Official Title: **A SINGLE-CENTER, NON-RANDOMIZED, OPEN-LABEL, PARALLEL GROUP, TWO-TREATMENT STUDY INVESTIGATING THE ABSOLUTE ORAL BIOAVAILABILITY OF BALOVAPTAN AFTER SINGLE AND MULTIPLE DAILY ORAL DOSES OF BALOVAPTAN IN HEALTHY VOLUNTEERS**

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Statistical Analysis Plan

Sponsor:	F. Hoffmann-La Roche Ltd
Protocol No:	WP40607
Protocol Title:	A SINGLE-CENTER, NON-RANDOMIZED, OPEN-LABEL, PARALLEL GROUP, TWO-TREATMENT STUDY INVESTIGATING THE ABSOLUTE ORAL BIOAVAILABILITY OF BALOVAPTAN AFTER SINGLE AND MULTIPLE DAILY ORAL DOSES OF BALOVAPTAN IN HEALTHY VOLUNTEERS
PRA Project ID:	RPU014EC-180141
Version Date:	25-Feb-2019

1.0 Approvals

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

Name of Sponsor Representative / Title:	[redacted] / Biostatistician
Signature of Sponsor Representative / Date:	27 Feb 2019 [redacted]
Name of Author / Title:	[redacted] / Biostatistician
Signature of Author / Date:	[redacted] Signed by: [redacted] Biostatistician Reason: I am the author of this document. Date & Time: 27 Feb 2019 10:08 AM +01:00 DocuSign

Pharmaceutical Research Associates Group B.V., a PRA Health Sciences company

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. Hoffmann-La Roche Ltd Protocol WP40607.

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 22-Oct-2018 (including all amendments up to this protocol date) and the final eCRF(s) dated 08-Jan-2019.

An approved and signed SAP is a requirement for database lock.

This SAP only covers the results that will be processed by the PRA Early Development Services (EDS) Biostatistics Department.

PRA EDS will perform the pharmacokinetic (PK) and 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 Section 9.8.2 of 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 study will evaluate the PK of balovaptan.

5.1 Primary Objective

- To determine the absolute oral bioavailability of a single dose of 10 mg balovaptan.

5.1.1 Primary Endpoints

- Absolute bioavailability (F) for balovaptan at the indicated dose level.

5.2 Secondary Objectives

- To determine the absolute oral bioavailability of a single dose of 50 mg balovaptan.
- To determine the absolute oral bioavailability of balovaptan after once daily doses of 10 mg or 50 mg for 14 days.
- To characterize the dose- and time-dependency of the PK of oral balovaptan.
- To determine plasma concentrations of [^{13}C]-labeled balovaptan, balovaptan, and its metabolites M2, and M3, following a single oral dose of balovaptan 10 mg or 50 mg together with a slow intravenous (IV) infusion of a microdose of [^{13}C]-labeled balovaptan.
- To determine plasma concentrations of [^{13}C]-labeled balovaptan, balovaptan, and its metabolites M2, and M3, following once daily oral dosing of 10 mg or 50 mg once daily (QD) for 14 days together with a slow IV infusion of a microdose of [^{13}C]-labeled balovaptan after the last oral dose.
- To evaluate the safety and tolerability of balovaptan 10 mg QD and 50 mg QD.

5.2.1 Secondary Endpoints

- Absolute bioavailability (F) for balovaptan at the indicated dose level.

- Plasma concentrations and associated PK parameters for [^{13}C]-labeled balovaptan, balovaptan, and its metabolites M2, and M3, including but not limited to C_{max}, t_{max}, $t_{1/2}$, AUC_{last}, AUC_{inf}, AUC_{0-24}, C_{last}, t_{last}, λ_z, and (for [^{13}C]-parent only) CL on Day 1 and Day 14 and V_{ss} on Day 1 and Day 14.

  Any other parameter as appropriate (see Table 1)

- Adverse events (AEs), clinical laboratory values, vital signs, electrocardiogram (ECG), and physical examination.

5.3 Tertiary/Exploratory Objectives

- To determine whether genes relating to drug-metabolizing enzymes and/or drug transporter enzymes affect the PK and/or the safety profile of balovaptan.
- [redacted]

5.3.1 Tertiary/Exploratory Endpoints

- The pharmacogenetics of metabolizing enzymes, transferases, transporters, etc., possibly involved in the absorption, distribution, metabolism and excretion of balovaptan and its major metabolites (e.g., CYP3A and P-glycoprotein). The results for pharmacogenetics may be pooled with data from other studies of balovaptan and will be reported separately.
- [redacted]

6.0 Study Design

This study is a non-randomized, open-label, parallel group, two-treatment study in healthy volunteers to investigate the absolute oral bioavailability of balovaptan. The study is conducted at 1 site in the Netherlands.

Two cohorts of 8 subjects each are included in the study. Cohort 1 receives 10 mg balovaptan and Cohort 2 receives 50 mg according to the following regimen:

Period 1:

- Day 1: a single oral dose of 10 or 50 mg balovaptan after at least 10 hours of fasting, followed 1.25 hours later by a 15-minute IV infusion of [^{13}C]-labeled balovaptan 0.1 mg.

