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: SAP Version 1: 07-May-2019

Statistical Analysis Plan

Sponsor:	F. Hoffmann-La Roche Ltd
Protocol No:	WP40734
Protocol Title:	A Single-Center, Multiple-Dose, Randomized, Double-Blind, Placebo-Controlled, Positive-Controlled, Crossover Study to Investigate the Effect of Balovaptan on the Qtc Interval in Healthy Subjects
PRA Project ID:	RPU029UF-180292
Version Date:	02-May-2019

1.0 Approvals

The undersigned have approved this Statistical Analysis Plan for use in this study.

Name of Sponsor Representative / Title:	[redacted] / Statistician
Signature of Sponsor Representative / Date:	[redacted] / May 7th 2019
Name of Author / Title:	[redacted] / [redacted]
Signature of Author / Date:	[redacted] 07-May-2019

2.0 Table of Contents

3.0 Introduction

This Statistical Analysis Plan (SAP) describes the statistical methods that will be used during the analysis and reporting of data collected under F. Hoffman-La Roche Ltd. Protocol WP40734.

This SAP should be read in conjunction with the study protocol and electronic case report form (eCRF). This version of the plan has been developed using the protocol dated 23-Apr-2019 (including all amendments up to this protocol date) and the final eCRF(s) dated 27-Feb-2019.

An approved and signed SAP is a requirement for database lock. An approved SAP is also required for unblinding of the study treatments.

This SAP defines the data and analyses that will be processed by the PRA Early Development Services (EDS) Biostatistics Department.

PRA EDS will perform the pharmacokinetic (PK), safety and tolerability evaluation.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. Any post-hoc or unplanned analyses, or significant changes from the planned analysis in this SAP performed to provide results for inclusion in the clinical study report (CSR) but not included in this SAP, will be clearly identified in the CSR. Changes to planned analyses do not require an updated SAP but should be included in the CSR if significant.

4.0 Changes from Previous Version of Approved SAP

This is the first version of the SAP.

5.0 Study Objectives

This SAP will cover secondary objectives/endpoints 4, 5, and 9 and exploratory objectives/endpoints 1 and 2. All other objectives and endpoints are noted within this section of this SAP for completeness; however an additional SAP authored by eResearch Technologies (ERT) will define the Cardiodynamic ECG analyses in detail .

5.1 Primary

1. To investigate the effect of balovaptan 50 mg qd on the QT interval corrected through use of Fridericia's formula (QTcF) after 14 days of dosing

5.2 Primary Endpoints

1. Placebo-corrected, change-from-baseline QTcF ($\Delta\Delta$QTcF) measured on 12-lead electrocardiograms (ECGs) extracted from continuous recordings at the specified time points on Day 14

5.3 Secondary

1. To investigate the effect of a single dose of balovaptan 50 mg on the QTcF interval at Day 1.
2. To investigate the effect of balovaptan 10 mg qd on the QTcF interval at 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.
4. To investigate the pharmacokinetics of balovaptan, M2 (as appropriate) and M3.
5. To investigate the pharmacokinetics of moxifloxacin.
6. To investigate the relationship between plasma concentrations of balovaptan, M2 (as appropriate), M3 and QTcF interval length.
7. To investigate the relationship between plasma concentrations of moxifloxacin and QTcF interval length (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.
9. To investigate the safety and tolerability of balovaptan and moxifloxacin at the dosing regimens scheduled.

5.3.1 Secondary Endpoints

1. ΔΔQTcF measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1.
2. ΔΔQTcF measured on 12-lead ECGs extracted from continuous recordings at the specified time points on Day 1 and Day 14.
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.
    a. Categorical outliers for QTcF, HR, PR, and QRS.
    b. Frequency of treatment emergent changes of T-wave morphology and U-wave presence.
4. Pharmacokinetic parameters estimation such as time to maximum concentration (Tmax), mean maximum concentration (Cmax), and area under the plasma concentration curve at 0-24 hours (AUC0-24h) for plasma concentrations of balovaptan, M2 (as appropriate) and M3. Other pharmacokinetic parameters may also be added.
5. Pharmacokinetic parameters estimation such as Tmax, Cmax, and AUC0-24h for plasma concentrations of moxifloxacin. Other pharmacokinetic parameters may also be added.
6. Pharmacokinetic-pharmacodynamic model of balovaptan, M2 (as appropriate) and M3 concentrations vs. QTcF changes.
7. Pharmacokinetic-pharmacodynamic model of moxifloxacin concentrations vs. QTcF changes (if warranted)
8. Placebo-corrected ΔΔQTcF measured on 12 lead ECGs extracted from continuous recordings at the specified time points on Day 2 and Day 15
9. Safety assessments: adverse events (AEs), physical examination, vital signs, laboratory tests, C-SSRS, and heart rate (HR) monitoring.

