Official Title: A Phase III, Randomized, Double-Blind, Placebo-Controlled, Efficacy, and Safety Study of Balovaptan in Adults With Autism Spectrum Disorder With a 2-Year Open-Label Extension

NCT Number: NCT03504917

Document Date: Protocol Version 3: 01-February-2019

PROTOCOL

TITLE:	**A PHASE III, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, EFFICACY, AND SAFETY STUDY OF BALOVAPTAN IN ADULTS WITH AUTISM SPECTRUM DISORDER WITH A 2-YEAR OPEN-LABEL EXTENSION**
PROTOCOL NUMBER:	WN39434
VERSION NUMBER:	3
EUDRACT NUMBER:	2017-004378-32
IND NUMBER:	116,483
TEST PRODUCT:	Balovaptan (RO5285119)
MEDICAL MONITOR:	[redacted], M.D.
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	Version 1: 3 April 2018
DATES AMENDED:	Version 2 (Canada): 3 July 2018 Version 3: See electronic date stamp below.

PROTOCOL AMENDMENT APPROVAL

Approver's Name	Title	Date and Time (UTC)
[redacted]	Company Signatory	01-Feb-2019 15:45:50

PROTOCOL AMENDMENT, VERSION 3: RATIONALE

Protocol WN39434 has been amended primarily to update the requirements for cardiac monitoring in response to specific requests received from the U.S. Food and Drug Administration (FDA) and to remove the capillary blood draw option. This Version 3 amendment includes revisions made in the Version 2 (Canada) amendment in response to specific requests from Health Canada. Changes to the protocol, along with a rationale for each change, are summarized below:

- [redacted]
- Capillary blood sampling has been removed. Capillary blood sample for hematologic and CPK monitoring at certain timepoints was allowed because neutrophil counts and CPK can be measured by a capillary blood sample and a capillary blood sample may be better tolerated than blood draws by venipuncture. However, hs cTnT has not been shown to be reliably measured from capillary blood. Thus, with the reduced CPK assessments and the increased hs cTnT assessments, capillary blood sampling has been removed (Section 4.5.6 and Appendices 1, 2, and 3).
- As requested by Health Canada, an exclusion criterion has been added to exclude patients with an unexplained syncopal episode within the last 12 months in order to exclude individuals who may have other underlying cardiac disease or condition that might not have been diagnosed (Section 4.1.2.3).

Additional changes to the protocol, along with a rationale for each change, are summarized below:

- [redacted]

- Clarification has been made that the screening period may be extended for a total period of 6 weeks when laboratory tests need to be repeated or for other clinical, administrative, or operational reasons (Section 3.1.1.1). Additionally, if re-screening is necessary, disease-specific assessments (e.g., Autism Diagnostic Observation Schedule™, Second Edition [ADOS™-2] score, SRS, WASI-II) should not be repeated (Section 3.1.1.2).
- Timing of the final follow-up visit has been specified (Section 3.1.1.5).
- Description of the balovaptan tablets as "dispersible" has been removed (Sections 3.3.1 and 4.3).
- The contraception language in the inclusion criteria have been corrected to at least "28 days" after the last dose of study drug, instead of 30 days (Section 4.1.1). This update is to align the requirement with Study BP30153.
- Further explanation has also been given for hepatitis B and C testing at screening (Section 4.1.2.4). This was previously provided in Section 4.5.6 of the protocol.
- Use of tetrahydrocannabinol has been prohibited (Sections 4.1.3 and 4.4.3).
- A formatting error has been corrected. Aripiprazole and risperidone are now correctly listed as examples of atypical antipsychotics (Section 4.4.1).
- Language has been added to clarify that, after withdrawal of consent for participation in the RBR, remaining RBR samples will be destroyed or will no longer be linked to the patient (Section 4.5.9.6).
- [redacted]
- The Medical Monitor has been changed from [redacted], M.D. to [redacted], M.D. (Section 5.4.1).
- Text has been modified to account for the fact that special situations (i.e., overdoses, medication errors, drug abuse, and drug misuse) are not required to be reported within 24 hours (Sections 5.3.5.11 and 5.4). Note that serious adverse events associated with special situations are still required to be reported within 24 hours.
- Language has been updated to indicate that therapeutic or elective abortions are not considered adverse events unless performed because of an underlying maternal or embryofetal toxicity. In such cases, the underlying toxicity should be reported as a serious adverse event. Language has also been added to clarify that all abortions are to be reported on the paper Clinical Trial Pregnancy Reporting Form (Section 5.4.3.2).
- As requested by Health Canada, clarity has been provided as to when the interim futility analysis will be completed (Section 6.5.9).
- Language has been added for consistency with Roche's current data retention policy and to accommodate more stringent local requirements (if applicable) (Section 7.6).

- Language has been added to indicate that the study will comply with applicable local, regional, and national laws (Section 8.1).
- Language has been revised to clarify that data posting will not be limited to two clinical trial registries and to clarify that redacted CSRs are provided only if requirements of Roche's global policy on data sharing have been met (Section 9.5).

Additional minor changes have been made to improve clarity and consistency. Substantive new information appears in italics. This amendment represents cumulative changes to the original protocol.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

PROTOCOL AMENDMENT ACCEPTANCE FORM

TITLE:	**A PHASE III, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, EFFICACY, AND SAFETY STUDY OF BALOVAPTAN IN ADULTS WITH AUTISM SPECTRUM DISORDER WITH A 2-YEAR OPEN-LABEL EXTENSION**
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SPONSOR:	F. Hoffmann-La Roche Ltd

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

_____

Principal Investigator's Name (print)

_____ _____

Principal Investigator's Signature Date

Please return a copy of this signed form as instructed by your local study monitor and retain a signed copy for your study files.

PROTOCOL SYNOPSIS

TITLE:	**A PHASE III, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, EFFICACY, AND SAFETY STUDY OF BALOVAPTAN IN ADULTS WITH AUTISM SPECTRUM DISORDER WITH A 2-YEAR OPEN-LABEL EXTENSION**
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PHASE:	Phase III
INDICATION:	Autism spectrum disorders
SPONSOR:	F. Hoffmann-La Roche Ltd

Objectives and Endpoints

This study will evaluate the efficacy, safety, and pharmacokinetics of 10 mg of oral administration balovaptan once a day (QD) compared with matching placebo in adults (18 years and older) with autism spectrum disorder (ASD). Specific objectives and corresponding endpoints for the study are outlined in the following table.

Objectives and Corresponding Endpoints

Objective(s)	Corresponding Endpoint(s)
Primary Efficacy Objective	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo	• Change from baseline at Week 24 on the Vineland™-II 2DC score (defined as the mean of the Communication domain standard score and the Socialization domain standard score)
Secondary Efficacy Objectives	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo	• Change from baseline at Week 12 on the Vineland-II 2DC score • Change from baseline at Weeks 12 and 24 in the PedsQL™ Core module, Version 4.0, on summary and total scores • Change from baseline at Weeks 12 and 24 in the Vineland-II composite standard score • Change from baseline at Weeks 12 and 24 in the following: – Vineland-II Socialization domain standard score – Vineland-II Communication domain standard score – Vineland-II Daily Living Skills domain standard score
	• Change from baseline in severity of clinical impressions as measured by CGI-S after 12 weeks and 24 weeks of treatment • Improvements in clinical impressions, as measured by CGI-I after 12 weeks and 24 weeks of treatment • Change from baseline in the HAM-A total and domain scores *at Weeks 12 and 24* • Proportion of subjects with a ≥6-point improvement in Vineland-II 2DC score *at Weeks 12 and 24*

2DC=two-domain composite; ASD=autism spectrum disorder; $AUC_{0\text{-}24,\ ss}$=area under the concentration–time curve from Time 0 to 24 hours at steady state; *AVPR1A*=arginine vasopressin receptor 1A; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impressions–Improvement; CGI-S=Clinical Global Impressions–Severity; $C_{max,\ ss}$=maximum concentration at steady state; *CNTNAP2*=contactin-associated protein-like 2; C-SSRS=Columbia-Suicide Severity Rating Scale; $C_{trough,\ ss}$=trough concentration at steady state; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

Objectives and Corresponding Endpoints (cont.)

Objectives	Corresponding Endpoint(s)
Exploratory Efficacy Objectives	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo *(cont.)*	• Change from baseline at Weeks 12 and 24 on the following scales: – PedsQL™ Family Impact to assess impact on study partner and family (summary and total scores) – PedsQL™ Cognitive Functioning Scale to assess impact on cognitive functioning (summary and total scores) – RBS-R scores (summary and total scores) • Proportion of subjects with a ≥4-point improvement in Vineland-II 2DC score • Proportion of subjects with a ≥8-point improvement in Vineland-II 2DC score • *Proportion of subjects at each score level of* PGI-S and PGI-C • *Proportion of subjects at each score level of* CAGI-S and CAGI-C, as perceived by the study partner
	• *Change from baseline at Weeks 12* and *24* on the following *scales:* • *EQ-5D-5L score on the following*: – EQ-5D-5L (self-report version) completed by the study participant – EQ-5D-5L (self-report version) completed by the study partner • PSQI total score to assess impact on sleep • [redacted]

2DC=two-domain composite; ASD=autism spectrum disorder; $AUC_{0\text{-}24,\ ss}$=area under the concentration–time curve from Time 0 to 24 hours at steady state; *AVPR1A*=arginine vasopressin receptor 1A; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impressions–Improvement; CGI-S=Clinical Global Impressions–Severity; $C_{max,\ ss}$=maximum concentration at steady state; *CNTNAP2*=contactin-associated protein-like 2; C-SSRS=Columbia-Suicide Severity Rating Scale; $C_{trough,\ ss}$=trough concentration at steady state; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

Objectives and Corresponding Endpoints (cont.)