Period 2:

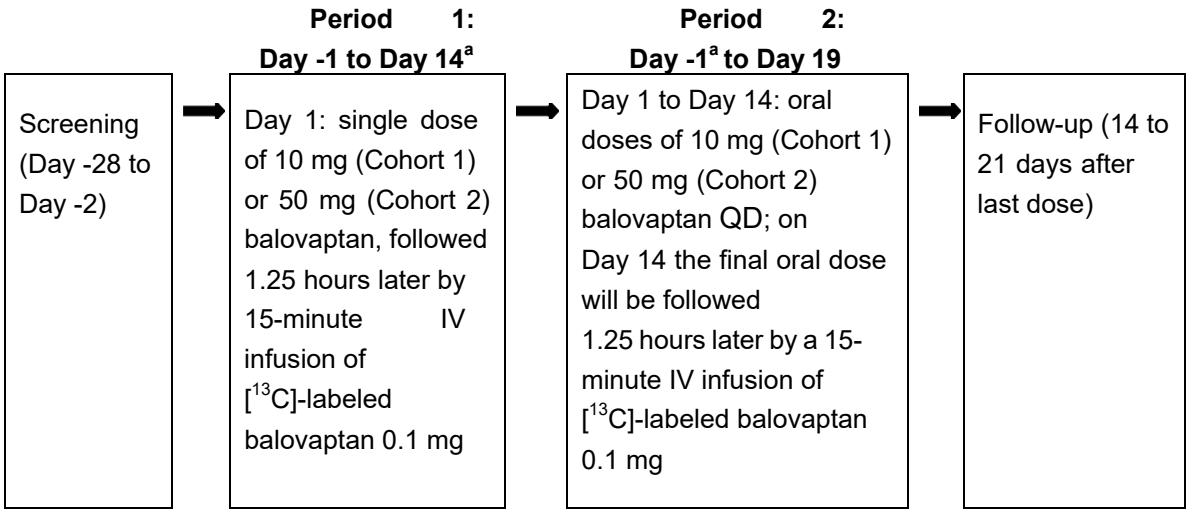
- Day 1 to Day 14: oral doses of 10 or 50 mg balovaptan QD; on Day 7 and Day 14 study drug administration will take place after at least 10 hours of fasting.
- Day 14: the final oral dosing will be followed 1.25 hours later by a 15-minute IV infusion of [^{13}C]-labeled balovaptan 0.1 mg.

[redacted]

The target population will be healthy male (at least 4 male subjects enrolled per cohort) and female subjects, aged 18 to 65 years, inclusive, at screening. At least 4 subjects enrolled per cohort should be aged 18 to 50 years, inclusive, at screening.

Figure 1 presents an overview of the study design.

Figure 1: Study Schema



IV = intravenous; QD = once daily

[a] Day 14 of Period 1 is Day -1 of Period 2

6.1 Sample Size Considerations

This is an exploratory study for which no formal statistical hypothesis will be tested, and therefore the sample size is chosen to estimate the absolute bioavailability of the 10 mg single dose with sufficient precision. A total of 8 subjects per cohort are to be enrolled with the target of at least 6 subjects available for the primary PK analysis (i.e., having taken a single dose of 10 mg on Day 1 and completed all PK assessment up to Day 10). If the number of subjects providing PK data from the multiple dose parts of the study is lower than 6 for either cohort more subjects may be enrolled.

Assuming a within-subject variability of around 35% it can be estimated that with 6 subjects the half-width of the 90% confidence interval (CI) for the ratio of geometric means of the microdose IV kinetics with that from the oral dose (i.e. CL versus CL/F) would be obtained by dividing/multiplying the ratio estimate by a factor of 1.50. The within-subject variability value of around 35% has been identified as a reasonable assumption: it is smaller than the observed 42% between-subject variability for the apparent clearance of distribution (CL/F) from a single oral 10 mg dose in study WP40038, and it considers that the reference will be obtained from IV kinetics, which is expected to be less variable than oral kinetics.

6.2 Randomization

Not applicable, this is a non-randomized study.

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

No interim reporting is planned for this study.

7.3 Final Analysis

Draft Tables, Figures and Listings (TFLs) will be provided with the draft CSR. After Sponsor comments have been incorporated, the TFLs will be finalized and incorporated in the final CSR.

8.0 Data Review

8.1 Data Management

Data handling, transfer and coding 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, all data (except the PK data) will be presented with the same precision as the original data. Derived data will be rounded for presentation purposes.

For descriptive statistics of safety data, range values will be presented with the same precision (number of decimals or significant digits) as the data they are calculated from, median, arithmetic mean values with 1 more decimal digit, standard deviation (SD) with 2 more decimal digits. Percentages will be rounded to integers.

For the rounding of derived PK parameters Appendix 2 of the Roche Clinical Pharmacology Guideline will be followed [3]. For all PK parameters except t_{max}, t_{last} and $t_{1/2}$; individual subject data, range values, median, arithmetic mean, geometric mean and SD will be presented with a precision of 3 significant digits. The coefficients of variation (CV) with 1 decimal digit.

All individual data and descriptive statistics for t_{max}, t_{last} and $t_{1/2}$ will be reported with 2 decimals, except for the CVs, which will be presented with 1 decimal digit.

9.1.2 Imputation

Except for the substitution of any PK concentrations below the lower limit of quantification (LLOQ) (see Section 16.2) and missing start or end date/times of AEs for the calculation of onset and duration (see Section 17.1.1), any missing data will not be imputed.

9.1.3 Descriptive Statistics

Unless otherwise indicated, continuous variables will be summarized with the following descriptive statistics:

- n (number of observations),
- nmiss (number of missing observations),
- (arithmetic) mean,
- SD,
- minimum (min) value,
- median, and
- maximum (max) value.

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 / Database.

9.1.4 Pooling

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

9.1.5 Unscheduled Measurements

Unscheduled measurements will be included in the listings. Except for 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 Period 1. The last observation can be an unscheduled / repeated measurement. Baseline for Electrocardiograms (ECGs) is derived as the mean of the triplicate pre-dose measurements of pre-dose assessments taken in Analysis Period 1.

