.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ABPM	ambulatory blood pressure monitoring
[redacted]	[redacted]
AE	adverse event
ALT	alanine transaminase
ANC	absolute neutrophil count
ASD	autism spectrum disorder
AST	aspartate transaminase
AUC	area under the curve
BMI	body mass index
BP	blood pressure
BPM	beats per minute
CI	confidence interval
Cmax	mean maximum observed plasma concentration
cTnT	cardiac troponin
CNS	central nervous system
CPK	creatine phosphokinase
CRO	contract research organization
CRU	clinical research unit
C-SSRS	Columbia-suicide severity rating scale (C-SSRS)
CTCAE	common terminology criteria for adverse events
Δ	change-from-baseline
ΔΔ	placebo-corrected change-from-baseline
DMC	data monitoring committee
EC	ethics committee
ECG	electrocardiogram
eCRF	electronic Case Report Form
EPQT	expert precision QT technique
EDC	electronic data capture
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GLP	good laboratory practice
HBsAg	hepatitis B virus surface antigen
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
hERG	human ether-à-go-go related gene

HR	heart rate
ICH	International Council for Harmonisation
IMP	investigational medicinal product
IND	Investigational New Drug (Application)
IRB	Institutional Review Board
LDH	lactate dehydrogenase
LPLV	last patient, last visit
LS	least squares
LVEF	left ventricular ejection fraction
MAD	multiple ascending dose
MDMA	3,4 Methylenedioxymethamphetamine
mEq/L	milliequivalents per liter
mmHg	millimeters of Mercury
NCI	National Cancer Institute
NT-proBNP	N-terminal pro B-type natriuretic peptide
NOAEL	no observed adverse effect level
NCI	National Cancer Institute
PAAT	pulmonary artery acceleration time
P-gp	P-Glycoprotein
PK	pharmacokinetic
PND	postnatal day
PR	PR interval of the ECG
PRO	patient-reported outcome
QRS	QRS interval of the ECG
QT	QT interval of the ECG
QTc	corrected QT interval
QTcF	QT interval corrected through use of Fridericia's formula
RR	RR interval of the ECG
RVOT	right ventricular outflow tract
SAD	single ascending dose
SAE	serious adverse event
SD	standard deviation
SE	standard error
SOA	schedule of activities
Tmax	time to reach Cmax
TT	trans thoracic
TVI	transvalvular impedance
ULN	upper limit of normal

1. BACKGROUND

1.1 AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by two core domains: impairments in social interaction and communication and the presence of repetitive or restricted behaviors, interests, or activities (American Psychiatric Association, Diagnostic and statistical manual of mental disorders (DSM-5®) 2013). The Autism and Developmental Disabilities Monitoring Network recently reported, based on children records, that the estimated prevalence of ASD in the United States for the year 2010 was 14.7 per 1000 (one in 68) children aged 8 years (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators, 2014). Core symptoms of ASD affecting domains of socialization, communication, and repetitive behavior are usually observed by 3 years of age, although typical language development might delay identification of symptoms.

Evidence from both human and animal studies strongly implicates the V1a receptor in mediating and modulating key social behaviors that are deficient in ASD. Together with the known negative effects of intranasal administration of vasopressin on emotional cognition, a V1a receptor antagonist may provide a novel and first approach to treat the deficits at the core of these disorders.

Balovaptan (RO5285119) is a potent and selective human V1a receptor antagonist that blocks the activation of the V1a G protein-coupled receptor. More detailed information around ASD and Balovaptan are presented in the Investigator's Brochure.

1.2 PRE-CLINICAL BACKGROUND ON BALOVAPTAN

The in vitro metabolism of balovaptan was studied in hepatic preparations from human, rat, dog, minipig, cynomolgus monkey, mouse, and rabbit. In microsomes from all species tested, major metabolism pathways were [redacted] (formation of M3) and [redacted] (formation of M1), whereas further secondary metabolites including oxidations and desaturations were found at low amounts.

The in vivo pharmacokinetic (PK) studies with balovaptan were conducted in rats, dogs, and cynomolgus monkeys. In animals, balovaptan showed a high (rats) to moderate or low (dogs and cynomolgus monkeys) plasma clearance and moderate extravascular distribution. High to moderate bioavailability, depending on the species, was observed after oral application. In human, 2 major metabolites, M2 [redacted] and M3 [redacted], have been identified, which are present in rats and dogs. [redacted] Balovaptan and M3 have been shown in vitro to be potentially strong, substrate dependent P-glycoprotein (P-gp) inhibitors. [redacted]

In vitro cardiovascular safety was assessed in a Good Laboratory Practice (GLP) human ether-à-go-go related gene (hERG) assay, and the balovaptan concentration required for 20% inhibition (IC20) was 4.4 µM, which is orders of magnitude above the free concentrations expected to be achieved in man at a 10 mg daily dose. Mild effects on cardiovascular functions observed in the pivotal dog telemetry study consisted of a mild sustained increase in systolic and mean blood pressures, paralleled by a left ventricular peak systolic increase. An additional mild sustained increase in positive and negative dP/dt and a mild transient heart rate (HR) increase was noted. The no observed effect level (NOEL) was 10 mg/kg. In the GLP safety pharmacology studies conducted with rats assessing CNS, respiratory, and renal function, 100 mg/kg was established as the NOEL/NOAEL.

Balovaptan was not irritating to the skin or eye in the rabbit, nor did the compound prove to be a skin sensitizer.

1.2.1 Previous and Ongoing Clinical Studies

To date, balovaptan has been investigated in 6 completed Phase I studies, including a proof-of-mechanism study in healthy volunteers (BP29412). A Phase II study in adult male ASD patients has also been completed (BP28420, also referred to as VANILLA). Balovaptan is currently being evaluated in 4 ongoing studies. Two of these are Phase I studies in healthy subjects examining pharmacokinetic CYP3A related drug-drug interactions (DDI), (WP40608 [rifampicin DDI] and WP40609 [itraconazole DDI]). Balovaptan is also currently being evaluated in a Phase II study (BP30153, also referred to as AV1ATION) in pediatric ASD patients and a Phase III study (WN39434 also referred to V1aduct) in adult ASD patients.

1.2.1.1 Clinical Pharmacology

Exposure of balovaptan increased in a greater than dose-proportional manner following single doses of 0.5 to 76 mg, whereas an approximately linear increase in exposure was observed after repeated dosing with 12 to 52 mg once daily (qd) for 14 days. Balovaptan was rapidly absorbed with a median time to maximum observed plasma concentration (Tmax) between 1 and 4.5 hours after administration of single doses in fasted state and between 3 and 4 hours in fed state following multiple dosing. Steady state was achieved after approximately 7 days; the mean apparent elimination half-life after the last dose (Day 14) was approximately 46 hours. Food had no relevant effect on the overall exposure of balovaptan.

In the human mass balance study (Study BP29279), an almost complete absorption of the study drug was observed within the first 72 hours following administration.

In plasma, steady state exposure to [redacted] M3 was similar to or slightly higher than that of balovaptan and exposure to [redacted] M2 was lower than that of parent. M3 and M2 qualify as major metabolites in plasma.

Mean cerebral spinal fluid (CSF)-to-plasma concentration ratios of balovaptan and M3 following 7 days of dosing of 10 and 52 mg qd balovaptan were independent of dose. The ratio was approximately 12% for balovaptan, which is comparable to the balovaptan plasma free fraction (approximately 13%), and 6% for M3, which is lower than the M3 plasma free fraction (15 to 17%) reflecting M3 being a P-gp substrate. Data regarding M2 exposure in CSF is not available at this time.

Hepatic metabolism was found to be the major pathway of elimination with the primary route of excretion via urine (approximately 53% of the material recovered, mostly composed of metabolites). A further 30% of the administered dose was recovered in feces. The majority of excretion occurred within the first 7 days.

There was no clinically/statistically relevant PK interaction with the P-glycoprotein substrate risperidone, one of only 2 approved medications for ASD symptoms in the US, and fluoxetine, a weak CYP3A4 inhibitor often used to treat associated symptoms of ASD. Administration of a single dose of balovaptan 12 mg in combination with itraconazole, a strong CYP3A4 inhibitor, resulted in an increase in the exposure of balovaptan by 36% for maximum observed plasma concentration (Cmax) and an approximately 3.1-fold increase for area under the concentration curve from time 0 to 120 hours (AUC0-120h).

1.2.1.2 Safety

To date, balovaptan has been dosed to 217 healthy subjects in 6 completed Phase 1 studies (Studies BP25694, BP28318, BP28977, BP29279, BP29412, and WP40038).

Additionally, balovaptan was investigated in a completed Phase II study in 148 adult male patients with ASD (BP28420, “VANILLA”), about 30 of whom received 10 mg qd for 12 weeks.

Balovaptan has been found to be safe and well tolerated in clinical trials. No particular safety concerns have emerged, and no adverse drug reactions have been identified per reference safety information (Section 6.4 of the IB). The maximum tolerated dose was not reached in either the single ascending dose (SAD) study with single doses (up to 76 mg) or the multiple ascending dose (MAD) study with multiple doses (up to 52 mg qd for 14 days) in healthy subjects. In order to keep an approximately 10-fold safety margin to observed in dogs, dose escalation was stopped so as not to exceed a Cmax (mean) of 810 ng/mL in the SAD and MAD studies which compares to Cmax of about 100 and 500 ng/mL after 10 and 50 mg qd dosing.

[redacted]

Balovaptan has also been found to be safe and well tolerated in male patients with ASD at doses up to 10 mg/day (highest dose administered) for a period of 12 weeks (Study BP28420). No particular safety patterns attributable to the administration of balovaptan were identified. A total of 8 serious adverse events (SAEs) (all but one [rhabdomyolysis] considered unrelated to study drug) in 4 patients and 1 adverse event (AE) of special interest (neutropenia) were reported in the study. No relevant safety concern has emerged from the ongoing study in pediatric ASD patients (BP30153) and the recently started Phase 3 study in adults ASD patients (WN39434).

Cardio-vascular SAEs in the clinical program refer to clinically asymptomatic sinus node dysfunction / arrhythmia observed in a subject diagnosed with ASD dosed with 1.5 mg od in the Phase 2 study BP28420 in whom sinus arrhythmia was interpreted to be pre-existing because follow-up examinations with 24-hour Holter ECG monitoring several weeks after the SAE showed the same pattern as seen when the SAE was reported. Second, another subject with ASD in Phase 2 study BP28420 suffered from syncope reported as SAE about a week after having voluntarily stopped treatment after 6 weeks of 4 mg qd. The same subject showed another syncope 4 weeks after the first one and died due to suspect heart failure about 16 weeks after last dosing. ECG monitoring and troponin measurements, which were part of the safety monitoring in this study, had not shown abnormalities at any time point. The event was considered “not related” to study treatment by the Investigator or designee. Of note, the subject had been taking multiple co-medications for several years including methylphenidate and aripiprazole, a combination that has been reported to be associated with a higher cardiovascular risk potentially.[1]

ECGs recorded in Phase I studies, including the MAD part of study BP25694 were analyzed by balovaptan concentration vs QTcF modelling. The preliminary data from these studies showed an increase in QTcF of < 5 ms for concentrations up to 550 ng/mL with an upper CI of < 13 ms at 550 ng/mL. In none of the subjects treated with balovaptan in the completed Phase I studies were QTcF values observed as QTcF > 450 ms or increases of QTcF from baseline of > 30 ms, except for a single observation but which was < 50 ms.

Refer to the Investigator's Brochure for more details on non-clinical and clinical studies.

1.3 STUDY RATIONALE AND BENEFIT-RISK ASSESSMENT

This study investigates the effect of balovaptan on cardiac repolarization by measuring ECGs in healthy subjects following multiple doses of the targeted therapeutic (10 mg) and supra-therapeutic (50 mg) doses.

The study will be performed in healthy subjects who will not receive any health benefit from participating in this study. The study is necessary for the further development of balovaptan and may be of benefit for future subjects.

The doses for the treatment periods planned in this study have been explored in the MAD part of study BP25694 and found to be safe and well tolerated. In proof-of-mechanism study BP29412 a dose of 52 mg, once daily (qd), was administered for 6 days to healthy volunteers (N=35) not revealing a balovaptan associated safety signal.

QTc analyses based on previous ECG recordings do not indicate relevant ECG changes.

The safety monitoring across all clinical studies has not identified a balovaptan linked adverse drug reaction.

Moxifloxacin, a fluoroquinolone antibiotic, was approved for use in the United States in 1999 and is currently on the World Health Organization's list of essential medicines. The dose of moxifloxacin planned for this study 400 mg is the recommended dosage for adults in the package insert. Moxifloxacin has been shown to increase HR and is associated with mild QT prolongation but only seldom with torsade de pointes.[2] The single dose of moxifloxacin planned for this study will be used as a positive control to evaluate the sensitivity of the study to detect increases in QT interval measurements. The FDA has proposed the use of moxifloxacin in this manner.[3]

While planned dosing of subjects in this study are expected to be well tolerated, diligent safety monitoring and in-house stays are included in this study during dosing. Subjects will be asked to report any events of feeling “not well” to study staff immediately.

In conclusion, any safety risk for the healthy volunteers participating in this study is considered minimal.

2. OBJECTIVES AND ENDPOINTS

This study will investigate the effect of balovaptan (RO5285119) on cardiac repolarization by measuring ECGs in healthy subjects following multiple doses of the targeted therapeutic and supra-therapeutic doses.

Table 1 Objectives and Corresponding Endpoints

Primary Objective	Corresponding Endpoint
1. To investigate the effect of balovaptan 50 mg qd on the QTcF interval after 14 days of dosing.	1. Placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 14
Secondary Objectives	**Corresponding Endpoints**
1. To investigate the effect of a single dose of balovaptan 50 mg on the QTcF interval at Day 1.	1. Placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1.
2. To investigate the effect of balovaptan 10 mg qd on the QTcF interval at Day 1 and Day 14.	2. Placebo-corrected, change-from-baseline QTcF (ΔΔQTcF) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1 and Day 14.
3. To investigate the effect of balovaptan on the following ECG parameters: PR, RR, QRS, QT, T-wave and U-wave morphology.	3. Placebo-corrected, change-from-baseline heart rate, PR and QRS interval (ΔΔHR, ΔΔPR and ΔΔQRS) measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1 and Day 14. • Categorical outliers for QTcF, HR, PR, and QRS. • Frequency of treatment emergent changes of T-wave morphology and U-wave presence.
4. To investigate the pharmacokinetics of balovaptan, M2 (as appropriate) and M3.	4. Pharmacokinetic parameters estimation such as Tmax, Cmax, and AUC0-24h for plasma concentrations of balovaptan, M2 (as appropriate) and M3. Other pharmacokinetic parameters may also be added.
5. To investigate the pharmacokinetics of moxifloxacin.	5. Pharmacokinetic parameters estimation such as Tmax, Cmax, and AUC0-24h for plasma concentrations of moxifloxacin. Other pharmacokinetic parameters may also be added.
6. To investigate the relationship between plasma concentrations of balovaptan, M2 (as appropriate), M3 and QTcF interval length.	6. Pharmacokinetic-pharmacodynamic model of balovaptan, M2 (as appropriate) and M3 concentrations vs. QTcF changes.

Table 1 Objectives and Corresponding Endpoints (cont.)

