Official Title: A SINGLE-CENTER, PART-RANDOMIZED, OPEN-LABEL, SINGLE-DOSE, THREE-PERIOD, CROSSOVER STUDY TO INVESTIGATE THE EFFECT OF ESOMEPRAZOLE AND THE EFFECT OF FOOD ON THE PHARMACOKINETICS OF BALOVAPTAN IN HEALTHY VOLUNTEERS

NCT Number: NCT04156646

Document Date: Protocol Version 1: 26-September-2019

PROTOCOL

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PROTOCOL NUMBER:	WP41047
VERSION NUMBER:	1.0
EUDRACT NUMBER:	NA
IND NUMBER:	116483
NCT NUMBER	To be determined
TEST PRODUCT:	Balovaptan (RO5285119)
MEDICAL MONITOR:	[redacted], MD
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	See electronic date stamp below

Date and Time (UTC)	**Title**	**Approver's Name**
26-Sep-2019 13:26:27	Company Signatory	[redacted]

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I agree to conduct the study in accordance with the current protocol.

_____

Principal Investigator's Name (print)

_____ _____

Principal Investigator's Signature Date

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

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

PROTOCOL SYNOPSIS

TITLE:	**A SINGLE-CENTER, PART-RANDOMIZED, OPEN-LABEL, SINGLE-DOSE, THREE-PERIOD, CROSSOVER STUDY TO INVESTIGATE THE EFFECT OF ESOMEPRAZOLE AND THE EFFECT OF FOOD ON THE PHARMACOKINETICS OF BALOVAPTAN IN HEALTHY VOLUNTEERS.**
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TEST PRODUCT:	Balovaptan (RO5285119)
PHASE:	Phase 1
INDICATION:	Not applicable
SPONSOR:	F. Hoffmann-La Roche Ltd

Objectives and Endpoints

This study will investigate the effect of food and the effect of esomeprazole on the pharmacokinetics (PK) of a single dose of balovaptan in healthy volunteers.

Objective	Endpoints
Primary Objectives	
• To investigate the effect of esomeprazole on the PK of balovaptan following a single oral dose of balovaptan in the fasted state • To investigate the effect of a high-fat meal compared to the fasted state on the PK of balovaptan following a single oral dose of balovaptan	• Plasma concentrations of balovaptan • Derived plasma PK parameters for balovaptan
Secondary Objectives	
• To investigate the effect of esomeprazole on the PK of M2 (as appropriate) and M3 following a single oral dose of balovaptan in the fasted state • To investigate the effect of a high-fat meal compared to the fasted state on the PK of M2 (as appropriate) and M3 following a single oral dose of balovaptan	• Plasma concentrations of M2 and M3 • Derived plasma PK parameters for M2 and M3
• To measure the PK of esomeprazole following 5 once daily doses of esomeprazole	• Plasma concentrations of esomeprazole • Derived plasma PK parameters for esomeprazole

• To investigate the safety and tolerability of a single 20 mg oral dose of balovaptan alone or in combination with esomeprazole in healthy subjects	• Incidence and severity of AEs, incidence of SAEs, non-serious AESIs, and AEs leading to treatment withdrawal • Results of vital signs, 12-lead ECGs, physical examinations, and clinical laboratory tests
Exploratory Objectives	
• To explore the effect of genetic variations in genes associated with drug ADME, and transport on the PK and/or safety profile of balovaptan.	• Genotyping data for study subjects. Data will be reported separately if analysis is conducted

Study Design

This will be a single-center, part-randomized, open-label, single-dose, 3-period crossover study to investigate the effect of food and of esomeprazole on the PK of balovaptan in healthy volunteers.

Screening will be conducted between Day -28 and Day -2.

In Period 1, eligible subjects will be admitted to the clinical research unit (CRU) on Day -1 (the day prior to balovaptan dosing on Day 1) and will remain confined in the CRU until discharge on the morning of Day 5.

In Period 2, subjects will be re-admitted to the CRU on Day -1 (the day prior to balovaptan dosing on Day 1) and will remain confined in the CRU until discharge on the morning of Day 5.

In Period 3, subjects will return to the CRU for re-admission 5 days prior to administration of balovaptan (Day 9 of Period 2) to begin esomeprazole dosing. Esomeprazole will be dosed for 6 days: for 4 days prior to co-administration with balovaptan on Day 1 of Period 3, plus an additional dose on Day 2. Subjects will be discharged on Day 5.

In each period, following discharge from the CRU on Day 5, subjects will return to the CRU on Day 7 and Day 9 for further PK sampling.

There will be a washout of at least 12 days and up to 21 days between administrations of balovaptan. Thus, re-admission for Period 2 may be between 3 to 12 days after Day 9 of Period 1, and readmission for Period 3 will be on Day 9 of Period 2.

Subjects will be randomized to 1 of 2 treatment sequences (ABC or BAC) on Day 1 of Period 1, with the treatments being as follows:

- Treatment A: 20 mg balovaptan administered as a single oral dose following consumption of high-fat, high-calorie meal
- Treatment B: 20 mg balovaptan administered as a single oral dose after a ≥ 10-hour fast
- Treatment C: 40 mg esomeprazole administered once daily for 6 days and with a single dose of balovaptan 20 mg 1 hour after the fifth esomeprazole dose in the fasted state

Subjects will return for a follow-up visit on Day 16 ± 2 of Period 3.

Number of Subjects

A total of approximately 16 healthy subjects is anticipated to be enrolled in this study to target 12 evaluable subjects. An attempt will be made to have 50% male and 50% female participants, and at least 30% of each sex.

Target Population

Healthy male and female subjects between 18 to 65 years of age, inclusive.

Inclusion Criteria

Subjects must meet the following criteria for study entry:

1. Healthy male and female subjects, aged 18 to 65 years, inclusive. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECGs, hematology, blood chemistry, urinalysis, and serology.
2. Body mass index (BMI) between 18 and 32 kg/m^2 inclusive, at screening.
3. For women of childbearing potential: if engaging in heterosexual activity, agreement to use at least two adequate forms of contraception during the entire study and for 90 days following the last dose of study drug. Contraceptive measures are not required for women with exclusively same-sex partners and for those for whom complete abstinence is their preferred and usual lifestyle.
    a. A woman is considered to be of childbearing potential if she is postmenarchal, has not reached a postmenopausal state (> 12 continuous months of amenorrhea with no identified cause other than menopause, confirmed with follicle-stimulating hormone (FSH) ≥35 IU/L), and has not undergone surgical sterilization (removal of ovaries and/or uterus).
    b. The following are adequate contraceptive methods: bilateral tubal ligation; sterilization of male 'partner; hormonal contraceptives; hormone-releasing intrauterine devices; copper intrauterine devices; Essure.
    c. Barrier methods alone are not considered an adequate form of contraception for this study and if utilized must be supplemented by one of the adequate methods described above. For this study barrier contraception methods are defined as: male or female condom with or without spermicide (male and female condom must not be used simultaneously); and cap, diaphragm, or sponge with spermicide.
    d. Hormonal contraceptive methods must be supplemented by a barrier method.
4. For men: agreement to use contraceptive measures, and agreement to refrain from donating sperm, as defined below:
    a. With female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during sexual activity for the entire study and for 90 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.
5. Able to participate, and willing to give written informed consent and to comply with the study restrictions.

Exclusion Criteria

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

Medical History

1. Female subjects who are pregnant or lactating.
2. If female, a positive serum pregnancy test at screening or at admission.
3. Any condition or disease detected during the medical interview/physical examination that would render the subject unsuitable for the study, place the subject at undue risk or interfere with the ability of the subject to complete the study in the opinion of the Investigator.
4. In the opinion of the Investigator, any major illness within 4 weeks prior to the screening examination or any febrile illness within 1 week prior to screening.
5. History of any clinically significant, as determined by the investigator, gastrointestinal, renal, hepatic, broncho-pulmonary, neurological, psychiatric, cardiovascular, endocrinological, hematological, lymphatic, musculoskeletal, genitourinary, immunological, dermatological, or connective tissue or allergic disease, metabolic disorder, or cancer.
6. Signs and symptoms potentially indicative of peripheral neuropathy.

7. History or evidence of any medical condition potentially altering the absorption, distribution, metabolism, or elimination of drugs. Surgical history of the gastrointestinal tract affecting gastric motility or altering the gastrointestinal tract, with the exception of uncomplicated appendectomy, which is allowed.
8. A history of clinically significant in the opinion of the Investigator hypersensitivity (e.g., to drugs, including balovaptan or its excipients, esomeprazole or its ingredients, or other proton pump inhibitors, e.g., pantoprazole, lansoprazole, rabeprazole, omeprazole) or allergic reactions.
9. History or presence of clinically significant ECG abnormalities before study drug administration.
10. Subjects with screening or predose baseline mean QT interval corrected using Fridericia's formula (QTcF) >450 msec or <300 msec (using the same upper QTcF limit in both males and females).
11. Notable resting bradycardia (mean HR <40 bpm) on screening or predose baseline ECG. Notable resting tachycardia (mean HR >100 bpm) on screening or predose baseline ECG.
12. Screening or baseline ECG with evidence of clinically relevant abnormalities, e.g., atrial fibrillation, atrial flutter, complete bundle branch block, Wolf-Parkinson-White Syndrome, or cardiac pacemaker. Isolated first degree atrioventricular block or right bundle branch block will not be exclusionary.
13. Confirmed (based on the average of $\geqslant$ 3 consecutive measurements) systolic blood pressure greater than 139 or less than 90 mmHg, and diastolic blood pressure greater than 89 or less than 45 mmHg.
14. Clinically significant abnormalities in laboratory test results (including hepatic and renal panels, complete blood count, chemistry panel, and urinalysis). In the case of uncertain or questionable results, screening tests may be repeated to confirm eligibility. For ANC, any result below the lower limit of normal.
15. History of coagulopathies, bleeding disorders, or blood dyscrasias.
16. Current suicidal risk, in the opinion of the Investigator.
17. Subjects with unexplained syncope during the 6 months prior to screening or with presyncopal and/or syncopal symptoms during orthostatic challenge testing.
18. Current smoker or user of tobacco or nicotine-containing products or subjects who have smoked or used tobacco or nicotine-containing products within 3 months prior to first study drug administration.
19. Suspicion of or presence of a clinically relevant history of or current alcohol and/or other substance abuse or addiction.
20. Alcohol consumption of >14 units per week for males and females. One unit of alcohol equals 12 ounces of regular beer, 5 ounces of wine or 1.5 ounces of 80-proof spirits within 3 months of first dose of study drug.
21. Positive urine alcohol test or urine drug screen at screening or Day -1 of any treatment period (amphetamines [including ecstasy], barbiturates, benzodiazepines, cannabinoids, cocaine, cotinine, methadone, methamphetamines, and opiates).
22. Subjects on hormone replacement therapy if postmenopausal status cannot be ascertained from medical history or FSH levels.
23. Clinically relevant deviation from normal in the physical examination including vital signs, as determined by the investigator.
24. Positive result for HIV 1, HIV 2, hepatitis C virus antibody, or hepatitis B core (HBc) antibody. Subjects with positive antiHBc but negative hepatitis B virus surface antigen and negative hepatitis B viral DNA will be allowed.
25. Participation in an investigational drug or device study within 4 weeks or 5 times the elimination half-life, whichever is longer, prior to first dosing, or within 5 months prior to first administration of study drug in case of a study with a biological, as calculated from the day of Follow-up visit from the previous study.
26. Any donation of blood or plasma or significant blood loss within 3 months prior to screening.