9.2.2 Treatment Grouping

Label	Grouping
Analysis Period	Period 1, Period 2
Study Drug	Cohort 1: 10 mg Balovaptan, 0.1 mg [^{13}C]-balovaptan Cohort 2: 50 mg Balovaptan, 0.1 mg [^{13}C]-balovaptan
Treatment	Treatment A: 10 mg balovaptan single dose + 0.1 mg [^{13}C]-balovaptan IV Treatment B: 10 mg balovaptan QD + 0.1 mg [^{13}C]-balovaptan IV on Day 14 Treatment C: 50 mg balovaptan single dose + 0.1 mg [^{13}C]-balovaptan IV on Day 14 Treatment D: 50 mg balovaptan QD + 0.1 mg [^{13}C]-balovaptan IV

IV= intravenous; QD = one daily

9.2.3 Common Variable Derivations

Variable	Data Type	Definition/Calculation	Note
Change from Baseline	All	Post-dose Observation minus Baseline Observation	
Analysis Period	All	Time period starting 1 day before dosing of each of the treatments and ends 1 day before dosing in next analysis period or at the start of the follow-up period for the last treatment.	
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 primary objective of this study is to characterize the PK the datasets considered critical are subject level and PK (ADSL, ADPC, ADPP). As these are related to the primary objectives these datasets will be double programmed per the QC Plan.

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. At least the following datasets will be created:

- ADSL
- ADAE
- Laboratory Analysis Dataset (ADLB)
- Vital Signs Analysis Dataset (ADVS)
- ECG Analysis Dataset (ADEG)
- ADPC
- ADPP

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 [redacted] 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

CYP3A4 genotype will be investigated as a factor in this study. PK tables will be presented by CYP3A4 genotype.

The pharmacogenetics of other isoforms, metabolizing enzymes, transferases, transporters, etc, possibly involved in the absorption, distribution, metabolism and excretion of balovaptan and its major metabolites (e.g., CYP3A and P-glycoprotein) may be investigated additionally (e.g. CYP3A5 genotype). Beside CYP3A, transporters that will be investigated (others may follow) are: P-glycoprotein (ABCB1), OCT2 (SLC22A2), OATP2B1 (SLCO2B1), AOT3 (SLC22A8). The results may be pooled with data from other studies of balovaptan and will be reported separately.

9.4.3 Hypothesis Testing

No formal hypothesis testing will be done.

9.5 TFL Layout

Report layout will be according to the PRA EDS – ICH E3 compliant – CSR Template. The layout of TFLs will be according to the PRA EDS standards.

No table shells will be provided. The TFLs will be provided in Adobe PDF format.

Format:

- Page size: A4
- Data in listings will be sorted by subject number and time point.
- Data in tables will be sorted by treatment and time point.
- Column titles will be in title case letters.
- All tables and listings will be in landscape format.
- The treatment labels will be as outlined in Section 9.2.2 Treatment/Subject Grouping Definition.

10.0 Analysis Populations

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

10.1 Safety Analysis Population

The safety set will consist of subjects who receive at least one dose of balovaptan. This set will be used for the safety data summaries and baseline characteristic summaries.

10.2 Pharmacokinetic Analysis Population

The PK set will consist of all subjects who receive at least 1 dose of balovaptan. Subject will be excluded from the PK set 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 in the CSR. All decisions on exclusions from the analysis will be made prior to database closure.

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. If the reason for discontinuation is “other”, further specifications will only be listed.

12.0 Protocol Deviations and Violations

Protocol deviations/violations will be included in the CSR.

13.0 Demographic and Baseline Characteristics

13.1 Demographics

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

Subject demographics will be summarized descriptively for all subjects by genotype and overall. The summary will include the subjects’ age (in years), gender, race, ethnicity, weight (in kg), height (in cm), and body mass index (BMI) (in kg/m^2) at baseline. Demographics will be summarized for the safety and (if necessary) for the PK sets.

13.2 Medical History

Medical history will be listed including the coding according to the Medical Dictionary for Regulatory Activities (MedDRA; latest version).

13.3 Other Baseline Characteristics

- Drug and alcohol screen: The results of urine drug screen (barbiturates, benzodiazepines, methadone, amphetamines [including ecstasy], methamphetamines, opiates, cocaine, and cannabinoids and urine alcohol test will be listed.
- Serology: The results of serology (human immunodeficiency virus (HIV)-1 and HIV-2, hepatitis B virus surface antigen (HBsAg), hepatitis C virus (HCV) antibody) at screening will be listed.
- Pregnancy test (females only): Serum pregnancy test results (beta-human chorionic gonadotropin) and follicle-stimulating hormone (FSH) test results will be listed for each female subject at screening. All other timepoints for serum pregnancy test will be listed as well.
- Non-compliance to inclusion or exclusion criteria (if any) will be listed.

14.0 Concomitant Medications

Concomitant medications, categorized by medication group and subgroup according to GNE Drug dictionary, will be summarized. The number and percentage of subjects using each medication will be displayed with the number and percentage of subjects using at least one medication within each medication group and subgroup, by treatment.

Concomitant medication will be listed. 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

Exposure data will be listed by subject.

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

16.0 Pharmacokinetic Analyses

PK concentrations will be collected in plasma.

16.1 Pharmacokinetic Variables

16.1.1 Concentrations

- Plasma concentration of balovaptan
- Plasma concentration of [^{13}C]-balovaptan
- Plasma concentration of metabolites (M2, M3)
- [redacted]
- [redacted]

16.1.2 Parameters

- PK Parameters for balovaptan, [^{13}C]-balovaptan, M2 and M3 as defined in Table 1 and Table 2.