Objective	Corresponding Endpoint(s)
Safety Objective	
• To evaluate the safety of 10 mg of balovaptan compared with placebo	Safety will be assessed through the following: • Occurrence, nature, and intensity of adverse events, serious adverse events, and non-serious adverse events of special interest, as determined using the Adverse Event Severity Grading Scale • Physical and neurologic examinations, vital signs, hematology, blood chemistry, and urinalyses • [redacted] • C-SSRS
Pharmacokinetic and Pharmacodynamic Objectives	
• To characterize the plasma pharmacokinetics of balovaptan and balovaptan related metabolites	• The exposure at steady state (e.g., $AUC_{0\text{-}24ss}$, $C_{max,ss}$, $C_{trough,ss}$, and other variables) for balovaptan and its major metabolites, as applicable • Population-PK parameters and variability estimates for balovaptan • The observed plasma concentrations of balovaptan and its metabolites M3 and M2 (as applicable) (and other metabolites as appropriate) summarized by observation time, and the ratios of metabolite to parent drug concentration at trough
• To explore the exposure–response and safety relationships of balovaptan	• Exploratory graphical analysis of Vinland-II 2-DC and selected other clinical efficacy and safety endpoints
Exploratory Biomarker Objective	
• To evaluate biomarkers that may be predictive of benefit from balovaptan	• Change from baseline in efficacy, safety, PK, immunogenicity, or other biomarker endpoints according to genetic variants in the *AVPR1A* or *CNTNAP2* gene, performed on DNA extracted from blood

2DC=two-domain composite; ASD=autism spectrum disorder; $AUC_{0\text{-}24,\ ss}$=area under the concentration–time curve from Time 0 to 24 hours at steady state; *AVPR1A*=arginine vasopressin receptor 1A; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impressions–Improvement; CGI-S=Clinical Global Impressions–Severity; $C_{max,\ ss}$=maximum concentration at steady state; *CNTNAP2*=contactin-associated protein-like 2; C-SSRS=Columbia-Suicide Severity Rating Scale; $C_{trough,\ ss}$=trough concentration at steady state; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

Study Design

Description of Study

Study WN39434 is a Phase III, randomized, double-blind, placebo-controlled, parallel-group, multicenter, 24-week, efficacy, and safety study of 10 mg of oral balovaptan QD in adults (18 years old or older) with ASD, followed by a 2-year open-label extension period for subjects completing the double-blind treatment period.

Subjects providing informed consent will undergo screening within 4 weeks prior to the first double-blind study drug (balovaptan or placebo) administration. Eligible subjects will be randomized in a 1:1 ratio in a blinded fashion to either 10 mg of balovaptan or matching oral placebo QD for 24 weeks. The primary efficacy endpoint is the change from baseline at Week 24 on the Vineland™ Adaptive Behavior Scales (Vineland™-II) two-domain composite (2DC) score.

Thus, for subjects remaining in the full study, the approximate length of the study will be 144 weeks or 34 months (screening [approximately 4 weeks], double-blind treatment [24 weeks], and an open-label extension period [104 weeks] with a follow-up period of 12 weeks after completion of the treatment phase).

Subjects will be recruited from international sites, including sites in North America and limited rest of the world (ROW) countries. Subjects who prematurely discontinue from study treatment or from the study will not be replaced. However, all subjects should be followed and their data *(specifically, vital sign measurements, adverse events, concomitant treatment, Vineland-II, CGI-S, CGI-I, PedsQL 4.0 Core)* collected up to and including the Week 24 visit, regardless of their adherence to treatment.

Randomization will be stratified by baseline Vineland-II two domain composite (2DC) (the average of Communication and Socialization domains) (<60 vs. ≥ 60), sex (male vs. female), geographical region (North America vs. ROW), and age (<25 years vs. ≥ 25 years). The main analysis of the primary and secondary efficacy endpoints will occur once the final data from all visits, up to and including Week 24, have been collected and cleaned and the database has been locked. An interim futility analysis *will* be performed by an independent Data Monitoring Committee (iDMC) when approximately 50% of subjects complete the Week 24 visit.

The iDMC will meet regularly to oversee safety throughout the trial and its activities will be described in a separate iDMC charter.

Secondary and exploratory endpoints will examine core autism symptoms (social interaction, social communication, and functional deficits), associated symptoms, impact on health-related quality of life, and broader impacts on study partners and family. Safety will be examined via adverse events, clinical laboratory values, *electrocardiograms (*ECGs*)*, physical and neurologic examinations, and safety outcome assessments such as suicidality.

Screening Period

Subjects who are willing and who have given their consent to participate in the study will undergo a screening procedure within 4 weeks before the first study drug administration (Day 1). Subjects must meet all of the eligibility criteria in order to qualify for the study (see the inclusion and exclusion criteria).

The screening period can be extended to a total period of 6 weeks in cases when a laboratory blood test needs to be repeated for confirmation during the screening interval or for other relevant clinical, administrative, or operational reasons. Subjects must fulfill all the entry criteria for participation in the study.

Double-Blind Treatment Period

All subjects will undergo 24 weeks of double-blind treatment. The study will be unblinded following the primary database lock when the last enrolled subject has completed the double-blind treatment period and all the data have been collected and cleaned.

Randomization (Day 1) will occur only after a subject has met all eligibility criteria. At the baseline visit (Day 1), subjects will undergo a series of assessments outlined in the schedule of activities. Breaks should be allowed between tests when necessary. If necessary, the baseline visit can be split into 2 consecutive days, and the second day will be considered the baseline visit date (Day 1). If either the Week 12 or Week 24 visit is split over 2 days, study drug (balovaptan or placebo) must be administered QD after the completion of all assessments.

After the predose assessments have been completed, each subject will receive an oral dose of balovaptan or placebo according to randomization. After completion of the postdose evaluations and following a medical check-up by a physician, the subject will be discharged from the study center.

Although study visits will be conducted on site, checks for signs and symptoms of infection will be conducted every 2 weeks either at a site visit or by telephone call conducted by site staff.

As a general rule, the test sequence for all assessments should remain the same for a given subject as established at screening and baseline. At all visits, patient-reported outcome (PRO) assessments should be completed prior to any other assessments. All assessments must be performed before administration of study drug.

For informant-based assessments, the following order of assessments will be followed:

- The Vineland-II will be the first informant-based assessment with a subject's study partner.
- For all other informant-based assessments: It is strongly recommended that the sequence provided in Section 3.1.3.1, Table 3, is followed. It will be possible for assessments (e.g., the Vineland-II interview and subject assessments) to be conducted in parallel if the site has sufficient qualified raters available. Importantly, the sequence established at screening and baseline should remain the same for a given subject; however, subject assessments should be prioritized.

At visits when blood samples are obtained, the order of assessments should be as follows:

- 12-Lead ECGs
- Vital signs
- Physical and neurologic examinations
- Blood samples

Note: ECGs and vital sign assessments should be completed before other invasive assessments such as blood samples and should occur following a short period when the study participant is able to rest (typically, at least 5 minutes),

Open-Label Extension Period

All subjects who complete the 24-week double-blind treatment period are eligible for participation in the open-label extension period of the study. Subjects who are not willing to participate in the open-label extension period of the study will complete the safety follow-up visits. Subject treatment allocation during the double-blind treatment period will not be unblinded regardless of a subject's participation in the open-label extension period.

The 2-year (104-week) duration of the open-label extension period serves to evaluate the long-term safety, tolerability, and efficacy of balovaptan treatment in subjects with ASD. All subjects will receive 10 mg of oral balovaptan QD.

After the predose assessments have been completed, each subject will receive an oral dose of balovaptan. After completion of the postdose evaluations and following a medical check-up by a physician, the subject will be discharged from the study center. If the visit is split over 2 days, study drug (balovaptan) must be administered QD after the completion of all assessments.

Checks for signs and symptoms of infection will be conducted according to the schedule of activities.

As a general rule, the test sequence for all assessments should remain the same for a given subject as previously established at screening and baseline. At all visits, PRO assessments should be completed prior to any other assessments. All assessments must be performed before administration of study drug.

For informant-based assessments, the following order of assessments will be followed:

- The Vineland-II will be the first informant-based assessment with a subject's study partner.

- For all other informant-based assessments, there is no restriction in the order, a sequence established at screening and baseline should remain the same for a given subject; however, subject assessments should be prioritized. The suggested sequence is provided in Section 3.1.3.1, Table 3. It will be possible for assessments (e.g., the Vineland-II interview and subject assessments) to be conducted in parallel if the site has sufficient qualified raters available.

At visits when blood samples are obtained, the order of assessments should be as follows:

- 12-Lead ECGs
- Vital signs
- Physical and neurologic examinations
- Blood samples

Note: ECGs and vital sign assessments should be completed before other invasive assessments such as blood samples and should occur following a short period when the study participant is able to rest (typically, at least 5 minutes),

Follow-Up Period

In order to evaluate the long-term effects of balovaptan, subjects will enter a follow-up period, consisting of a telephone call at Week 1 and site visits at Weeks 2 and 12. The final follow-up visit for safety and limited efficacy will be conducted 12 weeks after the *Week 24 visit* for subjects who do not enter the open-label extension period or at the end of the open-label extension period for all other subjects. *For subjects who discontinue treatment prematurely and will not return for the Week 24 visit, the final follow-up visit should occur 12 weeks after the final dose.*

Independent Data Monitoring Committee

All available safety data will be assessed on a regular basis by an iDMC. It is anticipated that these assessments will occur approximately every 6 months.

The iDMC will also undertake evaluation of any efficacy interim analyses.

Details about the iDMC will be provided in the iDMC Charter.

Number of Subjects

Approximately 350 subjects will be enrolled in the study.

Target Population

Inclusion Criteria

Subjects must meet the following criteria for study entry:

- Signed Informed Consent Form
- Males or females, age 18 years or older at time of signing Informed Consent Form
- Subject meets the Diagnostic and Statistical Manual of Mental Disorders, Version 5 (DSM-5) criteria for ASD for an autism diagnosis and is confirmed using Autism Diagnostic Observation Schedule™, Second Edition (ADOS™-2) criteria

  If the ADOS-2 assessment has been performed by a certified rater and documented within 12 months of the screening visit, there is no requirement to repeat it.
- Social Responsiveness Scale™, Second Edition (SRS™-2), proxy version, total *t*-score ≥66 at screening
- A full scale IQ score ≥70 on the Wechsler *Abbreviated* Scale of Intelligence®, Second Edition (WASI®-II)

  The score should be confirmed by assessment during screening. Previous test results on the WASI or equivalent scales such as Wechsler Adult *Intelligence* Scale, Fourth Edition (*WAIS*-IV) (including all subdomain scores) are accepted if the test has been performed within 12 months prior to screening by an appropriately qualified rater.
- Ability and willingness to fully comply with study visit schedule and regular assessments and fluency in the language of the site

- Subject's participation in the study or discontinuation of prohibited medication will not pose undue risks to the subject, in the investigator's opinion
- Subject has an appropriate study partner, in the opinion of the investigator

  The study partner is someone who has regular and sufficient periods of contact *(including regular conversations and face-to-face interactions)* with the subject to be able to report on the subject's status on relevant study assessments.

  The study partner should have sufficient capacity to evaluate social and communication changes in the subject and should be available throughout the entire duration of the study.

  The study partner will not be eligible to be a study participant in this study.

  Only at visits that do not require a study partner's input on assessments can a subject be accompanied by someone other than the study partner if the study partner is not available.

  Every effort should be made to have the same study partner participate throughout the subject's participation in the study.
- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use a contraceptive method with a failure rate of <1% per year during the treatment period and for at least *28* days after the last dose of study drug

  A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (≥12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

  Examples of contraceptive methods with a failure rate of <1% per year include bilateral tubal ligation, male sterilization, hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices.