27. Dietary restrictions that would prohibit the consumption of standardized meals or the high-fat, high-calorie meal planned for this study.
28. Use of any prohibited medications or food before study start or subjects who do not agree to refrain from consuming prohibited medications or food during the study.
29. Subjects likely to need concomitant medication during the study (including for dental conditions).
30. Subjects who have received any prescribed systemic or topical medication within 4 weeks (or within 5 times the elimination half-life of the medication, whichever is longer) of the first administration of study drug, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety, and subjects who have received slow release medicinal formulations considered to still be active within 4 weeks (or within 5 times the elimination half-life of the medication, whichever is longer) of the first administration of study drug will also be excluded unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety.
31. Used the following within 7 days before the first study drug administration, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety:
    - any nonprescribed systemic or topical medication
    - herbal remedies

    Paracetamol (up to 4 g/day within 7 days before study drug administration and 2 g/day within 48 hours before study drug administration), dietary supplements, vitamins and minerals, hormonal contraceptives, and hormone replacement therapy are allowed.
32. Received any medications known to chronically alter drug absorption or elimination processes within 4 weeks before the first administration of study drug, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety.
33. Use of any drugs or substances, including herbal treatments such as St John's wort, that are known to be substrates, inducers, or inhibitors of CYP3A4 within 4 weeks before the first administration of study drug.
34. Use of any drugs or substances, including herbal treatments, such as fluoxetine, fluvoxamine, aspirin, norethisterone, rifampicin, etc that are known to be substrates, inducers, or inhibitors of CYP2C19 within 4 weeks before the first administration of study drug.
35. Subjects under judicial supervision, guardianship, or curatorship.
36. Poor venous access for blood sampling.
37. Subjects who are intolerant to sucrose.
38. Previous exposure to balovaptan.

End of Study

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

Length of Study

The total length of the study, from screening of the first subject to the end of the study, is expected to be approximately 11 weeks.

Investigational Medicinal Products

Test Product (Investigational Drug)

Balovaptan 20-mg dispersible tablets (Ro 528-5119/F41), administered as a single oral dose after a high-fat, high-calorie meal (Treatment A), after a 10-hour fast (Treatment B), and after a 10-hour fast with esomeprazole 40 mg (Treatment C).

Esomeprazole 40 mg capsule administered once daily for 6 days and with a single dose of balovaptan 20 mg 1 hour after the fifth esomeprazole dose.

Statistical Methods

Primary Analysis

Analysis of variance models will be applied to the logarithmically transformed PK parameters of balovaptan $AUC_{0\text{-}inf}$ and C_{max}, to investigate 1) the relative bioavailability of balovaptan 20-mg dispersible tablets administered following a high-fat, high-calorie meal vs in the fasted state, and 2) the relative bioavailability of balovaptan 20-mg dispersible tablets administered in the fasted state either alone or in combination with esomeprazole.

Determination of Sample Size:

Sixteen subjects will be enrolled with the target of at least 12 subjects available for the final PK analysis. Considering the observed residual CV% for $AUC_{0\text{-}inf}$ from Study WP40038 of approximately 14%, with 12 subjects the 90% CI would be obtained by dividing/multiplying the ratio estimate by a factor of 1.11.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
ADME	absorption, distribution, metabolism, excretion
ALT	alanine transaminase
ANC	absolute neutrophil count
ASD	autism spectrum disorder
AST	aspartate transaminase
AUC	area under the curve
BMI	body mass index
BP	blood pressure
C_{max}	maximum observed plasma concentration
CNS	central nervous system
CPK	creatine phosphokinase
CRO	contract research organization
CRU	clinical research unit
CSF	cerebral spinal fluid
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HIPAA	Health Insurance Portability and Accountability Act
HR	heart rate
IB	Investigator's Brochure
ICH	International Council for Harmonisation
IMP	investigational medicinal product
IND	Investigational New Drug (Application)
IRB	Institutional Review Board
LLN	lower limit of normal
LS	least squares
MAD	multiple ascending dose
MATE	multidrug and toxin extrusion
mmHg	millimeters of mercury
NOEL	no observed effect level

Abbreviation	Definition
NOAEL	no observed adverse effect level
OAT	organic anion transporter
OATP	organic anion-transporting polypeptide
P-gp	P-glycoprotein
PK	pharmacokinetic
PND	postnatal day
PRA	PRA Health Sciences
qd	once daily
SAD	single ascending dose
SAE	serious adverse event
SOA	schedule of activities
Tmax	time to reach C_{max}
TQT	thorough QT
ULN	upper limit of normal
USPI	US package insert
WBC	white blood cells

1. BACKGROUND

1.1 AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by two core domains: impairments in social interaction and communication and the presence of repetitive or restricted behaviors, interests, or activities (American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 2013). The Autism and Developmental Disabilities Monitoring Network recently reported, based on pediatric records, that the estimated prevalence of ASD in the US for the year 2010 was 14.7 per 1000 (1 in 68) children aged 8 years (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators, 2014). Core symptoms of ASD affecting domains of socialization, communication, and repetitive behavior are usually observed by 3 years of age, although typical language development might delay identification of symptoms.

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

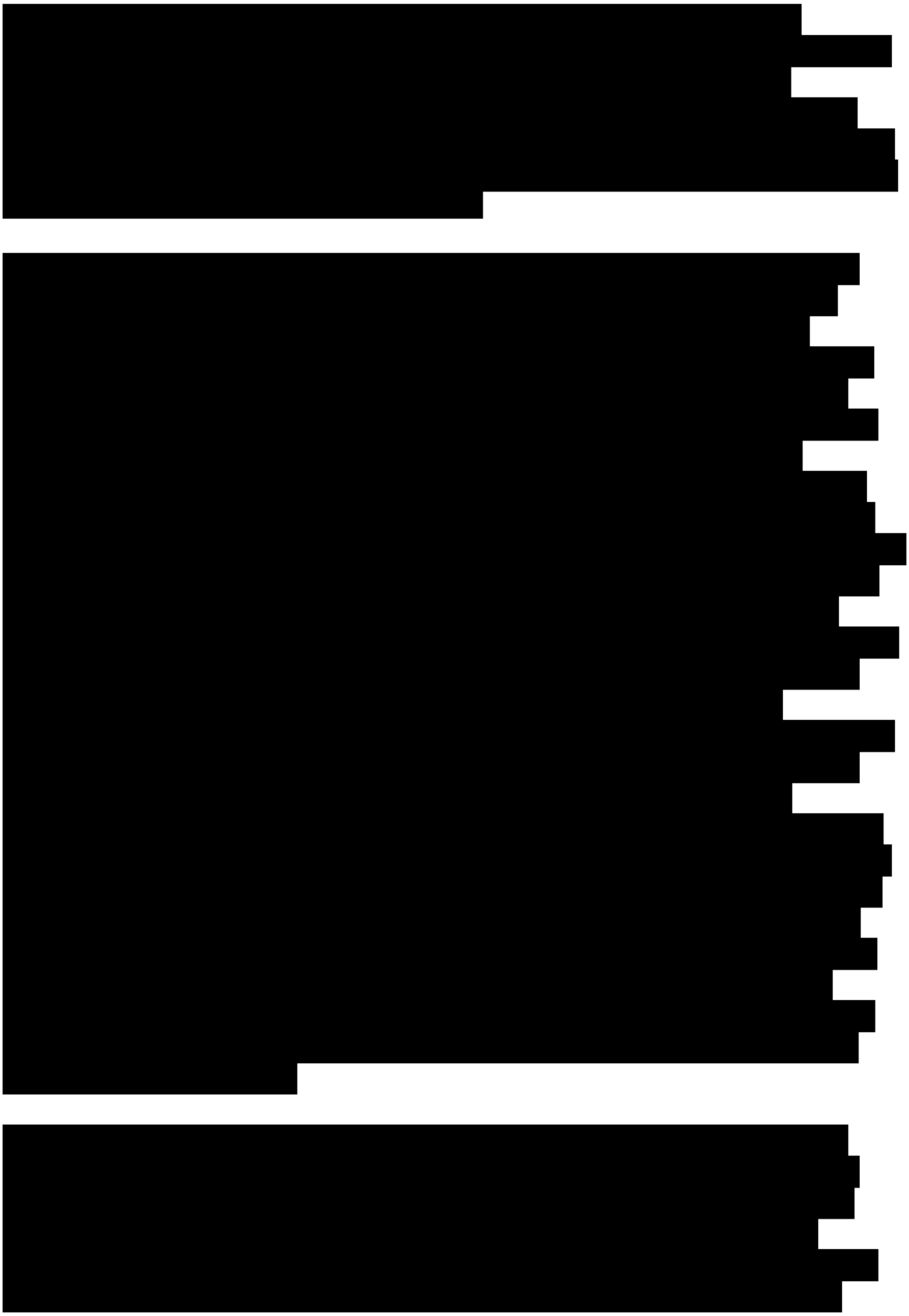
Balovaptan (RO5285119) is a potent and selective human V1a receptor antagonist that blocks the activation of the V1a G protein-coupled receptor. More detailed information regarding ASD and balovaptan are presented in the Investigator's Brochure.

1.2 PRECLINICAL BACKGROUND ON BALOVAPTAN

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

Balovaptan up to 20 mg has biopharmaceutics classification system class 1-like properties but has pH dependent solubility which may affect its absorption at values closer to neutral pH.

[redacted]



[redacted]

1.2.1 Previous and Ongoing Clinical Studies

The clinical development program of balovaptan started with the entry-into-human Study BP25694, which assessed the safety, tolerability, PK, and pharmacodynamics of escalating single and multiple doses of balovaptan. Subsequently, the excretion balance, PK, and metabolism of balovaptan were investigated in a mass balance study (Study BP29279). Four drug-drug interaction studies assessed the interaction of balovaptan as a P-gp inhibitor with the P-gp substrate risperidone (Study BP28318), the effect of the CYP3A4 inhibitors itraconazole and fluoxetine on the PK of a single dose of balovaptan (Study BP28977), and the effect of itraconazole and rifampicin on the PK of multiple doses of balovaptan (Studies WP40609 and WP40608, respectively). A study comparing the Phase 3 tablet formulation with formulations used in Phase 1 and 2 studies has been completed (Study WP40038). A proof-of-mechanism study (Study BP29412) to investigate the effect of balovaptan on vasopressin pathway activation in healthy male volunteers has also been completed. Additionally, a thorough QT (TQT) study (WP40734) and an absolute bioavailability study (WP40607) are clinically complete, with reporting ongoing at the time of protocol writing.