Table 1: Plasma Parameters

Parameter	Description	Analyte/Analysis Period	SAS Programming Notes
C_{max}	Maximum plasma concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units	All analytes	Cmax from WNL
C_{trough}	Concentration at the end of the dosing interval (tau)	All analytes/ Period 2	The concentration at 24 h calculated in SAS
C_{last}	Last measurable plasma concentration	All analytes	Calculated in SAS
t_{max}	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 analytes	Tmax from WNL
$AUC_{0\text{-last}}$	Area under the concentration-time curve (time 0 to time of last quantifiable concentration).	All analytes/ Period 1	AUClast from WNL
$AUC_{0\text{-inf}}$	Area under the serum concentration-time curve (time 0 to infinity). Percent extrapolation less than or equal to 20% is required to obtain a reliable AUCinf.	All analytes/ Period 1	AUCINF_obs from WNL If AUC_%Extrap_obs >20% then parameter is not included
$AUC_{0\text{-24h}}$	Area under the serum concentration-time curve from time 0 to 24 hours post-dose	All analytes/ Period 1 and 2	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
λz	Terminal phase rate constant calculated by linear regression of the terminal log-linear portion of the concentration vs. time curve. Linear regression of at least three points and an adjusted R^2 greater than 0.70 are required to obtain a reliable λz.	All analytes	Lambda_z from WNL If adjusted $R^2 \leq 0.7$ the parameter is not estimated
$t_{1/2}$	Terminal phase half-life expressed in time units. Percent extrapolation <20% and adjusted R^2 greater than 0.7 is required to obtain a reliable $t_{1/2}$.	All analytes	HL_Lambda_z from WNL If adjusted $R^2 \leq 0.7$ the parameter is not estimated
t_{last}	Time of last measurable plasma concentration	All analytes	Determined in SAS
R_{AUC}	Accumulation ratio for $AUC_{0\text{-24h}}$	All analytes	Day 14 $AUC_{0\text{-24h}}$ Period 2/ Day 1 $AUC_{0\text{-24h}}$ Period 1 Calculated in SAS

R_{Cmax}	Accumulation ratio for C_{max}	All analytes	Day 14 C_{max} Period 2/ Day 1 C_{max} Period 1 Calculated in SAS
$R_{Ctrough}$	Accumulation ratio for C_{trough}	All analytes	Day 14 C_{trough} Period 2/ Day 1 C_{trough} Period 1 (24 h sample on Day 2) Calculated in SAS
F	Absolute bioavailability (oral versus IV in %)	Balovaptan ([^{13}C]-balovaptan)	Calculated in SAS as $F = \frac{AUCoral*DIV}{AUCIV*Doral} * 100$ $AUC_{0\text{-}inf}$ of Balovaptan as AUC_{oral} and $AUC_{0\text{-}inf}$ of [^{13}C]-Balovaptan as AUC_{IV}
CL	Total body clearance (IV administration only) obtained by dividing the total dose of parent drug by its corresponding $AUC_{0\text{-}inf}$	[^{13}C]-balovaptan	CL_obs from WNL
CL/f	Apparent total plasma clearance of drug after oral administration obtained by dividing the total dose of parent drug by its corresponding $AUC_{0\text{-}inf}$	balovaptan	CL_F_obs from WNL
V_{ss}	Volume of distribution at steady state (IV administration only)	[^{13}C]-balovaptan	Vss_obs from WNL

Note: AUCs will be calculated using linear up / log down, expressed in units of concentration x time.

Table 2: [redacted] Parameters

Parameter	Description	Analyte/Analysis Period	SAS Programming Notes
R[redacted]/plasma	[redacted]/plasma concentration ratios	Balovaptan, M2, M3	Calculated in SAS, using the plasma concentration from the corresponding timepoint of the [redacted] sample.

16.2 Pharmacokinetic Summaries

16.2.1 Pharmacokinetic Concentrations

Plasma concentrations for balovaptan, [^{13}C]-balovaptan, M2 and M3 below the quantifiable limit (BQL) will be set to ½ lower limit of quantification (LLOQ) in the computation of mean concentration values.

Descriptive statistics (number of subjects, arithmetic mean, geometric mean, SD, coefficient of variation, median, min, and max) will be used to summarize the plasma concentrations by treatment and analyte (i.e. balovaptan, [^{13}C]-balovaptan, M2 and M3) at each scheduled time point. 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 minimum. With the exception of maximum all other statistics will be missing.

Linear and semi-logarithmic plots of the geometric mean plasma concentration by scheduled sampling time will be provided by analyte, cohort, treatment and analysis day. Separate plots will be created by analyte and cohort (dose level), with the analysis days (SD and QD treatment) displayed in the same plot. These plots will show time in hours. The plots will match the summary table results and will not have an observation at a given time point if more than half of the subjects have values BQL. Geometric mean trough balovaptan, M2 and M3 concentrations (Period 2) will be plotted separately.

Combined individual (spaghetti) plots of the individual plasma balovaptan, [^{13}C]-balovaptan, M2 and M3 concentration versus actual sampling time will be provided by treatment and day. These plots will show time in hours.

Separate plots will be created for the individual plasma balovaptan, [^{13}C]-balovaptan, M2 and M3 profiles by treatment. These plots will show period 1 (single dose), day 1 and period 2 (multiple dose), day 14 per subject in the same plot. Individual plots will use the BQL handling procedure described below for "Pharmacokinetic Parameters".