  The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

  Women must agree to use an effective form of contraception during all phases of the study.

  No specific contraception methods for males are required.

  Note: The Sponsor does not require the use of male contraception because of the minimal seminal dose transmitted via sexual intercourse.
- Treatment with permitted medications (at a stable dose for 12 weeks before screening) and behavioral therapy regimens (regimens stable for 6 weeks before screening), with the intent that such treatments remain stable throughout the study and with no expected changes before the Week 24 visit

Exclusion Criteria

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

General Exclusion Criteria

Subjects who meet the following general exclusion criterion will be excluded from study entry:

- Pregnancy or breastfeeding, or intention to become pregnant during the study

  Females of childbearing potential must have a negative urine pregnancy test result immediately prior to initiation of study drug.

Neurologic and Psychiatric Exclusion Criteria

Subjects who meet any of the following neurologic and psychiatric exclusion criteria will be excluded from study entry:

- Previous initiation of new or major change in psychosocial intervention (including investigational) within 6 weeks prior to screening

Minor changes in ongoing treatment (e.g., missed therapy sessions because of holiday or vacation, planned break in therapy due to school holidays, or changes in college or school programs) are not considered significant.

- Unstable or uncontrolled clinically significant affective or psychotic disorders and/or neurologic disorder that may interfere with the assessment of safety or efficacy endpoints
- *Substance use disorders (including a*lcohol or substance abuse or dependence disorder*)* during the last 12 months, as defined *by* the DSM-5 criteria
- Significant risk for suicidal behavior, in the opinion of the investigator
- Epilepsy or seizure disorder considered not well controlled within the past 6 months or changes in anticonvulsive therapy within the last 6 months
- Clinical diagnosis of peripheral neuropathy

Exclusions Related to Cardiovascular Disorders

Subjects who meet any of the following exclusion criteria related to cardiovascular disorders will be excluded from study entry:

- Within the last 2 years, unstable or clinically significant cardiovascular disease (e.g., myocardial infarction, angina pectoris, or New York Heart Association Class II or higher cardiac failure)
- Uncontrolled hypertension (e.g., blood pressure repeatedly >160 mmHg systolic or >95 mmHg diastolic)
- *Unexplained syncopal episode within the last 12 months*
- *Confirmed elevation above upper limit of normal (ULN) of CK-MB (electrophoretic measurement), high sensitivity cardiac troponin T (hs cTnT), cardiac troponin I (cTnI), and/or N-terminal pro B-type natriuretic peptide (NT-proBNP)*

Exclusions Related to Other Organ Systems

Subjects who meet any of the following exclusion criteria related to other organ systems will be excluded from study entry:

- Positive serology results for hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus 1 or 2

  All subjects must have a negative HBsAg result and negative hepatitis C antibody screening tests prior to enrollment. If total HBcAb is positive at screening, hepatitis B virus DNA measured by polymerase chain reaction must be negative to be eligible.

- History of coagulopathies, bleeding disorders, blood dyscrasias, hematological malignancies, myelosuppression (including iatrogenic), or current major bleeding event (e.g., gastrointestinal bleeding)
- Concomitant disease or condition that could interfere with, or treatment of which might interfere with, the conduct of the study, or what would, in the opinion of the investigator, pose an unacceptable risk to the subject in this study
- Confirmed clinically significant abnormality in parameters of hematology [redacted]
- Confirmed clinically significant abnormality in parameters of clinical chemistry, coagulation, or urinalysis

  If CPK is increased above 3× upper limit of normal at screening, additional samples to measure CPK, creatinine, and potassium levels should also be obtained at Week 2 of treatment.

- Medical history of malignancy, if not considered cured

Additional Exclusion Criteria

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

- Allowed medications have not been stable for 12 weeks prior to screening
- Previous treatment with prohibited medications or herbal remedies within 2 weeks prior to randomization or 5 half-lives (whichever is longer)
- Blood donation or loss of blood >500 mL within 3 months prior to randomization
- Previous participation in an investigational drug or device study within 60 days prior to randomization or previous enrollment in investigational trials of balovaptan

Prohibited Medications and Food Products

Prior use of certain therapies is permitted if the individual has undergone a washout period of 2 weeks prior to randomization or 5× the half-life (whichever is longer).

The following therapies are prohibited throughout the study and include:

- Moderate and strong inhibitors of CYP3A4 (e.g., ketoconazole, clarithromycin, erythromycin, ciprofloxacin, diltiazem, and grapefruit juice)

  Moderate inhibitors of CYP3A4 (e.g., erythromycin, ciprofloxacin, diltiazem) may be allowed for a treatment duration of no more than approximately 10 days (e.g., in the context of an adverse event) after discussion with the Medical Monitor or designee. The reason for and approval of such treatment is to be documented.
- Moderate and strong inducers of CYP3A4 (e.g., carbamazepine, phenytoin, St. John's Wort), with the following exceptions:

  Weak inducers are permitted.
- Concomitant oral medication that are P-gp substrates:
  - Quinidine should not be given with balovaptan.
  - Risperidone and cetirizine are allowed.
  - All other clinically relevant substrates of P-gp: An interaction cannot be ruled out and caution is advised in particular for those medications that have a narrow therapeutic window (e.g., loperamide). However, when balovaptan is administered 5 or more hours prior to such a P-gp substrate, the risk of pharmacokinetic (PK) interaction is predicted to be small.
  - Chronic adrenocorticoid or glucocorticoid use, with the following exceptions: Inhaled and topical formulations are permitted.
- Oxytocin
- Desmopression (DDAVP®)
- Bumetanide
- Agents inhibiting vasopressin receptors (e.g., tolvaptan, conivaptan)
- Agents that have been associated with significant and/or irreversible hematological toxicity and therefore require frequent hematologic monitoring (e.g., clozapine)
- Use of any concomitant medication known to potentially cause peripheral neuropathy per the Warnings and Precautions section of the U.S. label or the corresponding section of the local label
- Agents that have been associated with significant and/or irreversible hematologic toxicity and therefore require frequent monitoring (e.g., clozapine)
- *Use of tetrahydrocannabinol due its psychoactive effects (e.g., marijuana)*

End of Study

The end of the double-blind treatment period of the study is defined as the time when subjects have either transitioned to the open-label extension period or, for subjects not entering the open-label extension period, when the follow-up period has been completed.

The end of study is defined as the date when the last subject, last visit occurs in the open-label extension period or the follow-up period.

Length of Study

It is anticipated that it may take from 18 to 28 months to recruit all subjects for the study. Hence, the total length of the study is estimated to be approximately 4–5 years.

Investigational Medicinal Products

The investigational medicinal products for this study are balovaptan and matching placebo.

Test Product (Investigational Drug)

One tablet of study drug (balovaptan or placebo) should be taken orally QD in the morning, with or without food. The tablet should be swallowed whole with something to drink.

The first dose of the study drug will be administered on Day 1 after all predose baseline assessments have been conducted. At subsequent visits, study drug should not be taken until all protocol-mandated study assessments are completed*, unless otherwise specified in the protocol*.

Statistical Methods

Database lock to enable the analysis of the 24-week, placebo-controlled phase of the study will occur once all subjects have either completed the 24-week assessment or withdrawn from the study early, and all data required for analysis have been cleaned and verified. After database lock, treatment assignments will be unblinded to the Sponsor.

The final analysis of the 24-week placebo-controlled period will be performed after database lock. The final analysis of all data collected in the study, including the open-label extension, will occur after all subjects have completed the study.

Details of the planned statistical analyses mentioned below in this section will be fully specified in the Statistical Analysis Plan, which will be finalized prior to the locking the study database and unblinding the study.

Primary Analysis

The primary efficacy analysis for this trial will compare balovaptan with placebo at Week 24. The following null (H_0) and alternative (H_a) hypotheses will be tested at a two-sided α=0.05 level:

- H_0: $MEAN_{balovaptan} = MEAN_{placebo}$ versus
- H_a: $MEAN_{balovaptan} \neq MEAN_{placebo}$

for which the $MEAN_{RO}$ and $MEAN_{placebo}$ refer to the mean change from baseline for balovaptan and placebo, respectively.

The analysis of the primary endpoint, the change from baseline at Week 24 on the Vineland-II 2DC score, will be performed by means of analysis of covariance. The model will include the corresponding endpoint baseline score as covariate and treatment, age group, sex, and geographical region as fixed effects. Based on this analysis, least square means, standard errors, treatment difference, and corresponding 95% confidence intervals will be reported.

The effects on continuous secondary endpoints, as well as the short-term effect at 12 weeks will be similarly analyzed.

As a supplementary analysis, a mixed-model repeated measurement (MMRM) analysis, will be performed. The MMRM model will include the corresponding endpoint baseline score as a covariate, with visit, treatment, age group, sex, and geographical region as fixed effects, and treatment by visit interaction; visit will be fitted as a repeated effect with unstructured correlation across visits within each subject.

Determination of Sample Size

The sample size of 350 randomized subjects (175 subjects per arm) provides 85% power to detect a difference in means between treatments in the change from baseline at Week 24 on the Vineland-II 2DC score of at least 4.0 points, assuming a two-sided 5% significance level and a standard deviation of about 12.5 points. A blinded interim analysis of the change in Vineland-II 2DC score from baseline may be performed by the Sponsor to allow an increase in the sample size if necessary to achieve appropriate study power.

Futility Analyses

One or more interim futility analyses *will* be performed during the study. The analysis will be performed by the external independent Data Coordinating Center that generates the unblinded results reviewed by the iDMC on a regular basis.

The iDMC will review the unblinded results for the primary efficacy endpoint (change from baseline at Week 24 in the Vineland-II 2DC score) and for the Adaptive Behavior Composite and the three individual Vineland-II domains standard scores. *The iDMC will be given clear criteria, detailed in the Statistical Analysis Plan, regarding what would constitute futility, and it is expected to recommend stopping the trial if the futility analysis results meet those criteria.* If the analysis of the primary endpoint results at the time of this interim analysis indicates that the conditional probability of success for the trial is less than a prespecified threshold, then the committee may recommend that the trial be terminated for futility. The iDMC will communicate to the Roche Data Review Board *(DRB)* only this recommendation (whether or not to stop the study for futility); detailed results of the futility analysis will not be shared with the Sponsor unless specifically requested by the *DRB. Roche DRB Chair (or designate) will make the final decision regarding stopping the trial.*

No adjustment for multiple comparisons will be made to the α-level for this analysis, as the decision rules for the futility analysis will not allow for the opportunity to stop the study early for overwhelming efficacy.