A Phase 2 study investigating the efficacy and safety of balovaptan in male adults with ASD (Study BP28420) has been completed. In total 223 patients were enrolled into this study at 26 sites in the US. The patients were randomized 2:1 to balovaptan or placebo. In total, 192 patients completed 12 weeks of treatment; of these 192 patients, 25, 69, and 30 patients received 1.5 mg, 4 mg, and 10 mg balovaptan, respectively, and 67 patients received placebo.

Balovaptan is currently being investigated in a Phase 2 pediatric study to assess the efficacy, safety, and PK of balovaptan in adolescents (13 to 17 years of age) and children (5 to 12 years of age) with ASD (Study BP30153). Recruitment into this study started in November 2016 and was completed in September 2019 (339 enrolled). A Phase 3 study investigating the efficacy and safety of balovaptan in adults with ASD (Study WN39434) is underway. Recruitment into this study started in August 2018 and was ongoing at the time of protocol writing. A Phase 1b study investigating the pharmacokinetics, safety and tolerability in children (2-4 years of age) with ASD (WP40877) is also ongoing.

1.2.1.1 Clinical Pharmacology

Exposure of balovaptan increased in a greater than dose-proportional manner following single doses of 0.5 to 76 mg, whereas an approximately linear increase in exposure was observed after repeated dosing with 12 to 52 mg once daily (qd) for 14 days. Balovaptan was rapidly absorbed with a median time to maximum observed plasma concentration (Tmax) between 1 and 4.5 hours after administration of single doses in fasted state and between 3 and 4 hours in the fed state following multiple dosing. Steady state was achieved after approximately 7 days; the mean apparent elimination half-life after the last dose (Day 14) was approximately 46 hours. Food had no relevant effect on the overall exposure of balovaptan. The terminal half-lives after 14 once daily dosing of balovaptan 10 mg for balovaptan, [redacted], respectively

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

[redacted]

Mean cerebral spinal fluid (CSF)-to-plasma concentration ratios of balovaptan [redacted] following 7 days of dosing of 10 and 52 mg qd balovaptan were independent of dose.

[redacted]

Hepatic metabolism was found to be the major pathway of elimination [redacted] The majority of excretion occurred within the first 7 days.

There was no clinically/statistically relevant PK interaction with the P-glycoprotein substrate risperidone, one of only 2 approved medications for ASD symptoms in the US, and fluoxetine, a weak CYP3A4 inhibitor often used to treat associated symptoms of ASD. The presence of itraconazole led to a 5.6-fold increase in balovaptan exposure (AUC_{tau}) and a 4.5-fold increase in C_{max} following multiple doses of 5 mg of balovaptan. [redacted] The presence of rifampicin led to a 15-fold decrease in balovaptan exposure (AUC_{tau}) and a 7.3-fold decrease in C_{max} following multiple doses of 10 mg of balovaptan. [redacted]

1.2.1.2 Safety

To date, balovaptan has been dosed to 294 healthy subjects in 10 completed or clinically complete, reporting ongoing Phase 1 studies (Studies BP25694, BP28318, BP28977, BP29279, BP29412, WP40038, WP40608, WP40609, WP40607, WP40734).

Additionally, balovaptan was investigated in a completed Phase 2 study in 148 adult male patients with ASD (BP28420, "VANILLA"), about 30 of whom received 10 mg qd for 12 weeks.

Balovaptan has been found to be safe and well tolerated in clinical trials. No particular safety concerns have emerged, and no adverse drug reactions have been identified per reference safety information (Section 6.4 of the IB). The maximum tolerated dose was not reached in either the single ascending dose (SAD) study with single doses (up to 76 mg) or the multiple ascending dose (MAD) study with multiple doses (up to 52 mg qd for 14 days) in healthy subjects. [redacted]

[redacted]

Balovaptan has been found to be safe and well tolerated in male patients with ASD at doses up to 10 mg/day (highest dose administered) for a period of 12 weeks (Study BP28420). No particular safety patterns attributable to the administration of balovaptan were identified. [redacted]

[redacted]

[redacted]

[redacted]

Refer to the IB for more details on non-clinical and clinical studies.

1.3 STUDY RATIONALE AND BENEFIT-RISK ASSESSMENT

This study will investigate the effect of esomeprazole and the effect of food on the PK of balovaptan in healthy volunteers. Balovaptan has shown pH dependent solubility, which may affect its absorption at values closer to neutral pH. The 20 mg tablet used in this study is the highest possible dose strength of the to-be-marketed formulation.

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

The balovaptan dose planned for this study is 20 mg with a single tablet of the highest dose strength of the final formulation that may be used in pivotal studies. Plasma exposures with a single dose of balovaptan 20 mg are predicted to be approximately 8-fold below the concentrations observed after the once daily balovaptan doses for 14 days in Study BP25694 (52 mg qd) and Studies WP40607 and WP40734 (50 mg qd; both studies are clinically completed but have not yet reported). The SAD part in the entry-into-human study (BP25694) applied single doses up to 76 mg. No safety concern for the administration of a single dose of 20 mg have emerged from these trials or from any other clinical trial.

Esomeprazole is an approved medication, and the dose planned for this study, 40 mg qd, is the recommended dose for the treatment of duodenal and gastric ulcers.

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

2. OBJECTIVES AND ENDPOINTS

This study will investigate the effect of esomeprazole and the effect of food on the pharmacokinetics of balovaptan in healthy volunteers. Study objectives are presented in Table 1.

Table 1 Objectives and Corresponding Endpoints

Objective	Endpoints
Primary Objectives	
• To investigate the effect of esomeprazole on the PK of balovaptan following a single oral dose of balovaptan in the fasted state • To investigate the effect of a high-fat meal compared to the fasted state on the PK of balovaptan following a single oral dose of balovaptan	• Plasma concentrations of balovaptan • Derived plasma PK parameters for balovaptan
Secondary Objectives	
• To investigate the effect of esomeprazole on the PK of M2 (as appropriate) and M3 following a single oral dose of balovaptan in the fasted state • To investigate the effect of a high-fat meal compared to the fasted state on the PK of M2 (as appropriate) and M3 following a single oral dose of balovaptan • To measure the PK of esomeprazole following 5 once daily dosing of esomeprazole	• Plasma concentrations of M2 and M3 • Derived plasma PK parameters for M2 and M3 • Plasma concentrations of esomeprazole • Derived plasma PK parameters for esomeprazole

Objective	Endpoints
• To investigate the safety and tolerability of a single 20 mg oral dose of balovaptan alone or in combination with esomeprazole in healthy subjects	• Incidence and severity of AEs, incidence of SAEs, nonserious AESIs, and AEs leading to treatment withdrawal • Results of vital signs, 12-lead ECGs, physical examinations, and clinical laboratory tests
Exploratory Objectives	
• To explore the effect of genetic variations in genes associated with drug ADME and transport on the PK and/or safety profile of balovaptan.	• Genotyping data for study subjects. Data will be reported separately if analysis is conducted

ADME=absorption, distribution, metabolism, excretion; AE=adverse event; AESI=adverse event of special interest; ECG=electrocardiogram; PK=pharmacokinetics; SAE=serious adverse event;

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

This will be a single-center, part-randomized, open-label, single-dose, 3-period crossover study to investigate the effect of food and of esomeprazole on the PK of balovaptan in healthy volunteers.

Screening will be conducted between Day -28 and Day -2.

In Period 1, eligible subjects will be admitted to the clinical research unit (CRU) on Day -1 (the day prior to balovaptan dosing on Day 1) and will remain confined in the CRU until discharge on the morning of Day 5.

In Period 2, subjects will be re-admitted to the CRU on Day -1 (the day prior to balovaptan dosing on Day 1) and will remain confined in the CRU until discharge on the morning of Day 5.

In Period 3, subjects will return to the CRU for re-admission 5 days prior to administration of balovaptan (Day 9 of Period 2) to begin esomeprazole dosing. Esomeprazole will be dosed for 6 days: for 4 days prior to co-administration with balovaptan on Day 1 of Period 3, plus an additional dose on Day 2. Subjects will be discharged on Day 5.

In each period, following discharge from the CRU on Day 5, subjects will return to the CRU on Day 7 and Day 9 for further PK sampling.

There will be a washout of at least 12 days and up to 21 days between administrations of balovaptan. Thus, re-admission for Period 2 may be between 3 to 12 days after Day 9 of Period 1, and re-admission for Period 3 will be on Day 9 of Period 2.

Subjects will be randomized to 1 of 2 treatment sequences (ABC or BAC) on Day 1 of Period 1, with the treatments being as follows:

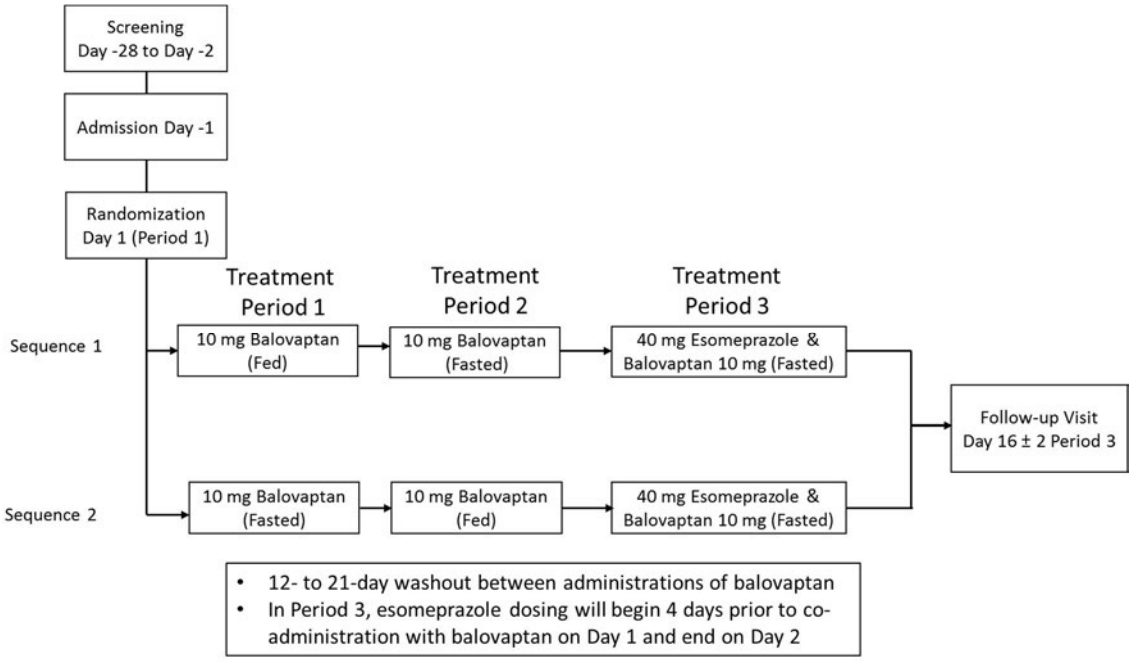
- Treatment A: 20 mg balovaptan administered as a single oral dose following consumption of high-fat, high-calorie meal
- Treatment B: 20 mg balovaptan administered as a single oral dose following a ≥ 10-hour fast
- Treatment C: 40 mg esomeprazole administered once daily for 6 days and with a single oral dose of balovaptan 20 mg in the fasted state 1 hour after the fifth esomeprazole dose

During treatment periods 1 and 2, subjects will receive each of Treatment A or B, according to the randomization schedule. All subjects will receive Treatment C in Treatment Period 3.