Individual plasma concentration data will be presented together with descriptive statistics by analyte and treatment. [redacted] concentration data will be listed only.

16.2.2 Pharmacokinetic Parameters

Plasma PK parameters for balovaptan, [^{13}C]-balovaptan, M2 and M3, will be estimated using non-compartmental methods with WinNonlin®.

The plasma PK parameters will be estimated from the concentration-time profiles. In estimating the PK parameters, BQL values will be set to zero for the pre-dose PK sample, as well as for all other samples being BLQ and occurring before t_{max}. For subsequent time points, the result will be set to missing. 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.

Descriptive statistics (number of subjects, arithmetic mean, geometric mean, SD, geometric and arithmetic CV, median, min, and max) will be used to summarize the calculated PK parameters by treatment, day and analyte. For t_{max}, only median, min and max will be presented.

The points to be included in the λz range will be determined by the pharmacokineticist after inspection of the semi-log concentration-time profiles. At least 3 points will be required to be used and the range should ideally be spread over a time interval representing at least 2 half-lives. The C_{max} data point will not be included.

Parameters based on R^2 below 0.70 or $\%AUC_{extra}$ above 20% will be flagged and excluded from descriptive statistics.

The ratio of geometric means from the microdose IV kinetics with that from the oral dose will be estimated using an analysis of variance (ANOVA) of the natural logarithm transformed clearance (CL and CL/F). A mixed model will be used, separately for the 2 cohorts with the 2 doses of 10mg and 50mg, and will include fixed effects for route (oral or iv) and for day (1 and 14) and a random effect for subject. From this model the least-squares means (LSMeans) for clearance (CL) of [13C]-balovaptan iv and for CL/F of oral balovaptan will be presented. The ratios of the LSMeans for the comparison of CL to CL/F (IV/Oral) will be obtained by exponentiating the difference of the LSMeans on the log scale. A 90% confidence interval for the ratios of the treatment means will be calculated.

17.0 Safety Analyses

17.1 Safety Variables

Physical examination and body weight (including BMI) will be listed according to scheme times.

The following safety variables will be summarized:

- AEs
- Vital Signs
  - Supine Blood Pressure
    - Systolic Blood Pressure (SBP)
    - Diastolic Blood Pressure (DBP)
  - Pulse rate
  - Body temperature (tympanic)
  - Respiratory rate
- Electrocardiograms (ECG)
  - Heart Rate
  - PR Interval
  - QRS-Duration
  - QT Interval
  - QTc (Fridericia) Interval
  - T-Wave
  - U-Wave
- Clinical Laboratory Evaluations
  - Serum Chemistry
  - Hematology
  - Urinalysis
  - Coagulation
- Columbia-Suicide Severity Rating Scale (C-SSRS)
  - Suicidal ideation
  - Suicidal behavior

17.1.1 Adverse Events

All AEs will be coded to the current version of Medical Dictionary for Regulatory Activities (MedDRA, the latest version) by F. Hoffmann-La Roche, Ltd.

All AE summaries will include only treatment emergent adverse events (TEAEs). Treatment-emergent adverse events are those which occur after the first dose of study drug. AEs starting prior to medication dosing in the first period will be regarded as pre-dose AEs (i.e. non-TEAEs).

TEAEs occurring following dosing in a specific analysis period but before dosing in the next analysis period will be attributed to that specific analysis period, thus to the last received treatment. 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.

All AEs (including non-TEAEs) recorded on the eCRF will be listed (including analysis period and treatment information). Pre-dose AEs will be presented in a separate listing. In addition, a separate listing of AEs leading to withdrawal from study will be provided.

TEAEs will be tabulated by system organ class and preferred term: one table with all TEAEs (number and percentage of subjects) overall and by treatment; one table with related TEAEs (number and percentage of subjects) overall and by treatment; one table with all TEAEs (number and percentage of subjects) by treatment and relationship to study drug; one table with TEAEs (number and percentage of subjects) by treatment and severity.

Subjects are counted once, per preferred term per treatment, for the most severe of multiple occurrences (in case of severity) or most drug-related event (in case of relationship) of a specific MedDRA term. AEs whose causal relationship was characterized as ‘Yes’/’No’ will be regarded as being related/not related to the study medication.

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 attributed to the treatment from analysis period 1 unless the AE end date occurs before first IMP administration

17.1.2 Deaths and Serious Adverse Events

A listing of deaths and other serious adverse events (SAE) 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.

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

Descriptive statistics summarizing continuous laboratory results of clinical chemistry, hematology and urinalysis (observed and derived changes from baseline) by treatment and scheduled time will be provided.

Additionally, for CPK (chemistry) a frequency table for the following ranges will be added:

- ≤ 2 times upper limit of normal (ULN)
- > 2 and ≤5 times ULN
- > 5 and ≤10 times ULN
- ≥10 times ULN

For categorical urinalysis parameters frequency tables, showing the number of subjects in a category (n) and the percentage of the total number of subjects per treatment (N), will be provided. Sporadic urinalysis tests that were performed when abnormalities were observed (i.e. microscopy/sediment) will only be listed.

17.1.4 Vital Signs

All vital signs data including derived changes from baseline will be listed. For timepoints with triplicate measurements the mean of the triplicate measurements and the change from baseline of the mean triplicate measurements will be listed by subject additionally.

Descriptive statistics will be provided to summarize vital signs including changes from baseline by treatment and scheduled time.

17.1.5 Electrocardiograms

All ECG parameters (including changes from baseline) and the corresponding abnormalities and physician's conclusions will be listed by subject.