The specifics of the interim futility analysis will be documented prior to its conduct in the Statistical Analysis Plan.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
2DC	two-domain composite (score)
ADAMS	Anxiety, Depression, and Mood Scale
ADOS™-2	Autism Diagnostic Observation Schedule™, Second Edition
ASD	autism spectrum disorder
AUC	area under the concentration–time curve
AVP	arginine vasopressin
AVPR1A	arginine vasopressin receptor 1A (gene)
CaGI-C	Caregiver Global Impression–Change
CaGI-S	Caregiver Global Impression–Severity
CDC	Centers for Disease Control and Prevention
CGI-I	Clinical Global Impression–Improvement
CGI-S	Clinical Global Impression–Severity
CK-MB	*cardiac preferred isoform of CPK*
CNTNAP2	contactin-associated protein–like 2 (gene)
CPK	creatinine phosphokinase
C-SSRS	Columbia-Suicide Severity Rating Scale
cTnI	*cardiac troponin I*
DRB	Data Review Board
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Version 5
EC	Ethics Committee
eCOA	electronic clinical outcome assessment
eCRF	electronic Case Report Form
EDC	electronic data capture
EQ-5D-5L	EuroQoL Five Dimensions Questionnaire–Five Levels
FDA	Food and Drug Administration
GI	gastrointestinal
GIFT	granulocyte immunofluorescence test
GLP	Good Laboratory Practice
HAM-A	Hamilton Anxiety Rating Scale
HBcAb	hepatitis B core antibody
HbsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HRQoL	health-related quality of life

Abbreviation	Definition
hs cTnT	*high sensitivity cardiac troponin T*
ICH	International Council for Harmonisation
iDMC	independent Data Monitoring Committee
IMP	investigational medicinal product
IND	Investigational New Drug (Application)
IQ	intelligence quotient
ITT	intent to treat
IRB	Institutional Review Board
IxRS	interactive voice or web-based response system
LLN	lower limit of normal
MMRM	mixed-model repeated measurement
NOAEL	no-observed-adverse-effect level
NT-proBNP	N-terminal pro b–type natriuretic peptide
ObsRO	observer-reported outcome
PedsQL™	Pediatric Quality of Life Inventory™
PedsQL™4.0 Core	Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0
PGI-C	Patient Global Impression–Change
PGI-S	Patient Global Impression–Severity
PK	pharmacokinetic
PRO	patient-reported outcome
PSQI	Pittsburgh Sleep Quality Index
QD	once a day
QTcF	QT interval corrected through use of Fridericia's formula
RBR	Research Biosample Repository
RBS-R	Repetitive Behavior Scale–Revised
ROW	rest of the world
SAP	Statistical Analysis Plan
SRS™-2	Social Responsiveness Scale™, Second Edition
ULN	upper limit of normal
V1a	vasopressin 1a
V1b	vasopressin *1b*
V2	vasopressin 2
Vineland™-II	Vineland™ Adaptive Behavior Scales, Second Edition
VPA	valproic acid

Abbreviation	*Definition*
WAIS®-IV	*Wechsler Adult Intelligence Scale®, Fourth Edition*
WASI®-II	Wechsler Abbreviated Scale of Intelligence®, Second Edition
WGS	whole genome sequencing

1. BACKGROUND

1.1 BACKGROUND ON AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and social interaction and repetitive patterns of behaviors, interests, or activities. The prevalence of ASD is between 1 in 50 and 1 in 88 children (Levy et al. 2009; Centers for Disease Control and Prevention [CDC] 2012) and is approximately 1 in 42 boys and 1 in 189 girls (CDC 2016). Core symptoms of ASD are usually observed by 3 years of age, although typical language development might delay identification of symptoms. It is estimated that approximately 1.2% of the overall population in the United States lives with ASD (Buescher et al. 2014), and it has been reported to be similar in the population in the United Kingdom (Brugha et al. 2011). Diagnosis rates in the United Kingdom for ASD in children are currently estimated to be 1.1% of the total population, thus suggesting that the prevalence rates between adults and children do not differ. ASD is a disorder diagnosed in children that continues to have a life-long burden that follows the patient from childhood throughout adulthood.

Although initial symptoms may be identified between 12 and 24 months, typically ASD does not manifest as a formal diagnosis, and even if a diagnosis is made, this may not be stable (Kleinman et al. 2008; Chawarska et al. 2009). Deficits in social interaction manifest themselves as impaired use of non-verbal communication, delayed and reduced interactions with peers, absent sharing of enjoyable experiences and interest with peers, and lack of social judgment. Abnormalities in communication include a delay in verbal language development, impaired expressive language, deficient language pragmatics, as well as stereotyped, repetitive, or idiosyncratic use of language. Females with impairments in social and communication function are typically more intellectually impaired (Fombonne et al. 2011; Stacy et al. 2014). Stereotyped and repetitive behavior manifests as a preoccupation with stereotyped or restricted interests, adherence to routines, rigidity, perseverative, motor mannerisms, and preoccupation or fascination with parts of items, and unusual visual exploration.

In addition to these core deficits, individuals with ASD suffer from a range of co-morbid conditions, including irritability, depression or anxiety, attention deficits, obsessive-compulsive symptoms, seizures, and sleep disruption. Furthermore, adults with ASD are at increased risk of suicide compared with the general population. Individuals with ASD die on average 18 years before the general population (Hirvikoski et al. 2016). In addition, autistic adults without a learning disability were 9 times more likely to die by suicide. This could be a reflection of the isolation and depression many people with ASD experience as data show that approximately 35% of young adults (ages 19–23 years) with ASD are unemployed or do not complete postgraduate education after leaving high school (Shattuck et al. 2012).

The etiology of ASD is highly genetic, although environmental factors also contribute. Heritability estimates from family and twin studies suggest that about 90% of variance can be attributed to genetic factors (Levy et al. 2009).

At present, no pharmacological treatment exists for the core deficits of ASD, and currently available treatments address only associated behavioral problems (Wink et al. 2010). Non-pharmacological treatments have been developed to address the core symptoms; however, efficacy has not been proven in large clinical trials (Warren et al. 2011).

The hypothalamic neuropeptides vasopressin (also known as anti-diuretic hormone) and oxytocin, in addition to their well-defined roles in the control of osmotic balance and in reproduction, appear to have prominent roles in the regulation of higher brain functions, such as learning and memory, emotional control, and social behaviors. Vasopressin mediates its effect via vasopressin receptors (vasopressin 1a [V1a], vasopressin 1b [V1b], and vasopressin 2 [V2]), which are all members of the G protein–coupled receptor family. V1a and V1b lead to intracellular increases in calcium through the phosphatidyl-inositol pathway, whereas V2 is coupled to adenylyl-cyclase and cyclic adenosine monophosphate production. V1a receptors are the primary subtype found in the CNS, expressed in several areas of the limbic system (hypothalamus, septum, hippocampus, and amygdala) but are also present in several tissues (vascular smooth muscle, liver, kidney, platelets, and spleen) (Loup et al. 1991; Ostrowski et al. 1994; Ostrowski 1998). V1b receptors are also present in several brain regions but appear to be the most important for the increase in corticotropin-releasing hormone–induced adrenocorticotropic hormone secretion. V2 receptors are present in the renal collecting duct and mediate the antidiuretic effects of vasopressin.

Studies in animals and humans have implicated the vasopressin system in the modulation of behaviors related to both core and associated symptoms of ASD. In non-human mammals, V1a receptors are distributed in brain regions associated with control of stress and anxiety and social and affiliative behaviors, including parental care, pair-bonding, social memory, and social aggression. Vasopressin levels have been shown to be elevated during stress, as induced by the forced swim test in rats (Ebner et al. 2002). Central administration of a V1 peptide antagonist has shown anxiolytic effects in an elevated plus-maze test, a standard animal model of anxiety (Liebsch et al. 1996), and antidepressant-like effects in the forced swim test, a model of depressive behavior. Similarly, V1a receptor knockout mice also show reduced anxiety in open-field, light-dark box, and elevated plus-maze tests (Bielsky et al. 2004; Egashira et al. 2007). In addition, central injection of arginine vasopressin (AVP) in rodents (voles and hamsters) has been shown to induce offensive aggressive behavior (Winslow et al. 1993; Delville et al. 1996), which can be prevented by a V1a receptor antagonist (Ferris et al. 2006). Scratching and grooming, reminiscent of obsessive-compulsive behavior, can also be observed in mice after central injection of vasopressin (Meisenberg 1988).

In humans, support for a role of the vasopressin system in ASD is provided by studies on the arginine vasopressin receptor 1A (*AVPR1A*) gene that encodes the V1a receptor and is located on chromosome 12q. Multiple studies have shown genetic associations of the *AVPR1A* gene with ASD, mainly with genetic markers in the promoter region of the gene, which includes microsatellites. However, it is unclear which microsatellite alleles show association with ASD or specific clinical phenotypes (Kantojärvi et al. 2015). Consistent with behavioral studies in animals (see above), these risk alleles have been found to modulate activation of the amygdala during emotional face processing (Meyer-Lindenberg et al. 2009) and to be associated with specific personality traits in healthy volunteers (Ebstein et al. 2012). Similarly, intranasal administration of vasopressin was shown to modulate the activity of a network involved in the processing of emotional information with specific effects in the subgenual cingulate regions (Zink et al. 2010).