Subjects will return for a follow-up visit on Day 16 ± 2 of Period 3. Assessments during the study will be conducted according to the schedule of assessments (Appendix 1).

Figure 1 presents an overview of the study design.

Figure 1 Study Schema



3.2 END OF STUDY AND LENGTH OF STUDY

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

The total length of the study, from screening of the first subject to the end of the study, is expected to be approximately 11 weeks. For each enrolled subject the duration of the study from screening to last subject observation will be approximately 6 to 11 weeks.

- Screening: Approximately 4 weeks (Day -28 through Day -2)
- Treatment periods:
  - Treatment Period 1: up to 3 weeks (Day -1 – Day 13, 14, 15, 16, 17, 18, 19, 20, or 21 [12 to 21 day washout between balovaptan administrations])
  - Treatment Period 2: 2 weeks (Day -1 – Day 13)
  - Treatment Period 3: 1.5 weeks (Day -1 – Day 9)
- Follow-up: up to 5 days (Day 16 ± 2 of Period 3) (at least 14 days after the final dose of balovaptan)

3.3 RATIONALE FOR STUDY DESIGN

This study is designed to evaluate the effect of food and modification of gastric pH by esomeprazole on the PK of balovaptan, with the highest dose strength of the final formulation of balovaptan that may be used in pivotal studies. The PK parameters of balovaptan administered fasted with or without administration of esomeprazole, a gastric pH modifier, or balovaptan administered following a high-fat, high-calorie meal, will be compared. A crossover design was chosen to allow for within-subject assessments, minimizing the impact of variability across subjects on the planned analyses. An open-label study was considered appropriate because of the objective nature of the PK measures.

3.3.1 Rationale for Balovaptan and Esomeprazole Dose

3.3.1.1 Balovaptan

A dose of 20 mg balovaptan is the highest daily dose that is currently foreseen to be used in further clinical development.

Based on previous clinical experience, single doses of balovaptan 20 mg are expected to be well tolerated. The effect of food on the plasma exposure of balovaptan has previously been shown to be small, with in particular, a reduction in C_{max}; esomeprazole is expected to have either no effect on or to reduce the exposure of balovaptan.

3.3.1.2 Esomeprazole

The study will examine the effect of esomeprazole 40 mg on the PK of a single dose of 20 mg balovaptan. Esomeprazole is an approved medication, and the dose planned for this study, 40 mg qd, is approved for the treatment of gastric and duodenal ulcers and has an acceptable safety profile. During repeated once-daily dosing with an enteric-coated granule capsule omeprazole formulation, inhibition of acid secretion increases initially, and stabilizes within about 4 days (Olbe, 1989). The mean percentage of time with intragastric pH > 4 increases significantly after 5 days of dosing compared to after a single dose with esomeprazole 40 mg (Röhss, 2002). Intragastric pH rises to

above 4 about 1 hour to 2 hours after the morning of Day 5 (Lind, 2000). In the current study, esomeprazole 40 mg will be dosed for 4 consecutive days prior to coadministration with a single dose of balovaptan on the fifth day of esomeprazole dosing (Day 1), followed by a further single dose of esomeprazole on Day 2, to ensure that during any recirculation of balovaptan in the first 48 hours after administration, inhibition of acid production is maintained.

3.3.2 Rationale for Study Population

Healthy male or female volunteers aged 18 to 65 years inclusive have been chosen as the study population due to the low risk of this study. Moreover, use of healthy subjects, as opposed to patients, will allow a clearer interpretation of the study results, as there will be no confounding factors that result from changes in disease state and/or concomitant medication use.

4. MATERIALS AND METHODS

4.1 SUBJECTS

The target population will be healthy male or female volunteers aged 18 to 65 years, inclusive. An attempt will be made to have 50% male and 50% female participants, and at least 30% of each sex.

Approximately 16 subjects will be enrolled, with the goal of obtaining 12 evaluable subjects. Enrolled subjects who withdraw from the study may be replaced at the discretion of the Investigator (or designee) and Sponsor to ensure adequate numbers of evaluable subjects.

4.1.1 Inclusion Criteria

Subjects must meet the following criteria for study entry:

1. Healthy male and female subjects, aged 18 to 65 years, inclusive. Healthy status is defined by absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead ECGs, hematology, blood chemistry, urinalysis, and serology.
2. Body mass index (BMI) between 18 and 32 kg/m^2 inclusive, at screening.
3. For women of childbearing potential: if engaging in heterosexual activity, agreement to use at least two adequate forms of contraception during the entire study and for 90 days following the last dose of study drug. Contraceptive measures are not required for women with exclusively same-sex partners and for those for whom complete abstinence is their preferred and usual lifestyle.

a. A woman is considered to be of childbearing potential if she is postmenarchal, has not reached a postmenopausal state (> 12 continuous months of amenorrhea with no identified cause other than menopause, confirmed with follicle-stimulating hormone (FSH) ≥35 IU/L), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

b. The following are adequate contraceptive methods: bilateral tubal ligation; sterilization of male 'partner; hormonal contraceptives; hormone-releasing intrauterine devices; copper intrauterine devices; Essure.

c. Barrier methods alone are not considered an adequate form of contraception for this study and if utilized must be supplemented by one of the adequate methods described above. For this study barrier contraception methods are defined as: male or female condom with or without spermicide (male and female condom must not be used simultaneously); and cap, diaphragm, or sponge with spermicide.

d. Hormonal contraceptive methods must be supplemented by a barrier method.

4. For men: agreement to use contraceptive measures, and agreement to refrain from donating sperm, as defined below:

    a. With female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during sexual activity for the entire study and for 90 days after the last dose of study drug to avoid exposing the embryo. Men must refrain from donating sperm during this same period.

5. Able to participate, and willing to give written informed consent and to comply with the study restrictions.

4.1.2 Exclusion Criteria

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

Medical History

1. Female subjects who are pregnant or lactating.
2. If female, a positive serum pregnancy test at screening or at admission.
3. Any condition or disease detected during the medical interview/physical examination that would render the subject unsuitable for the study, place the subject at undue risk or interfere with the ability of the subject to complete the study in the opinion of the Investigator.
4. In the opinion of the Investigator, any major illness within 4 weeks prior to the screening examination or any febrile illness within 1 week prior to screening.

5. History of any clinically significant, as determined by the investigator, gastrointestinal, renal, hepatic, broncho-pulmonary, neurological, psychiatric, cardiovascular, endocrinological, hematological, lymphatic, musculoskeletal, genitourinary, immunological, dermatological, or connective tissue or allergic disease, metabolic disorder, or cancer.
6. Signs and symptoms potentially indicative of peripheral neuropathy.
7. History or evidence of any medical condition potentially altering the absorption, distribution, metabolism, or elimination of drugs. Surgical history of the gastrointestinal tract affecting gastric motility or altering the gastrointestinal tract, with the exception of uncomplicated appendectomy, which is allowed.
8. A history of clinically significant in the opinion of the Investigator hypersensitivity (e.g., to drugs, including balovaptan or its excipients, esomeprazole or its ingredients, or other proton pump inhibitors, e.g., pantoprazole, lansoprazole, rabeprazole, omeprazole) or allergic reactions.
9. History or presence of clinically significant ECG abnormalities before study drug administration.
10. Subjects with screening or predose baseline mean QT interval corrected using Fridericia's formula (QTcF) >450 msec or <300 msec (using the same upper QTcF limit in both males and females).
11. Notable resting bradycardia (mean HR <40 bpm) on screening or predose baseline ECG. Notable resting tachycardia (mean HR >100 bpm) on screening or predose baseline ECG.
12. Screening or baseline ECG with evidence of clinically relevant abnormalities, e.g., atrial fibrillation, atrial flutter, complete bundle branch block, Wolf-Parkinson-White Syndrome, or cardiac pacemaker. Isolated first degree atrioventricular block or right bundle branch block will not be exclusionary.
13. Confirmed (based on the average of $\geqslant$ 3 consecutive measurements) systolic blood pressure greater than 139 or less than 90 mmHg, and diastolic blood pressure greater than 89 or less than 45 mmHg.
14. Clinically significant abnormalities in laboratory test results (including hepatic and renal panels, complete blood count, chemistry panel, and urinalysis). In the case of uncertain or questionable results, screening tests may be repeated to confirm eligibility. For ANC, any result below the lower limit of normal.
15. History of coagulopathies, bleeding disorders, or blood dyscrasias.
16. Current suicidal risk, in the opinion of the Investigator.
17. Subjects with unexplained syncope during the 6 months prior to screening or with presyncopal and/or syncopal symptoms during orthostatic challenge testing.
18. Current smoker or user of tobacco or nicotine-containing products or subjects who have smoked or used tobacco or nicotine-containing products within 3 months prior to first study drug administration.

19. Suspicion of or presence of a clinically relevant history of or current alcohol and/or other substance abuse or addiction.
20. Alcohol consumption of >14 units per week for males and females. One unit of alcohol equals 12 ounces of regular beer, 5 ounces of wine or 1.5 ounces of 80-proof spirits within 3 months of first dose of study drug.
21. Positive urine alcohol test or urine drug screen at screening or Day -1 of any treatment period (amphetamines [including ecstasy], barbiturates, benzodiazepines, cannabinoids, cocaine, cotinine, methadone, methamphetamines, and opiates).
22. Subjects on hormone replacement therapy if postmenopausal status cannot be ascertained from medical history or FSH levels.
23. Clinically relevant deviation from normal in the physical examination including vital signs, as determined by the investigator.
24. Positive result for HIV 1, HIV 2, hepatitis C virus antibody, or hepatitis B core (HBc) antibody. Subjects with positive antiHBc but negative hepatitis B virus surface antigen and negative hepatitis B viral DNA will be allowed.
25. Participation in an investigational drug or device study within 4 weeks or 5 times the elimination half-life, whichever is longer, prior to first dosing, or within 5 months prior to first administration of study drug in case of a study with a biological, as calculated from the day of Follow-up visit from the previous study.
26. Any donation of blood or plasma or significant blood loss within 3 months prior to screening.
27. Dietary restrictions that would prohibit the consumption of standardized meals or the high-fat, high-calorie meal planned for this study.
28. Use of any prohibited medications or food before study start or subjects who do not agree to refrain from consuming prohibited medications or food during the study.
29. Subjects likely to need concomitant medication during the study (including for dental conditions).
30. Subjects who have received any prescribed systemic or topical medication within 4 weeks (or within 5 times the elimination half-life of the medication, whichever is longer) of the first administration of study drug, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety, and subjects who have received slow release medicinal formulations considered to still be active within 4 weeks (or within 5 times the elimination half-life of the medication, whichever is longer) of the first administration of study drug will also be excluded unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety.
31. Used the following within 7 days before the first study drug administration, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety:
    - any nonprescribed systemic or topical medication
    - herbal remedies

Paracetamol (up to 4 g/day within 7 days before study drug administration and 2 g/day within 48 hours before study drug administration), dietary supplements, vitamins and minerals, hormonal contraceptives, and hormone replacement therapy are allowed.