The observed measurements for all ECG parameters (including T-wave, U-wave) and the corresponding abnormalities will be listed for all time points. The means of triplicate measurements for continuous parameters and the change from baseline of the mean triplicate measurements at each scheduled time point will be listed by subject.

Descriptive statistics will be provided to summarize mean continuous ECG parameters (observed and changes from baseline) by treatment and scheduled time. A frequency table will be provided as well to summarize the ECG parameters by physicians conclusion (normal, abnormal not clinically significant, abnormal clinically significant). Additionally a frequency table for the following pre-specified QTcF ranges (for the rounded values) will be added:

ECG Parameter	Raw Value	(Positive) Change from Baseline
QTcF (msec)	≤ 450, > 450 and ≤ 480, > 480 and ≤ 500, > 500	≤ 20 > 20 and ≤ 30, > 30 and ≤ 60, > 60

17.1.6 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). It captures the occurrence, severity and frequency of suicide-related thoughts and behaviors during the assessment period. All individual C-SSRS results will be listed.

18.0 References

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

2. Clinical Study Protocol. A SINGLE-CENTER, NON-RANDOMIZED, OPEN-LABEL, PARALLEL GROUP, TWO-TREATMENT STUDY INVESTIGATING THE ABSOLUTE ORAL BIOAVAILABILITY OF BALOVAPTAN AFTER SINGLE AND MULTIPLE DAILY ORAL DOSES OF BALOVAPTAN IN HEALTHY VOLUNTEERS Version 1.0, Final, 22 Oct 2018.

3. F. Hoffmann-La Roche LTD
CLINICAL PHARMACOLOGY GUIDING PRINCIPLES CALCULATION AND ANALYSES OF NON-COMPARTMENTAL PHARMACOKINETIC PARAMETERS Version 4.0, Jul 2015

Appendix 1: Glossary of Abbreviations

Glossary of Abbreviations:	
AE	Adverse event
ADAE	Adverse Event Analysis Dataset
ADaM	Analysis data model
ADPC	PK Concentrations Analysis Dataset
ADPP	PK Parameters Analysis Dataset
ADSL	Subject Level Analysis Dataset
BMI	Body mass index
BQL	Below the quantifiable limit
CDISC	Clinical Data Interchange Standard Consortium
CI	Confidence interval
CRU	Clinical research unit
[redacted]	[redacted]
CSR	Clinical study report
C-SSRS	Columbia Suicide Severity Rating Scale
CV	Coefficient of variation

DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
eCRF	Electronic case report form
EDS	Early Development Services
FSH	Follicle-stimulating hormone
HBsAg	Hepatitis B virus surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICH	The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IV	Intravenous
LLOQ	Lower limit of quantification
M2, M3	Metabolites [redacted]
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetic
QA'd	Quality assured
QC'd	Quality controlled
QD	Once daily
SAP	Statistical analysis plan
SAE	Serious adverse event
SBP	Systolic Blood Pressure
SD	Standard deviation
SDTM	Study data tabulation model
TEAE	Treatment-emergent adverse event
TFL(s)	Tables, figures and listings
ULN	Upper limit of normal
WNL	WinNonlin

Appendix 2: Schedule of Assessments

Assessments	Screening	Period 1 (Single Dose)			Period 2 (Multiple Doses)			Follow-up
Day	-28 to -2	-1	1	2 to 10	-1	1 to 14	15 to 19	28 to 35
Resident at study center[a]		X			X			
Written informed consent	X							
Inclusion and exclusion criteria	X							
Demographics	X							
Medical history	X							
Medication history	X							
Blood sampling for CG		X						
Study drug: oral administration			X			X		
Study drug: IV administration[b]			X			X		
Standardized meals[c]			X			X		
PK sampling			X	X		X	X	
[redacted]						[redacted]		
Physical examination and measurement of body weight	X[f]	X			X	X (D11)	X (D18)	X
C-SSRS	X				X		X (D18)	
12-lead ECG[g]	X	X		X[g]	X	X (D6, D11)	X (D18)	X
Vital signs[h]	X	X	X	X (D2, D5)	X	X (D1, D7, D14)	X (D18)	X
Urine drug screen including alcohol	X	X			X			
Serology	X							
Pregnancy test[i]	X	X			X			X
Hematology[j]	X	X			X	X (D6, D11)	X (D18)	X
Biochemistry[k]	X	X			X	X (D11)	X (D18)	X
Urinalysis	X	X			X			X
Coagulation[l]	X					X (D11)[l]	X (D18)[l]	X
AE monitoring	←							→
Review concomitant medications	←							→

AE = adverse event; BMI = body mass index; CG = clinical genotyping; CRU = clinical research unit; [redacted]; C-SSRS = Columbia-Suicide Severity Rating Scale; D = Day; ECG = electrocardiogram; FSH = follicle-stimulating hormone; IV = intravenous; PK = pharmacokinetic.