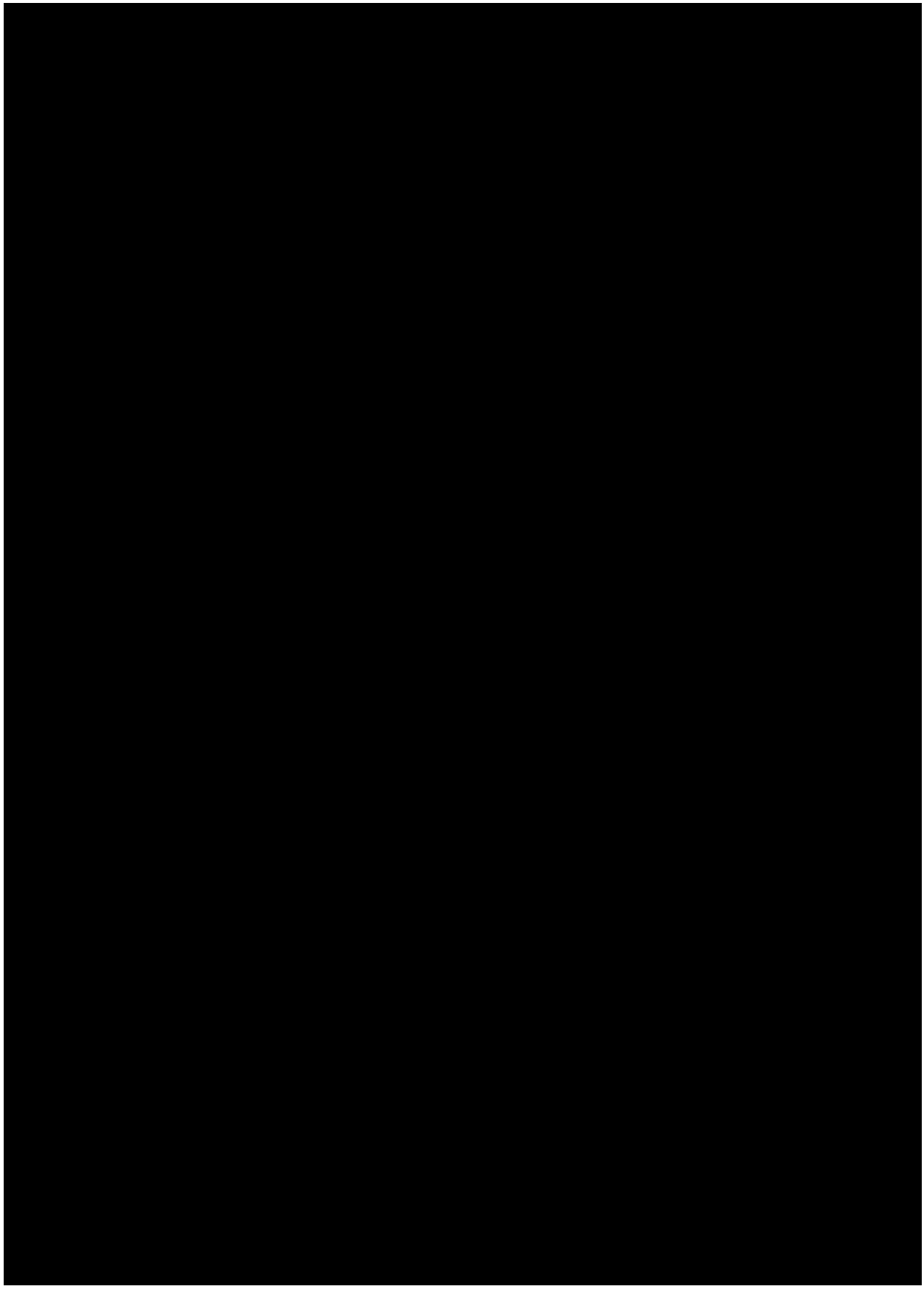
Additional evidence for a role of vasopressin in modulating behaviors of relevance to ASD is provided by studies showing increased cerebrospinal levels of vasopressin in obsessive-compulsive disorder and aggressive behavior (Zink et al. 2010).
Also, increased levels of AVP in plasma of subjects with ASD have been reported (Boso et al. 2007), although other researchers have been unable to replicate this observation.

In summary, there is a high unmet medical need for pharmacological treatments of these core symptoms of the disorder.

1.2 BACKGROUND ON BALOVAPTAN

Balovaptan is a potent and highly selective human V1a receptor antagonist that blocks the activation of the V1a G protein–coupled receptor.

[redacted]



[redacted]

1.2.2 Clinical Studies

1.2.2.1 Safety and Tolerability

To date, balovaptan has been investigated in five completed Phase I studies in healthy volunteers (Studies BP25694, BP28318, BP28977, BP29279, and WP40038), and one completed proof-of-mechanism study (BP29412). *Clinical pharmacology studies investigating the effect of CYP3A modulators on the pharmacokinetics of multiple balovaptan doses (WP40608 and WP40609) are clinically complete. Please refer to the Balovaptan Investigator's Brochure for detailed information.*

A Phase II study (BP28420, "Vanilla") in adult subjects with ASD has been completed and the recruitment for a Phase II study (BP30153, "aV1ation") in 5- to 17-year-old subjects with ASD was started in November 2016 and is ongoing.

Study BP28420 was a proof-of-concept, randomized, double-blind, parallel-group study that evaluated the efficacy, and safety of 1.5, 4, and 10 mg/day of balovaptan compared with placebo in male adults (aged 18–45 years) with ASD. The scheduled duration of treatment was 12 weeks. Key inclusion criteria included a Social Responsiveness Scale™, Second Edition (SRS™-2) total *t*-score ≥66, a Clinical Global Impression–Severity (CGI-S) Scale score ≥4 (moderately ill), and an intelligence quotient (IQ) score ≥70 generated on the Wechsler Abbreviated Scale of Intelligence®, Second Edition (WASI®-II).

The study was conducted in a staged fashion as outlined in Figure 1.

Figure 1 Study BP28420: Overview of Study Design

Note: RO5285119 is the former name for balovaptan.

The primary endpoint was the change from baseline in social communication deficits in adult individuals with ASD, as measured by the SRS-2. In addition, a number of secondary efficacy endpoints were also evaluated, including the change from baseline in adaptive functioning and skills, as measured by the Vineland™ Adaptive Behavior Scales, Second Edition (Vineland™-II), change from baseline in behavior and symptoms

as measured by the Aberrant Behavior Checklist (ABC), the Repetitive Behavior Scale–Revised (RBS-R), the Anxiety, Depression, and Mood Scale (ADAMS), and the Clinical Global Impressions–Improvement (CGI-I).

In all, 223 subjects were enrolled in the study and 186 subjects completed the study. Of the 186 subjects who completed the study, 67 subjects received placebo, 26 received 1.5 mg once a day (QD), 69 subjects received 4 mg QD, and 30 subjects received 10 mg QD. Two hundred thirteen subjects were included in the intent-to-treat (ITT) analysis dataset.

The primary efficacy endpoint was the change from baseline in social communication deficits, as measured by the SRS-2 total *t*-score after 12 weeks of treatment. The data analysis showed no significant treatment effects on the SRS-2 total *t*-score. The estimated effects for placebo in the three analysis datasets were –13.1, –11.4, and –8.3, which are in agreement with the placebo effect observed in recent trials (Berry-Kravis et al. 2016). An effect size of –0.3 favoring balovaptan was observed between 10 mg and placebo.

Several assessments were analyzed as secondary outcome measures of core symptoms, including the following: the Vineland-II Adaptive Behavior Standard Composite score (hereafter referred to as Vineland-II composite score), the ABC, CGI-I, RBS-R, and ADAMS.

The outcome of the assessment of the Vineland-II Composite score demonstrated a dose-dependent improvement in the composite score. The three individual domains of the Vineland-II Composite were also analyzed. Results show meaningful treatment effects, especially with the 10-mg dose, for the Socialization and Communication domains.

Balovaptan appeared to be safe and well tolerated in adult male subjects with ASD. No treatment-emergent safety concerns were identified. [redacted]

A total of eight serious adverse events were reported for 4 subjects in Study BP28420. The serious adverse events included sinus node dysfunction (at the 1.5-mg dose), syncope (4-mg dose), and suicidal ideation (placebo). One subject (in the 1.5-mg arm) experienced four serious adverse events: two episodes of rhabdomyolysis, acute psychosis, and agitation. No serious adverse events were reported in the 10-mg/day balovaptan treatment group. One death due to suspected heart failure was reported 16 weeks after treatment was withdrawn in the individual with the reported serious adverse event of syncope. Except for the case of rhabdomyolysis, all other serious adverse events, including the syncopal event and death due to suspected heart failure, were assessed by the respective investigators as not related to study treatment.

Additional information is provided in Section 5.1 of the Balovaptan Investigator's Brochure.

1.2.2.2 Pharmacokinetics

In a single-ascending dose study, exposure to balovaptan increased in a greater than dose-proportional manner following doses of 0.5–76 mg, whereas an approximately linear increase in exposure was observed after repeated dosing with 12–52 mg/day for 14 days. Balovaptan was rapidly absorbed with a median time to maximum concentration between 1 and 4.5 hours after administration of single doses and between 3 and 4 hours after multiple doses. Steady state was achieved after approximately 7 days of daily dosing. Food had no effect on the pharmacokinetics of balovaptan.

Renal excretion is the major pathway of elimination (approximately 53% of the drug material recovered), with most of the drug-related material in urine being composed of metabolites. An additional 30% of the administered dose was recovered in feces.

2. OBJECTIVES AND ENDPOINTS

This study will evaluate the efficacy, safety, and pharmacokinetics of 10 mg of oral administration balovaptan QD compared with matching placebo in adults (18 years and older) with ASD. Specific objectives and corresponding endpoints for the study are outlined below.

Table 1 Objectives and Corresponding Endpoints

Objective(s)	Corresponding Endpoint(s)
Primary Efficacy Objective	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo	• Change from baseline at Week 24 on the Vineland™-II 2DC score (defined as the mean of the Communication domain standard score and the Socialization domain standard score)
Secondary Efficacy Objectives	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo	• Change from baseline at Week 12 on the Vineland-II 2DC score • Change from baseline at Weeks 12 and 24 in the PedsQL™ Core module, Version 4.0, on summary and total scores • Change from baseline at Weeks 12 and 24 in the Vineland-II A composite standard score • Change from baseline at Weeks 12 and 24 in the following: – Vineland-II Socialization domain standard score – Vineland-II Communication domain standard score – Vineland-II Daily Living Skills domain standard score

2DC=two-domain composite; ASD=autism spectrum disorder; $AUC_{0\text{-}24,\ ss}$=area under the concentration–time curve from Time 0 to 24 hours at steady state; *AVPR1A*=arginine vasopressin receptor 1A; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impressions–Improvement; CGI-S=Clinical Global Impressions–Severity; $C_{max,\ ss}$=maximum concentration at steady state; *CNTNAP2*=contactin-associated protein-like 2; C-SSRS=Columbia-Suicide Severity Rating Scale; $C_{trough,\ ss}$=trough concentration at steady state; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

Table 1 Objectives and Corresponding Endpoints (cont.)

Objectives	Corresponding Endpoint(s)
Secondary Efficacy Objectives (cont.)	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo (cont.)	• Change from baseline in severity of clinical impressions as measured by CGI-S after 12 weeks and 24 weeks of treatment • Improvements in clinical impressions, as measured by CGI-I after 12 weeks and 24 weeks of treatment • Change from baseline in the HAM-A total and domain scores at Weeks 12 and 24 • Proportion of subjects with a ≥6-point improvement in Vineland-II 2DC score at Weeks 12 and 24
Exploratory Efficacy Objectives	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo	• Change from baseline at Weeks 12 and 24 on the following scales: – PedsQL™ Family Impact to assess impact on study partner and family (summary and total scores) – PedsQL™ Cognitive Functioning Scale to assess impact on cognitive functioning (summary and total scores) – RBS-R scores (summary and total scores) • Proportion of subjects with a ≥4-point improvement in Vineland-II 2DC score • Proportion of subjects with a ≥8-point improvement in Vineland-II 2DC score • Proportion of subjects at each score level of PGI-S and PGI-C • Proportion of subjects at each score level of CaGI-S and CaGI-C, as perceived by the study partner

2DC=two-domain composite; ASD=autism spectrum disorder; $AUC_{0\text{-}24,\ ss}$=area under the concentration–time curve from Time 0 to 24 hours at steady state; *AVPR1A*=arginine vasopressin receptor 1A; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impressions–Improvement; CGI-S=Clinical Global Impressions–Severity; $C_{max,\ ss}$=maximum concentration at steady state; *CNTNAP2*=contactin-associated protein-like 2; C-SSRS=Columbia-Suicide Severity Rating Scale; $C_{trough,\ ss}$=trough concentration at steady state; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

Table 1 Objectives and Corresponding Endpoints (cont.)