32. Received any medications known to chronically alter drug absorption or elimination processes within 4 weeks before the first administration of study drug, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety.
33. Use of any drugs or substances, including herbal treatments such as St John's wort, that are known to be substrates, inducers, or inhibitors of CYP3A4 within 4 weeks before the first administration of study drug.
34. Use of any drugs or substances, including herbal treatments, such as fluoxetine, fluvoxamine, aspirin, norethisterone, rifampicin, etc that are known to be substrates, inducers, or inhibitors of CYP2C19 within 4 weeks before the first administration of study drug.
35. Subjects under judicial supervision, guardianship, or curatorship.
36. Poor venous access for blood sampling.
37. Subjects who are intolerant to sucrose.
38. Previous exposure to balovaptan.

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

This is a single-center, part-randomized, open-label, 3-treatment, 3-period crossover study. Subjects will be randomly assigned in equal proportions to one of the 2 treatment sequences according to a computer-generated randomization scheme.

After informed consent is obtained, subjects will be screened according to the inclusion and exclusion criteria. Subjects who have met all eligibility criteria will receive a randomization number on Day 1 of the first treatment period and according to the randomization code generated by the Biostatistics Department of PRA Health Sciences (PRA). Replacement subjects will receive the randomization number of the subject to be replaced, increased by a fixed unit (e.g., 1901 replacement number for randomization number 1001), and will be assigned to the same treatment sequence.

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

4.2.1 Blinding

Not applicable; this is an open-label study.

4.3 STUDY TREATMENT AND OTHER TREATMENTS RELEVANT TO THE STUDY DESIGN

The investigational medicinal products (IMPs) for this study are balovaptan and esomeprazole.

4.3.1 Study Treatment Formulation, Packaging, and Handling

4.3.1.1 Balovaptan

Balovaptan 20-mg dispersible tablets (Ro 528-5119/F41) will be supplied by the Sponsor in high-density polyethylene bottles. Balovaptan must be stored and handled according to the details specified on the product label. For additional details, refer to the balovaptan IB.

4.3.1.2 Esomeprazole

Esomeprazole 40-mg capsules will be obtained commercially by the site. Esomeprazole must be stored and handled according to the specifications in the US package insert (USPI).

4.3.2 Study Treatment Dosage, Administration, and Compliance

The treatment regimens are summarized in Section 3.1

Refer to the balovaptan Investigator Brochure or esomeprazole USPI for detailed instructions on drug preparation, storage, and administration.

Cases of accidental overdose or medication error along with any associated adverse events, should be reported as described in Section 5.4.4.

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

4.3.2.1 Balovaptan

Single oral doses of balovaptan 20 mg (1 × 20 mg tablet) will be administered following consumption of a high-fat, high-calorie meal (Treatment A), after a ≥ 10 h fast (Treatment B), and following a ≥ 10 h fast and coadministered with esomeprazole 40 mg (Treatment C) in a fasted state. Balovaptan will be administered with approximately 240 mL of water.

For the fed treatment, balovaptan will be administered within 30 minutes of the start of the meal and after all of the meal has been consumed.

For fasted treatments, subjects are to continue to fast for 4 hours following dosing and refrain from drinking for 1 hour prior to and 1 hour after balovaptan administration, except for water provided to take study medication.

4.3.2.2 Esomeprazole

Single doses of esomeprazole 40 mg will be administered alone for 4 days prior to coadministration with balovaptan 20 mg on Day 1 of Period 3, and administered alone on Day 2 of Period 3. When esomeprazole is coadministered with balovaptan, esomeprazole will be administered 1 hour prior to balovaptan in a fasted state. Esomeprazole will be administered with approximately 240 mL of water.

4.3.3 Investigational Medicinal Product Accountability

Balovaptan will be provided by the Sponsor. Esomeprazole will be procured by the study site. The study site will acknowledge receipt of balovaptan by returning to the Sponsor the appropriate documentation form to confirm the shipment condition and content. Esomeprazole shipping documents will be filed at the site. Any damaged shipments will be replaced.

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

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

4.4 CONCOMITANT THERAPY, PROHIBITED FOOD, AND ADDITIONAL RESTRICTIONS

Concomitant therapy consists of any medication (e.g., prescription drugs, over-the-counter drugs, vaccines, or homeopathic remedies, nutritional supplements) used by a subject in addition to protocol-mandated treatment from 4 weeks prior to initiation of study drug to the study completion/ discontinuation visit. All such medications should be reported to the Investigator or designee and recorded on the Concomitant Medications eCRF.

4.4.1 Permitted Therapy

Existing hormonal contraceptives and hormone replacement therapy regimens may remain unchanged during the conduct of the study.

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

4.4.2 Prohibited Therapy

Participation in an investigational drug study within 4 weeks or 5 times the elimination half-life, whichever is longer, prior to first dosing, or within 5 months prior to first dosing in case of a study with a biologic, as calculated from the day of follow-up from the previous study. Participation in an investigational device study within 4 weeks prior to first dosing is prohibited.

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

The following concomitant medications in particular are prohibited, unless an exception has been agreed as mentioned above.

- P-gp substrates
- Any inhibitor of CYP3A or CYP2C19 taken within 4 weeks (i.e. more than 5 half-lives) prior to study drug administration, including but not limited to the following drugs: ketoconazole, miconazole, itraconazole, erythromycin, clarithromycin, ranitidine, cimetidine, fluoxetine, fluconazole, fluvoxamine, until follow-up.

Any inducer of CYP3A or CYP2C19 taken within 4 weeks prior to study drug administration, including but not limited to the following drugs: rifampicin, rifabutin, glucocorticoids, carbamazepine, phenytoin, phenobarbital, and St. John's wort, until follow-up. Use of any nonprescribed systemic or topical medication or herbal remedies within 7 days before the first study drug administration through follow-up is prohibited, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise safety.

4.4.3 Prohibited Food

- Consumption of methylxanthine-containing products (e.g., coffee, tea, cola, chocolate) will be forbidden from 48 hours prior to day of check-in to the CRU and during the stay.
- Alcohol consumption will not be permitted 96 hours prior to admission in the clinical unit and until discharge from the CRU.
- It is not permitted to take any nutrients known to modulate cytochrome CYP3A activity (e.g., grapefruit juice; Seville orange) within 2 weeks prior to first dosing until discharge from the CRU at the end of the study.

- To avoid false positive drug screen results, participants should refrain from consuming any foods containing poppy seeds within 48 hours (2 days) prior to screening and day of check-in for each treatment period.

4.4.4 Additional Restrictions/Considerations

- For Treatment A, high-fat, high-calorie breakfast with calculated calorific content will be provided. An example of a high-fat, high-calorie meal is provided in Table 2; components of the high-fat breakfast may be substituted as long as the total calorie content from fat, carbohydrates, and protein is approximately the same as listed. The high-fat, high-calorie meal should be consumed within 30 minutes.
- On Day 1 of each study period, meals standardized with regard to timing and content will be provided at approximately 4 and 8 hours post-balovaptan dosing.
- On all other days during confinement, there are no specific meal requirements; meals and snacks will be provided according to PRA SOPs.
- Subjects should refrain from strenuous exercise at least 72 hours prior to dosing until discharge from the unit in each period.
- While in the CRU, subjects will be allowed to consume water ad libitum, except for 1 hour before and 1 hour after oral dosing with balovaptan. Subjects will be provided guidance at CRU admission, for each treatment period, that they should avoid excessive fluid intake during the study. Excessive fluid intake for this study is defined as 3 L (100 fl oz) per day. Clinic staff will provide guidance as to approximate number of drinks in 3 liters as part of the subject orientation process at admission. However, the clinical site will not be required to measure, or record fluid intake.
- Alcohol consumption will not be allowed 96 hours before the day of admission to the CRU and while staying in the CRU. Additional alcohol/drugs of abuse testing may be employed throughout the study to verify compliance. For other days during the study, alcohol must be restricted to no more than 2 drinks per day (one drink is equal to 12 ounces of beer, 5 ounces of wine, or 1.5 ounces of 80 proof spirits).

Table 2 Example of a Standard High-Fat, High-Calorie Breakfast

Food item	Calories	Fat (g)	Carbohydrates (g)	Protein (g)
2 large eggs fried in 14 g (1 Tbsp) of butter	219	19	0	12
4 strips bacon	140	12	0	8
½ cup (115 g) fried hash brown potatoes	128	0	26	6
2 slices toasted wheat bread	129	1	22	8
4 packets of butter, 5 g each	144	16	0	0
8 oz whole milk	148	8	11	8
Totals	**908**	**56**	**59**	**42**

4.5 STUDY ASSESSMENTS

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

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

4.5.1 Informed Consent Forms and Screening Log

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

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

4.5.2 Medical History, Baseline Conditions, Concomitant Medication, and Demographic Data

Medical history, including clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and use of alcohol and drugs of abuse, will be recorded at baseline. In addition, all medications (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by the subject within 4 weeks prior to initiation of study treatment will be recorded.

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

4.5.3 Physical Examinations

A complete physical examination will be performed at screening and at the follow-up visit (Appendix 1). The examination should include an evaluation of the head, eyes, ears, nose, and throat; and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, and genitourinary (at the Investigator discretion) systems. Any clinically significant abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF. Changes from baseline abnormalities should be recorded in subject notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF and source form.

4.5.4 Vital Signs

Oral body temperature, pulse rate, blood pressure, and respiratory rate will be measured at the time points described in Appendix 1. Blood pressure and pulse rate measurements will be performed after the subject has rested in a supine position for at least 5 minutes.

4.5.4.1 Orthostatic Challenge Testing

Orthostatic challenge testing will be performed at Screening.

During a 15-minute period during which subjects will remain in a supine position, blood pressure according to the Riva-Rocci method and pulse rate will be measured after 10 and 15 minutes. If the second blood pressure measurement deviates from the previous measurement by more than 5 mmHg, another 5-minute extension period will be added, and blood pressure and pulse rate will be obtained after 20 minutes in the supine position.

The subject will then be asked to erect rapidly into a standing position, and blood pressure and pulse rate will be assessed again after 3 minutes of standing.

Standing blood pressure and pulse rates will be compared against the latest blood pressure and pulse rate values obtained in the supine position. Orthostatic hypotension is defined as a decrease in systolic blood pressure by at least 20 mmHg and/or a decrease in diastolic blood pressure by at least 10 mmHg.