a Subjects will be admitted to the CRU on Day -1 and will be allowed to leave when all assessments on Day 10 have been completed. Subjects will return in the afternoon of Day 14 of Period 1 (which is also Day -1 of Period 2) and will be allowed to leave when all assessments on Day 19 have been completed.
b The IV infusion of the microdose will start 1.25 hours after the oral study drug administration on Day 1 of Period 1 and Day 14 of Period 2.
c On Day 1 of Period 1, and Days 7 and 14 of Period 2, subjects are fasted for at least 10 hours prior to dosing. Water will be allowed ad libitum until 1 hour prior to dosing and from 1 hour after dosing. No food is allowed for at least 4 hours postdose. Standardized lunch and dinner are provided 4 and 10 hours postdose, respectively, on Day 1 of Period 1, and Days 7 and 14 of Period 2. Standardized afternoon and evening snacks will be provided as well on these days. On the other dosing days in the multiple dosing period, subjects will be dosed at least 30 minutes prior to breakfast. On these dosing days, meals will not be standardized.
d All PK sampling times are relative to oral dosing (see also Appendix 3).
[redacted]
f Includes measurement of height and BMI.
g Triplicate ECGs will be collected at screening and Day -1 of Period 1 only. At all other time points a single ECG will be collected (Period 1: Day 10; Period 2: Day -1 of Period 2; predose on Days 6 and 11; Day 18 (96 hours after last dosing); follow-up).
h Vital signs will be measured at screening (in triplicate), and in Period 1: on Day -1 (in triplicate), Day 1 at 3 hours postdose, Day 2, Day 5; in Period 2: on Day -1, predose on Days 1, 7 and 14; on Day 18 (96 hours after last dosing); follow-up. At screening and on Day -1 of Period 1, vital signs will be measured in triplicate; at other time-points, vital signs will be single measurements.
i For females only: FSH and serum pregnancy test at screening, and serum pregnancy test only at all other time points.
j Hematology samples will be collected at screening, Day -1 of Period 1, Day -1 of Period 2; predose on Days 6 and 11 of Period 2; Day 18; follow-up.
k Biochemistry samples will be collected at screening, Day -1 of Period 1, Day -1 of Period 2; predose on Day 11 of Period 2; Day 18; follow-up.
[redacted]

Appendix 3: Schedule of Pharmacokinetic Blood [redacted] Samples

Procedure		Blood sampling for balovaptan and M2, M3 metabolites								Blood sampling for [^{13}C]-balovaptan		[redacted]
Period		Period 1	Period 2							Period 1	Period 2	Period 2
Study Day		1	1	2	7	8	12	13	14	1	14	12
Predose		X	X	X	X	X	X	X	X			
Hours Post Oral Dose	0.25	X										
	0.5	X							X			
	1	X							X			
	1.25	X							X	X[a]	X[a]	
	1.33	X							X	X	X	
	1.5	X							X	X	X	
	1.75	X							X	X	X	
	2	X			X				X	X	X	
	2.25	X							X	X	X	
	2.5	X							X	X	X	
	3	X			X				X	X	X	X[b]
	3.5	X							X	X	X	
	4	X			X				X	X	X	
	4.5	X							X	X	X	
	5	X							X	X	X	
	6	X			X				X	X	X	
	8	X							X	X	X	
	12	X			X				X	X	X	
	16 (P1/D2 or 2/D15)	X (D2)				X			X (D15)	X	X (D15)	
	24 (P1/D2 or 2/D15)	X (D2)							X (D15)	X	X (D15)	
	48 (P1/D3 or 2/D16)	X (D3)							X (D16)	X	X (D16)	
	60 (P1/D3 or 2/D16)	X (D3)							X (D16)	X	X (D16)	
	72 (P1/D4 or 2/D17)	X (D4)							X (D17)	X	X (D17)	
	96 (P1/D5 or 2/D18)	X (D5)							X (D18)	X	X (D18)	
	108 (P1/D5 or 2/D18)	X (D5)							X (D18)	X	X (D18)	
	120 (D6)	X (D6)										
	144 (D7)	X (D7)										
	168 (D8)	X (D8)										
	192 (D9)	X (D9)										
	216 (D10)	X (D10)										

[redacted]; P1 = Period 1; P2 = Period 2.

[a] Sample to be taken as shortly as possible prior to start of intravenous infusion.

[redacted]

Appendix 4: List of End of Text Outputs

List of End of Text Tables and Figures:		
Output	**Title**	**Population Set**
Section 14.1 – Disposition and Demographic Data		
Table 14.1.1	Summary of Subject Disposition	Safety
Table 14.1.2.1	Summary of Demographics	Safety
Table 14.1.2.2	Summary of Demographics (if applicable)	PK
Section 14.2 – Pharmacokinetic Data		
Table 14.2.1	Individual Values and Descriptive Statistics of Balovaptan and [^{13}C]-Balovaptan Plasma Concentrations by Treatment, Analysis Day and Time-Point	PK
Table 14.2.2	Individual Values and Descriptive Statistics of Balovaptan and [^{13}C]-Balovaptan Plasma PK Parameters by Treatment and Analysis Day	PK
Table 14.2.3	Individual Values and Descriptive Statistics of Balovaptan Metabolite (M2 and M3) Plasma Concentrations by Treatment, Analysis Day and Time-Point	PK
Table 14.2.4	Individual Values and Descriptive Statistics of Balovaptan Metabolite (M2 and M3) Plasma PK Parameters by Treatment and Analysis Day	PK
Table 14.2.5	Individual Values of Balovaptan and Balovaptan Metabolite (M2 and M3) [redacted] Concentrations by Treatment, Analysis Day and Time-Point	PK
Table 14.2.6	Individual Values of Balovaptan and Balovaptan Metabolite (M2 and M3) [redacted] Ratios	PK
Table 14.2.7	Ratio of geometric means of microdose IV kinetics (clearance) with that of oral dose	PK
Figure 14.2.8.1	Geometric Mean Balovaptan Plasma Concentrations versus Time (Linear and Semi-Logarithmic Scale) by Treatment and Analysis Day	PK
Figure 14.2.8.2	Geometric Mean [^{13}C]-Balovaptan Plasma Concentrations versus Time (Linear and Semi-Logarithmic Scale) by Treatment and Analysis Day	PK
Figure 14.2.8.3	Geometric Mean Balovaptan Metabolite (M2 and M3) Plasma Concentrations versus Time (Linear and Semi-Logarithmic Scale) by Treatment and Analysis Day	PK
Figure 14.2.8.4	Geometric Mean Balovaptan and Balovaptan Metabolite (M2 and M3) Plasma Trough Concentrations (Linear and Semi-Logarithmic Scale) by Treatment	PK
Figure 14.2.9.1	Combined Individual Balovaptan Plasma Concentrations versus Time (Linear and Semi-Logarithmic Scale)	PK