7. To investigate the relationship between plasma concentrations of moxifloxacin and QTcF interval length (if warranted).	7. Pharmacokinetic-pharmacodynamic model of moxifloxacin concentrations vs. QTcF changes (if warranted)
8. To investigate the ECG assay sensitivity to detect a change in the QTcF interval, using moxifloxacin 400 mg as an active control.	8. Placebo-corrected, change-from-baseline QTcF ($\Delta\Delta$QTcF) measured on 12 lead ECGs extracted from continuous recordings at the specified time points on Day 2 and Day 15
9. To investigate the safety and tolerability of balovaptan and moxifloxacin at the dosing regimens scheduled.	9. Safety assessments: adverse events, physical examination, vital signs, laboratory tests, C-SSRS, and heart rate (HR) monitoring.
Exploratory Objectives	**Corresponding Endpoints**
1. To investigate any effect of balovaptan at 50 mg QD and 10 mg QD doses on pulmonary blood pressure as assessed by the use of TT-doppler echocardiography.	1. TT-Doppler echocardiography: pulmonary artery acceleration time (PAAT), right ventricular outflow tract - velocity time integral (RVOT-VTI) and derived mean pulmonary arterial pressure as change to baseline relative to placebo treatment.(objectives 1 & 2)
2. To investigate any effect of 50 mg qd and 10 mg qd on right and left heart as assessed by TT-Doppler echo.	2. TT-Doppler-echocardiography: LVEF and additional secondary TT-Doppler-echocardiography parameters of left and right heart, as described in Section 4.5.7 (objectives 1 & 2)
3. To investigate the effect 10 mg and 50 mg QD balovaptan on blood pressure and heart rate as measured by the use of Holter-ECG and ABPM.	3. Ambulatory blood pressure monitoring (ABPM): mean diastolic and systolic blood pressure (daytime and nighttime mean). • Heart rate will also be analyzed as derived from Holter ECG. – Heart rate as derived from ABPM and/or Holter ECG may be explored against fluctuations in blood pressure measured by ABPM (if warranted).
4. To investigate whether genetic variants in genes are associated with natural or potential drug induced neutropenia; with differences in pharmacokinetics of balovaptan, M2 or M3, or with any potential effect of balovaptan on ECG parameters.	4. The genetic variants in genes that are associated with natural or potential drug induced neutropenia; genetic variants in genes that are associated with pharmacogenetics of metabolizing enzymes, transferases, transporters, etc, (eg,CYP3A4 and P-glycoprotein); genetic variants in genes that may be associated with a potential effect of balovaptan on ECG parameters.

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

This will be a single-center, multiple-dose, randomized, double-blind, placebo-controlled, positive-controlled, twelve sequence, 3-period cross-over study to investigate the effect of balovaptan on the QTc interval in healthy subjects.

Screening

Screening will be conducted up to 28 and 3 days (inclusive) before each subject's first dosing day (Day 1 of Treatment Period 1).

In-Clinic Confinement

Day -2

Subjects will be confined to the CRU starting on Day -2 until the morning of Day 16 during each Treatment Period. Subjects are entered into CRU on Day -2 so that subjects are rested and prepared for the baseline ABPM and baseline TT-Doppler echocardiogram on Day -1. Eligibility assessments will be performed on Day -2 as outlined in the Schedule of Activities (SoA), Appendix 1.

Day -1

On Day -1 of each Treatment Period, ABPM and TT-Doppler echocardiogram assessments will be performed. These results will serve as the baseline for the subjects. On days when TT-Doppler echocardiogram and ABPM assessments are performed subjects will be given low salt standardized meals.

Randomization (Day -1)

At the start of the study Treatment Period 1, at the end of the day of Day -1, subjects will be randomized to one of the 12 treatment sequences comprised of the 4 treatments described:

Treatments

Treatment A (balovaptan therapeutic dose)

Days 1-14: A single once daily oral dose of 10 mg (1 × 10 mg tablet) of balovaptan and 4 tablets of matching placebo for balovaptan for 14 days.

Day 2 and 15: A single oral dose of a matching placebo capsule for moxifloxacin.

Treatment B (balovaptan supra-therapeutic dose)

Days 1-14: A single once daily oral dose of 50 mg (5 x 10 mg tablets) of balovaptan for 14 days.

Day 2 and 15: A single oral dose of a matching placebo capsule for moxifloxacin.

Treatment C (active control [400 mg moxifloxacin] on Day 2)

Days 1-14: A single once daily oral dose of 5 tablets of matching placebo for balovaptan for 14 days.

Day 2: A single oral dose of 400 mg moxifloxacin capsule

Day 15: A single oral dose of a matching placebo capsule for moxifloxacin

Treatment D (active control [400 mg moxifloxacin] on Day 15)

Days 1-14: A single once daily oral dose of 5 tablets of matching placebo for balovaptan for 14 days.

Day 2: A single oral dose of a matching placebo capsule for moxifloxacin

Day 15: A single oral dose of 400 mg moxifloxacin capsule.

Treatment Sequences

1. A, B, C
2. A, C, B
3. B, A, C
4. B, C, A
5. C, A, B
6. C, B, A
7. A, B, D
8. A, D, B
9. B, A, D
10. B, D, A
11. D, A, B
12. D, B, A

Dosing (Days 1-15)

Each Treatment Period will involve 15 days of dosing (Day 1 – Day 15). Subjects must fast for at least 8 hours prior to and 4 hours after dosing on study days when 24-hour Holter ECG assessments will be performed (study Days 1, 2, 14 and 15). Dosing on

study days with 24-hour Holter ECG assessment should be at approximately the same time of day ± 1h.

Prior to dosing on Day 1 subjects will undergo pre-dose assessments as outlined in the SoA (Appendix 1). Following pre-dose assessments subjects will receive a single dose of study medication preceded by a ≥ 8 hour fast the night before. Study drugs will be taken with 240 mL of water and be followed by a 4-hour post-dose fast. If subjects require more liquid to take all study drugs, additional water may be provided in aliquots of 50 mL. If additional water is provided the amount should be captured. Water provided for study drug administration will be identical on all dosing days. Identical fasting procedures from Day 1 will be followed on study Days 2, 14 and 15. No fasting will be required on other dosing days. Subjects will receive standardized low salt meals on study days when 24-hour Holter ECGs (Days 1, 2, 14 and 15), ABPM (Days -1 and 12) or TT-Doppler echocardiogram (Days -1 and 13) assessments will be performed. Meals will be given at approximately the same time on each of these study days.

Days 1 and 2: 24-hour Holter ECG Assessments

On study Days 1 and 2, of each Treatment Period, 24-hour ECG assessments will be performed. Subjects will be administered study drug following a ≥ 8 hour fast and continue to be fasted for 4 hours post-dose on these days. Subject will be provided with standardized low-salt meals on these days. Subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Day 12

On study Day 12 of each Treatment Period, ABPM assessments will be performed for each subject. On study days when ABPM assessments are performed subjects will be provided with standardized low salt meals. Subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Day 13

On study Day 13, of each Treatment Period, at approximately the same time of day as on Day -1, TT-Doppler echocardiogram will be performed to assess any effects of steady state balovaptan levels on cardiac parameters. Subjects will receive standardized low salt meals on this study day. In addition to TT-Doppler echocardiogram subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Days 14 and 15- 24-hour Holter ECG Assessments

On study Days 14 and 15 of each Treatment Period, 24-hour ECG assessments will be performed. Subjects will be administered study drug following a ≥ 8 hour fast and continue to be fasted for 4 hours post-dose. Subjects will be provided with standardized

low salt meals on these days. Subjects will undergo pre and post-dose assessments as outlined in the SoA (Appendix 1).

Day 16

Subjects will be discharged from the CRU on the morning of Day 16 after final assessments as described in the SoA (Appendix 1).

Washout Period:

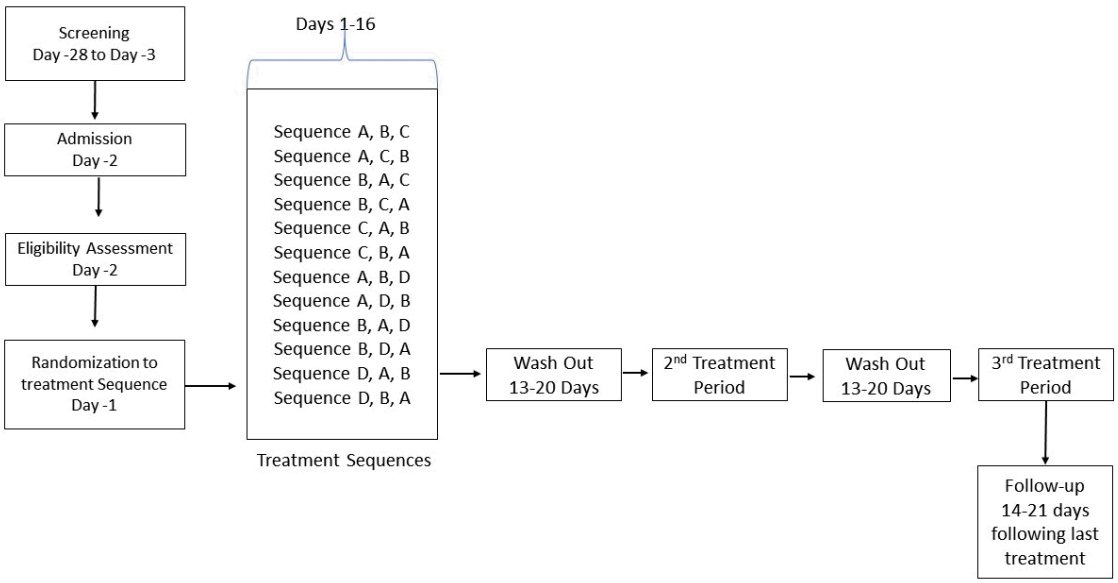
Following each Treatment Period subjects will undergo a washout period lasting a minimum of 13 and a maximum of 20 days from the last dose of study drug.

Follow-up:

Subjects will return to the unit for a follow-up visit between 14 and 21 days after the final dose of study drug. This will include a general physical examination, 12-lead ECG, vital signs, C-SSRS, adverse event review, body weight and laboratory safety tests.

Figure 1 presents an overview of the study design. A SoA is provided in Appendix 1.

Figure 1 Study Schema



3.2 END OF STUDY AND LENGTH OF STUDY

The end of this study is defined as the date when the last subject, last visit occurs, or safety follow-up is received from the last subject, whichever occurs later.

The total length of the study, from screening of the first subject to the end of the study, is expected to be approximately 14 - 20 weeks. For each enrolled subject the duration of the study from Screening to the end of Follow-up will be a minimum of 122 days or a maximum of 143 days.

- Screening: up to 28 days
- Dosing periods including washout: 80 - 94 days
    - Treatment Period 1: 18 Days (Day -2 – Day 16)
    - Washout: 13 - 20 Days
    - Treatment Period 2: 18 Days (Day -2 – Day 16)
    - Washout: 13 - 20 Days
    - Treatment Period 3: 18 Days (Day -2 – Day 16)
- Follow up: 14 - 21 days after last dosing

3.3 RATIONALE FOR STUDY DESIGN

The study will utilize a positive-control, 400 mg moxifloxacin, to assure study design and measurements will be able to detect potential ECG effects of balovaptan. Moxifloxacin, a fluoroquinolone antibiotic, when utilized at therapeutic doses (400 mg) is associated with QT prolongation and regulatory authorities have recommended it as a positive control to evaluate the sensitivity of clinical studies designed to detect small but significant increases in QT interval measurements.[4]

Across clinical trials, no balovaptan associated changes in blood pressure or HR are apparent. Therefore, this study uses QTcF as primary parameter rather than an individualized corrected QTc. Since a cross-over design corrects for diurnal fluctuations of QTc, a separate Day -1 reading is not needed, 3 time points (replicates at each time point) pre-dose are used as baseline.

3.3.1 Rationale for Balovaptan and Moxifloxacin Dose and Schedule

3.3.1.1 Balovaptan

A dose of 10 mg balovaptan has shown statistically significant changes in efficacy parameters in adult male patients with ASD (Phase 2) and is the current recommended Phase 3 dose.

Balovaptan is being developed for use in ASD, so it is expected that patients will need to take it for prolonged periods of time, likely for years or for their lifetime. Hence multiple dose testing is relevant from a clinical point of view. The multiple dose PK of balovaptan with 14 days of dosing was previously characterized in humans and was found to be time-dependent. Accumulation was observed with multiple dosing, and steady state of

balovaptan was observed after approximately 7 days of dosing and after 10 days for M3. The plasma PK time course of M2 has not been closely studied but steady state has been demonstrated after 2 weeks of once daily dosing with 10 mg balovaptan.

The primary objective of this study is to investigate the effect of balovaptan on cardiac repolarization by measuring ECGs in healthy subjects at both therapeutic and supra-therapeutic doses. To this end subjects will be dosed once daily with balovaptan for 14 days at either the projected therapeutic dose of 10 mg or a supra-therapeutic dose of 50 mg and ECG parameters monitored.

In the MAD part of study BP25694 multiple doses of 12 mg (just above the clinical dose of 10 mg) resulted in a Cmax of 99 ng/mL. In Study BP28977 co-administration of itraconazole led to a 36% increase in Cmax. In the MAD part of study BP25694 multiple doses of 52 mg qd in healthy subjects were well tolerated and resulted in a Cmax of 484 ng/mL. In light of convulsions observed in dogs, dose escalation in study BP25694 was stopped so as not to exceed a Cmax of 810 ng/mL. In conclusion, based on previous clinical experience a once-daily dose of 50 mg balovaptan for 14 days is expected to be well tolerated considering the short duration of dosing in this study.

3.3.1.2 Moxifloxacin

The study will utilize moxifloxacin (400 mg) as a positive-control to assure the study as designed will be able to detect potential ECG effects of balovaptan. Moxifloxacin, a fluoroquinolone antibiotic, when utilized at its recommended therapeutic dose of 400 mg qd is associated with QT prolongation. Regulatory authorities have recommended moxifloxacin as a positive control to evaluate the sensitivity of clinical studies designed to detect small but significant increases in QT interval measurements. Subjects will receive 400 mg oral moxifloxacin in a nested cross-over fashion using a joint placebo/positive control period. In this period, half of the subjects will be dosed with moxifloxacin on Day 2 and the other half dosed on Day 15. To keep the blind between balovaptan and placebo, this nested design has 4 Holter extraction days and moxifloxacin is blinded to avoid unblinding of the placebo period. The moxifloxacin dosing day is the same number apart from the baseline as in the balovaptan periods to maintain the blind.

3.3.2 Rationale for Study Population

Healthy male or female volunteers aged 18 to 60 years inclusive have been chosen as the study population due to the low risk of clinically significant toxicity at anticipated exposure levels. Moreover, use of healthy subjects, as opposed to patients, will allow a clearer interpretation of the study results, as there will be no confounding factors that result from changes in disease state and/or concomitant medication use.

3.3.3 Rationale for Transthoracic Doppler-echocardiogram and Ambulatory Blood Pressure Monitoring

As an exploratory marker transthoracic Doppler echo-cardiography is included to estimate pulmonary blood pressure with the a priori expectation that no changes in pulmonary circulatory dynamics will be seen.

In animals as well as preparations of pulmonary vasculature, vasopressin has been shown to decrease pulmonary blood pressure and to relax arterial constriction through V1a receptors and in animals these changes were accompanied by systemic blood pressure increases.[8,9] However, GLP toxicology animal studies with balovaptan have not pointed to a right heart challenge strain nor to pulmonary fibrosis. No systemic blood pressure changes with balovaptan were noted in clinical studies.

In clinical trials, blood pressure monitoring has not revealed apparent blood pressure changes. However, blood pressure monitoring focused on few time points around pharmacokinetic Tmax and no night-time blood pressure data are available. Hence, ABPM is included in this study to supplement the existing blood pressure profiling, exploring in a more intense way any effects of balovaptan on daytime blood pressure (9:00 am to 9:00 pm) and to collect data for nighttime blood pressure (1:00 am to 5:30 am).