5.3.2 Exploratory Objectives

1. To investigate any effect of balovaptan at 50 mg once per day (QD) and 10 mg QD doses on pulmonary blood pressure as assessed by the use of TT-doppler echocardiography.
2. To investigate any effect of 50 mg qd and 10 mg qd on right and left heart as assessed by TT-Doppler echo.
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 ambulatory blood pressure monitoring (ABPM).

5.3.3 Exploratory Endpoints

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. TT-Doppler-echocardiography: left ventricular ejection fraction (LVEF) and additional secondary TT-Doppler-echocardiography parameters of left and right heart.
3. ABPM: mean diastolic and systolic blood pressure (daytime and nighttime mean).
    a. Heart rate will be analyzed as derived from Holter ECG.
        i. Heart rate as derived from ABPM and/or Holter ECG may be explored against fluctuations in blood pressure measured by ABPM (if warranted).

6.0 Study Design

This is a single-center, multiple-dose, randomized, double-blind, placebo-controlled, positive-controlled, twelve sequence 3-period cross-over study.

At the start of treatment period 1, at the end of Day -1, subjects will be randomized to one of the 12 treatment sequences comprised of 3 of the 4 treatments described in 6.2. All subjects will receive treatments A and B, half will receive treatment C, and the other half will receive treatment D. Each treatment period consists of 15 dosing days. 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.

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)

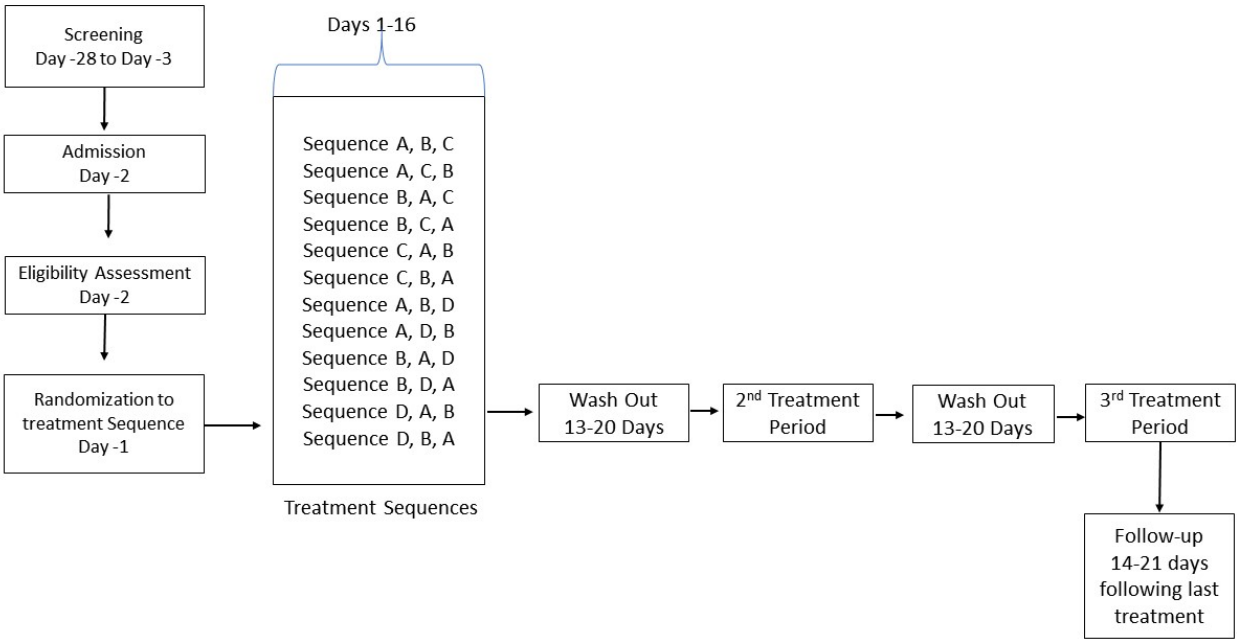
Days 1-14: A single once daily oral dose of 5 tablets of matching placebo for balovaptan for 14 days.

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

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

Figure 1 presents an overview of the study design.

Figure 1: Study Schema



6.1 Sample Size Considerations

A sample size of 56 subjects was chosen to obtain 48 evaluable subjects. Based on the calculation of the sample size for a TQT study (Zhang and Machado, 2008), assuming a 1-sided 5% significance level and a within-subject SD of 8 ms for change from baseline QTcF (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).

6.2 Randomization

Subjects who meet all eligibility criteria will be randomized to 1 of the following 12 sequences using a computer-generated randomization scheme produced by a PRA statistician prior to the start of the study.