Such changes used to define orthostatic hypotension or pre-syncopal/syncopal events during standing will result in exclusion.

4.5.5 Clinical Laboratory Samples

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

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

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

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

Laboratory samples will be collected at the time points indicated in Appendix 1. Additional blood or urine samples may be taken at the discretion of the Investigator or designee if the results of any test falls outside the reference ranges, or clinical symptoms necessitate additional testing to monitor subject safety. Subjects must fast for at least 8 hours prior to laboratory safety assessments (except for urine assessments).

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

- Hematology: leucocytes, erythrocytes, hemoglobin, hematocrit, platelets, MCH, MCV, WBC and differential count, including absolute counts and percentages [neutrophils, eosinophils, basophils, monocytes, lymphocytes]

- Urinalysis: A midstream, clean-catch urine specimen will be collected for dipstick analysis of pH, glucose, leucocytes, nitrites, protein, and blood. Microscopy to be performed if abnormalities are observed and deemed necessary by the Investigator, in particular when blood or protein is positive or strong positive. If there is an explanation for the positive dipstick result, it should be recorded, and there is no need to perform laboratory for microscopy and culture
- Coagulation: prothrombin time (international normalized ratio) and activated thromboplastin time
- Blood chemistry panel: sodium, potassium, glucose (fasting), creatinine, albumin, total and conjugated bilirubin, alkaline phosphatase, alkaline aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl-transferase, creatine phosphokinase (CPK), lactate dehydrogenase, total cholesterol and, triglycerides
- Drugs of abuse (including amphetamines [including ecstasy], barbiturates, benzodiazepines cannabinoids, cocaine, cotinine, opiates, methamphetamines, and methadone) and alcohol will be measured in urine at Screening and day of admission for each treatment period. Further testing may be performed throughout the study to confirm compliance, at the discretion of the Investigator.
- Viral serology: HIV -1 and -2 antibodies, hepatitis B core antibody (anti-HBc), hepatitis C virus antibody at screening; hepatitis B surface antigen and hepatitis B viral DNA will be assessed at screening if anti-HBc is positive
- Pregnancy test: beta-human chorionic gonadotropin (serum pregnancy test) will be performed at screening and day of admission of each treatment period and at follow-up for all female subjects.
- Follicle-stimulating hormone: at screening for postmenopausal women to confirm menopause

4.5.6 Pharmacokinetic Samples

PK samples for analysis of balovaptan and for analysis of esomeprazole will be taken via an indwelling catheter or by direct venipuncture at the time points indicated in Appendix 1.

For additional sampling procedures, storage conditions, and shipment instructions, see the Pharmacokinetic and Clinical Genotyping Sample Manual.

4.5.7 Genotyping Samples

A mandatory whole blood sample will be taken for DNA extraction from every subject during the study on Day -1 of Treatment Period 1. The DNA may be used to determine whether genes relating to drug-metabolizing enzymes and/or drug transporter enzymes affect the PK and/or the safety profile of balovaptan. Genotyping samples will be sent to the Sponsor or a designee for analysis. This specimen will be destroyed no later than 2 years after the final Clinical Study Report has been issued.

The Sponsor maintains confidentiality standards by coding each subject enrolled in the study through assignment of a unique subject identification number. This number is used to code the samples. Subject names are not included in samples that are sent for analysis.

4.5.8 Electrocardiograms

Safety 12-lead ECG assessments will be performed in this study at the timepoints indicated in Appendix 1. Safety 12-lead ECGs will be performed in triplicate at Screening and Day-1; single ECGs will be performed at all other time points. These ECG recordings will only be used to assess/define a safety baseline and determine the immediate safety of each subject during each treatment period. Safety ECG recordings may be obtained at unscheduled timepoints as required by the Investigator, or designee, to ensure the safety of study subjects. For safety monitoring purposes, the Investigator, or designee, must review, sign, and date all ECG tracings.

4.5.9 Drug and Metabolite Concentration Measurements

For each treatment period plasma samples will be collected, as described in Appendix 1, to determine concentrations of balovaptan and its metabolites M2 (as appropriate) and M3 utilizing a specific and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. In Period 3, plasma samples to determine concentrations of esomeprazole will be collected, and concentrations will be determined using a separate specific and validated LC-MS/MS method.

Timing of Study Assessments

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

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

For PK assessments, the following windows are recommended: -30 minutes for predose time points; ± 5 minutes for time points within the first 36 hours post dose; ±10 minutes for the next time points up to 96 hours post dose; and ±12 hours for Day 7 and Day 9 samples. Actual sampling times will be recorded.

4.6 TREATMENT, SUBJECT, STUDY, AND SITE DISCONTINUATION

4.6.1 Study Treatment Discontinuation

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

- Any medical condition that the Investigator, designee or Sponsor determines may jeopardize the subject's safety if he or she continues to receive study treatment. Section 5.1.1 provides additional guidance on the management of toxicities and of subjects who experience AEs within the outline of specific stopping rules.
- Investigator, designee or Sponsor determines it is in the best interest of the subject
- Pregnancy
- QTcF > 500 ms or QTcF increase from baseline > 60 ms
- [redacted]

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

Subjects who terminate the study early while admitted to the CRU will have safety assessments performed as indicated for the Follow-up visit in Appendix 1 prior to discharge. In addition, subjects will be encouraged to return to the clinic for a Follow-up visit at least 14 days after the final dose of balovaptan.

4.6.2 Subject Discontinuation from the Study

Subjects will return to the CRU for a follow-up visit on Day 16 ± 2 of Period 3.

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

- Subject withdrawal of consent
- Study termination or site closure
- Adverse event
- Loss to follow-up
- Subject noncompliance, defined as failure to comply with protocol requirements as determined by the Investigator, designee or Sponsor

Every effort should be made to obtain a reason for subject discontinuation from the study. The primary reason for withdrawal from the study should be documented on the

appropriate eCRF. Subject requests to be withdrawn from the study must be documented in the source documents and signed by the Investigator (or designee). Subjects who withdraw from the study may be replaced at the discretion of the Investigator or Sponsor.

4.6.3 Study Discontinuation

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

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

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

4.6.4 Site Discontinuation

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

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

5. ASSESSMENT OF SAFETY

5.1 SAFETY PLAN

Balovaptan is not approved in any country and clinical development is ongoing. The safety plan for subjects in this study is based on clinical experience with balovaptan in completed and ongoing studies. Please refer to the balovaptan (RO5285119) IB for a complete summary of the safety information available for the balovaptan.

[redacted]

[redacted]

Several general measures will be taken to ensure the safety of subjects participating in this study. Eligibility criteria have been designed to exclude subjects at higher risk for toxicities. Subjects will undergo safety monitoring during the study, including assessment of the nature, frequency, and severity of adverse events.

In addition, guidelines for managing adverse events, including criteria for treatment discontinuation, are provided below.

5.1.1 Management of Subjects Who Experience Adverse Events

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

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

[redacted]

[redacted]

[redacted]

5.2 SAFETY PARAMETERS AND DEFINITIONS

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

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

5.2.1 Adverse Events

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

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition) (Section 5.3.5.8).
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

5.2.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

An SAE is any AE that meets any of the following criteria:

- Is fatal (i.e., the AE actually causes or leads to death)
- Is life threatening (i.e., the AE, in the view of the Investigator or designee, places the subject at immediate risk of death)
    - This does not include any AE that, had it occurred in a more severe form or was allowed to continue, might have caused death.
- Requires or prolongs subject hospitalization (Section 5.3.5.9)

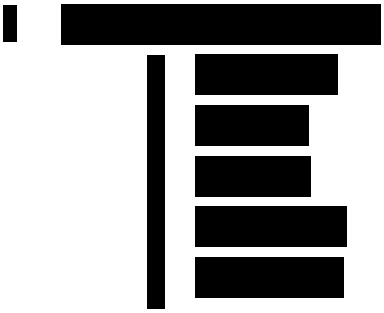
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drugs
- Is a significant medical event in the Investigator or designee's judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above)

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

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

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

[redacted]



5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

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

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

5.3.1 Adverse Event Reporting Period

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

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

After initiation of study drug, all AEs will be reported until up to 14 days after the final dose of study drug.

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

5.3.2 Eliciting Adverse Event Information

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

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

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

5.3.3 Assessment of Severity of Adverse Events

Table 3 provides guidance for assessing AE severity.

Table 3 Adverse Event Severity Grading Scale

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

Note: Regardless of severity, some events may also meet seriousness criteria. Refer to definition of an SAE (see Section 5.2.2).

5.3.4 Assessment of Causality of Adverse Events

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

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

Table 4 Causal Attribution Guidance

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

For subjects receiving combination therapy, causality will be assessed individually for each protocol-mandated therapy.

5.3.5 Procedures for Recording Adverse Events

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

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

5.3.5.1 Diagnosis versus Signs and Symptoms

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

5.3.5.2 Adverse Events That Are Secondary to Other Events

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

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and consequent fracture, all 3 events should be reported separately on the eCRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the eCRF.

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

5.3.5.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between subject evaluation timepoints. Such events should only be recorded once on the Adverse Event eCRF and source form. The initial severity (intensity or grade) of the event will be

recorded at the time the event is first reported. If a persistent AE becomes more severe, the most extreme severity should also be recorded on the Adverse Event eCRF and source form. If the event becomes serious, it should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning that the event became serious; see Section 5.4.2 for reporting instructions). The Adverse Event eCRF and source form should be updated by changing the event from "nonserious" to "serious," providing the date that the event became serious, and completing all data fields related to SAEs.

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

5.3.5.4 Abnormal Laboratory Values

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

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

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

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

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

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

5.3.5.5 Abnormal Vital Sign Values

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

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

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

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

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

5.3.5.6 Abnormal Liver Function Tests

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

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

The most appropriate diagnosis or (if a diagnosis cannot be established) the abnormal laboratory values should be recorded on the Adverse Event eCRF and source form (see Section 5.3.5.1) and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event), either as a SAE or an AESI (see Section 5.4.2).

5.3.5.7 Deaths

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

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical

concept on the Adverse Event eCRF and source form. Generally, only one such event should be reported. If the cause of death is unknown and cannot be ascertained at the time of reporting, **"unexplained death"** should be recorded on the Adverse Event eCRF and source form. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death. The term **"sudden death"** should not be used unless combined with the presumed cause of death (e.g., "sudden cardiac death").

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

5.3.5.8 Preexisting Medical Conditions

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

A preexisting medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF and source form, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

5.3.5.9 Hospitalization or Prolonged Hospitalization

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

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

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

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

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

5.4 IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

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

- SAEs (defined in Section 5.2.2; see Section 5.4.2 for details on reporting requirements)
- [redacted] see Section 5.4.2 for details on reporting requirements)
- Pregnancies (see Section 5.4.3 for details on reporting requirements)

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

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

Investigator or designees must also comply with local requirements for reporting SAEs to the local health authority and Institutional Review Board (IRB)/Ethics Committee (EC).