Figure 14.2.9.2	Combined Individual [^{13}C]-Balovaptan Plasma Concentrations versus Time (Linear and Semi-Logarithmic Scale)	PK
Figure 14.2.9.3	Combined Individual Balovaptan Metabolite (M2 and M3) Plasma Concentrations versus Time (Linear and Semi-Logarithmic Scale)	PK
Figure 14.2.10	Individual Balovaptan, [^{13}C]-Balovaptan, M2 and M3 Plasma Concentrations versus Time (Linear and Semi-Logarithmic Scale)	PK
Section 14.3 – Safety Data		
Section 14.3.1 Adverse Events		
Table 14.3.1.1	Summary of TEAEs for Each System Organ Class and Preferred Term (Number and Percentage of Subjects) by Treatment	Safety
Table 14.3.1.2	Summary of Related TEAEs for Each System Organ Class and Preferred Term (Number and Percentage of Subjects) by Treatment	Safety
Table 14.3.1.3	Summary of TEAEs for Each System Organ Class and Preferred Term (Number and Percentage of Subjects) by Treatment and Relationship to Study Drug	Safety
Table 14.3.1.4	Summary of TEAEs for Each System Organ Class and Preferred Term (Number and Percentage of Subjects) by Treatment and Severity	Safety
Section 14.3.2 Lists of Deaths, Other Serious and Significant Adverse Events		
Table 14.3.2.1	Listing of Deaths and Other Serious Adverse Events	Safety
Section 14.3.3 Clinical Laboratory		
Table 14.3.3.1	Listing of Abnormal Laboratory Values	Safety
Table 14.3.3.2	Descriptive Statistics of Clinical Laboratory Results - Hematology	Safety
Table 14.3.3.3	Descriptive Statistics of Clinical Laboratory Results – Chemistry	Safety
Table 14.3.3.4	Descriptive Statistics of Clinical Laboratory Results - Urinalysis	Safety
Table 14.3.3.5	Frequency Table CPK	
Section 14.3.4 Other Safety		
Table 14.3.4.1	Descriptive Statistics of Vital Signs	Safety
Table 14.3.4.2	Summary of 12-Lead Electrocardiogram	Safety
Table 14.3.4.3	Frequency of 12-Lead Electrocardiogram Physicians Conclusion	Safety
Table 14.3.4.4	Frequency Table QTcF	Safety

List of End of Text Listings:	
Output	**Title**
Section 16.2.1 – Disposition	
Listing 16.2.1	Subject Disposition

Section 16.2.2 – Protocol Deviations	
	Not part of TFL – Reserved for protocol deviations in CSR
Section 16.2.3 – Excluded Subjects	
Listing 16.2.3.1	Analysis Sets
Section 16.2.4 – Demographics and Baseline Characteristics	
Listing 16.2.4.1	Subject Demographics (including genotype)
Listing 16.2.4.2	Medical History
Listing 16.2.4.3	Prior and Concomitant Medications
Listing 16.2.4.4	Drug and Alcohol Screen
Listing 16.2.4.5	Serology Test Results
Listing 16.2.4.6	Pregnancy and Serum FSH Test Results
Listing 16.2.4.7	Genotyping Results
Section 16.2.5 – Compliance and Drug Concentration Data	
Listing 16.2.5.1	Study Dates
Listing 16.2.5.2	Study Drug Administration
Listing 16.2.5.3	Deviations from Inclusion/Exclusion criteria
Listing 16.2.5.4	PK Blood Sampling Time Deviations and Comments
Listing 16.2.5.5	Food Intake
Section 16.2.7 – Adverse Events Data	
Listing 16.2.7.1	Adverse Events
Listing 16.2.7.2	Adverse Events Leading to Withdrawal
Section 16.2.8 – Laboratory Data	
Listing 16.2.8.1	Clinical Laboratory Results – Hematology
Listing 16.2.8.2	Clinical Laboratory Results – Chemistry
Listing 16.2.8.3	Clinical Laboratory Results – Urinalysis
Listing 16.2.8.4	Clinical Laboratory Results – Coagulation
Section 16.2.9 – Other Safety Data	
Listing 16.2.9.1	Vital Sign Results
Listing 16.2.9.2	12-Lead Electrocardiogram Results
Listing 16.2.9.3	Abnormalities and Changes in Findings at the Physical Examinations
Listing 16.2.9.4	Columbia-Suicide Severity Rating Scale
Listing 16.2.9.5	Weight and BMI

Other Appendix Outputs:	
Output	**Title**
Appendix 16.1.7	Randomization
Appendix 16.1.9.2	Statistical Appendices

Document History

Version Date	Modified/Reviewed By	Brief Summary of Changes (if created from a template, include template code)
19-Nov-2018	[redacted]	Created first draft from EDSREP 009 T 01 G template
28-Nov-2018	[redacted]	Comments from internal review
18-Jan-2019	[redacted]	Comments from sponsor review
04-Feb-2019	[redacted]	Comments from sponsor review
25-Feb-2019	[redacted]	Minor additions after sponsor review