A, B, C

A, C, B

B, A, C

B, C, A

C, A, B

C, B, A

A, B, D

A, D, B

B, A, D

B, D, A

D, A, B

D, B, A

Randomization numbers will range from [redacted] to [redacted]. Replacement subjects will receive the number of the subject to be replaced increased by 1000 (eg, replacement number [redacted] for subject [redacted]), and will be administered the same treatment sequence. The randomization schedule will alternate between sequences containing treatment C and sequences containing treatment D to ensure a balance between the number of subjects who receive treatment C and D.

7.0 Overview of Planned Analysis

7.1 Changes from Protocol

There are no changes from the protocol.

7.2 Interim Analysis and Key Results

There will be no interim analyses or summaries of data provided prior to the delivery of the full set of post-lock tables, figures and listings (TFLs).

7.3 Final Analysis

Draft TFLs will be provided after database lock. After Sponsor comments have been incorporated, the TFLs will be finalized and incorporated in the first draft CSR.

8.0 Data Review

8.1 Data Management

Data handling and transfer will take place under the PRA Data Management Plan for the study.

8.2 Acceptance of Data for Summarization

Programming of analysis datasets and TFLs may be ongoing during the data management of the study. However, programming of analysis datasets and TFLs will be completed and quality controlled (QC'd) after database lock. Only quality assured (QA'd) results released by the Safety Laboratory, Bioanalytical Laboratory, or other external data source will be used for the programming of analysis datasets and TFLs for the final report. Any data values requiring investigation or corrections that are identified while programming the analysis datasets and TFLs will be sent to the project Data Manager. If the issue affects the TFLs the Programmer or Statistician who identified the issue will follow it to resolution.

9.0 Definitions and General Analysis Methods

9.1 Analysis Data Presentation

9.1.1 Rounding

In listings, data will be presented with the same precision as the original data. Derived data will be rounded for presentation purposes.

For all summaries, the mean and median will be presented to one decimal place greater than the data, standard deviation (SD) to two greater than the data, and the minimum (min) and maximum (max) will be presented to the same number of decimal places as the data. Percentages will be presented with one decimal.

The above rule can be applied directly to collected data. For derived data rounding will occur prior to summarization so a specific number of decimal places will have to be assumed to apply the above rounding rules for summary statistics.

PK parameters will be rounded in the derived dataset as determined by the pharmacokineticist. Each parameter will have a fixed number of decimals. The pharmacokineticist will follow this following rule when deciding the number of decimals for each parameter: no decimal if data for a parameter are generally greater than or equal to 100; 1 decimal if data are generally between 10 and 100; 2 decimals if data are generally between 1 and 10; 3 decimals if data are generally below 1.

The Tmax will be reported with 2 decimals.

Additional derived data will be rounded in the derived dataset as determined by the statistician.

9.1.2 Imputation

Unless otherwise noted, data will not be imputed.

9.1.3 Daylight Savings Time Adjustments

On March 10, 2019 at 2:00 am the clocks change to 3:00 am for Daylight Savings Time. All clinic procedures for the remainder of the treatment period will be moved forward by one hour after daylight savings time occurs. All duration calculations for times post-daylight savings time that will be relative to a time prior to daylight savings will need to be programmatically adjusted for the hour that was lost on the morning of March 10.

9.1.4 Descriptive Statistics

Unless otherwise indicated, continuous variables will be summarized with the following descriptive statistics: n (number of observations), (arithmetic) mean, SD, min, median, and max.

Categorical data will be summarized with frequencies and percentages. Percentages by categories will be based on the number of subjects exposed within a treatment.

For categorical data the categories will be presented in the tables exactly as they appear in the CRF.

9.1.5 Pooling

Summary statistics will be calculated by treatment (and timepoint, if applicable).

9.1.6 Unscheduled Measurements

Unscheduled and early termination measurements will be included in the listings. With the exception of unscheduled measurements used for baseline, unscheduled measurements will be excluded from the descriptive statistics and statistical analysis.

9.2 Analysis Data Definitions

9.2.1 Baseline Definition

Unless otherwise stated, baseline for post-dose evaluations within each period is defined as the last observation recorded before the first study drug administration in each period. The last observation can be an unscheduled / repeated measurement. If a pre-treatment observation is missing in a given period then the screening value may be used.

9.2.2 Treatment/Subject Grouping

Label	Grouping
Study Drug	Balovaptan, matching placebo for balovaptan, moxifloxacin, matching placebo for moxifloxacin
Treatment	A: 10mg balovaptan B: 50mg balovaptan C: 400mg moxifloxacin on day 2 D: 400mg moxifloxacin on day 15
Dose Level	10mg balovaptan, 50mg balovaptan, 400mg moxifloxacin

9.2.3 Common Variable Derivations

Variable	Data Type	Definition/Calculation
Change from baseline	All	Post-dose Observation minus baseline Observation within period
Change from baseline relative to placebo	TT-Doppler Echocardiogram Parameters	Change from baseline for the treatment C or D period minus the change from base for the treatment A or B period
Analysis Study Day (Prior to first Dose)	All	Date of Measurement minus Dose Date
Analysis Study Day (Post first Dose)	All	Date of Measurement minus Dose Date +1

9.2.4 QC

The analysis datasets and the TFLs will be QC'd according to the general PRA EDS QC plan.