5.4.1 Emergency Medical Contacts

Medical Monitor Contact Information:

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

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

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

5.4.2.1 Events that Occur prior to Study Drug Initiation

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

5.4.2.2 Events that Occur after Study Drug Initiation

After initiation of study drug, SAEs and AEs of special interest will be reported until up to 14 days after the final dose of study drug. Investigator or designees should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators and submitting the report, either by faxing or by scanning and emailing the form using the fax number or email address provided to investigators.

Instructions for reporting SAEs that occur > 14 days after the last dose of study treatment are provided in Section 5.6.

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Subjects

Female subjects of childbearing potential will be instructed to immediately inform the Investigator or designee if they become pregnant during the study or within 90 days after the final dose of study drug. A paper Clinical Trial Pregnancy Reporting Form should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy), either by faxing or by scanning and emailing the form using the fax number or email address provided to the Investigator. Pregnancy should not be recorded on the Adverse Event eCRF and source form. The Investigator or designee should discontinue the study drug and counsel the subject, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the subject

should continue until conclusion of the pregnancy. Any SAE associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event eCRF and source form. In addition, the Investigator or designee will submit a Clinical Trial Pregnancy Reporting Form when updated information on the course and outcome of the pregnancy becomes available.

5.4.3.2 Pregnancies in Female Partners of Male Subjects

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

5.4.3.3 Abortions

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

If a therapeutic or elective abortion was performed because of an underlying maternal or embryofetal toxicity, the toxicity should be classified as an SAE, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2). A therapeutic or elective abortion performed for reasons other than an underlying maternal or embryofetal toxicity is not considered an AE.

All abortions should be reported as pregnancy outcomes on the paper Clinical Trial Pregnancy Reporting Form.

5.4.3.4 Congenital Anomalies/Birth Defects

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

5.4.4 Reporting Requirements for Cases of Accidental Overdose or Medication Error

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

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

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

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

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

In addition, all special situations associated with balovaptan, regardless of whether they result in an AE, should be recorded on the Adverse Event eCRF as described below:

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

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

As an example, an accidental overdose that resulted in a headache would require 2 entries on the Adverse Event eCRF, one entry to report the accidental overdose and one entry to report the headache. The "Accidental overdose" and "Medication error" boxes would need to be checked for both entries.

5.5 FOLLOW-UP OF SUBJECTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

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

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

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

5.5.2 Sponsor Follow-Up

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

5.6 ADVERSE EVENTS THAT OCCUR AFTER THE ADVERSE EVENT REPORTING PERIOD

The Sponsor should be notified if the Investigator or designee becomes aware of any SAE that occurs after the end of the AE reporting period (defined as 14 days after the final dose of study drug), if the event is believed to be related to prior study drug treatment. These events should be reported through use of the Adverse Event eCRF and source form. The Investigator or designee should report these events directly to the Sponsor or its designee, either by faxing or by scanning and emailing the paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form using the fax number or email address provided to investigators.

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

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

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

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events through use of the reference safety information in the documents listed below:

Drug	Document
Balovaptan	RO5285119 V1a Receptor Antagonist (Balovaptan) Investigator's Brochure
Esomeprazole	Esomeprazole US Package Insert

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

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

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

6. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

A statistical analysis plan will be generated and will be finalized prior to database lock.

6.1 DETERMINATION OF SAMPLE SIZE

Sixteen subjects will be enrolled with the target of at least 12 evaluable subjects available for the final PK analysis. Considering the observed residual CV% for $AUC_{0\text{-}inf}$ from Study WP40038 of approximately 14%, with 12 subjects the 90% CI would be obtained by dividing/multiplying the ratio estimate by a factor of 1.11.

6.2 SUMMARIES OF CONDUCT OF STUDY

The number of subjects who enroll, discontinue, or complete the study will be summarized. Reasons for premature study withdrawal will be listed and summarized.

Enrollment and major protocol deviations will be listed and evaluated for their potential effects on the interpretation of study results.

6.3 SUMMARIES OF DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Demographic and baseline characteristics will be summarized using means, standard deviations, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. Summaries will be presented overall and by treatment sequence.

6.4 EFFICACY ANALYSES

Not Applicable

6.5 SAFETY ANALYSES

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

Safety will be assessed through summaries of exposure to study treatment, adverse events, changes in laboratory test results, and changes in vital signs and ECGs.

Study treatment exposure (such as treatment, duration [as applicable], and total dose received) will be summarized with descriptive statistics.

All verbatim AE terms will be mapped to Medical Dictionary for Regulatory Activities thesaurus terms, and AE severity will be graded according to Table 3. All AEs, SAEs, AEs leading to death, AESIs, and AEs leading to study treatment discontinuation that occur on or after the first dose of study treatment (i.e., treatment-emergent adverse events) will be summarized by mapped term, appropriate thesaurus level, and severity grade. For events of varying severity, the highest grade will be used in the summaries. Deaths and cause of death will be summarized.

Relevant laboratory, vital sign (pulse rate, respiratory rate, blood pressure, and temperature), and ECG data will be displayed by time, with grades identified where appropriate. Additionally, a shift table of selected laboratory tests will be used to summarize the baseline and maximum postbaseline severity grade. Changes in vital signs and ECGs will be summarized.

6.6 PHARMACOKINETICS ANALYSES

The PK population will include all subjects who receive at least 1 dose of balovaptan and have sufficient evaluable concentrations to determine the overall exposure to balovaptan.

Subjects will be excluded from the PK analysis population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol, or if data are

unavailable or incomplete. Excluded cases will be documented together with the reason for exclusion. All decisions on exclusions from the analysis will be made prior to database lock.

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

- C_{max}: maximum plasma concentration
- $AUC_{(0\text{-}inf)}$: area under the concentration-time curve from time extrapolated to infinity
- $AUC_{(0\text{-}24h)}$: area under the concentration-time curve from time 0 to 24 hours
- AUC_{last}: area under the concentration-time curve from time 0 to the time of the last quantifiable concentration
- T_{max}: time to reach C_{max} in plasma
- C_{last}: last quantifiable concentration
- T_{last}: time to the last quantifiable concentration
- Tlag: time between dosing and time of first balovaptan plasma concentration.
- Cl/F: apparent clearance
- Vd/F: apparent volume of distribution
- $T_{1/2}$: terminal elimination phase half-life

Individual and mean concentration versus time data will be plotted on linear and semilogarithmic scales. Plasma concentrations and derived plasma PK parameters will be listed for balovaptan and its metabolites. Summary statistics of PK parameters, including means, geometric means, medians, ranges, SD, CV% will be presented for each treatment. Analysis of variance models will be applied to the logarithmically transformed PK parameters of balovaptan $AUC_{0\text{-}inf}$ and C_{max}, to investigate 1) the relative bioavailability of balovaptan 20-mg dispersible tablets administered following a high-fat, high-calorie meal vs in the fasted state, and 2) the relative bioavailability of balovaptan 20-mg dispersible tablets administered in the fasted state either alone or in combination with esomeprazole.

The effect of food on the PK of balovaptan will be assessed using the ratio and 90% CIs of the geometric least squares (LS) means of the plasma PK parameters C_{max} and $AUC_{0\text{-}inf}$ for balovaptan. A linear mixed model with fixed effects for treatment, period, sequence, and a random effect for subject nested in sequence will be used on the natural log-transformed parameters. Point estimates on the natural log scale will be exponentiated to obtain point estimates on the original scale of measurement. Geometric LS means will be provided for each treatment. Food had no relevant effect on the overall exposure of the capsule formulation of balovaptan but T_{max} was shifted from 1 hour after dosing under fasted conditions to approximately 3 hours in fed conditions.

The effect of esomeprazole on the PK of balovaptan will be assessed using the ratio and 90% CIs of the geometric LS means of the plasma PK parameters: C_{max} and $AUC_{0\text{-}inf}$ for balovaptan. A linear mixed model with a fixed effect for treatment and a random effect for subject will be used on the natural log-transformed parameters. Point estimates on the natural log scale will be exponentiated to obtain point estimates on the original scale of measurement. LS geometric means will be provided for each treatment.

In all comparisons, balovaptan under fasted conditions will be used as the reference. No adjustments will be made for multiplicity.

PK parameters will be estimated using standard noncompartmental methods.

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

6.7 OTHER ANALYSES

6.7.1 Clinical Genotyping

The effect of genetic variations in genes associated with drug ADME (see Appendix 2) on the PK and/or the safety profile of balovaptan may be evaluated.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

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

PRA will produce a Data Management Plan that describes the quality checking to be performed on the data. Central laboratory data (safety) will be sent directly to PRA using PRA's standard procedures to handle and process the electronic transfer of these data.

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

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

7.2 ELECTRONIC CASE REPORT FORMS

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

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

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

7.3 SOURCE DATA DOCUMENTATION

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

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

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

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

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

7.4 USE OF COMPUTERIZED SYSTEMS

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

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

7.5 RETENTION OF RECORDS

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

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

Roche will retain study data for 25 years after the final study results have been reported or for the length of time required by relevant national or local health authorities, whichever is longer.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the ICH E6 guideline for GCP and the principles of the Declaration of Helsinki, or the applicable laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the US or under a US Investigational New Drug Application will comply with US FDA regulations and applicable local, state, and federal laws. Studies conducted in the EU or European Economic Area will comply with the EU Clinical Trial Directive (2001/20/EC) and applicable local, regional, and national laws.

8.2 INFORMED CONSENT

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

submission. The final IRB/EC–approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

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

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

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

If the Consent Forms are revised (through an amendment or an addendum) while a subject is participating in the study, the subject or a legally authorized representative must re-consent by signing the most current version of the Consent Forms or the addendum, in accordance with applicable laws and IRB/EC policy. For any updated or revised Consent Forms, the case history or clinical records for each subject shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

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

Each Consent Form may also include subject authorization to allow use and disclosure of personal health information in compliance with the US Health Insurance Portability and Accountability Act (HIPAA) of 1996. If the site utilizes a separate Authorization Form for subject authorization for use and disclosure of personal health information under the HIPAA regulations, the review, approval, and other processes outlined above apply except that IRB review and approval may not be required per study site policies

8.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the subject, and relevant supporting information must be submitted to the IRB/EC by the Principal

Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any subject recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigator or designees are also responsible for promptly informing the IRB/EC of any protocol amendments (see Section 9.7).

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

8.4 CONFIDENTIALITY

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

Subject medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the subject, unless permitted or required by law.

Medical information may be given to a subject's personal physician or other appropriate medical personnel responsible for the subject's welfare, for treatment purposes.

Given the complexity and exploratory nature of exploratory biomarker analyses, data derived from these analyses will generally not be provided to study investigators or subjects unless required by law. The aggregate results of any conducted research will be available in accordance with the effective Sponsor policy on study data publication (see Section 9.6).

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

Study data, which may include data on genomic variants, may be submitted to government or other health research databases or shared with researchers, government agencies, companies, or other groups that are not participating in this study. These data may be combined with or linked to other data and used for research purposes, to

advance science and public health, or for analysis, development, and commercialization of products to treat and diagnose disease. In addition, redacted Clinical Study Reports and other summary reports will be provided upon request (see Section 9.6).