Objective	Corresponding Endpoint(s)
Exploratory Efficacy Objectives (cont.)	
• To evaluate the efficacy of 10 mg of balovaptan compared with placebo (cont.)	• Change from baseline at Weeks 12 and 24 on the following scales: – EQ-5D-5L score on the following: EQ-5D-5L (self-report version) completed by the study participant EQ-5D-5L (self-report version) completed by the study partner – PSQI total score • [redacted]
Safety Objective	
• To evaluate the safety of 10 mg of balovaptan compared with placebo	Safety will be assessed through the following: • Occurrence, nature, and intensity of adverse events, serious adverse events, and non-serious adverse events of special interest, as determined using the Adverse Event Severity Grading Scale • Physical and neurologic examinations, vital signs, hematology, blood chemistry, and urinalyses • [redacted] • C-SSRS

2DC=two-domain composite; ASD=autism spectrum disorder; $AUC_{0\text{-}24,\ ss}$=area under the concentration–time curve from Time 0 to 24 hours at steady state; *AVPR1A*=arginine vasopressin receptor 1A; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impressions–Improvement; CGI-S=Clinical Global Impressions–Severity; $C_{max,\ ss}$=maximum concentration at steady state; *CNTNAP2*=contactin-associated protein-like 2; C-SSRS=Columbia-Suicide Severity Rating Scale; $C_{trough,\ ss}$=trough concentration at steady state; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

Table 1 Objectives and Corresponding Endpoints (cont.)

Objectives	Corresponding Endpoint(s)
Pharmacokinetic and Pharmacodynamic Objectives	
• To characterize the plasma pharmacokinetics of balovaptan and balovaptan related metabolites	• The exposure at steady state (e.g., $AUC_{0\text{-}24ss}$, $C_{max,ss}$, $C_{trough,ss}$, and other variables) for balovaptan and its major metabolites, as applicable • Population-PK parameters and variability estimates for balovaptan • The observed plasma concentrations of balovaptan and its metabolites M3 and M2 (as applicable) (and other metabolites as appropriate) summarized by observation time, and the ratios of metabolite to parent drug concentration at trough
• To explore the exposure–response and safety relationships of balovaptan	• Exploratory graphical analysis of Vineland-II 2DC and selected other clinical efficacy and safety endpoints
Exploratory Biomarker Objective	
• To evaluate biomarkers that may be predictive of benefit from balovaptan	• Change from baseline in efficacy, safety, PK, immunogenicity, or other biomarker endpoints according to genetic variants in the *AVPR1A* or *CNTNAP2* gene, performed on DNA extracted from blood

2DC=two-domain composite; ASD=autism spectrum disorder; $AUC_{0\text{-}24,\ ss}$=area under the concentration–time curve from Time 0 to 24 hours at steady state; *AVPR1A*=arginine vasopressin receptor 1A; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impressions–Improvement; CGI-S=Clinical Global Impressions–Severity; $C_{max,\ ss}$=maximum concentration at steady state; *CNTNAP2*=contactin-associated protein-like 2; C-SSRS=Columbia-Suicide Severity Rating Scale; $C_{trough,\ ss}$=trough concentration at steady state; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

The proposed biomarkers for exploratory research are presented in Table 2.

Table 2 Proposed Biomarkers for Exploratory Research

Sample Type	Timing	Proposed Biomarkers
Clinical genotyping of DNA extracted from blood	Baseline	Polymorphisms of the *AVPR1A* receptor gene (microsatellites RS1, AVR, and rs1587097) and the *CNTNAP2* gene (rs7456839)

AVPR1A=arginine vasopressin receptor 1A; *CNTNAP2*=contactin-associated protein–like 2.

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

Study WN39434 is a Phase III, randomized, double-blind, placebo-controlled, parallel-group, multicenter, 24-week, efficacy, and safety study of 10 mg of oral balovaptan QD in adults (18 years old or older) with ASD, followed by a 2-year open-label extension period for subjects completing the double-blind treatment period.

Subjects providing informed consent will undergo screening within 4 weeks prior to the first double-blind study drug (balovaptan or placebo) administration. Eligible subjects will be randomized in a 1:1 ratio in a blinded fashion to either 10 mg of balovaptan or matching oral placebo QD for 24 weeks. The primary efficacy endpoint is the change from baseline at Week 24 on the Vineland-II two-domain composite (2DC) score.

Thus, for subjects remaining in the full study, the approximate length of the study will be 144 weeks or 34 months (screening [approximately 4 weeks], double-blind treatment [24 weeks], and open-label extension period [104 weeks] with a safety follow-up period of 12 weeks after completion of double-blind treatment period or open-label extension period).

Subjects will be recruited from international sites, including sites in North America and limited rest of the world (ROW) countries. Subjects who prematurely discontinue from study treatment or from the study will not be replaced. However, all subjects should be followed and their data (specifically, vital sign measurements, adverse events, concomitant treatment, Vineland-II, CGI-S, CGI-I, PedsQL 4.0 Core) collected up to and including the Week 24 visit, regardless of their adherence to treatment.

Randomization will be stratified by baseline 2DC score of the Vineland-II score ($<$60 vs. $\geq$60), sex (male vs. female), geographical region (North America vs. ROW), and age ($<$25 years vs. $\geq$25 years). The main analysis of the primary and secondary efficacy endpoints will occur once the final data from all visits, up to and including Week 24, have been collected and cleaned, and the database has been locked. An interim futility analysis $will$ be performed by an independent Data Monitoring Committee (iDMC) when approximately 50% of subjects complete the Week 24 visit.

The iDMC will meet regularly to oversee safety throughout the trial and its activities will be described in a separate iDMC charter (see Section 3.1.1.5 for further details).

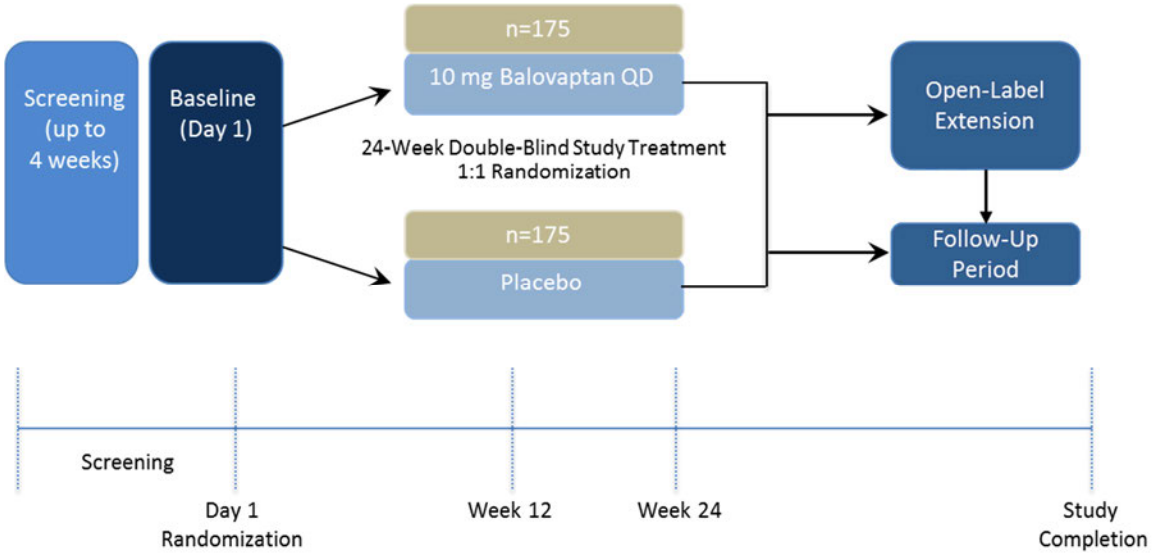
Secondary and exploratory endpoints will examine core autism symptoms (social interaction, social communication, and functional deficits), associated symptoms, impact on health-related quality of life (HRQoL), and broader impacts on study partners and family. Safety will be examined via adverse events, clinical laboratory values, electrocardiograms (ECGs), physical and neurologic examinations, and safety outcome assessments such as suicidality.

Any substudies associated with Study WN39434 will be detailed in separate protocols, with the exception of the Granulocyte Immunofluorescence Test (GIFT) substudy, which is presented in Appendix 6.

3.1.1 Overview of Study Design

This study will enroll approximately 350 adult subjects (18 years or older) to either placebo or active treatment in a 1:1 ratio. All subjects completing 24 weeks of double-blind treatment will be eligible to enter the open-label treatment period with 10 mg of balovaptan QD (see Figure 2).

Figure 2 Study Schema



QD=once a day.

A schedule of activities for the double-blind and open-label extension treatment periods are provided in Appendix 1 and Appendix 3, respectively. The schedule of activities for the follow-up period is provided in Appendix 2.

The total duration of the study (from screening through to study completion) for each subject will be approximately 144 weeks as described in the following sections.

3.1.1.1 Screening Period

Subjects who are willing and who have given their consent to participate in the study will undergo a screening procedure within 4 weeks before the first study drug administration (Day 1). Subjects must meet all of the eligibility criteria in order to qualify for the study (see Sections 4.1.1 and 4.1.2).

The screening period can be extended to a total period of 6 weeks in cases when a laboratory blood test needs to be repeated for confirmation during the screening interval

or for other relevant clinical, administrative, or operational reasons. Subjects must fulfill all the entry criteria for participation in the study.

3.1.1.2 Re-Screening

In exceptional cases, subjects not meeting the eligibility criteria can be re-screened once and uncertainties *should* be discussed with the Medical Monitor or designee. Re-screening will not be allowed if a subject previously does not meet the disease-specific inclusion criteria (e.g., Autism Diagnostic Observation Schedule™, Second Edition [ADOS™-2] score*, SRS, WASI-II). In cases where re-screening occurs, these assessments should not be repeated.*

3.1.1.3 Double-Blind Treatment Period

All subjects will undergo 24 weeks of double-blind treatment. The study will be unblinded following the primary database lock when the last enrolled subject has completed the double-blind treatment period and all the data have been collected and cleaned.