This study provides an opportunity for these exploratory measures considering a homogeneous population of healthy volunteers and the inclusion of a multiple dose higher than the dose of 10 mg qd tested in the ongoing Phase 3 study WN39434.

4. MATERIALS AND METHODS

4.1 SUBJECTS

The target population will be healthy male or female volunteers aged 18 to 60 years inclusive. An attempt will be made to have each Treatment population contain at least 1/3 male subjects by ensuring that a minimum of 40% of enrolled population are male.

Initially 56 subjects will be enrolled with the goal of obtaining 48 evaluable subjects. Enrolled subjects who withdraw from the study may be replaced at the discretion of the Investigator (or designee) and Sponsor to ensure adequate numbers of evaluable subjects.

4.1.1 Inclusion Criteria

Subjects must meet the following criteria for study entry:

1. Healthy male or female volunteers aged 18 to 60 years inclusive, at Screening. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical

examination including vital signs, 12-lead ECG, hematology, blood chemistry, urinalysis, and serology.

2. Body Mass Index (BMI) between 18 and 32 kg/m^2 inclusive, at Screening.
3. For women of childbearing potential: agreement to use at least 1 acceptable form of contraception during the entire study and for 90 days following last dose of study drug.
    a. A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (> 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).
    b. The following are acceptable contraceptive methods: abstinence from heterosexual activity; bilateral tubal ligation; sterilization of male ‘partner; hormonal contraceptives; hormone-releasing intrauterine devices; copper intrauterine devices; Essure.
    c. Barrier methods alone are not considered an acceptable form of contraception for this study and if utilized must be supplemented by one of the acceptable methods described above. For this study barrier contraception methods are defined as: male or female condom with or without spermicide (male and female condom must not be used simultaneously); and cap, diaphragm, or sponge with spermicide.
4. For men: Vasectomized, agreement to remain abstinent (refrain from heterosexual intercourse) or use of a condom during intercourse. Must also agree to refrain from donating sperm, as defined below:
    a. With female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during the entire study and for 90 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.
5. Able to participate, and willing to give written informed consent and to comply with the study restrictions.
6. Fluent in English.

4.1.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from study entry:

Past Medical History

1. History of any clinically significant gastrointestinal, renal, hepatic, broncho-pulmonary, neurological, psychiatric, cardiovascular, endocrinological, hematological, lymphatic, musculoskeletal, genitourinary, immunological, dermatological or connective tissue or allergic disease, metabolic disorder, or cancer.
2. Signs and symptoms potentially indicative of peripheral neuropathy.

3. Unexplained orthostatic reaction with syncope within last 6 months.
4. A history of clinically significant hypersensitivity (e.g., to drugs, including moxifloxacin or any quinolone antibiotics and balovaptan, and excipients) or allergic reactions.
5. History of alcohol and/or drug abuse or addiction within the last 2 years before study enrollment.
6. Subjects on hormone replacement therapy if post-menopausal status cannot be documented from medical history.
7. Previous treatments with iron for iron deficiency anemia within three months of screening.
8. History of coagulopathies, bleeding disorders, or blood dyscrasias.
9. History of porphyria, active liver disease or permanent or unexplained elevation of serum transaminases.
10. In the opinion of the Investigator or designee, any major illness within one month before the Screening examination or any febrile illness within one week prior to Screening and up to first study drug administration.

Current Status

1. Clinically relevant deviation from normal in the physical examination including vital signs.
2. Positive result on human immunodeficiency virus (HIV) 1, HIV 2, hepatitis C virus (HCV) antibody, or hepatitis B virus surface antigen (HBsAg).
3. Clinically significant abnormalities in laboratory test results (including complete blood count, chemistry panel, and urinalysis). In the case of uncertain or questionable results, tests performed during Screening may be repeated on Day -1 or Day -2 to confirm eligibility. Repeat of abnormal test results are allowed during Screening and eligibility assessment at the discretion of the Investigator or designee provided results will be available prior to Randomization on Day -1.
4. If female, a positive pregnancy test at screening or prior to Day 1 of any Treatment Period.
5. Lactating women.
6. Any condition or disease detected during the medical interview / physical examination that would render the subject unsuitable for the study, place the subject at undue risk or interfere with the ability of the subject to complete the study in the opinion of the Investigator or designee.
7. Subjects with CPK values > 2x upper limit of normal (ULN), confirmed by repeat sample.
8. Positive urine alcohol test or urine drug test at screening or Day -2 of each treatment period (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, methadone, methamphetamines, and 3,4-methylenedioxymethamphetamine [MDMA]).

9. Subject has been a regular smoker (> 5 cigarettes, > 1 pipeful, > 1 cigar per day or equivalent as determined by Investigator or designee) within 6 months of study enrollment.
10. Coffee (or tea) consumption > 5 cups per day or methylxanthine-containing drinks > 1.5 liter/day or more than 250 g/day of chocolate within 3 months of first dose of study drug.
11. Alcohol consumption of > 18 units per week for males and females. One unit of alcohol equals 12 ounces of regular beer, 5 ounces of wine or 1.5 ounces of 80 proof spirits within 3 months of first dose of study drug.
12. Subjects who have lost or donated in excess of ~450 mL blood in the 3 months prior to Day 1 of Treatment Period 1.
13. Subjects under judicial supervision, guardianship, or curatorship.
14. Dietary restrictions that would prohibit the consumption of standardized meals.
15. Poor venous access for blood sampling.

Concomitant Medication

1. Taken greater than 2 g/day paracetamol within 48 hours of study drug administration or greater than 4 g/day paracetamol within 7 days of study drug administration.
2. Any nonprescribed systemic or topical medication or herbal remedies are prohibited for 7 days prior to the first study drug administration through Follow-up visit.
3. Participation in an investigational drug study within 60 days or 5 times the elimination half-life, whichever is longer, prior to first dosing, or within 5 months prior to first dosing in case of a study with a biological, as calculated from the day of Follow-up visit from the previous study.
4. Participation in an investigational device study within 60 days of first dosing of study drug.
5. Subjects who have received any prescribed systemic or topical medication within 14 days of the first dose administration are excluded, unless in the opinion of the Investigator (or designee) the medication will not interfere with the study procedures or compromise safety.
6. Any slow release medicinal formulations considered to still be active within 4 weeks (or within 5 times the elimination half-life of the medication, whichever is longer) prior to the first study drug administration are prohibited, unless in the opinion of the Investigator or designee the medication will not interfere with the study procedures or compromise safety.
7. Taking any inhibitor of CYP3A taken within 4 weeks (or within 5 half-lives, whichever is longer) prior to study drug administration or taking any inducer of CYP3A within 4 weeks of the first dose of study drug.
8. Use of any prohibited medications and food before study start and subjects who do not agree to refrain from consuming prohibited medications or food during the study.

9. Subjects who have previously enrolled in, or withdrawn after enrollment, from this study.

TQT-related Exclusion Criteria:

1. Personal history of unexplained black-outs or faints.
2. Personal history of reactions or faints at having their blood taken, unexplained black-outs or faints or any condition which could relapse during or immediately after the study which in the opinion of the Investigator or designee makes the subject unsuitable to participate in this study.
3. Subjects experiencing fainting or pre-syncopal events during orthostatic challenge testing, performed according to site SOP, at Screening and Day -2 of Treatment Period 1.
4. Subjects with Screening or Treatment Period 1 (Day -2) baseline mean QT interval corrected using Fridericia's formula (QTcF) > 450 ms or < 300 ms (using the same upper QTcF limit in both males and females) or > 30 ms difference between the highest and the lowest readings of the triplicate ECGs recorded.
5. Screening or baseline (Day -2 Treatment Period 1) ECG evidence of atrial fibrillation, atrial flutter, second or third-degree AV block, complete right or left bundle branch block, Wolff-Parkinson-White-syndrome, or cardiac pacemaker.
6. Screening or baseline ECGs (Day -2 of Treatment Period 1) with QRS and/or T-wave judged to be unfavorable for a consistently accurate QT measurement (i.e., neuromuscular artifact that cannot be readily eliminated, arrhythmias, indistinct QRS onset, low amplitude T-wave, merged T- and U-waves, prominent U-waves, etc.).
7. Confirmed (based on the average of ≥ 3 consecutive measurements) systolic blood pressure greater than 139 or less than 90 mmHg, and diastolic blood pressure greater than 89 or less than 45 mmHg at Screening and Day -2 of Treatment Period 1.
8. Confirmed (based on the average of ≥ 3 consecutive measurements) resting HR > 100 bpm or < 40 bpm at Screening or Day -2 of Treatment Period 1.
9. Notable resting bradycardia (HR < 40 bpm) on ECG at Screening or Day -2 of Treatment Period 1. Notable resting tachycardia (mean HR > 100 bpm) on ECG at Screening or Day -2 of Treatment Period 1.
10. Family history of congenital long QT syndrome or sudden death.
11. History or presence of clinically significant ECG abnormalities, as determined by study Investigator or designee, before study drug administration.

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

This is to be a randomized, double-blind study.

4.2.1 Treatment Assignment

After obtaining informed consent, subjects will be screened according to the inclusion and exclusion criteria. Subjects who have met all eligibility criteria will receive a

randomization number on Day -1 of the first treatment period and according to the randomization code generated by the Biostatistics Department of PRA. The randomization number will ensure identification throughout the study. Replacement subjects will receive the randomization number of the subject to be replaced, increased by 1000 (e.g., 1101 replacement number for randomization number 101), and will be administered the same treatment.

Subjects who drop out or withdraw for any reason without completing all screening evaluations successfully, will be considered “screening failures”. Such subjects will not receive a randomization number, and only applicable data will be entered in the electronic case report forms (eCRFs).

Subjects will be randomly assigned to one of the 12 treatment sequences according to the randomization code.

4.2.2 Blinding

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

A double dummy design will be used, all treatments are blinded (balovaptan and matching placebo, moxifloxacin and matching placebo). Groups will receive placebo and study drugs as described in Section 3.1. The oral tablets/capsules will be indistinguishable in appearance and taste to balovaptan and moxifloxacin, respectively. The number of tablets and capsules will be identical for each subject regardless of treatment and dose.

Study site personnel (with the exception of the unblinded pharmacy staff) and subjects will be blinded to treatment assignment during the study. The Sponsor and its agents will also be blinded to treatment assignment, with the exception of individuals who require access to subject treatment assignments to fulfill their job roles during a clinical trial.

As per health authority reporting requirements, the Sponsor's Drug Safety representative will break the treatment code for all serious, unexpected suspected adverse reactions that are considered by the Investigator or designee or Sponsor to be related to study drug. The subject may continue to receive treatment, and the Investigator or designee, subject, and Sponsor personnel, with the exception of the Drug Safety representative and personnel who must have access to subject treatment assignments to fulfill their roles (as defined above), will remain blinded to treatment assignment.

4.3 STUDY TREATMENT AND OTHER TREATMENTS RELEVANT TO THE STUDY DESIGN

The investigational medicinal products (IMPs) for this study are balovaptan, moxifloxacin and placebo.

4.3.1 Study Treatment Formulation, Packaging, and Handling

4.3.1.1 Balovaptan and Placebo

RO5285119/F17 10 mg film-coated dispersible tablets and matching placebo tablets will be supplied by the Sponsor in high-density polyethylene bottles. The balovaptan and matching placebo tablets must be stored and handled according to the details specified on the product label. For additional details, refer to the balovaptan Investigator's Brochure.

4.3.1.2 Moxifloxacin Active Control and Placebo

Moxifloxacin (400 mg qd, administered as 400 mg over-encapsulated tablets) will be supplied by the Sponsor. The over-encapsulated moxifloxacin and matching placebo must be stored and handled according to the details specified on the product label.

4.3.2 Study Treatment Dosage, Administration, and Compliance

The treatment regimens are summarized in Section 3.1

Any overdose or incorrect administration of any of the study treatments should be noted on the Study Drug Administration electronic Case Report Form (eCRF). In this study an overdose is defined as any dose of balovaptan greater than 50 mg per day and any dose of moxifloxacin greater than 400 mg per day. Adverse events associated with an overdose or incorrect administration of any of the study treatments should be recorded on the Adverse Event Report Form.

Guidelines for treatment interruption or discontinuation for subjects who experience adverse events are provided in Section 5.1.

4.3.2.1 Balovaptan and Placebo

Treatment A: A single once daily oral dose of 10 mg (1 × 10 mg tablet) of balovaptan and 4 tablets of matching placebo for balovaptan for 14 days.

Treatment B: A single once daily oral dose of 50 mg (5 x 10 mg tablets) of balovaptan for 14 days.

Treatment C: A single once daily oral dose of 5 tablets of matching placebo for balovaptan for 14 days.

Treatment D: A single once daily oral dose of 5 tablets of matching placebo for balovaptan for 14 days

Doses of balovaptan, and matching placebos on Days 1, 2, 14 or 15 will be administered with approximately 240 mL of water (at room temperature) after at least 8 hours of fasting. Dosing on these days will be at approximately the same time of day ± 1h. Post-dose subjects will maintain a 4-hour fast during which no food will be allowed.

While in the clinical research unit, subjects will be allowed to consume water ad libitum, except for 2 hours before and 2 hours after oral dosing. However, excessive consumption of liquids should be avoided (Section 4.4.4). Liquids are permitted with the breakfast meal on non-fasting days.

4.3.2.2 Moxifloxacin (Active Control) and Placebo

Treatment A: A single oral dose of a matching placebo capsule for moxifloxacin on Day 2 and 15.

Treatment B: A single oral dose of a matching placebo capsule for moxifloxacin on Day 2 and 15.

Treatment C: A single oral dose of 400 mg moxifloxacin capsule on Day 2. A single oral dose of a matching placebo capsule for moxifloxacin on Day 15.

Treatment D: A single oral dose of a matching placebo capsule for moxifloxacin on Day 2. A single oral dose of 400 mg moxifloxacin capsule on Day 15.

The moxifloxacin and matching placebos on Day 2 and 15 will be administered with approximately 240 mL of water (at room temperature) after at least 8 hours of fasting. On Day 2, moxifloxacin or matching placebo will be administered immediately after the balovaptan or placebo dose with the same 240 mL of water. Dosing on these days will be at approximately the same time of day ± 1h. Post-dose subjects will maintain a 4-hour fast during which no food will be allowed.

4.3.3 Investigational Medicinal Product Accountability

Balovaptan and placebo will be provided by the Sponsor. The study site will acknowledge receipt of IMPs supplied by the Sponsor by returning the appropriate documentation form to confirm the shipment condition and content. Any damaged shipments will be replaced. Moxifloxacin and matching placebo will be provided by the Sponsor.

The IMPs will either be disposed of at the study site according to the study site's institutional standard operating procedure or be returned to the Sponsor with the appropriate documentation. The site's method of destroying Sponsor-supplied IMPs must be agreed to by the Sponsor. The site must obtain written authorization from the Sponsor before any Sponsor-supplied IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Accurate records of all IMPs received at, dispensed from, returned to, and disposed of by the study site should be recorded on the Drug Inventory Log.

4.4 CONCOMITANT THERAPY, PROHIBITED FOOD, AND ADDITIONAL RESTRICTIONS

Concomitant therapy consists of any medication (e.g., prescription drugs, over-the-counter drugs, vaccines, or homeopathic remedies, nutritional supplements) used by a subject in addition to protocol-mandated treatment from 30 days prior to initiation of study drug to the study completion/ discontinuation visit. All such medications should be reported to the Investigator or designee and recorded on the Concomitant Medications eCRF.