9.2.4.1 Critical Data

The QC plan requires datasets be classified as critical or non-critical. As the objectives of this study are to characterize the pharmacokinetics and assess safety and tolerability the datasets considered critical are subject level, pharmacokinetic, and adverse events (ADSL, ADPC, ADPP, and ADAE).

9.2.5 ADaM Datasets and Metadata

The analysis datasets will be generated in accordance with Clinical Data Interchange Standard Consortium (CDISC) Analysis Data Model (ADaM) Version 2.1.

ADaM compliant datasets will be delivered to the sponsor. A define.xml file version 2 with the corresponding metadata will be included. Analysis results metadata are excluded.

9.3 Software

The statistical analysis and reporting will be done using SAS® for Windows™ Version 9.4 or higher (SAS Institute, Inc.).

PK parameter calculations will primarily be done using Phoenix® WinNonlin® version 8.1 or higher (Certara, L.P.). Additional PK computations may be performed in SAS®.

9.4 Statistical Methods

9.4.1 Statistical Outlier Determination

No statistical outlier analysis is planned.

9.4.2 Predetermined Covariates and Prognostic Factors

There are no predetermined covariates or prognostic factors.

9.4.3 Hypothesis Testing

No formal hypothesis testing will be done.

9.5 TFL Layout

Table shells are provided with and approved as part of this SAP. Small changes to shell layout due to the nature of the data may be required after lock at the discretion of the PRA project statistician. Other changes to the shells may be out of scope. The TFLs will be provided as a single document in Adobe PDF format (in Letter format), and as individual files for each table, figure or listing in Rich Text Format (.rtf).

10.0 Analysis Sets

Analyses	Safety Set	PK Set
Disposition Summaries	✓	✓
Baseline Characteristics	✓	✓
Safety Assessments	✓	
Pharmacokinetic Concentrations	✓	
Pharmacokinetic Parameters		✓

10.1 Safety Set

The Safety Set will consist of subjects who are randomized and receive at least one dose of study drug (either balovaptan, moxifloxacin or matching placebo), whether prematurely withdrawn from the study or not. This set will be used for the safety data summaries, baseline characteristic summaries, and PK concentration summaries. This set will be analyzed as treated.

10.2 Pharmacokinetic Set

The PK Set will consist of all subjects in the safety set with the exception of subjects who significantly violated the inclusion or exclusion criteria, deviated significantly from the protocol, or have unavailable or incomplete data. This set will be analyzed as treated.

11.0 Subject Disposition

The number and percentage of subjects randomized, dosed, and members of each analysis set will be presented. The number and percentage of subjects who withdrew from the study prematurely and a breakdown of the corresponding reasons for withdrawal will also be presented.

12.0 Protocol Deviations

Protocol deviations will be included in the CSR.

13.0 Demographic and Baseline Characteristics

13.1 Demographics

Subject demographics will be summarized by analysis set. The summary will include the subjects' age (years), sex, race, ethnicity, weight (kg), height (cm), and BMI (kg/m^2).

All demographic data as collected during the screening visit will be listed by subject.

13.2 Medical History

Medical history, categorized by preferred term according to MedDRA, will be listed by subject.

14.0 Concomitant Medications

Concomitant medications, categorized by medication group and subgroup according to WHO Drug Dictionary, will be listed by subject. Medications with an end date prior to the first dose of study drug will be considered prior medications and will be noted in the listing. If a partial date allows a medication to be considered concomitant it will be categorized as such.

15.0 Treatment Compliance and Exposure

The number of subjects receiving each dose of study drug will be summarized by visit.

Exposure data will be listed by subject.

16.0 Pharmacokinetic Analyses

16.1 Pharmacokinetic Variables

Concentrations of balovaptan, M2, M3, and moxifloxacin will be collected in plasma.

PK parameters of balovaptan, M2 (as appropriate), M3, and moxifloxacin will be calculated for plasma.

16.2 Plasma Pharmacokinetic Summaries

16.2.1 Plasma Concentrations

Plasma concentrations for balovaptan, M2 (as appropriate), M3, and moxifloxacin below the quantifiable limit (BQL) will be set to 0 in the computation of mean concentration values. Descriptive statistics (n, arithmetic mean, geometric mean, SD, coefficient of variation, median, min, and max) will be used to summarize the plasma concentrations by treatment and visit for balovaptan and by treatment for moxifloxacin at each scheduled timepoint. If over ½ the subjects in a given cell have values BQL then the descriptive statistics will not be presented and will instead display as BQL for the mean and min. With the exception of min and max, all other statistics will be missing.