Randomization (Day 1) will occur only after a subject has met all eligibility criteria. At the baseline visit (Day 1), subjects will undergo a series of assessments outlined in the schedule of activities (see Appendix 1). Breaks should be allowed between tests when necessary. If necessary, the baseline visit can be split into 2 consecutive days, and the second day will be considered the baseline visit date (Day 1). If either the Week 12 or Week 24 visit is split over 2 days, study drug (balovaptan or placebo) must be administered QD after the completion of all assessments.

After the predose assessments have been completed, each subject will receive an oral dose of balovaptan or placebo according to randomization. After completion of the postdose evaluations and following a medical check-up by a physician, the subject will be discharged from the study center.

Although study visits will be conducted on site, checks for signs and symptoms of infection will be conducted every 2 weeks either at a site visit or by telephone call conducted by site staff.

As a general rule, the test sequence for all assessments should remain the same for a given subject as established at screening and baseline. At all visits, patient-reported outcome (PRO) assessments should be completed prior to any other assessments. All assessments must be performed before administration of study drug.

For informant-based assessments, the following order of assessments will be followed:

- The Vineland-II will be the first informant-based assessment with a subject's study partner.
- For all other informant-based assessments: It is strongly recommended that the sequence provided in Table 3 is followed. It will be possible for assessments

(e.g., the Vineland-II interview and subject assessments) to be conducted in parallel if the site has sufficient qualified raters available. Importantly, the sequence established at screening and baseline should remain the same for a given subject; however, subject assessments should be prioritized.

Table 3 Sequence of Scale Administration

Order	Scale	Respondent(s)		
1	Vineland-II		Study partner	Qualified rater
2	PedsQL 4.0 Core	Subject		
3	PedsQL Cognitive Functioning	Subject		
4	PSQI	Subject		
5	EQ-5D-5L	Subject		
6	PGI-S	Subject		
7	PGI-C	Subject		
8	HAM-A	Subject		Qualified rater
9	C-SSRS	Subject		Qualified rater
10	PedsQL Family Impact		Study partner	
11	RBS-R		Study partner	
12	EQ-5D-5L		Study partner	
13	CaGI-S		Study partner	
14	CaGI-C		Study partner	
15	CGI-S			Qualified rater
16	CGI-I			Qualified rater

CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impression–Improvement; CGI-S=Clinical Global Impression–Severity; C-SSRS=Columbia-Suicide Severity Rating Scale; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; PedsQL=Pediatric Quality of Life Inventory™; PedsQL 4.0 Core=Pediatric Quality of Life Inventory Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; Vineland-II=Vineland Adaptive Behavior Scales, Second Edition.

The last assessments to be completed are the Clinical Global Impression scales.

At visits when blood samples are obtained, the order of assessments should be as follows:

- 12-Lead ECGs
- Vital signs
- Physical and neurologic examinations
- Blood samples

Note: ECGs and vital signs assessments should be completed before other invasive assessments such as blood samples and should occur following a short period when the study participant is able to rest (typically, at least 5 minutes).

3.1.1.4 Open-Label Extension Period

All subjects who complete the 24-week double-blind treatment period are eligible for participation in the open-label extension period of the study. Subjects who are not willing to participate in the open-label extension period of the study will complete the safety follow-up visits. Subject treatment allocation during the double-blind treatment period will not be unblinded regardless of a subject's participation in the open-label extension period.

The 2-year (104-week) duration of the open-label extension period serves to evaluate the long-term safety, tolerability, and efficacy of balovaptan treatment in subjects with ASD. All subjects will receive 10 mg of oral balovaptan QD. For the schedule of activities to be performed during the open-label extension, see Appendix 3.

After the predose assessments have been completed, each subject will receive an oral dose of balovaptan. After completion of the postdose evaluations and following a medical check-up by a physician, the subject will be discharged from the study center. If the visit is split over 2 days, study drug (balovaptan) must be administered QD after the completion of all assessments.

Checks for signs and symptoms of infection will be conducted according to the schedule of activities.

As a general rule, the test sequence for all assessments should remain the same for a given subject as previously established at screening and baseline. At all visits, PRO assessments should be completed prior to any other assessments. All assessments must be performed before administration of study drug.

For informant-based assessments, the following order of assessments will be followed:

- The Vineland-II will be the first informant-based assessment with a subject's study partner.
- For all other informant-based assessments, there is no restriction in the order, a sequence established at screening and baseline should remain the same for a given subject; however, subject assessments should be prioritized. The suggested sequence is provided in Table 3. It will be possible for assessments (e.g., the Vineland-II interview and subject assessments) to be conducted in parallel if the site has sufficient qualified raters available.

The last assessments to be completed are the Clinical Global Impression scales.

At visits when blood samples are obtained, the order of assessments should be as follows:

- 12-Lead ECGs
- Vital signs
- Physical and neurologic examinations
- Blood samples

Note: ECGs and vital signs assessments should be completed before other invasive assessments such as blood samples and should occur following a short period when the study participant is able to rest (typically, at least 5 minutes).

3.1.1.5 Follow-Up Period

In order to evaluate the long-term effects of balovaptan, subjects will enter a follow-up period, consisting of a telephone call at Week 1 and site visits at Weeks 2 and 12. The final follow-up visit for safety and limited efficacy will be conducted 12 weeks after the *Week 24 visit* for subjects who do not enter the open-label extension period or at the end of the open-label extension period for all other subjects. *For subjects who discontinue treatment prematurely and will not return for the Week 24 visit, the final follow-up visit should occur 12 weeks after the final dose.* For the list of assessments, see Appendix 2.

3.1.1.6 Independent Data Monitoring Committee

The incidence and nature of adverse events, serious adverse events, adverse events of special interest, abnormalities from ECG recordings, vital sign assessments, laboratory parameters, as well as the results of neurologic examinations will be assessed on a regular basis by an iDMC. It is anticipated that these assessments will occur approximately every 6 months or at the discretion of the iDMC.

The iDMC will also undertake evaluation of any efficacy interim analyses (see Section 6.5.9).

Details about the iDMC will be provided in the iDMC Charter.

3.2 END OF STUDY AND LENGTH OF STUDY

The end of the double-blind treatment period of the study is defined as the time when subjects have either transitioned to the open-label extension period or, for subjects not entering the open-label extension period, when the follow-up period has been completed.

The end of study is defined as the date when the last subject, last visit occurs in the open-label extension period or the follow-up period.

It is anticipated that it may take from 18 to 28 months to recruit all subjects for the study. Hence, the total length of the study is estimated to be approximately 4–5 years.

3.3 RATIONALE FOR STUDY DESIGN

3.3.1 Rationale for Balovaptan Dose and Schedule

The double-blind treatment period of this study will allow the evaluation of the safety and efficacy of 10 mg of oral balovaptan tablets QD compared with placebo.

Results from Study BP28420 suggest that 10 mg of balovaptan QD is the only dose level of the dose levels tested to have shown consistent efficacy across multiple domains of the Vineland-II and on other instruments explored. The safety profile of balovaptan across dose cohorts was similar.

The 10-mg balovaptan QD dose was associated with the largest improvement on the 2DC score of the Vineland-II, as well as individually on the Vineland-II Composite and on the Socialization and Communication domains of the Vineland-II scales in Study BP28420. The 10-mg dose was also associated with greater concordance across the domains of the Vineland-II compared with other doses.

Furthermore, a graphical evaluation of patient-level responses in Study BP28420 shows that the 10-mg balovaptan QD dose was associated with a greater proportion of positive response outcomes on the Vineland-II Composite score compared with either of the other two doses tested. Although the 4-mg balovaptan QD dose group had more subjects and resulted in a meaningful effect on the Vineland-II Composite score, the outcomes were overall less consistent than those observed with the 10-mg QD dose of balovaptan.

Additional support for the use of a single-dose arm of 10 mg of balovaptan QD comes from the PedsQL 4.0 Core results in Study BP28420. The PedsQL 4.0 Core is a generic assessment of HRQoL for children, adolescents, and adults (Varni et al. 1999, 2001) and provides subject insights into perceived treatment benefit (self-reported assessment), whereas the Vineland-II Composite offers the external perspective provided by a study partner during the structured interview and completion of the survey form. The PedsQL 4.0 Core was an exploratory measure in Study BP28420. Clinically meaningful improvements, as measured by the PedsQL 4.0 Core, were seen only with the 10-mg QD dose, which produced a clinically relevant change above the minimal clinically important difference threshold of 4.4 points on self-report at Week 12 (Day 84) (Varni et al. 2003).

[redacted]

[redacted]

Furthermore, the Sponsor's internal study data suggest that occupancy of the V1a receptor reaches near maximal saturation with the 10-mg dose of balovaptan.

3.3.2 Rationale for Use of Vineland-II Adaptive Behavior Scales 2DC Score as the Primary Efficacy Endpoint

The Vineland-II has been used extensively in ASD research and includes standardization and validation in diverse populations, including in individuals with autism, Asperger syndrome, or pervasive developmental disorder (Sparrow et al. 2005; Dawson et al. 2010; Virues-Ortega et al. 2013; Aman et al. 2015). A recent Autism Speaks–sponsored Working Group panel recommended the Vineland-II scales as suitable (adequate reliability and validity and responsiveness to intervention) to quantify social communication deficits in clinical trials of subjects with ASD (Anagnostou et al. 2015). The Vineland-II is organized into five domains measuring adaptive behavior of individuals from birth to 90 years old, including assessment of the performance of daily activities required for personal and social sufficiency (Sparrow et al. 2005). To support reliability in interview conduct and structure, the Vineland-II Survey Interview, which is a semi-structured interview of the study partner administered by a trained interviewer and takes approximately 45–60 minutes to complete, will be used. The Vineland-II will be administered and scored according to the manual (Sparrow et al. 2005).

In this study, a novel composite score, the Vineland-II 2DC score, has been selected as the primary endpoint, defined as the arithmetic mean of the Communication domain standard score and the Socialization domain standard score. Concepts measured in the Communication and Socialization domains of the Vineland-II map closely to the respective communication and socialization deficits in the subject-centered conceptual model of ASD generated through interviews conducted with people with ASD and their parents (Willgoss et al. 2017). As such, the Vineland-II 2DC score enables a comprehensive assessment of social communication deficits in people with ASD. Vineland-II Socialization and Communication domain standard scores are reliable and valid (Sparrow et al. 2005) and have been used independently as endpoints in clinical trials of ASD (Scahill et al. 2016). To explore the measurement properties of the new Vineland-II 2DC score, which combines these two independently validated scales into a single score, the Sponsor has conducted a psychometric analysis of the score using data from Study BP28420 (Roche internal data). The data show that the Vineland-II 2DC score is reliable, valid, and sensitive to change and supports the Sponsor's

proposal to combine the two domains into a single endpoint. Furthermore, the data were discussed with the U.S. Food and Drug Administration (FDA) and European Medicines Agency who have provisionally approved the use of the Vineland-II 2DC score as the primary endpoint for Phase III trials.