4.4.1 Permitted Therapy

Existing hormonal contraceptives and hormone replacement therapy regimens may remain unchanged during the conduct of the study. Women of childbearing potential: agreement to use at least 1 acceptable form of contraception during the entire study and for 90 days following last dose of study drug.

Acetaminophen/paracetamol is allowed up to a maximum dose of 4 g/day within 7 days before study drug administration and 2 g/day within 48 hours before study drug administration, but total should not exceed 4 g during the week prior to dosing. Subjects will be instructed not to exceed these limits.

4.4.2 Prohibited Therapy

Participation in an investigational drug study within 60 days or 5 times the elimination half-life, whichever is longer, prior to first dosing, or within 5 months prior to first dosing in case of a study with a biological, as calculated from the day of follow-up from the previous study. Participation in an investigational device study within 60 days prior to first dosing is prohibited.

Any non-prescribed systemic, topical medication or herbal remedies are prohibited for 7 days prior to the first study drug administration through follow-up.

Any slow release medicinal formulations considered to still be active within 4 weeks (or within 5 times the elimination half-life of the medication, whichever is longer) prior to the first study drug administration are prohibited, unless in the opinion of the Investigator or designee the medication will not interfere with the study procedures or compromise safety.

Any prescribed systemic or topical medication taken or applied within 14 days prior to the first dose of study drug through the follow-up visit (with the exception of permitted therapies per Section 4.4.1).

Additional exceptions maybe made for medications to treat AEs, if the rationale for the exception is discussed and approved by the Investigator (or designee) as well as the Roche medical monitors. All medication administered to manage AEs should be recorded on the Adverse Event Report Form.

The following concomitant medications in particular are prohibited, unless an exception has been agreed to by the Investigator (or designee), and the Roche medical monitors.

- Any inhibitor of CYP3A taken within 4 weeks (or within 5 half-lives, whichever is longer) prior to study drug administration, including but not limited to the following drugs: ketoconazole, miconazole, itraconazole, fluconazole, erythromycin, clarithromycin, ranitidine, and cimetidine, until follow-up.
- Any inducer of CYP3A taken within 4 weeks prior to study drug administration, including but not limited to the following drugs: rifampicin, rifabutin, glucocorticoids, carbamazepine, phenytoin, phenobarbital and St. John's wort, until follow-up.

4.4.3 Prohibited Food

- Consumption of methylxanthine-containing products (e.g., coffee, tea, cola, chocolate) will be forbidden from 48 hours prior to Day -2 check-in to CRU and during the stay.
- It is not permitted to take any nutrients known to modulate cytochrome CYP3A activity (e.g., grapefruit juice; Seville orange) within 2 weeks prior to first dosing until discharge from the CRU at the end of the study.
- Subjects should refrain from consumption of any foods containing poppy seeds within 48 hours (2 days) prior to screening and each admission to the clinical research center to avoid false positive drug screen results.
- Alcohol consumption will not be allowed 48 hours before Day -2 admission to CRU and while staying in the CRU

4.4.4 Additional Restrictions/Considerations

- While in the clinical research unit, subjects will be allowed to consume water ad libitum, except for 2 hours before and 2 hours after oral dosing. Subjects will be provided guidance at CRU Admission, for each Treatment Period, that they should avoid excessive fluid intake during the study. Excessive fluid intake for this study is defined as 3 L (100 fl oz.) per day. Clinic staff will provide guidance as to approximate number of drinks in 3 liters as part of the subject orientation process at Admission. However, the clinical site will not be required to measure, or record fluid intake.
- Subjects must fast for at least 8 hours prior to and 4 hours after dosing on Days 1, 2, 14, and 15. Dosing on these days will be at approximately the same time of day (± 1h). Standardized low salt meals should be provided at approximately the same time of day on Days -1, 1, 2, 12, 13, 14 and 15.
- Between the screening visit and follow-up visit, alcohol must be restricted to no more than 2 drinks per day (one drink is equal to 12 ounces of beer, 5 ounces of wine or 1.5 ounces of 80 proof spirits). Alcohol consumption will not be allowed 48 hours before Day -2 admission to the CRU and while staying in the CRU. Additional alcohol/drugs of abuse testing may be employed throughout the study to verify compliance.

- Use of tobacco will not be permitted 48 hours prior to admission and until discharge from the CRU in each study period.
- Subjects should refrain from strenuous exercise for at least 96 hours prior to dosing until discharge from the CRU at the end of the study.

4.4.4.1 Herbal Therapies

Any herbal remedies are prohibited for 7 days prior to the first study drug administration through follow-up.

4.5 STUDY ASSESSMENTS

The schedule of activities to be performed during the study is provided in Appendix 1. All activities must be performed and documented for each subject.

Subjects will be closely monitored for safety and tolerability throughout the study.

4.5.1 Informed Consent Forms and Screening Log

Written informed consent for participation in the study must be obtained before performing any study-related procedures (including screening evaluations). Informed Consent Forms for enrolled subjects and for subjects who are not subsequently enrolled will be maintained at the study site.

All screening evaluations must be completed and reviewed to confirm that subjects meet all eligibility criteria before enrollment. The Investigator or designee will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

4.5.2 Medical History, Concomitant Medication, and Demographic Data

Medical history, including clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and use of alcohol and drugs of abuse, will be recorded at baseline. In addition, all medications (e.g., prescription drugs, over-the-counter drugs, vaccines, or homeopathic remedies, nutritional supplements, or herbal) used by the subject within 30 days prior to initiation of study treatment will be recorded. Any nonprescribed systemic or topical medication or herbal remedies are prohibited for 7 days prior to the first study drug administration through Follow-up. At the time of each Follow-up visit physical examination, an interval medical history should be obtained and any changes in medications and allergies should be recorded.

Demographic data will include age, sex, and self-reported race/ethnicity.

4.5.3 Physical Examinations

A complete physical examination, performed at Screening and other specified visits (Appendix 1), should include an evaluation of the head, eyes, ears, nose, and throat,

and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, genitourinary (at Investigator or designee's discretion). Any clinically significant abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF. Changes from baseline abnormalities should be recorded in subject notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF and source form.

4.5.4 Vital Signs

Oral body temperature, pulse rate, and supine blood pressure will be collected as described in the SoA (Appendix 1). ABPM assessment will be performed on study Days -1 and 12.

4.5.5 Laboratory, Genotyping, and Pharmacokinetic Samples

For sampling and sample processing procedures, storage conditions, and shipment instructions, see the Laboratory Manuall.

Biological samples will be destroyed when the final Clinical Study Report has been issued, except where specified otherwise.

When a subject withdraws from the study, samples collected prior to the date of withdrawal may still be analyzed, unless the subject specifically requests that the samples be destroyed, or local laws require destruction of the samples. However, if samples have been tested prior to withdrawal, results from those tests will remain as part of the overall research data.

Data arising from sample analysis will be subject to the confidentiality standards described in Section 8.4.

4.5.5.1 Laboratory Samples

Laboratory samples will be collected at the time points indicated in the SoA (Appendix 1). Samples will be collected at Screening, Day -2, Day 3, Day 7, Day 15 and Follow-up visit for each Treatment Period. Additional blood or urine samples may be taken at the discretion of the Investigator or designee if the results of any test falls outside the reference ranges, or clinical symptoms necessitate additional testing to monitor subject safety. Subjects must fast for at least 8 hours prior to laboratory safety assessments (except for urine assessments).

Samples for the following laboratory tests will be sent to the study site's local laboratory for analysis:

- Hematology: leukocytes, erythrocytes, hemoglobin, hematocrit, platelets, absolute and percent differential WBC count (neutrophils, eosinophils, basophils, monocytes, lymphocytes).
- Urinalysis: A midstream, clean-catch urine specimen will be collected for dipstick analysis of pH, glucose, leukocytes, nitrites, protein and blood. Microscopy will be performed if abnormalities are observed in initial analysis.

- Coagulation: prothrombin time (international normalized ratio) and activated thromboplastin time.
- Blood chemistry panel: sodium, potassium, glucose (fasting), creatinine, total and conjugated bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Biomarkers for cardiac damage: samples will be taken for troponin I, cardiac troponin T (cTnT) and N-terminal pro b-type natriuretic peptide (NT-proBNP) but these samples will be stored, and assays only run if requested for specific subject(s) due to concerns regarding cardiac safety.
- Drugs of abuse (including amphetamines, barbiturates, benzodiazepines cannabinoids, cocaine, opiates, methamphetamines, methadone and 3,4-methylenedioxymethamphetamine (MDMA), will be measured in urine at Screening and on Day -2 of each Treatment Period. Further testing may be performed throughout the study to confirm compliance at the discretion of the Investigator or designee.
- Urine alcohol test at Screening and on Day -2 of each study period. Further testing may be performed throughout the study to confirm compliance at the discretion of the Investigator or designee.
- Viral serology: human immunodeficiency virus (HIV) -1 and -2 antibodies, hepatitis C antibody, hepatitis B surface antigen and hepatitis C virus (HCV) antibody will be assessed at Screening.
- Pregnancy test: beta-human chorionic gonadotropin (serum pregnancy test) will be performed at Screening and Day -2 for all female subjects at Treatment Period 1 and Day -2 for subsequent Treatment Periods.
- Follicle-stimulating hormone (FSH) will be assessed at Screening for all females

4.5.5.2 Pharmacokinetic Samples

Pharmacokinetic samples will be taken via an indwelling catheter or by direct venipuncture at the time points in Appendix 1 and Appendix 2.

Three (3) aliquoted plasma samples will be prepared for each time point. Two (2) of the aliquoted PK plasma samples will be shipped to the designated bioanalytical laboratory for analysis. The third aliquot will be frozen and stored at PRA (-80°C) for up to 2 years after issue of the CSR to allow for repeat analysis if required.

For additional sampling procedures, storage conditions, and shipment instructions, see the Laboratory Manual.

4.5.5.3 Optional Samples for genetic analyses

An optional whole blood sample will be taken for DNA extraction and clinical genotyping from subjects consenting to this additional sample. See Appendix 1 for collection timepoint and associated footnote.

The DNA may be used for:

- [redacted]
- to determine if genetic variants of cytochrome P450s (e.g., CYP3A4), transporters, or receptors may affect the metabolism, pharmacokinetics of balovaptan, or be associated with any potential effect on ECG parameters.

This sample may be stored for up to two years following the issue of the CSR.

Data arising from all biosamples including samples for analyses of inherited DNA will be subject to the confidentiality standards described in Section 8.4.

4.5.6 Electrocardiograms

Safety and diagnostic ECG assessments will be performed in this study.

4.5.6.1 12-lead Safety Electrocardiograms

Safety ECGs will be performed at defined time points (Appendix 1). Safety ECG performed at Screening and Day -2 of each treatment period will be done in triplicate. All other safety ECG readings will be single recordings. These ECG recordings will only be used to assess/define a safety baseline and determine the immediate safety of each subject during each Treatment Period. Safety ECG recordings may be obtained at unscheduled timepoints as required by the Investigator, or designee, to ensure the safety of study subjects. For safety monitoring purposes, the Investigator, or designee, must review, sign, and date all ECG tracings.

All safety ECG recordings must be performed using a standard high-quality, high-fidelity digital electrocardiograph machine equipped with computer-based interval measurements. Lead placement should be as consistent as possible. ECG recordings must be performed after the subject has been resting in a supine position for at least 10 minutes. All ECGs are to be obtained prior to other procedures scheduled at that same time (e.g., vital sign measurements, blood draws) and should not be obtained within 3 hours after any meal, when possible. Circumstances that may induce changes in HR, including environmental distractions (e.g., television, radio, conversation), should be avoided during the pre-ECG resting period and during ECG recording.

Paper copies of ECG tracings will be kept as part of the subjects' permanent study file at the site. The following should be recorded in the appropriate eCRF: HR, QRS interval, PR duration, uncorrected QT interval, and QTcF based on the machine readings of the individual ECG tracings. Any morphologic waveform changes or other ECG

abnormalities must be documented on the eCRF. If considered appropriate by the Sponsor, ECGs may be analyzed retrospectively at a central laboratory.

If at a post-dose timepoint the mean QTcF is > 500 ms and/or 60 ms longer than the baseline value, another ECG must be recorded, ideally within the next 5 minutes, and ECG monitoring should continue until QTcF has stabilized on two successive ECGs. The Medical Monitor should be notified. Standard-of-care treatment may be instituted per the discretion of the Investigator or designee. If a PK sample is not scheduled for that timepoint, an unscheduled PK sample should be obtained. A decision on study drug discontinuation should be made, as described in Section 4.6.1. The Investigator or designee should also evaluate the subject for potential concurrent risk factors (e.g., electrolyte abnormalities, co-medications known to prolong the QT interval, severe bradycardia).

4.5.6.2 Cardiodynamic ECG Evaluation

12-lead ECGs will be recorded continuously using a Holter device from at least 1 hour before to at least 24 hours after drug administration on Day 1, 2, 14 and 15. The placement of electrodes for each subject must be marked on the skin with permanent marker, to allow electrodes to be positioned as consistently as possible throughout the study (Appendix 1 and Appendix 2).

ECGs will be extracted from Holter devices at 3 time points pre-dose (-45, -30 and -15 minutes) on Days 1, 2, 14, and 15. Post-dose ECGs will be extracted at 0.5, 1.0, 2.5, 4, 8, 12 and 24 hours post-dose on Days 1, 2, 14 and 15 (Appendix 1 and Appendix 2). The 24 h Holter extraction time point for Days 1 and 14 can be taken from the 3 pre-dose Holter time points on Days 2 and 15. 12-lead ECGs will be extracted from the continuous recording in up to 10 replicates from a 5-minute window during a 15-minute supine period pre-dose and at the nominal Holter timepoints post-dose. Whenever ECG extraction and PK sample timepoints are close together the ECG extraction will be performed prior to PK sample collection.

During days with Holter ECG monitoring subjects (Appendix 1), will be fed standardized low-salt meals. To reduce variability, Holter recording and supine periods have to be performed according to PRA Work Instruction EDSEXE 009 W 131.

The 12-lead Holter will be supplied and supported by ERT. All ECG data will be collected using a [redacted] USA) M12R ECG continuous 12-lead digital recorder. The continuous 12-lead digital ECG data will be stored onto SD memory cards. ECGs to be used in the analyses will be selected by pre-determined time points as defined in the SoA (Appendix 1) and will be read centrally by ERT.

The following principals will be followed in ERT's core laboratory:

- ECG analysts will be blinded to the subject, visit and treatment allocation.

- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analyzed by the same reader.
- The primary analysis lead is lead II. If lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire subject data set.

TQT Plus ECG Extraction Technique

Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the 'TQT Plus method', a computer-assisted and statistical process utilized by ERT. The method enables extraction of ECGs with the lowest HR variability and noise within the protocol-specified extraction time window (e.g., the HR and QT changes from beat-to-beat in the range of < 10%). At each protocol-specified timepoint, 10 ECG replicates will be extracted from a 5-minute "ECG window" (the last 5 minutes of the 15-minute period when the subject is maintained in a supine quiet position).

Expert-Precision QT Analysis

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

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

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

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

Table 2 T-wave Morphology Categories (Assessed Manually)

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

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

4.5.7 Transthoracic-Doppler Echocardiography

Transthoracic (TT)-Doppler echocardiograms will be performed on Day -1 and Day 13 of each Treatment Period by Diagnostic Partners.

TT-Doppler echocardiograms should be performed at approximately the same time on Days -1 and 13.