Linear and semi-logarithmic plots of the arithmetic mean plasma concentration by scheduled sampling time will be provided by treatment for balovaptan on day 1 and day 14 and by treatment for moxifloxacin on day 2 for treatment C and day 15 for treatment D. These plots will show time in hours. The plots will match the summary table results and will not have an observation at a given timepoint if more than half of the subjects have values BQL.

Linear and semi-logarithmic plots of the individual plasma concentration by actual sampling time will be provided by subject (one subject per page). These plots will show time in hours. Individual plots will use the BQL handling procedure described below for "Plasma Pharmacokinetic Parameters".

All individual subject plasma concentration data will be listed by subject.

16.2.2 Plasma Pharmacokinetic Parameters

Plasma PK parameters for balovaptan, M2, M3, and moxifloxacin will be estimated using non-compartmental methods with WinNonlin®. The plasma PK parameters will be estimated from the concentration-time profiles, and AUCs will be calculated using linear up / log down. In estimating the PK parameters, BQL values at the beginning of the profile will be set to zero. BQL values that occur after the first quantifiable point will be considered missing. Values that are embedded between BQLs, or quantifiable values occurring after two or more BQLs, will be set to missing at the discretion of the pharmacokineticist. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. If the actual time or dose time is missing, the scheduled time may be substituted in order to calculate the PK parameter.

Parameter	Description	Analytes	Treatment: Day	SAS Programming Notes
Cmax	Maximum plasma concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units	All	A: 1 and 14 B: 1 and 14 C: 2 D: 15	Cmax from WNL
Tmax	Time to maximum plasma concentration. First observed time to reach peak analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	All	A: 1 and 14 B: 1 and 14 C: 2 D: 15	Tmax from WNL
AUC0-24	Area under the plasma concentration-time curve from time 0 to 24 hours post-dose. If the actual 24 hr concentration was taken at or after 24 hr post-dose then AUC0-24 will be calculated by WNL to 24 hr . If the actual 24 hr concentration was taken before nominal 24 hr and a valid λz is available then the data will be extrapolated to 24 hr. If the actual 24 hr concentration was taken before 24 hr and a valid λz is not available, then AUC0-24 hr will be calculated to actual time closest to 24 hr within 10% of 24 (i.e.,t >21.6 hr) If the actual 24 hr concentration was not taken and the last sample was taken prior to the nominal 24 hr and a valid λz is not available then AUC0-24 hr	All	A: 1 and 14 B: 1 and 14 C: 2 D: 15	AUC0-24 from WNL where partial time =24, if missing for a subject then AUC at nominal time 24 hr from summary file is used for AUC0-24

	will not be calculated.			
Ctrough	Predose concentration prior to each daily dose from Day 11 to Day 14	Balovaptan, M2, M3	A: 11 to 14 B: 11 to 14	Taken directly from the observed plasma concentration-time data

Descriptive statistics (n, mean, geometric mean, SD, %CV, median, min, and max) will be used to summarize the calculated PK parameters by treatment. For Tmax, only median, min and max will be presented.

16.2.2.1 Steady State

A linear plot of the arithmetic mean trough (predose) plasma concentrations versus time will be provided for Balovaptan on days 11 through 14 to visually assess the achievement of steady state. This plot will show time in days.

In addition, a statistical evaluation of steady state will also be made by dose level using the trough plasma concentrations. All valid trough concentrations on the natural-log scale will be analyzed using an analysis of variance (ANOVA) model with study day as a fixed effect. From this model, orthogonal contrasts with 95% confidence intervals (CIs) will be formed between the adjusted mean trough concentration at each study day and the mean trough concentrations for all the following study days using Helmert contrasts. Precisely, Day 11 will be compared to Day 12 through Day 14 combined; Day 12 will be compared to Day 13 through Day 14 combined; and so on.

The following SAS PROC MIXED pseudo-code may be used:

```
proc mixed data= adpc;
   by treatment;
   class day;
   model ln(trough concentration) = day / solution;
   lsmeans day / cl alpha=.05;
   estimate "Day 11 vs Days 12 - 14" day 3 -1 -1 -1 / e cl alpha=0.05
   divisor=3;
   estimate "Day 12 vs Days 13 - 14" day 0 2 -1 -1 / e cl alpha=0.05
   divisor=2;
   estimate "Day 13 vs 14" day 0 0 1 -1 / e cl alpha=0.05 divisor=2;
run;
```