8.5 FINANCIAL DISCLOSURE

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

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

9.1 STUDY DOCUMENTATION

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

9.2 PROTOCOL DEVIATIONS

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

9.3 MANAGEMENT OF STUDY QUALITY

The Sponsor will implement a system to manage the quality of the study, focusing on processes and data that are essential to ensuring patient safety and data integrity. The Sponsor will identify potential risks associated with critical trial processes and data and will implement plans for evaluating and controlling these risks. Risk evaluation and control will include the selection of risk-based parameters (e.g., adverse event rate, protocol deviation rate) and the establishment of quality tolerance limits for these parameters. Detection of deviations from quality tolerance limits will trigger an evaluation to determine if action is needed. Details on the establishment and monitoring of quality tolerance limits will be provided in a Quality Tolerance Limit Management Plan.

9.4 SITE INSPECTIONS

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

9.5 ADMINISTRATIVE STRUCTURE

This trial will be sponsored and managed by F. Hoffmann-La Roche Ltd. The Sponsor has contracted PRA Health Science, which will be delegated responsibility for various aspects of this clinical trial.

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

9.6 DISSEMINATION OF DATA AND PROTECTION OF TRADE SECRETS

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, at scientific congresses, in clinical trial registries, and in peer-reviewed journals. The Sponsor will comply with all requirements for publication of study results. Study data may be shared with others who are not participating in this study (see Section 8.4 for details), and redacted Clinical Study Reports and other summary reports will be made available upon request. For more information, refer to the Roche Global Policy on Sharing of Clinical Trials Data at the following website:

www.roche.com/roche_global_policy_on_sharing_of_clinical_study_information.pdf

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

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

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

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

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

9.7 PROTOCOL AMENDMENTS

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

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

10. REFERENCES

Shin JY, Roughead EE, Park BJ, et al. Cardiovascular safety of methylphenidate among children and young people with attention-deficit/hyperactivity disorder (ADHD):nationwide self-controlled case series study. BMJ. 2016;353:i2550.

Al-Mosalem OA, El-Ansary A, Attas O, Al-Ayadhi L. Metabolic biomarkers related to energy metabolism in Saudi autistic children. Clinical biochemistry. 2009 Jul 1;42(10-11):949-57.

World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Jama. 2013 Nov 27;310(20):2191.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E3: Structure and Content of Clinical Study Reports (CPMP/ICH/137/95), November 1995

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Note for Guidance on Clinical Safety Data Management, June 1995

Olbe L, Cederberg C, Lind T, Olausson M. Effect of omeprazole on gastric acid secretion and plasma gastrin in man. Scand J Gastroenterol Suppl. 1989;166:27-32; discussion 41-2. Review.

Röhss K, Hasselgren G, Hedenström H. Effect of esomeprazole 40 mg vs omeprazole 40 mg on 24-hour intragastric pH in patients with symptoms of gastroesophageal reflux disease. Dig Dis Sci. 2002 May;47(5):954-8.

Lind T, Rydberg L, Kyleback A, Jonsson A, Andersson T, Hasselgren G, Holmberg J, Röhss K: Esomeprazole provides improved acid control vs. omeprazole in patients with symptoms of gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2000;14:861–867. 12

Appendix 1
Schedule of Activities

	Scr	Period 1								Period 2									Period 3							F/U
	D -28 to -2	D -1	D 1	D 2	D 3 & 4	D 5	D 7	D 9	w/ o	D -1	D1	D 2	D 3 & 4	D 5	D 7	D 9	D10 to D12	D 13[a]	D -1[a]	D 1	D 2	D 3 & 4	D 5	D 7	D 9	D16 ±2 or ET
Written informed consent	X								12 to 21 days between balovaptan administrations																	
Inclusion and exclusion criteria	X	X								X									X							
Demographics	X																									
Medical history	X																									
Medication history	X																									
Confinement		X	X	X	X					X	X	X	X			X	X	X		X	X	X				
Discharge						X								X									X			
Ambulatory visits							X	X							X									X	X	X
Randomization			X																							
Balovaptan administration[b]			X								X									X						
Esomeprazole administration																	X	X		X[c]	X					
Standardized meals[d]			X								X									X						
PK sampling balovaptan[e]			X	X	X	X	X	X			X	X	X	X	X	X				X	X	X	X	X	X	
PK sampling esomeprazole[f]																				X						
Physical examination	X																									X
Height, weight, and BMI[g]	X																									X
12-lead ECG[h]	X	X				X								X		X							X			X
Vital signs[i]	X[j]	X	X[k]			X				X	X[k]			X		X			X	X[k]			X			X
Hematology	X	X				X				X				X		X			X				X			X

Appendix 1: Schedule of Activities

	Scr	Period 1								Period 2									Period 3							F/U
	D -28 to -2	D -1	D 1	D 2	D 3 & 4	D 5	D 7	D 9	w/o	D -1	D1	D 2	D 3 & 4	D 5	D 7	D 9	D10 to D12	D 13[a]	D -1[a]	D 1	D 2	D 3 & 4	D 5	D 7	D 9	D16 ±2 or ET
Biochemistry	X	X				X				X				X		X			X				X			X
Urinalysis	X	X																								X
Coagulation	X	X																								X
Urine drug and alcohol screen[l]	X	X								X						X										
Serology	X																									
Pregnancy test[m]	X	X								X						X										X
FSH[n]	X																									
Genotyping sample		X																								
Adverse event monitoring	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X

ET=early termination; F/U= follow-up; FSH=follicle-stimulating hormone; w/o=washout

a. Day 13 of Period 2 is Day -1 of Period 3.
b. Balovaptan will be administered following a high-fat, high-calorie meal (Treatment A) or after a ≥ 10 h fast (Treatments B and C). Subjects should fast for 4 hours after balovaptan administration and refrain from drinking for 1 hour prior to and 1 hour post dose.
c. Esomeprazole will be administered 1 hour prior to balovaptan.
d. Standardized meals will be provided at approximately 4 and 8 hours post-balovaptan dosing in each treatment period
e. Samples at predose, and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 16, 24, 36, 48, 72, 96, 144, and 192 hours post dose
f. Samples at predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post dose of esomeprazole
g. Height, weight, and BMI calculation at screening; only weight at all follow-up.
h. Safety ECG will be performed in triplicate at Screening and on Day -1 of each period; at all other time points single ECGs will be performed.
i. Body temperature and respiratory rate will be measured at Screening and Day -1 of each period only.
j. Orthostatic challenge testing with be performed at screening.
k. On Day 1, vital signs measurements will be performed at 1 hour post dose.
l. Drugs of abuse (amphetamines [including ecstasy], barbiturates, benzodiazepines cannabinoids, cocaine, cotinine, opiates, methamphetamines, and methadone).
m. Serum pregnancy test performed on all female subjects at Screening and Day -1 of each treatment period (Day 13 of Period 2 is Day -1 of Period 3) and at follow-up.
n. In postmenopausal women to confirm menopause

Appendix 2: List of DMET™ (Drug Metabolism Enzymes and Transporters) Genes

Genes represented by DMET™ Plus Panel								
ABCB1	ALDH2	COMT	CYP4F3	EPHX2	MAOA	RPL13	SLC22A14	TBXAS1 *
ABCB4	ALDH3A1	CROT	CYP4F8	FAAH	MAOB	RXRA	SLC25A27	TPMT *p
ABCB7	ALDH3A2	CYP1A1 *	CYP4F11	FMO1	MAT1A	SERPINA7	SLC28A1	TPSG1
ABCB11	AOX1	CYP1A2 *p	CYP4F12	FMO2 *	METTL1	SLC5A6	SLC28A2	TYMS
ABCC1	APOA2	CYP1B1 *	CYP4Z1	FMO3	NAT1 *p	SLC6A6	SLC28A3	UGT1A1 *p
ABCC2	ARNT	CYP2A6 *p	CYP7A1	FMO4	NAT2 *p	SLC7A5	SLC29A1	UGT1A3 *
ABCC3	ARSA	CYP2A7	CYP7B1	FMO5	NNMT	SLC7A7	SLC29A2	UGT1A4 *
ABCC4	ATP7A	CYP2A13*	CYP8B1	FMO6	NQO1	SLC7A8	SLCO1A2	UGT1A5
ABCC5	ATP7B	CYP2B6 *p	CYP11A1	G6PD	NR1I2	SLC10A1	SLCO1B1*p	UGT1A6 *
ABCC6	CA5P	CYP2B7P1	CYP11B1	GSTA1	NR1I3	SLC10A2	SLCO1B3	UGT1A7 *
ABCC8	CBR1	CYP2C8 *p	CYP11B2	GSTA2	NR3C1	SLC13A1	SLCO2B1*	UGT1A8 *
ABCC9	CBR3	CYP2C9 *p	CYP17A1	GSTA3	ORM1	SLC15A1	SLCO3A1	UGT1A9 *
ABCG1	CDA *	CYP2C18	CYP19A1*	GSTA4	ORM2	SLC15A2*	SLCO4A1	UGT1A10*
ABCG2	CES2	CYP2C19*p	CYP20A1	GSTA5	PGAP3	SLC16A1	SLCO5A1	UGT2A1
ABP1	CHST1	CYP2D6 *p	CYP21A2	GSTM1 *p	PNMT	SLC19A1	SPG7	UGT2B4
ADH1A	CHST2	CYP2E1 *p	CYP24A1	GSTM2	PON1	SLC22A1	SPN	UGT2B7 *p
ADH1B	CHST3	CYP2F1 *	CYP26A1	GSTM3	PON2	SLC22A2*	SULT1A1*	UGT2B11
ADH1C	CHST4	CYP2J2 *	CYP26C1	GSTM4	PON3	SLC22A3	SULT1A2	UGT2B15*
ADH4	CHST5	CYP2S1 *	CYP27A1	GSTM5	POR	SLC22A4	SULT1A3	UGT2B17*
ADH5	CHST6	CYP3A4 *p	CYP27B1	GSTO1	PPARD	SLC22A5	SULT1B1	UGT2B28
ADH6	CHST7	CYP3A5 *p	CYP39A1	GSTP1 *p	PPARG	SLC22A6	SULT1C2	UGT8
ADH7	CHST8	CYP3A7 *p	CYP46A1	GSTT1 *	PPP1R9A	SLC22A7	SULT1C4	VKORC1 *p
AHR	CHST9	CYP3A43*	CYP51A1	GSTT2	PRSS53	SLC22A8	SULT1E1	XDH
AKAP9	CHST10	CYP4A11	DCK	GSTZ1	PTGIS *	SLC22A11	SULT2A1	
ALB	CHST11	CYP4B1 *	DPYD *	HMGCR	QPRT	SLC22A12	SULT2B1	
ALDH1A1	CHST13	CYP4F2 *	EPHX1	HNMT	RALBP1	SLC22A13	SULT4A1	

* = translated to star allele classification p = translated to predicted phenotype/metabolizer status