Parameters to be collected by TT-Doppler echocardiography (details will be provided in a separate manual):

Primary Assessments:

- Doppler-echo parameters for evaluation of right heart:
  - pulmonary artery acceleration time (PAAT), used to estimate mean PAP
  - right ventricular outflow tract- velocity time integral (RVOT-VTI)

Secondary Assessments:

- Doppler-echo parameters for evaluation of right heart:
  - Inferior vena cava diameter (IVC)
  - right atrial size
  - right-ventricular index of myocardial performance (RIMP)
  - right ventricle: end-systolic pressure
  - right ventricle end-diastolic pressure
  - tricuspidal regurgitation- absent or present. If present, used to estimate systolic PAP

- pulmonary valve regurgitation- absent or present. If present, used to estimate diastolic PAP
- Right ventricular wall thickness
- Right ventricular size; diameter

- Doppler-echo parameters for evaluation of left heart:
  - left ventricular ejection fraction (LVEF)
  - left ventricular wall thickness
  - Ratio of transmitral Doppler early filling velocity (E) to tissue Doppler early diastolic mitral annular velocity (E')
  - aortic valve insufficiency: if present, categorization as mild, moderate, severe
  - mitral valve regurgitation: if present, categorization as mild, moderate, severe per definition in the procedure manual.

4.5.8 Ambulatory Blood Pressure Monitoring

Ambulatory Blood Pressure Monitoring (ABPM) will be performed on Day -1 and Day 12 and mean daytime and mean nighttime blood pressure will be determined to assess the effect of balovaptan on blood pressure. For this study the daytime period is defined as 9:00 am to 9:00 pm and the nighttime period is defined as 1:00 am to 5:30 am. The ABPM device needs to be active during these periods.

The blood pressure cuff for the ABPM device will be placed on the non-dominant arm of the subjects. This means the majority of subjects will likely have the cuff fastened to their left arm. Since the TT-Doppler echocardiogram requires access to the left side of the subject and manipulation of the left arm the APBM device might be turned off during this procedure. On completion of the TT-Doppler echocardiogram the ABPM device will be reactivated, and assessments collected as usual for the remainder of the testing period. All deactivations and or removals of the APBM device during Day -1 and during Day 12 should be captured for each subject.

During the period of time that no assessments are planned on Day -1 and Day 12, i.e., 9:00 pm to 1:00 am, subjects may remove the ABPM device to perform activities that would be hindered by the device, e.g., to shower or change clothing.

Daytime period: Daytime is defined from 9 am to 9 pm for this study and blood pressure will be measured in 20-minute intervals during this period. Unusual or strenuous physical activities are not allowed during assessment periods. Subjects will be instructed not to sleep during this assessment period. The ABPM device maybe deactivated due to the need to perform other assessments including TT-Doppler echocardiograms.

Nighttime period: Nighttime for this study is defined as from 1 am to 5:30 am and blood pressure will be measured in 20-minute intervals during this period. It is expected that subjects will remain asleep during this assessment period. Subjects will be instructed to try and remain asleep during this period and not leave the bed.

Pulse rate will be recorded by ABPM device during each assessment period at the times of blood pressure measurements.

Further details of ABPM analysis will be provided in a separate manual.

4.5.9 Columbia-Suicide Severity Rating Scale

The Columbia-Suicide Severity Rating Scale (C-SSRS) is a clinical tool used to assess the lifetime suicidality of a subject (C-SSRS lifetime version) as well as any new instances of suicidality (C-SSRS since last visit).

The structured interview prompts recollection of suicidal ideation, including the intensity of the ideation, behavior, and attempts with actual/potential lethality.

C-SSRS assessments will be completed by the Investigator or designee at Screening, Day -2, Day 16 and at Follow-up visits (Appendix 1).

4.5.10 Drug and Metabolite Concentration Measurements

For each Treatment Period plasma samples will be collected, as described in Appendix 1 and Appendix 2, to determine concentrations of balovaptan and its metabolites M2 (as appropriate) and M3 utilizing a specific and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Plasma concentrations of moxifloxacin will also be measured using a separate, specific and validated LC-MS/MS method.

Moxifloxacin PK samples: will only be analyzed if the moxifloxacin assay sensitivity test fails; in such case, moxifloxacin plasma levels matched to the QT data by timepoint across subjects will be used for exposure response analysis of the QT effect.

4.5.11 Timing of Study Assessments

If performed at the same timepoint, assessments should be prioritized as follows, while ensuring PK blood sampling is conducted at the scheduled time, when possible:

- 12-lead ECGs
- Vital signs measurements
- PK blood sampling
- Laboratory tests

4.6 TREATMENT, SUBJECT, STUDY, AND SITE DISCONTINUATION

4.6.1 Study Treatment Discontinuation

Subjects must permanently discontinue study treatment if they experience any of the following:

- Any medical condition that the Investigator, designee or Sponsor determines may jeopardize the subject's safety if he or she continues to receive study treatment. Section 5.1.2 provides additional guidance on the management of toxicities and of subjects who experience AEs within the outline of specific stopping rules.
- Investigator, designee or Sponsor determines it is in the best interest of the subject
- Pregnancy

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF. Subjects who discontinue study treatment may be replaced.

Subjects who terminate the study early while admitted to the CRU will have safety assessments performed as indicated for the Follow-up visit as described in SoA (Appendix 1) prior to discharge. In addition, subjects will be encouraged to return to the clinic for a Follow-up visit 14 to 21 days after the last dose of study drug (Appendix 1).

4.6.2 Subject Discontinuation from Study

Subjects will return to the clinic for a Follow-up visit 14 to 21 days after the last dose of study drug.

Subjects have the right to voluntarily withdraw from the study at any time for any reason. In addition, the Investigator or designee has the right to withdraw a subject from the study at any time. Reasons for withdrawal from the study may include, but are not limited to, the following:

- Subject withdrawal of consent
- Study termination or site closure
- Subject non-compliance, defined as failure to comply with protocol requirements as determined by the Investigator, designee or Sponsor

Every effort should be made to obtain information on subjects who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. Subject requests to be withdrawn from the study must be documented in the source documents and signed by the Investigator (or designee). Subjects who withdraw from the study may be replaced at the discretion of the Investigator or Sponsor.

4.6.3 Study Discontinuation

The Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory

The Sponsor will notify the Investigator if the Sponsor decides to discontinue the study.

4.6.4 Site Discontinuation

The Sponsor has the right to close a site at any time. Reasons for closing a site may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Council for Harmonisation (ICH) guideline for Good Clinical Practice
- No study activity (i.e., all subjects have completed the study and all obligations have been fulfilled)

5. ASSESSMENT OF SAFETY

5.1 SAFETY PLAN

Balovaptan is not approved in any country and clinical development is ongoing. The safety plan for subjects in this study is based on clinical experience with balovaptan in completed and ongoing studies. Potential safety risks for balovaptan are outlined below. Please refer to the balovaptan (RO5285119) Investigator's Brochure for a complete summary of the safety information available for the molecule.

Several measures will be taken to ensure the safety of subjects participating in this study. Eligibility criteria have been designed to exclude subjects at higher risk for toxicities. Subjects will undergo safety monitoring during the study, including assessment of the nature, frequency, and severity of adverse events. In addition, guidelines for managing adverse events, including criteria for dosage modification and treatment interruption or discontinuation, are provided below.

[redacted]

[redacted]

[redacted]

5.1.1.4 Pulmonary Blood Pressure

In animals as well as preparations of pulmonary vasculature, vasopressin has been shown to decrease pulmonary blood pressure and to relax arterial constriction through V1a receptors and in animals these changes were accompanied by systemic blood pressure increases.[8,9]

GLP toxicology animal study have not pointed to a right heart challenge strain nor to pulmonary fibrosis. The cardiomyopathy seen in rats refers to the right and left heart and mirrors cardiomyopathy with advanced age.

No systemic blood pressure changes with balovaptan were noted in clinical studies.

Hence, the a priori expectation is to not see changes in pulmonary circulatory dynamics and with a N of 48, this study is powered to detect a change in pulmonary blood pressure representing half of 1 SD at α of 0.05 and with a power of 80 %.

As an accompanied monitoring measure, the current database on systemic blood pressure monitoring is supplemented by ABPM monitoring as scheduled within this study.

[redacted]

5.1.1.6 Orthostasis

Inhibition of V1a receptors by balovaptan may impair orthostatic regulation with blood pressure decreases, particularly when moving rapidly from a supine or sitting position to a standing position.

However, various biological systems besides the vasopressinergic system are involved in regulating blood pressure control and the relevance of isolated V1a receptor inhibition may be minor. Safety pharmacology studies in dogs have not pointed to blood pressure lowering effect of balovaptan.

Orthostatic challenge testing was included in the Phase 2 study BP28420 and no imbalance was noted between placebo- and balovaptan-treated cohorts in the percentage of subjects (approximately 8%) showing relevant blood pressure changes.

No respective safety signals emerged from the MAD arm of study BP25694 nor from the proof-of-mechanism study (BP29412) when balovaptan doses of 52 mg qd were administered for several days. However, no orthostatic challenge testing was included in these trials.

Given that a dose of either 10 mg or 50 mg qd balovaptan is estimated to block the V1a receptor fully throughout the dosing interval, together with the clinical trial experience outlined above, the risk of balovaptan-related orthostatic impairment in this study is considered low.

As indicated above, this study monitors systemic blood pressure by the means of ABPM.

5.1.2 Management of Subjects Who Experience Adverse Events

5.1.2.1 Management Guidelines

In general, any emerging AEs must diligently be watched, treated as medically indicated according to common medical practice and documented in terms of onset-date, intensity, off-set date, and any measures taken in order to treat the AE.

No specific treatment guidance to any potentially emerging AEs in balovaptan clinical trials exist and treatment should be according to the common medical practice for the given AE.

Temporary dosing interruptions must be discussed with the Sponsor's Medical Monitor in the sense that temporary interruptions will have to be very limited in order to nor jeopardize the trial's pharmacokinetic objectives. Dose modifications because of an AE are not allowed in this study.

[redacted]

[redacted]

[redacted]

5.1.2.5 Changes in Pulmonary / Systemic Blood Pressure

While no relevant changes in pulmonary blood pressure are expected, should the TT-Doppler echocardiogram examination unexpectedly reveal such a change all available cardio-vascular and potential pulmonary data should be reviewed by Investigator (or designee) and cardiologist to assess the subject's safety and to decide on next actions which may include treatment interruption and / or permanent treatment stop.

An increase in mean pulmonary blood pressure of 25 mm/Hg will result in termination of study drug dosing and referral of the subject to a cardiologist. If logistically possible abnormal TT-Doppler echocardiograms should be repeated as soon as possible to confirm results.

The relevance of changes in the parameters obtained by TT-Doppler echocardiogram will be interpreted by the cardiologist. A change in PAAT by 50% or more in a subject, is a safety signal that will result in a discussion of discontinuing treatment for the affected subject.

Given current clinical experience for systemic blood pressure and HR, relevant changes in standard and ambulatory blood pressure monitoring (ABPM) monitoring are not expected. However, repeated blood pressure changes exceeding the BP systolic > 160 mm Hg and/or BP diastolic > 100 mm Hg and that cannot be explained by any circumstances or low blood pressure linked with moderate to severe clinical AEs related to low blood pressure may lead to treatment interruption or permanent withdrawal from study treatment. For orthostatic events, please see also Section 5.1.2.6.

5.1.2.6 Management of Orthostatic Hypotension

Subjects with unexplained orthostatic reaction with syncope within the last 6 months are excluded from this study.

Vital signs will be monitored frequently throughout the study as indicated in the SoA (Appendix 1).

Subjects will be instructed to avoid rapid body movements from supine to standing positions, and to inform the Investigator or designee as soon as possible should they experience dizziness, nausea, sweating, pale face, or even fainting with such body movements.

5.1.2.7 Management of Signs and Symptoms Indicative for Peripheral Neuropathy

Subjects with signs and symptoms potentially indicative of peripheral neuropathy are excluded from this study.

As outlined above, the risk of peripheral neuropathy is minimal in study.

Should however signs and symptoms potentially indicative of peripheral neuropathy occur, an expert in the field of peripheral neuropathy should be consulted to discuss next diagnostic steps as well as the need to interrupt study treatment. Additional diagnostic tests may include a nerve conduction study.

5.2 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording AEs, including SAEs and AEs of special interest (AESI), performing protocol-specified safety laboratory assessments, measuring protocol-specified vital signs, and conducting other protocol-specified tests that are deemed critical to the safety evaluation of the study. The schedule for conducting these activities is provided in the SoA (Appendix 1).

Certain types of events require immediate reporting to the Sponsor, as outlined in Section 5.4.

5.2.1 Adverse Events

According to the ICH guideline for Good Clinical Practice, an AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

5.2.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

A serious adverse event is any adverse event that meets any of the following criteria:

- Is fatal (i.e., the AE actually causes or leads to death)
- Is life threatening (i.e., the AE, in the view of the Investigator or designee, places the subject at immediate risk of death)
    - This does not include any AE that, had it occurred in a more severe form or was allowed to continue, might have caused death.
- Requires or prolongs subject hospitalization (Section 5.3.5.9)
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drugs
- Is a significant medical event in the Investigator or designee's judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (e.g., rated as mild, moderate, or severe, or according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE]; see Section 5.3.3); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

Serious adverse events are required to be reported by the Investigator or designee to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2 for reporting instructions).

5.2.3 Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

Adverse events of special interest are required to be reported by the Investigator (or designee) to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2 for reporting instructions). AESIs for this study are as follows:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's Law (see Section 5.3.5.6)
- Suspected transmission of an infectious agent by the study drug, as defined below

    Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be

suspected from clinical symptoms or laboratory findings that indicate an infection in a subject exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

- Clinically relevant decreases in ANC and for which no alternative explanation has been identified (i.e., the relationship to study drug is rated "yes").
- CPK values that exceed 10× ULN for several days without a trend of normalization or are associated with relevant dysfunction of kidneys, and if no alternative explanation exists.
- Adverse events such as:
    - Arrhythmia
    - Syncope
    - Dyspnea
    - Palpitations
    - Chest pain.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The Investigator is responsible for ensuring that all AEs are recorded on the Adverse Event eCRF and source form and reported to the Sponsor in accordance with instructions provided in this Section and in Sections 5.4-5.6.

For each AE recorded on the Adverse Event eCRF and source form, the Investigator or designee will make an assessment of seriousness (see Section 5.2.2), severity (see Section 5.3.3), and causality (see Section 5.3.4).

5.3.1 Adverse Event Reporting Period

All AEs, whether reported by the subject or noted by study personnel, will be recorded in the subject's medical record and on the Adverse Event eCRF and source form.

After informed consent has been obtained but prior to initiation of study drug,only SAEs caused by a protocol-mandated intervention (e.g., invasive procedures such as biopsies, discontinuation of medications) should be reported (see Section 5.4.2 for instructions for reporting SAEs).

After initiation of study drug, all AEs will be reported until 21 days after the last dose of study drug.

Instructions for reporting AEs that occur after the AE reporting period are provided in Section 5.6.

5.3.2 Eliciting Adverse Event Information

A consistent methodology of non-directive questioning should be adopted for eliciting AE information. Examples of non-directive questions include the following:

"How have you felt since your last clinic visit?"

"Have you had any new or changed health problems since you were last here?"

5.3.3 Assessment of Severity of Adverse Events

Table 3 provides guidance for assessing AE severity.

Table 3 Adverse Event Severity Grading Scale

Severity	Description
Mild	Discomfort noticed, but no disruption of normal daily activity
Moderate	Discomfort sufficient to reduce or affect normal daily activity
Severe	Incapacitating with inability to work or to perform normal daily activity

Note: Regardless of severity, some events may also meet seriousness criteria. Refer to definition of a serious adverse event (see Section 5.2.2).