17.0 Safety Analyses

17.1 Safety Variables

- AEs
- Clinical Laboratory Evaluations
  - Clinical Chemistry
  - Hematology
  - Urinalysis
  - Myocyte Biomarkers
- Vital Signs
  - Supine Blood Pressure
    - Systolic Blood Pressure
    - Diastolic Blood Pressure
  - Pulse rate

  - Oral body temperature
- Electrocardiograms (ECG)
  - Heart Rate
  - PR Interval
  - QRS-Duration
  - QT Interval
  - QTc (Frederica) Interval
  - T-Waves
  - U-Waves
- Echocardiograms
  - PAAT
  - RVOT-VTI
  - Inferior Vena Cava Diameter (IVC)
  - Right Atrial Size
  - Right-Ventricular Index of Myocardial Performance (RIMP)
  - Right Ventricle (RV): End-Systolic Pressure
  - RV: End-Diastolic Pressure
  - Tricuspidal Regurgitation
  - Systolic PAP
  - Pulmonary Valve Regurgitation
  - Diastolic PAP
  - Right Ventricular Wall Thickness
  - Right Ventricular Size
  - 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
  - Mitral Valve Regurgitation
  - Number of subjects per treatment with mPAP exceeding 25 mm Hg
- Physical Examination
- Columbia-Suicide Severity Rating Scale (C-SSRS)

17.1.1 Adverse Events

Treatment emergence will be evaluated for all AEs. Treatment-emergent adverse events (TEAE) are those which occur after the first dose of study drug.

TEAEs occurring following dosing in a specific period but before dosing in the next period will be attributed to the treatment in that period. If the time is missing for an AE on a dosing day then the AE will be attributed to the treatment given on that day.

The following missing data will be imputed as defined (for calculations only / will not be presented):

- Missing AE start and / or end times for the calculation of onset and duration will be assumed to be at 00:01 for a start time and 23:59 for end times
- Missing AE severity or relationship will be assumed to be severe or related, respectively
- Missing AE start times for the determination of treatment emergence will be assumed to occur after treatment unless partial date documents the AE as happening prior to treatment
- Missing AE start times for the determination of treatment assignment will be assumed to occur after treatment on the recorded date one minute after dosing
- Missing AE start date will be assumed to be after treatment for the determination of TEAE but will not be attributed to a specific treatment

A summary of number and percentage of subjects reporting TEAEs, TEAEs by severity and relationship, serious AEs (SAEs), and subjects who discontinued study drug due to an AE will be provided.

A summary of the number and percentage of subjects reporting each TEAE, categorized by system organ class and preferred term coded according to the Medical Dictionary for Regulatory Activities (MedDRA), will be presented by treatment and overall. Counting will be done by subject only, not by event; subjects will only be counted once within each body system or preferred term.

A summary of the number and percentage of subjects reporting each TEAE will be presented by relationship to study drugs (as recorded on the eCRF) and by treatment and overall. Subjects with multiple events within a system organ class or preferred term will be counted under the category of their most drug-related event within that system organ class or preferred term.

A summary of the number and percentage of subjects reporting each TEAE will be presented by severity (as recorded on eCRF) and by treatment and overall. Subjects with multiple events within a system organ class or preferred term will be counted under the category of their most severe event within that system organ class or preferred term.

A summary presenting the preferred terms for the events in descending order of frequency for the overall treatment group will also be presented.

All AEs (including non-treatment-emergent events) recorded on the eCRF will be listed by subject.

A separate listing of AEs leading to study drug discontinuation will be provided.

17.1.2 Serious Adverse Events

A listing of SAEs will be provided by subject.

17.1.3 Laboratory Data

Clinical laboratory data will be presented using units from the study data tabulation model (SDTM) Controlled Terminology.

Descriptive statistics summarizing continuous laboratory results of clinical chemistry, hematology, urinalysis, and myocyte by treatment and visit will be provided.

All laboratory data will be listed by subject, including laboratory variables not listed in the protocol. A separate listing of out-of-range values will also be provided. Normal ranges will be used directly from the clinical laboratory and will be included in the listings for reference.

17.1.4 Vital Signs

Descriptive statistics summarizing vital signs by treatment and scheduled time will be provided.

All vital signs will be listed by subject, including orthostatic challenge testing taken at supine and standing positions.

Orthostatic change will be calculated for orthostatic challenge testing results for Systolic Blood Pressure, Diastolic Blood Pressure, and Pulse Rate as: Orthostatic Vital Sign = Standing Vital Sign – Supine/Sitting Vital Sign.

17.1.5 12-Lead Safety Electrocardiograms

Descriptive statistics summarizing ECG parameters by treatment and visit will be provided.

All ECG parameters and the corresponding abnormalities will be listed by subject.