5.3.4 Assessment of Causality of Adverse Events

Investigator or designees should use their knowledge of the subject, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether an AE is considered to be related to the study drug, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration (see also Table 4)

- Temporal relationship of event onset to the initiation of study drug
- Course of the event, with special consideration of the effects of dose reduction, discontinuation of study drug, or reintroduction of study drug (as applicable)
- Known association of the event with the study drug or with similar treatments
- Known association of the event with the disease under study
- Presence of risk factors in the subject or use of concomitant medications known to increase the occurrence of the event
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event

Table 4 Causal Attribution Guidance

Is the AE suspected to be caused by the study drug on the basis of facts, evidence, science-based rationales, and clinical judgment?	
YES	There is a plausible temporal relationship between the onset of the AE and administration of the study drug, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the study drug; and/or the AE abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.
NO	An AE will be considered related, unless it fulfills the criteria specified below. Evidence exists that the AE has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to administration of the study drug (e.g., cancer diagnosed 2 days after first dose of study drug).

For subjects receiving combination therapy, causality will be assessed individually for each protocol-mandated therapy following conclusion of the study and unblinding of study data.

5.3.5 Procedures for Recording Adverse Events

Investigator or designees should use correct medical terminology/concepts when recording AEs on the Adverse Event eCRF and source form. Avoid colloquialisms and abbreviations.

Only one AE term should be recorded in the event field on the Adverse Event eCRF and source form.

5.3.5.1 Diagnosis versus Signs and Symptoms

A diagnosis (if known) should be recorded on the Adverse Event eCRF and source form rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded on the Adverse Event eCRF and source form. If a diagnosis is subsequently established, all previously reported AEs based on signs and symptoms should be nullified and replaced by one AE report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

5.3.5.2 Adverse Events That Are Secondary to Other Events

In general, AEs that are secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. A medically significant secondary AE that is separated in time from the initiating event should be recorded as an independent event on the Adverse Event eCRF and source form. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and consequent fracture, all three events should be reported separately on the eCRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the eCRF.

All AEs should be recorded separately on the Adverse Event eCRF and source form if it is unclear as to whether the events are associated.

5.3.5.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between subject evaluation timepoints. Such events should only be recorded once on the Adverse Event eCRF and source form. The initial severity (intensity or grade) of the event will be recorded at the time the event is first reported. If a persistent AE becomes more severe, the most extreme severity should also be recorded on the Adverse Event eCRF and source form. If the event becomes serious, it should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning that the event became serious; see Section 5.4.2 for reporting instructions). The Adverse Event eCRF and source form should be updated by changing the event from "non-serious" to "serious," providing the date that the event became serious, and completing all data fields related to SAEs.

A recurrent adverse event is one that resolves between subject evaluation timepoints and subsequently recurs. Each recurrence of an adverse event should be recorded as a separate event on the Adverse Event eCRF and source form.

5.3.5.4 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an AE. A laboratory test result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)

- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy
- Is clinically significant in the Investigator or designee's judgment

It is the Investigator or designee's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 × ULN associated with cholestasis), only the diagnosis (i.e., cholestasis) should be recorded on the Adverse Event eCRF and source form.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the Adverse Event eCRF and source form, along with a descriptor indicating whether the test result is above or below the normal range (e.g., "elevated potassium," as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the AE. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit should only be recorded once on the Adverse Event eCRF and source form (see Section 5.3.5.3 for details on recording persistent AEs).

5.3.5.5 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an AE. A vital sign result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Is clinically significant in the Investigator or designee's judgment

It is the Investigator or designee's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an AE.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (i.e., hypertension) should be recorded on the Adverse Event eCRF and source form.

Observations of the same clinically significant vital sign abnormality from visit to visit should only be recorded once on the Adverse Event eCRF and source form (see Section 5.3.5.3 for details on recording persistent AEs).

5.3.5.6 Abnormal Liver Function Tests

The finding of an elevated ALT or AST ($>3\times$ULN) in combination with either an elevated total bilirubin ($>2\times$ULN) or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia is considered to be an indicator of severe liver injury (as defined by Hy's Law). Therefore, Investigator or designees must report as an AE the occurrence of either of the following:

- Treatment-emergent ALT or AST $>3\times$ULN in combination with total bilirubin $>2\times$ULN
- Treatment-emergent ALT or AST $>3\times$ULN in combination with clinical jaundice

The most appropriate diagnosis or (if a diagnosis cannot be established) the abnormal laboratory values should be recorded on the Adverse Event eCRF and source form (see Section 5.3.5.2) and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event), either as a SAE or an AESI (see Section 5.4.2).

5.3.5.7 Deaths

All deaths that occur during the protocol-specified adverse event reporting period (see Section 5.3.1), regardless of relationship to study drug, must be recorded on the Adverse Event eCRF and source form and immediately reported to the Sponsor (see Section 5.4.2).

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the Adverse Event eCRF and source form. Generally, only one such event should be reported. If the cause of death is unknown and cannot be ascertained at the time of reporting, **"unexplained death"** should be recorded on the Adverse Event eCRF and source form. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death. The term **"sudden death"** should not be used unless combined with the presumed cause of death (e.g., "sudden cardiac death").

Deaths that occur after the adverse event reporting period should be reported as described in Section 5.6.

5.3.5.8 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded on the General Medical History and Baseline Conditions eCRF.

A preexisting medical condition should be recorded as an adverse event only if the frequency, severity, or character of the condition worsens during the study. When

recording such events on the Adverse Event eCRF and source form, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

5.3.5.9 Hospitalization or Prolonged Hospitalization

Any AE that results in hospitalization (i.e., inpatient admission to a hospital) or prolonged hospitalization should be documented and reported as a serious adverse event (per the definition of serious adverse event in Section 5.2.2), except as outlined below.

An event that leads to hospitalization under the following circumstances should not be reported as an AE or a SAE:

- Hospitalization for respite care
- Planned hospitalization required by the protocol
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
    - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease
    - The subject has not experienced an AE

An event that leads to hospitalization under the following circumstances is not considered to be a SAE, but should be reported as an AE instead:

- Hospitalization that was necessary because of subject requirement for outpatient care outside of normal outpatient clinic operating hours

5.4 IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The Investigator or designee must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the Investigator or designee learns of the event. The following is a list of events that the Investigator or designee must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- Serious adverse events (defined in Section 5.2.2; see Section 5.4.2 for details on reporting requirements)
- Adverse events of special interest (defined in Section 5.2.3; see Section 5.4.2 for details on reporting requirements)
- Pregnancies (see Section 5.4.3 for details on reporting requirements)

The Investigator or designee must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

Investigator or designees must also comply with local requirements for reporting serious adverse events to the local health authority and IRB/EC.

5.4.1 Emergency Medical Contacts

Medical Monitor Contact Information:

Medical Monitor:	[redacted], M.D. (Primary)
Telephone No.:	[redacted]
Mobile Telephone No.:	[redacted]
Medical Monitor:	[redacted], M.D. (Secondary)
Telephone No.:	[redacted]
Mobile Telephone No.:	[redacted]

To ensure the safety of study subjects, an Emergency Medical Call Center Help Desk will access the Roche Medical Emergency List, escalate emergency medical calls, provide medical translation service (if necessary), connect the Investigator or designee with a Roche Medical Responsible (listed above and/or on the Roche Medical Emergency List), and track all calls. The Emergency Medical Call Center Help Desk will be available 24 hours per day, 7 days per week. Toll-free numbers for the Help Desk, as well as Medical Monitor and Medical Responsible contact information, will be distributed to all Investigators or designees.

5.4.2 Reporting Requirements for Serious Adverse Events and Adverse Events of Special Interest

5.4.2.1 Events That Occur prior to Study Drug Initiation

After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. The paper Clinical Trial Serious Adverse Event (SAE)/AE of Special Interest Reporting Form provided to Investigator or designees should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the event), either by faxing or by scanning and emailing the form using the fax number or email address provided to Investigator.

5.4.2.2 Events That Occur after Study Drug Initiation

After initiation of study drug, SAEs and AEs of special interest will be reported until 21 days after the last dose of study drug. Investigator or designees should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators and submitting the report, either by faxing or by scanning and emailing the form using the fax number or email address provided to investigators.

Instructions for reporting SAEs that occur >21 days after the last dose of study treatment are provided in Section 5.6.

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Subjects

Female subjects of childbearing potential will be instructed to immediately inform the Investigator or designee if they become pregnant during the study or within 90 days after the last dose of study drug. A paper Clinical Trial Pregnancy Reporting Form should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy), either by faxing or by scanning and emailing the form using the fax number or email address provided to the Investigator. Pregnancy should not be recorded on the Adverse Event eCRF and source form. The Investigator or designee should discontinue the study drug and counsel the subject, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the subject should continue until conclusion of the pregnancy. Any SAE associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event eCRF and source form. In addition, the Investigator or designee will submit a Clinical Trial Pregnancy Reporting Form when updated information on the course and outcome of the pregnancy becomes available.

5.4.3.2 Pregnancies in Female Partners of Male Subjects

Male subjects will be instructed through the Informed Consent Form to immediately inform the Investigator or designee if their partner becomes pregnant during the study or within 90 days after the last dose of study drug. A paper Clinical Trial Pregnancy Reporting Form should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy), either by faxing or by scanning and emailing the form using the fax number or email address provided to investigators. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male subject exposed to study drug. When permitted by the site, the pregnant partner would need to sign an Authorization for Use and Disclosure of Pregnancy Health Information to allow for follow-up on her pregnancy. If the authorization has been signed, the Investigator or designee should submit a Clinical Trial Pregnancy Reporting Form when updated information on the course and outcome of the pregnancy becomes available. An

Investigator or designee who is contacted by the male subject or his pregnant partner may provide information on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the treating physician and/or obstetrician.

5.4.3.3 Abortions

Any abortion should be classified as a SAE (as the Sponsor considers abortions to be medically significant), recorded on the Adverse Event eCRF and source form, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

5.4.3.4 Congenital Anomalies/Birth Defects

Any congenital anomaly/birth defect in a child born to a female subject exposed to study drug or the female partner of a male subject exposed to study drug should be classified as a serious adverse event, recorded on the Adverse Event form, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

5.4.4 Reporting Requirements for Cases of Accidental Overdose or Medication Error

Accidental overdose and medication error (hereafter collectively referred to as "special situations"), are defined as follows:

- Accidental overdose: accidental administration of a drug in a quantity that is higher than the assigned dose
- Medication error: accidental deviation in the administration of a drug

  In some cases, a medication error may be intercepted prior to administration of the drug.

Special situations are not in themselves adverse events but may result in adverse events. Each adverse event associated with a special situation should be recorded separately on the Adverse Event eCRF. If the associated adverse event fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2) Special situations should be recorded as described below:

- Accidental overdose: Enter the drug name and "accidental overdose" as the event term. Check the "Accidental overdose" and "Medication error" boxes.
- Medication error that does not qualify as an overdose: Enter the name of the drug administered and a description of the error (e.g., wrong dose administered, wrong dosing schedule, incorrect route of administration, wrong drug, expired drug administered) as the event term. Check the "Medication error" box.
- Medication error that qualifies as an overdose: Enter the drug name and "accidental overdose" as the event term. Check the "Accidental overdose" and "Medication error" boxes. Enter a description of the error in the additional case details.

- Intercepted medication error: Enter the drug name and "intercepted medication error" as the event term. Check the "Medication error" box. Enter a description of the error in the additional case details.

For study drug, each AE associated with a special situation should be recorded separately on the Adverse Event eCRF and source form. If the associated AE fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2). Study drug AEs associated with special situations should be recorded as described below for each situation:

- Accidental overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.
- Medication error that does not qualify as an overdose: Enter the adverse event term. Check the "Medication error" box.
- Medication error that qualifies as an overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.

As an example, an accidental overdose that resulted in a headache would require the completion of two Adverse Event eCRF and source form, one to report the accidental overdose and one to report the headache. The "Accidental overdose" and "Medication error" boxes would need to be checked on both eCRF pages.

5.5 FOLLOW-UP OF SUBJECTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

The Investigator or designee should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the Investigator or designee, the subject is lost to follow-up, or the subject withdraws consent. Every effort should be made to follow all SAEs considered to be related to study drug or trial-related procedures until a final outcome can be reported.

During the study period, resolution of adverse events (with dates) should be documented on the Adverse Event eCRF and source form and in the subject's medical record to facilitate source data verification.

All pregnancies reported during the study should be followed until pregnancy outcome.

5.5.2 Sponsor Follow-Up

For SAEs, AESI, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, email, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

5.6 ADVERSE EVENTS THAT OCCUR AFTER THE ADVERSE EVENT REPORTING PERIOD

The Sponsor should be notified if the Investigator or designee becomes aware of any SAE that occurs after the end of the AE reporting period (defined as 21 days after the last dose of study drug), if the event is believed to be related to prior study drug treatment. These events should be reported through use of the Adverse Event eCRF and source form. The Investigator or designee should report these events directly to the Sponsor or its designee, either by faxing or by scanning and emailing the paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form using the fax number or email address provided to investigators.

5.7 EXPEDITED REPORTING TO HEALTH AUTHORITIES, INVESTIGATORS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

The Sponsor will promptly evaluate all SAEs and AESI against cumulative product experience to identify and expeditiously communicate possible new safety findings to Investigator (or designee), IRBs, ECs, and applicable health authorities based on applicable legislation.

Independent from the causality assessment, the following SAEs and non-SAEs of special interest will be reported to the FDA on an expedited basis: death, arrhythmia, syncope, dyspnea, palpitations, and chest pain.

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events using the following reference documents:

- RO5285119 (Balovaptan) Investigator's Brochure
- Moxifloxacin package insert as relevant in the United States of America

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the Investigator or designee's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

An aggregate report of any clinically relevant imbalances that do not favor the test product will be submitted to health authorities.

6. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

A statistical analysis plan (SAP) will be generated by the biostatistics department of the Sponsor and will be finalized prior to database lock.

6.1 DETERMINATION OF SAMPLE SIZE

A sample size of 56 subjects was chosen to obtain 48 evaluable subjects. Subjects are evaluable if ECGs are available for all three periods to allow (1) the derivation of the primary endpoint (i.e., change from baseline on Day 14), and (2) the assessment of the effect of moxifloxacin.

Based on the calculation of the sample size for a TQT study (Zhang and Machado, 2008)[6], assuming a 1-sided 5% significance level and a within-subject SD of 8 ms for ΔQTcF and a true mean difference of 3 ms in ΔQTcF between balovaptan and placebo, a sample size of 48 evaluable subjects who complete the study will provide a power of 96.6% to demonstrate that the upper bound of all the 2-sided 90% CIs on ΔΔQTcF will fall below 10 ms for up to 7 post-dose time points (0.5, 1.25, 2.5, 4, 8, 12 and 24 hours).

Sample Size Determination for Assay Sensitivity:

Based on the calculation of the sample size for a TQT study from Zhang and Machado, 2008[6], as the test is performed at three time points separately (1.25, 2.5,and 4 hours), a one-sided 5% significance level (with adjusted one-sided significance levels of 5%, 2.5% and 1.67%) is used along with a within-subject SD of 8 ms for ΔQTcF, a sample size of 48 evaluable subjects who complete the study will provide a power of 99% to prove a mean difference of 5 ms in ΔQTcF between moxifloxacin and placebo (i.e., ΔΔQTcF) at 1 ≥ of the 3 specified time points.

6.2 SUMMARIES OF CONDUCT OF STUDY

The number of subjects who enroll, discontinue, or complete the study will be summarized. Reasons for premature study withdrawal will be listed and summarized. Enrollment and major protocol deviations will be listed and evaluated for their potential effects on the interpretation of study results.

6.3 SUMMARIES OF DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Demographic and baseline characteristics (including age, sex) will be summarized using means, standard deviations, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. Summaries will be presented overall and by treatment group.

6.4 EFFICACY ANALYSES

Not Applicable

6.5 SAFETY ANALYSES

All subjects who have received at least one dose of balovaptan, whether prematurely withdrawn from the study or not, will be included in the safety analysis. The safety data, including AEs, laboratory data, and vital signs, will be listed and summarized by study treatment. Reasons for withdrawal from study will be listed and summarized.

Concomitant medications, 12-lead ECG data and clinically significant neurological examinations will be listed. Adverse events will be listed and summarized by treatment, body system and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). All verbatim AE terms will be mapped to MedDRA thesaurus terms, and AE severity will be graded according to Table 3. For laboratory and 12-lead ECG data, subject listings will be presented with abnormalities flagged.

For statistical analyses of results from TT-Doppler echocardiogram and ABPM please see Sections 6.8 and 6.9 below.

6.6 PHARMACOKINETIC ANALYSES

Subjects will be excluded from the PK analysis population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol, or if data are unavailable or incomplete. Excluded cases will be documented together with the reason for exclusion. All decisions on exclusions from the analysis will be made prior to database closure.

Pharmacokinetics are a secondary objective of this study. The specific objectives are

- To investigate the pharmacokinetics of balovaptan, M2 (as appropriate), and M3.
- To investigate the pharmacokinetics of moxifloxacin.

Individual plasma concentrations at each sampling timepoint for balovaptan, M2 (as appropriate), M3, and moxifloxacin will be presented by listings and descriptive summary statistics by treatment, including means, geometric means, ranges, standard deviations and coefficients of variation. Individual and mean concentration versus time will be plotted on linear and semi-logarithmic scales.

PK parameters will be estimated using standard non-compartmental methods. All PK parameters for balovaptan, M2 (as appropriate), and M3 will be presented by individual listings and summary statistics by treatment including means, geometric means, medians, ranges, standard deviations and coefficients of variation.

The following balovaptan, M2 (as appropriate), M3 PK parameters will be estimated:

- Cmax: Day 1 and Day 14 for Treatment A and B
- AUC0-24h: Day 1 and Day 14 for Treatment A and B
- Tmax: Day 1 and Day 14 for Treatment A and B
- Ctrough: Day 11, 12, 13, 14 for Treatment A and B
- Time to steady state for Treatment A and B

The following moxifloxacin PK parameters will be estimated:

- Cmax, AUC0-24h, and Tmax will be estimated on Days 2 (Treatment C) or 15 (Treatment D)

Additional PK analyses maybe be conducted as appropriate for balovaptan, M2 (as appropriate), M3 and moxifloxacin.

Subjects will be excluded from the PK analysis population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol, or if data are unavailable or incomplete. Excluded cases will be documented together with the reason for exclusion. All decisions on exclusions from the analysis will be made prior to database closure.

6.7 CARDIODYNAMIC ECG EVALUTAION

The following ECG parameters will be measured and calculated: HR, PR, QT, QTcF, and QRS. T-wave morphology and U-wave presence will be assessed.

The primary analysis will be based on by-time point analysis to evaluate the effect of balovaptan on the placebo-corrected change-from-baseline QTcF (ΔΔQTcF) at each post-dosing time point using the Intersection Union Test. The effect of balovaptan on placebo-corrected change-from-baseline in HR, PR, and QRS (ΔΔHR, ΔΔPR, and ΔΔQRS) will also be evaluated using the Intersection Union Test. In addition, the relationship between plasma concentrations of balovaptan and its metabolites (M2 [as appropriate] and M3) and ΔΔQTcF will be evaluated using a linear mixed-effects modeling approach. An analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence. Assay sensitivity will also be evaluated using by-time point analysis of the effect on ΔΔQTcF of moxifloxacin using a similar model as for the primary analysis.

6.7.1 Analysis Populations

The Safety population will include all subjects who are randomized and received at least one dose of the study medication (either balovaptan, moxifloxacin or matching placebo), whether prematurely withdrawn from the study or not.

The PK population will include all subjects who receive at least 1 dose of investigational product and have at least 1 evaluable PK plasma concentration of balovaptan (and moxifloxacin, when applicable).

The QT/QTc population will include all subjects in the Safety population with measurements at baseline as well as on-treatment with at least 1 post-dose time point with a valid ΔQTcF value. The QT/QTc population will be used for the by-time point and categorical analyses of cardiodynamic ECG parameters.

The PK/QTc population will include all subjects who are in both the QT/QTc and PK populations with at least 1 pair of post-dose PK and QTcF data from the same time point. The PK/QTc population will be used for the concentration-QTc analysis. PK/QTc population will be defined for balovaptan (and for moxifloxacin, when applicable).

6.7.2 Baseline

For the analysis of the ECG effect of balovaptan versus placebo, baseline is defined as the average of the measured ECG intervals from the 3 pre-dose time points on Day 1.

For the evaluation of assay sensitivity, the following baselines will be used:

- Treatment C (Moxifloxacin / Placebo): For moxifloxacin dosed on Day 2, baseline is defined as the average of the measured QTcF intervals from the 3 pre-dose time points on Day 15. For placebo–correction in this group, placebo values will be derived from Day 14, and baseline is defined as the average of the measured QTcF intervals from the 3 pre-dose time points on Day 1.

- Treatment D (Placebo / Moxifloxacin): For moxifloxacin dosed on Day 15, baseline is defined as the average of the measured QTcF intervals from the 3 pre-dose time points on Day 2. For placebo–correction in this group, placebo values will be derived from Day 1, and baseline is defined as the average of the measured QTcF intervals from the 3 pre-dose time points on Day 14.

6.7.3 By-Time Point Analysis

The statistical hypothesis to be tested for the primary assessment of QT prolongation for each dose of the balovaptan treatment is:

$$H_0: \cup \{\mu_{D(i)} - \mu_{P(i)}\} \geq 10, i = 1,2,\dots,7$$

$$H_1: \cap \{\mu_{D(i)} - \mu_{P(i)}\} < 10, i = 1,2,\dots,7$$

where $\mu_{D(i)}$ and $\mu_{P(i)}$are the least squares (LS) mean of ΔQTcF for each dose of balovaptan and placebo at time point i on Day 14, respectively.

The “by-time point” analysis for QTcF will be based on a linear mixed-effects model with change-from-baseline QTcF (ΔQTcF) as the dependent variable; gender, period, sequence, time (categorical), treatment (balovaptan, and placebo), and time-by-treatment interaction as fixed effects; and baseline QTcF as a covariate. An unstructured covariance matrix will be specified for the repeated measures at time points for subject within treatment period. If the model with an unstructured covariance matrix fails to converge, other covariance matrices such as compound symmetry and autoregressive will be considered. If the fixed effects for period and/or sequence should prove to be nonsignificant (i.e., if the p value > 0.1), these effects may be removed from the model

and the analysis will be repeated without those covariates. From this analysis, the LS mean and 2-sided 90 % CI will be calculated for the contrast "balovaptan versus placebo" at each dose of balovaptan and each post-dose time point on Day 1 and Day 14, separately. If the upper bound of the CI lies below 10 ms for the highest dose group of balovaptan at all seven post-dose time points (0.5, 1.25, 2.5, 4, 8, 12, and 24 hours) on Day 14 (steady state), balovaptan will be concluded not to have a significant effect on QT interval prolongation.

6.7.4 Secondary Analysis

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

6.7.5 Assay Sensitivity

In order to confirm that the assay is sufficiently sensitivity to detect an effect, the following statistical hypothesis is tested for values recorded at the three pre-defined post-dose time points (1.25, 2.5, and 4 hours):

$$H_0: \cap \left\{\mu_{M(i)} - \mu_{P(i)}\right\} \leq 5, i = 1,2,3$$

$$H_1: \cup \left\{\mu_{M(i)} - \mu_{P(i)}\right\} > 5, i = 1,2,3$$

where $\mu_{M(i)}$ and $\mu_{P(i)}$ are the LS mean of ΔQTcF for moxifloxacin and placebo at time point i, respectively.

The analysis to show assay sensitivity will be based on the change-from-baseline post-dosing QTcF of moxifloxacin. The same model will be used as described for the primary analysis. For the 3 predefined time points (1.25, 2.5, and 4 hours post-dosing), the contrast in treatment ΔΔQTcF = "moxifloxacin - placebo" will be tested against the 1-sided null hypothesis ΔΔQTcF ≤ 5 ms on the 5% level. Multiplicity will be controlled by using a Hochberg procedure.[10] If after this procedure, the LS mean of ΔΔQTcF is significantly larger than 5 ms for at least 1 time point of these 3 time points, assay sensitivity will be considered shown. In addition, 2-sided 90% CIs will be obtained for the contrast at all time points and used in the figures.

6.7.6 Categorical Analyses

The analyses results for categorical outliers, T wave morphology, and U-wave presence will be summarized in frequency tables with counts and percentages for both number of subjects and number of time points. For categorical outliers, the number (percentage) of subjects as well as time points who had increases in absolute QTcF values > 450 and

≤ 480 ms, > 480 and ≤ 500 ms, or > 500 ms, and changes from pre-dose baseline of > 30 and ≤ 60 ms, or > 60 ms; increase in PR from pre-dose baseline > 25% to a PR > 200 ms; increase in QRS from pre-dose baseline > 25% to a QRS > 120 ms; decrease in HR from pre-dose baseline > 25% to a HR < 50 bpm; and increase in HR from pre-dose baseline > 25% to a HR > 100 bpm will be determined. For T wave morphology and U-wave presence, the analyses will be focused on change from baseline (i.e., treatment-emergent changes).

6.7.7 Concentration-QTc Analysis (Secondary Analysis)

The concentration-QTc analysis will be based on placebo-corrected change-from-baseline QTcF (ΔΔQTcF). That is, for the placebo adjustment, the individual ΔQTcF for placebo calculated at a specific time point is subtracted from ΔQTcF for the same subject on balovaptan at the same time point to generate ΔΔQTcF. The relationship between plasma concentrations of balovaptan and its metabolites (M2 [as appropriate] and M3) and ΔΔQTcF will be investigated by linear mixed-effects modeling.

A full model will include ΔΔQTcF as the dependent variable, time-matched plasma concentrations of each analyte (balovaptan, M2 [as appropriate] and M3) as the covariates, centered baseline QTcF as an additional covariate (i.e., baseline QTcF for individual subject at each post-dose time point subtracting the population mean baseline QTcF for all subjects), and subject as a random effect for both intercept and slopes, when applicable. Assay sensitivity may also be evaluated by a concentration-QTc analysis of the effect on ΔΔQTcF of moxifloxacin using a similar model as the above one.

The following model selection procedure among the full model and reduced models from possible first order combinations (without quadratic and interaction terms) among the three analytes (including models with only one analyte and with any two analytes) will be conducted by using Akaike Information Criterion (AIC) and the t-value for the intercept estimator.[5] The selection will start from the full model and remove the analyte with the least significant slope from the model until only one analyte is left to evaluate the most appropriate model(s). Note that a significant intercept is not biologically plausible and therefore indicates model misfit. The selection of the final models will be performed among those models with an absolute t-value for the intercept estimate < 1.95. Nonlinear models (Emax model) will be included in this set only if the test for linearity indicates non-linearity. Among these models, the model with the smallest AIC will be selected as primary. If none of the models qualify according to the condition on the t-value for the intercept estimator, non-linear models will be considered.

If the primary model selected from model comparison includes two or more analytes, then all analytes will be used to predict QTcF effect. The geometric mean of the individual Cmax values of each analyte for subjects at each of balovaptan dose groups will be determined together with the geometric mean concentrations of the other two analytes at the Tmax of this analyte. The predicted effect and its 2-sided 90% CI for

ΔΔQTcF interval at these pairs of concentrations will be obtained for the models with two or three analytes.

The plot of the observed median-quantile balovaptan (M2 or M3) concentrations and associated mean ΔΔQTcF interval (90% CI) together with the regression line presenting the predicted ΔΔQTcF interval (90% CI) (Tornoe et al, 2011) will be used to evaluate the adequacy of the model fit of the primary model to the assumption of linearity and the impact on quantifying the concentration-QTcF relationship. For models with two or three analytes, the analyte not presented at the x-axis will be set to the arithmetic mean concentration across all subjects under this analyte at the Tmax of the analyte presented at the x-axis. For evaluation of the HR corrected QT interval, scatter and quantile plots of QTcF and HR intervals by treatment with regression line and linear mixed effects line (90% CI) will be also given, respectively. Additional exploratory analyses (via graphical displays and/or model fitting) will include accounting for a delayed effect (hysteresis) and the justification for the choice of PD model (linear versus nonlinear) as follows.

6.7.8 Investigation of Hysteresis

Hysteresis will be assessed based on joint graphical displays of the least squares (LS) mean difference between ΔQTcF under balovaptan and under placebo (ΔΔQTcF) for each post-dose timepoint and the mean concentrations of balovaptan, M2 and M3 at the same timepoints. In addition, hysteresis plots will be given for mean ΔΔQTcF and each of the mean concentrations of balovaptan, M2, and M3. If a QT effect (ΔΔQTcF) > 10 ms cannot be excluded from the by-timepoint analysis and if a delay between peak ΔΔQTcF and peak plasma concentration in any of these three plots (ΔΔQTcF versus balovaptan, ΔΔQTcF versus M2, and ΔΔQTcF versus M3) of more than 1 hour is observed, other concentration-QTc models, such as a model with an effect compartment, may be explored. With the provision stated above, hysteresis will be assumed if any of these three curves shows a counterclockwise loop.

Appropriateness of a linear model

To assess the appropriateness of a linear model, normal Q-Q plots for the standardized residuals and random effects, and plots of standard residuals versus concentration and versus fitted values and versus centered baseline QTcF will be produced. The scatter plots of standardized residuals versus concentration and versus centered baseline QTcF by LOESS fitting (i.e., locally weighted scatter plot smoothing as described by Cleveland 1979) will also be produced with optimal smoothing parameters selected by the Akaike information criterion with a correction.[12] In addition, a model with the original terms, and quadratic and interaction terms of the concentrations of balovaptan, M2 (as appropriate) and M3 will be fitted, and the quadratic and interaction terms will be tested on the 2-sided 5% level. If there is an indication that a linear model is inappropriate, additional models will be fitted, in particular an Emax model. The additional non-linear models (Emax) will be added to the candidate set for the primary model if the t-value for the

intercept estimate is < 1.95 (see above). The concentration-QTc analysis will then be repeated for the model found to best accommodate the nonlinearity detected.

6.8 TT-DOPPLER ECHOCARDIOGRAM

Results from TT-Doppler echocardiogram at each time point will be listed by subject for each time point. Descriptive summary statistics for each parameter by time point as well as its change from baseline and change relative to placebo will be provided and illustrated by plots by individual subject and treatment cohorts. A more detail discussion of statistics for this parameter will be provided in the SAP to be completed prior to database lock.

Categorical analysis will refer N subjects per treatment with mPAP exceeding 25 mm Hg.

Additional exploratory analyses may be made post-hoc.

6.9 BLOOD PRESSURE AND HEART RATE MONITORING

Blood pressure and HR will be analyzed combined and separated for 2 time periods, daytime and nighttime. For this study, daytime is defined as 9:00 am to 9:00 pm and nighttime is defined as 1:00 am to 5:30 am. Summary statistics will be provided for systolic and diastolic blood pressure as well as for HR and compared between treatments.

Systolic and diastolic blood pressure will be summarized combined and separate across daytime and nighttime assessment periods as mean of all valid measurements with only implausible measurement results being considered invalid and removed from analysis.