17.1.6 Echocardiograms

Descriptive statistics summarizing echocardiogram parameters, changes from baseline, and changes from baseline relative to placebo by treatment and visit will be provided. Treatments C and D will be pooled for this summary and displayed as “Placebo.” Change from baseline relative to placebo will be calculated as change from baseline for the placebo period minus the change from baseline for treatment A and B periods.

All ECG parameters and the corresponding categorical indicators will be listed by subject.

17.1.7 Other Observations Related to Safety

Physical examination results will be listed by subject. Any clinically significant findings will be reported as AEs.

C-SSRS results will be listed by subject.

18.0 References

SAS Institute, Inc., SAS® Version 9.4 software, Cary, NC.

Clinical Study Protocol. 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. Version 2.0, Final, 16 Jan 2018.

Zhang, Joanne and Machado, Stella G. (2008) 'Statistical Issues Including Design and Sample Size Calculation in Thorough QT/QTc Studies', Journal of Biopharmaceutical Statistics,18:3, 451 — 467

Appendix 1: Glossary of Abbreviations

Glossary of Abbreviations:	
AE	Adverse event
ADaM	Analysis data model
AUC	Area under the plasma concentration curve
BMI	Body mass index
BQL	Below the quantifiable limit
CDISC	Clinical Data Interchange Standard Consortium
Cmax	Maximum plasma concentration
CI	Confidence interval
CSR	Clinical study report
C-SSRS	Columbia Suicide Severity Rating Scale
%CV	Coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic case report form
EDS	Early Development Services
LLOQ	Lower limit of quantification
LVEF	Left ventricular ejection fraction
Min	Minimum
MedDRA	Medical Dictionary for Regulatory Activities
Max	Maximum
PAAT	Pulmonary artery acceleration time
PK	Pharmacokinetic
QA'd	Quality assured
QC'd	Quality controlled
QD	Once per day
QTcF	QT interval corrected through use of Fridericia's formula
ΔQTcF	Change from baseline QTcF
ΔΔQTcF	Placebo corrected change from baseline QTcF
RVOT-VTI	Right ventricular outflow tract – velocity time integral
SAP	Statistical analysis plan
SAE	Serious adverse event
SDTM	Study data tabulation model
TEAE	Treatment-emergent adverse event

TFL(s)	Tables, figures and listings
Tmax	Time to maximum plasma concentration
WHO-DDE	World Health Organization – Drug Dictionary Enhanced
WNL	WinNonlin

Appendix 2: Protocol Schedule of Assessments

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]	✓																			
Blood sampling for balovaptan PK[m]				✓	✓									✓	✓	✓	✓	✓		
Blood sampling for moxifloxacin PK[m]					✓													✓	✓	

Myocyte 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 collected at Screening. During Follow-up visit only weight will be recorded
c. Subjects will fast for a minimum of 8 hours predose 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 3 for details)
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 3 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 predose (-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 predose 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 3 for PK sampling details
n. Blood samples for the assessment of early markers of myocyte damage: Troponin I, Troponin T and NT-proBNP, Troponin I will be assessed following sample collection while Troponin T and NT--proBNP samples will be stored for potential future analysis.
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 feasible: ECG > Vital Signs > Blood Sampling > Lab Safety Tests

Appendix 3: Schedule of Pharmacokinetics, Vital Signs, and ECG

Assessments		Hours post-dose								
	Pre-dose	0.5	1.00	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 predose 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 predose 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 predose sample also serves as the 24 h post-dose sample for the previous days dosing. A moxifloxacin PK sample will be taken on Day 16 for the Day 15, 24 h post-dose.

Appendix 4: List of End of Text Outputs

List of End of Text Tables and Figures:		
Output	**Title**	**Analysis Set**
Section 14.1 – Disposition and Demographic Data		
Table 14.1.1	Summary of Subject Disposition	All
Table 14.1.2	Summary of Demographics	All
Table 14.1.3	Summary of Study Drug Administration	Safety
Section 14.2.2 – PK Data		
Table 14.2.2.1.1	Summary of Balovaptan Plasma Concentrations	PK
Table 14.2.2.1.2	Summary of Moxifloxacin Plasma Concentrations	PK
Table 14.2.2.2.1	Summary of Balovaptan Plasma Pharmacokinetic Parameters	PK
Table 14.2.2.2.2	Summary of Moxifloxacin Plasma Pharmacokinetic Parameters	PK
Table 14.2.2.3	Statistical Analysis to Determine Time to Steady State	PK
Figure 14.2.2.4.1	Plot of Mean (± SD) Balovaptan Plasma Concentrations on Day 1 versus Time on a Linear Scale	PK
Figure 14.2.2.4.2	Plot of Mean Balovaptan Plasma Concentrations on Day 1 versus Time on a Semi-Log Scale	PK
Figure 14.2.2.4.3	Plot of Mean (± SD) Balovaptan Plasma Trough Concentrations versus Time on a Linear Scale	PK
Figure 14.2.2.5.1	Plot of Mean (± SD) Moxifloxacin Plasma Concentrations versus Time on a Linear Scale	PK
Figure 14.2.2.5.2	Plot of Mean Moxifloxacin Plasma Concentrations versus Time on a Semi-Log Scale	PK
Figure 14.2.2.6.1	Plot of Individual Balovaptan Plasma Concentrations on Day 1versus Time on a Linear Scale	PK
Figure 14.2.2.6.2	Plot of Individual Balovaptan Plasma Concentrations on Day 1 versus Time on a Semi-Log Scale	PK
Figure 14.2.2.7.1	Plot of Individual Moxifloxacin Plasma Concentrations versus Time on a Linear Scale	PK
Figure 14.2.2.7.2	Plot of Individual Moxifloxacin Plasma Concentrations versus Time on a Semi-Log Scale	PK
Figure 14.2.2.8.1	Scatter Plot of Individual Balovaptan Plasma Pharmacokinetic Parameters	PK
Figure 14.2.2.8.2	Scatter Plot of Individual Moxifloxacin Plasma Pharmacokinetic Parameters	PK
Section 14.3 – Safety Data		
Table 14.3.1.1	Summary of Adverse Events	Safety