Categorical analyses for the mean blood pressure of respective period according to the following categories:

Table 5 Threshold for Hypertension Diagnosis Based on Ambulatory Blood Pressure Monitoring

Assessments Time Period	Ambulatory Blood Pressure
Daytime mean	≥ 130/85 mm Hg
Nighttime mean	≥ 120/70 mm Hg

In addition, categorical analyses for systolic and diastolic blood pressure as well for heart rate will be performed (details will be outlined in the SAP).

The categorical analyses include a comparison of the number of outliers by treatment with outliers defined as: A value for a subject is considered to be an outlier at an on-treatment time point if the mean daily systolic BP at the follow-up time point was ≥ 180 mm Hg and it was at least a 20 mm Hg increase from the subject's baseline mean daily systolic BP. A value for a subject is considered to be an outlier at an on-treatment time point if the mean daily diastolic BP at the follow-up time point was ≥ 105 mm Hg and it was at least a 15 mm Hg increase from the subject's baseline mean daily diastolic BP.

Additional exploratory analyses may be made post-hoc.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

PRA will be responsible for data management of this study, including quality checking of the data. Data entered manually will be collected through use of CRFs. In the event of discrepant data, the Sponsor will request data clarification from the sites.

PRA will produce a Data Quality Plan that describes the quality checking to be performed on the data. Central laboratory data will be sent directly to PRA using PRA's standard procedures to handle and process the electronic transfer of these data. CRFs and correction documentation will be maintained in the EDC system's audit trail. System backups for data stored by the Sponsor and records retention for the study data will be consistent with the Sponsor's standard procedures.

The Sponsor will perform oversight of the data management of this study, including approval of the PRA's data management plans and specifications. Data will be periodically transferred electronically from PRA to the Sponsor, and the Sponsor's standard procedures will be used to handle and process the electronic transfer of these data.

Electronic CRFs and correction documentation will be maintained in the EDC system's audit trail. System backups for data stored at the CRO and records retention for the study data will be consistent with the CRO's standard procedures. eCRFs and correction documentation will be maintained in the EDC system's audit trail. System backups for data stored at the CRO and records retention for the study data will be consistent with the CRO's standard procedures.

7.2 ELECTRONIC CASE REPORT FORMS

eCRFs are to be completed through use of a PRA EDC system. Sites will receive training and have access to a manual for appropriate eCRF completion. eCRFs will be submitted electronically to the Sponsor and should be handled in accordance with instructions from the Sponsor.

All eCRFs should be completed by designated, trained site staff. eCRFs should be reviewed and electronically signed and dated by the Investigator or a designee.

At the end of the study, the Investigator will receive subject data for his or her site in a readable format on a compact disc that must be kept with the study records. Acknowledgement of receipt of the compact disc is required.

7.3 SOURCE DATA DOCUMENTATION

Study monitors will perform ongoing source data verification and review to confirm that critical protocol data (i.e., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents.

Source documents (paper or electronic) are those in which subject data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subject-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, the types of source documents that are to be generated will be clearly defined in the Trial Monitoring Plan. This includes any protocol data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 7.5.

To facilitate source data verification and review, the Investigator (or designees) and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The study site must also allow inspection by applicable health authorities.

7.4 USE OF COMPUTERIZED SYSTEMS

When clinical observations are entered directly into a study site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve

as the source document if the system has been validated in accordance with health authority requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

7.5 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, electronic or paper subject reported outcome (PRO) data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 15 years after completion or discontinuation of the study or for the length of time required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual.[15] The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the United States or under a U.S. Investigational New Drug (IND) Application will comply with U.S. FDA regulations and applicable local, state, and federal laws.[16,17] Studies conducted in the European Union or European Economic Area will comply with the E.U. Clinical Trial Directive (2001/20/EC).

8.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Child's Informed Assent Form or Mobile Nursing Informed Consent Form, if applicable) will be provided to each site. If applicable, it will be provided in a certified translation of the local language. The Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC submission. The final IRB/EC–approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

If applicable, the Informed Consent Form will contain separate sections for any optional procedures. The Investigator or authorized designee will explain to each subject the objectives, methods, and potential risks associated with each optional procedure. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time for any reason. A separate, specific signature will be required to document a subject's agreement to participate in optional procedures. Subjects who decline to participate will not provide a separate signature.

The Consent Forms must be signed and dated by the subject or the subject's legally authorized representative before his or her participation in the study. The case history or clinical records for each subject shall document the informed consent process and that written informed consent was obtained prior to participation in the study.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the subject to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

Subjects must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each subject shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the subject or the subject's legally authorized representative. All signed and dated Consent Forms must remain in each subject's study file or in the site file and must be available for verification by study monitors at any time.

8.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the subject, and relevant supporting information must be submitted to the IRB/EC by the Principal Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any subject recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigator or designees are also responsible for promptly informing the IRB/EC of any protocol amendments (see Section 9.6).

In addition to the requirements for reporting all adverse events to the Sponsor, Investigator or designees must comply with requirements for reporting serious adverse

events to the local health authority and IRB/EC. Investigator or designees may receive written IND safety reports or other safety-related communications from the Sponsor. Investigator or designees are responsible for ensuring that such reports are reviewed and processed in accordance with health authority requirements and the policies and procedures established by their IRB/EC and archived in the site's study file.

8.4 CONFIDENTIALITY

The Sponsor maintains confidentiality standards by coding each subject enrolled in the study through assignment of a unique subject identification number. This means that subject names are not included in data sets that are transmitted to any Sponsor location.

Subject medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the subject, unless permitted or required by law.
Medical information may be given to a subject's personal physician or other appropriate medical personnel responsible for the subject's welfare, for treatment purposes.

Given the complexity and exploratory nature of exploratory biomarker analyses, data derived from these analyses will generally not be provided to study investigators or subjects unless required by law. The aggregate results of any conducted research will be available in accordance with the effective Sponsor policy on study data publication (see Section 9.5).

Data generated by this study must be available for inspection upon request by representatives of national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

Study data, which may include data on genomic variants, [applicable if the study is collecting mandatory (i.e., not requiring separate patient consent) samples for analysis of genomic variants]} may be submitted to government or other health research databases or shared with researchers, government agencies, companies, or other groups that are not participating in this study. These data may be combined with or linked to other data and used for research purposes, to advance science and public health, or for analysis, development, and commercialization of products to treat and diagnose disease. In addition, redacted Clinical Study Reports and other summary reports will be provided upon request (see Section 9.5).

8.5 FINANCIAL DISCLOSURE

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study (see definition of end of study in Section 3.2).

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

9.1 STUDY DOCUMENTATION

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including, but not limited to, the protocol, protocol amendments, Informed Consent Forms, and documentation of IRB/EC approval. In addition, at the end of the study, the Investigator will receive the subject data, including an audit trail containing a complete record of all changes to data.

9.2 PROTOCOL DEVIATIONS

The Investigator should document and explain any protocol deviations. The Investigator or designee should promptly report any deviations that might have an impact on subject safety and data integrity to the Sponsor and to the IRB/EC in accordance with established IRB/EC policies and procedures. The Sponsor will review all protocol deviations and assess whether any represent a serious breach of Good Clinical Practice guidelines and require reporting to health authorities. As per the Sponsor's standard operating procedures, prospective requests to deviate from the protocol, including requests to waive protocol eligibility criteria, are not allowed.

9.3 SITE INSPECTIONS

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, subjects' medical records, and eCRFs. The Investigator or designee will permit national and local health authorities; Sponsor monitors, representatives, and collaborators; and the IRBs/ECs to inspect facilities and records relevant to this study.

9.4 ADMINISTRATIVE STRUCTURE

This trial will be sponsored and managed by F. Hoffmann-La Roche Ltd. The Sponsor will provide clinical operations management, data management, and medical monitoring.

Central facilities will be used for certain study assessments throughout the study (e.g., specified laboratory tests, biomarker and PK analyses), as specified in Section 4.5. Accredited local laboratories will be used for routine monitoring; local laboratory ranges will be collected.

9.5 PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, both at scientific congresses and in peer-reviewed journals. The Sponsor will comply with all requirements for publication of study results. For more information, refer to the Roche Global Policy on Sharing of Clinical Trials Data at the following Web site:

www.roche.com/roche_global_policy_on_sharing_of_clinical_study_information.pdf

The results of this study may be published or presented at scientific congresses. For all clinical trials in subjects involving an IMP for which a marketing authorization application has been filed or approved in any country, the Sponsor aims to submit a journal manuscript reporting primary clinical trial results within 6 months after the availability of the respective Clinical Study Report. In addition, for all clinical trials in subjects involving an IMP for which a marketing authorization application has been filed or approved in any country, the Sponsor aims to publish results from analyses of additional endpoints and exploratory data that are clinically meaningful and statistically sound.

The Investigator must agree to submit all manuscripts or abstracts to the Sponsor prior to submission for publication or presentation. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the Investigator.

In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Any formal publication of the study in which contribution of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the Investigator and the appropriate Sponsor personnel.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of data from this study will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

9.6 PROTOCOL AMENDMENTS

Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to subjects or changes that involve logistical or administrative aspects only (e.g., change in Medical Monitor or contact information).

10. REFERENCES

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Appendix 1
Schedule of Activities

Assessments	Screening	Nominal Study Day for Each Treatment Period																		Follow-up[a]
	Days -28 to -3	Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	
Written informed consent	✓																			
Inclusion and exclusion criteria	✓																			
Randomization			✓																	
Medical history	✓																			
Conmeds review	✓	✓																		
Physical examination	✓	✓																		✓
Height and weight[b]	✓																			✓
Pre and post-dose fasting[c]				✓	✓												✓	✓		
Standardized low salt meals[d]			✓	✓	✓										✓	✓	✓	✓		
Vital signs[e]	✓	✓		✓		✓			✓			✓		✓		✓	✓	✓	✓	✓
TT-Doppler echocardiogram			✓													✓				
ABPM			✓												✓					
Laboratory safety tests[f]	✓	✓				✓				✓								✓		✓
12-lead safety ECG[g]	✓	✓		✓		✓			✓			✓		✓		✓	✓	✓	✓	✓
12-lead ECG Holter monitoring[h]				✓	✓												✓	✓		
Administration of investigational medicinal product				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Drugs of abuse screen[i]	✓	✓																		
Orthostatic challenge testing	✓	✓																		
Urine alcohol screen[j]	✓	✓																		
Pregnancy test[k]	✓	✓																		
Follicle stimulating hormone[l]	✓																			

Appendix 1
Schedule of Activities (cont.)

Blood sampling for balovaptan PK[m]				✓										✓	✓	✓	✓			
Blood sampling for moxifloxacin PK[m]					✓													✓		
Cardiac biomarkers[n]		✓														✓				
C-SSRS	✓	✓																	✓	✓
Adverse Events		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Blood sampling for Clinical Genotyping[o]																				

a. Follow-up visit should occur between 14 and 21 days after the final dose of study drug
b. Height and weight to calculate BMI will be collected at Screening. During Follow-up visit only weight will be recorded
c. Subjects will fast for a minimum of 8 hours pre-dose and 4 hours post-dose
d. Subjects will be fed standardized low salt meals
e. Assessment of supine blood pressure, pulse rate and body temperature. All measurements to be recorded after the subject has remained in a supine position for at least 10 minutes. (See Appendix 2 for details for vital signs taken on days with study drug treatment will be administered [Days 1, 3, 6, 9,11,13,14, and 15])
f. Includes hematology, biochemistry, urinalysis, coagulation and serology (coagulation and serology will be performed only during Screening)
g. Safety ECG assessments: Triplicate ECG measurements at Screening and Day -2. Single ECG measurements at Day 1, Day 3, Day 6, Day 9, Day 11, Day 13, Day 14, Day 15, Day 16 and follow-up visit. All measurements to be recorded after the subject has remained in a supine position for at least 10 minutes
h. ECG Holter monitoring assessments will be recorded on study Days 1, 2, 14 and 15 (See Appendix 2 for additional details).
   All ECG measurements are to be recorded while the subject remains supine for at least 15 minutes.
   ECGs will be extracted from Holters at 3 time points pre-dose (-45, -30 and -15 minutes) and 7 time points post-dose (0.5, 1.0, 2.5, 4, 8, 12 and 24 h post-dose). The 24-hour post-dose extraction time point for Days 1 and 14 can be taken from the 3 pre-dose time points on Days 2 and 15.
   12-lead ECGs will be extracted from the continuous recording in up to 10 replicates from a 5-minute window during a 15-minute supine period pre-dose and at the nominal Holter timepoints post-dose. Whenever ECG extraction and PK sample timepoints are close together the ECG extraction will be performed prior to PK sample collection.
i. Drugs of abuse: amphetamines, barbiturates, benzodiazepines cannabinoids, cocaine, opiates, methamphetamines, methadone and 3,4 Methylenedioxymethamphetamine (MDMA), will be measured in urine at Screening and Day 2. Additional testing may be performed during the study, at the discretion of the Investigator or designee, to confirm subject compliance.
j. Planned at Screening and Day -2, further testing may be performed during the study, at the discretion of the Investigator or designee, to confirm subject compliance.
k. Serum pregnancy test
l. Assessed for all females.
m. See Appendix 2 for PK sampling details
n. Blood samples for the assessment of early markers of cardiac damage: troponin I, cTnT and NT-proBNP, will be taken and stored but not analyzed unless requested for a specific subject.
o. Optional blood sample for Clinical Genotyping can be collected any time during the study. N.B. one sample only to be collected and only if consented for this sample

PRIORITY OF ASSESSMENTS: where appropriate and feas ble: ECG > Vital Signs > Blood Sampling > Lab Safety Tests

Appendix 2
Schedule of Pharmacokinetic, Vital Signs, and ECG

Assessments		Hours post-dose								
	Pre-dose	0.5	1.0	2.5	3	4	6	8	12	24[a]
12-lead ECG Holter monitoring[b]	✓	✓	✓	✓		✓		✓	✓	✓
Vital Signs	✓				✓					✓
Blood collection for pharmacokinetics[c]	✓	✓	✓	✓		✓	✓	✓	✓	✓

a. On days when consecutive 24 hour and pre-dose assessments are being done, only one assessment needs to be done prior to dose. This includes Holter extractions where the 24-hour post-dose extraction time point for Days 1 and 14 can be taken from the 3 pre-dose time points on Days 2 and 15.

b. Predetermined 24 h Holter ECG time points on Day 1, Day 2, Day 14 and Day 15. Holter ECG assessments will be performed in the 15 minutes prior to the nominal Holter timepoint but prior to the PK sample, if applicable. On Day 2 and 15 of each Treatment Period subjects are scheduled to have 24 h post-dose ECG Holter reading, PK sampling and daily dosing of study drug. These activities will be performed in the following order the 24 h post-dose Holter readings will be performed first followed by drawing of the 24 h post-dose PK sample and then dosing of study drug. Due to this study drug dosing on these days may be delayed relative to dosing on other study days.

c. Blood collection for PK assessments will be identical for Treatment Arms in all Treatment Periods as this is a blinded study. Intensive PK shown above, in Appendix 2, will take place on study Days 1, and 14 for balovaptan and Days 2 and 15 for moxifloxacin. On other study days with PK sampling and study drug dosing (Days 11, 12, 13) a single PK sample will be drawn prior to dosing with study drugs. This pre-dose sample also serves as the 24 h post-dose sample for the previous days dosing. A balovaptan PK sample will be taken on Day 2 and Day 15 for the Day 1 and Day 14, 24 h post-dose sample, respectively. A moxifloxacin PK sample will be taken on Day 16 for the Day 15, 24 h post-dose sample.