Table 14.3.1.2	Summary of Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Safety
Table 14.3.1.3	Summary of Treatment Emergent Adverse Events by Relationship to Study Drugs	Safety
Table 14.3.1.4	Summary of Treatment Emergent Adverse Events by Severity	Safety
Table 14.3.2	Listing of Serious Adverse Events	All Subjects
Table 14.3.3	Not part of TFL – Reserved for Narratives in CSR	
Table 14.3.4	Listing of Abnormal Laboratory Values	All Subjects
Table 14.3.5	Summary of Laboratory Results	Safety
Table 14.3.6	Summary of Vital Signs	Safety
Table 14.3.7	Summary of 12-Lead Safety Electrocardiogram Results	Safety
Table 14.3.8	Summary of TT-Doppler Echocardiogram Change from Baseline Relative to Placebo	Safety

List of End of Text Listings:	
Output	**Title**
Section 16.2.1 – Disposition	
Listing 16.2.1.1	Subject Disposition
Listing 16.2.1.2	Eligibility Criteria
Section 16.2.2 – Protocol Deviations	
Listing 16.2.2	Not part of TFL – Reserved for protocol deviations in CSR
Section 16.2.3 – Excluded Subjects	
Listing 16.2.3	Analysis Sets
Section 16.2.4 – Demographics and Baseline Characteristics	
Listing 16.2.4.1	Subject Demographics
Listing 16.2.4.2	Medical History
Listing 16.2.4.3	Prior and Concomitant Medications
Section 16.2.5 – Compliance	
Listing 16.2.5	Study Drug Administration
Section 16.2.6 – Response Data	
Listing 16.2.6.5.1	Balovaptan Plasma Concentrations
Listing 16.2.6.5.2	Moxifloxacin Plasma Concentrations
Listing 16.2.6.6.1	Balovaptan Plasma Pharmacokinetic Parameters
Listing 16.2.6.6.2	Moxifloxacin Plasma Pharmacokinetic Parameters
Section 16.2.7 – Adverse Events Data	

Listing 16.2.7.1	Adverse Events
Listing 16.2.7.2	Adverse Events Leading to Study Drug Discontinuation
Section 16.2.8 – Laboratory Data	
Listing 16.2.8.1	Clinical Laboratory Results – Chemistry
Listing 16.2.8.2	Clinical Laboratory Results – Hematology
Listing 16.2.8.3	Clinical Laboratory Results – Urinalysis
Listing 16.2.8.4	Clinical Laboratory Results – Myocyte Biomarkers
Listing 16.2.8.5	Clinical Laboratory Results – Additional Assessments
Section 16.2.9 Onward – Other Safety Data	
Listing 16.2.9	Vital Signs
Listing 16.2.10	12-Lead Safety Electrocardiogram Results
Listing 16.2.11	TT-Doppler Echocardiogram Results
Listing 16.2.12	Physical Examination Findings
Listing 16.2.13	Columbia-Suicide Severity Rating Scale Results

Appendix 5: Shells for Post-Text Tables, Figures and Listings

Shells are provided in a separate document.

19.0 Document History

Version Date	Modified/Reviewed By	Brief Summary of Changes (if created from a template, include template code)
20-Mar-2019	[redacted]	Created from template EDSREP 009 T 01 G
02-May-2019	[redacted]	Minor updates to account for the 23-Apr-2019 protocol amendment and updates to TFL numbering to align with ERT's SAP

Effective Date	Version	Modified/Reviewed By	Brief Summary of Changes (if created from a template, include template code)