3.3.3 Rationale for Use of Responder Definition

A clinically meaningful response is defined as a ≥6-point improvement on the Vineland-II 2DC score at Week 24. This responder threshold was determined through anchor- and distribution-based analyses conducted on data from Study BP28420. Estimates anchored to global clinical impressions of verbal communication, non-verbal communication, social skills, and overall ASD severity ranged from 6.0 to 7.2 points. Distribution-based estimates included 0.2 SD and 0.5 SD at baseline and 1× the standard error of the mean and ranged from 3.16 to 7.90 points. A threshold of ≥6 points was selected following discussions with a panel of global ASD experts who agreed that individual change of this magnitude over a 24-week period would be indicative of a clinically relevant response to treatment.

3.3.4 Rationale for Subject Population

Study WN39434 will include both males and females. The prevalence of ASD has been reported to be approximately 1 in 42 boys and 1 in 189 girls (CDC 2016). Furthermore, females with impairments in social and communication function are typically more intellectually impaired and are less likely to meet the eligibility criteria stipulating an IQ score ≥70; only 1 in 8 to 9 ASD subjects with IQ score ≥70 is reported to be female (Fombonne et al. 2011; Stacy et al. 2014). Therefore, the proportion of females who would be recruited is expected to be approximately 12% of the total study population. To ensure that the subject population is representative of ASD epidemiology the proportion of female subjects will be limited to a maximum of 20% of the entire study population. Randomization of subjects will be stratified by sex.

Study WN39434 is designed as an international study that will recruit subjects from regions, including North America and the ROW. The proportion of subjects from the ROW is expected to be approximately 20% of the total study population. Randomization of subjects will be stratified by region (North America vs. ROW) to increase the reliability of subgroup analyses within each region.

To limit the impact of diagnostic heterogeneity, eligible male and female subjects ages ≥18 must meet Diagnostic and Statistical Manual of Mental Disorders, Version 5 (DSM-5) criteria for ASD, which will be confirmed using the ADOS-2. The ADOS-2 is a semi-structured, standardized assessment of communication, social interaction, play/imaginative use of materials, and restricted and repetitive behaviors in ASD and is referred to as the "gold standard" observational assessment for diagnosing ASD (Ozonoff et al. 2005; Kanne et al. 2008).

Subjects with confirmed ASD who are exhibiting impairments in social communication will be studied. For this study, the SRS-2 will be performed during screening; only subjects with a study partner–completed (proxy) SRS-2 total *t*-score ≥66, consistent with moderate or severe symptoms, will be randomized. These assessments ensure that subjects demonstrate relevant deficits in social communication and social interactions and that, collectively, these symptoms result in a moderate-to-severe phenotype.

Another dimension along which subjects are commonly categorized is the degree to which intellectual disability is co-morbid with their ASD symptoms. From an assessment framework, there is no consensus on which cognitive measures are the most useful for subject categorization. In this study, the WASI®-II will be employed to ensure randomized subjects score above a "cognitive floor" (i.e., IQ score ≥70) and do not have intellectual disability, consistent with the ASD literature (Siegel et al. 1996). Full scale IQ and subscale scores will be collected.

3.3.5 Rationale for Use of Placebo

To date, no pharmacological therapies have shown effectiveness treating the core symptoms of ASD and none is registered for its treatment in any global region. Pursuant to the Helsinki Declaration, when standard treatment of a disease exists, placebo should generally not be used in clinical trials (ICH 2000). However, Paragraph 33 of the Declaration supports the stance that placebo can be acceptable in the setting where no proven standard therapy exists.

Given that no standard therapy exists for the treatment of ASD, a placebo-controlled trial is acceptable, provided that appropriate subject consent and safeguards are instituted to minimize the risk of serious or irreversible harm resulting from exposure to placebo.

3.3.6 Rationale for Study Duration

There are no published studies showing significant pharmacological effects on communication and social behaviors in ASD as measured by the Vineland-II. Nevertheless, behavioral interventions have shown benefits. For example, Virues-Ortega and colleagues reported a systematic review and meta-analysis of the effect of the TEACCH program (Virues-Ortega et al. 2013). Some of the included studies used the Vineland-II, and the duration of those studies ranged from 10 to 36 weeks, suggesting that the minimum length of a study to detect gains in adaptive behaviors in subjects with ASD, as measured by the Vineland-II, is in between these numbers.

It is thought that a treatment duration of 24 weeks would allow sufficient exposure to adequately and efficiently assess clinically meaningful responses in both social cognition and functional social outcomes as well as characterize the safety and tolerability profile of balovaptan.

3.3.7 Rationale for Biomarker Assessments

ASD is a heterogeneous but highly heritable disease, with heritability estimates ranging from 64% to 91% in twin studies and ranging from 31% to 71% in whole genome genotyping studies (Autism Spectrum Disorders Working Group of the Psychiatric Genomics Consortium 2017). Given that variants in the *V1A* receptor gene are among those previously associated with ASD risk (Tansey et al. 2011), not all subjects may be equally likely to benefit from treatment with a *V1A* antagonist like balovaptan. Additionally, *V1A* antagonism is associated with rescue of social impairments and repetitive behavior in the *CNTNAP2* knockout model of autism. This gene has also been strongly linked to ASD (Peñagarikano and Geschwind 2012). Therefore, biomarker samples that may be predictive of efficacy will be collected for genotyping the *V1A* receptor gene as well as the *CNTNAP2* gene, prior to dosing, and will be assessed in an effort to identify those subjects who are most likely to benefit from balovaptan.

Beyond the defined biomarkers above, available assessments from the optional Research Biosample Repository (RBR) (see Section 4.5.9) will be utilized to evaluate the potential of novel biomarkers unrelated to the proposed mechanisms.

4. MATERIALS AND METHODS

4.1 SUBJECTS

Approximately 350 adult ASD subjects will be enrolled in this study.

4.1.1 Inclusion Criteria

Subjects must meet the following criteria for study entry:

- Signed Informed Consent Form
- Males or females, age 18 years or older at time of signing Informed Consent Form
- Subject meets the DSM-5 criteria for ASD for an autism diagnosis and is confirmed using ADOS-2 criteria

  If the ADOS-2 assessment has been performed by a certified rater and documented within 12 months of the screening visit, there is no requirement to repeat it.
- SRS-2, proxy version, total *t*-score ≥66 at screening
- A full scale IQ score ≥70 on the WASI®-II

  The score should be confirmed by assessment during screening. Previous test results on the WASI or equivalent scales such as Wechsler Adult *Intelligence* Scale®, Fourth Edition (*WAIS*®-IV) (including all subdomain scores) are accepted if the test has been performed within 12 months prior to screening by an appropriately qualified rater.
- Ability and willingness to fully comply with study visit schedule and regular assessments and fluency in the language of the site

- Subject's participation in the study or discontinuation of prohibited medication will not pose undue risks to the subject, in the investigator's opinion
- Subject has an appropriate study partner, in the opinion of the investigator

  The study partner is someone who has regular and sufficient periods of contact (including regular conversations and face-to-face interactions) with the subject to be able to report on the subject's status on relevant study assessments.

  The study partner should have sufficient capacity to evaluate social and communication changes in the subject and should be available throughout the entire duration of the study.

  The study partner will not be eligible to be a study participant in this study.

  Only at visits that do not require a study partner's input on assessments can a subject be accompanied by others if the study partner is not available.

  Every effort should be made to have same study partner participate throughout the subject's participation in the study.
- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use a contraceptive method with a failure rate of $<$1% per year during the treatment period and for at least 28 days after the last dose of study drug

  A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state ($\geq$12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

  Examples of contraceptive methods with a failure rate of $<$1% per year include bilateral tubal ligation, male sterilization, hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices.

  The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

  Women must agree to use an effective form of contraception during all phases of the study.

  No specific contraception methods for males are required.

  Note: The Sponsor does not require male contraception because of the minimal seminal dose transmitted via sexual intercourse (Banholzer et al. 2012) (see Section 1.2.1).
- Treatment with permitted medications (at a stable dose for 12 weeks before screening) and behavioral therapy regimens (regimens stable for 6 weeks before screening), with the intent that such treatments remain stable throughout the study and with no expected changes before the Week 24 visit

4.1.2 Exclusion Criteria

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

4.1.2.1 General Exclusion Criteria

Subjects who meet the following general exclusion criterion will be excluded from study entry:

- Pregnancy or breastfeeding, or intention to become pregnant during the study

  Females of childbearing potential must have a negative urine pregnancy test result immediately prior to initiation of study drug.

4.1.2.2 Neurologic and Psychiatric Exclusion Criteria

Subjects who meet any of the following neurologic and psychiatric exclusion criteria will be excluded from study entry:

- Previous initiation of new or major change in psychosocial intervention (including investigational) within 6 weeks prior to screening

  Minor changes in ongoing treatment (e.g., missed therapy sessions because of holiday or vacation, planned break in therapy due to school holidays, or changes in college or school programs) are not considered significant.
- Unstable or uncontrolled clinically significant affective or psychotic disorders and/or neurologic disorder that may interfere with the assessment of safety or efficacy endpoints
- *Substance use disorders (including* alcohol or substance abuse or dependence disorder*)* during the last 12 months, as defined *by* the DSM-5 criteria
- Significant risk for suicidal behavior, in the opinion of the investigator
- Epilepsy or seizure disorder considered not well controlled within the past 6 months or changes in anticonvulsive therapy within the last 6 months
- Clinical diagnosis of peripheral neuropathy

4.1.2.3 Exclusions Related to Cardiovascular Disorders

Subjects who meet any of the following exclusion criteria related to cardiovascular disorders will be excluded from study entry:

- Within the last 2 years, unstable or clinically significant cardiovascular disease (e.g., myocardial infarction, angina pectoris, or New York Heart Association Class II or higher cardiac failure)
- Uncontrolled hypertension (e.g., blood pressure repeatedly >160 mmHg systolic or >95 mmHg diastolic)
- *Unexplained syncopal episode within the last 12 months*
- *Confirmed elevation above upper limit of normal (ULN) of CK-MB (electrophoretic measurement), high sensitivity cardiac troponin T (hs cTnT), cardiac troponin I (cTnI), and/or N-terminal pro B-type natriuretic peptide (NT-proBNP)*

4.1.2.4 Exclusions Related to Other Organ Systems

Subjects who meet any of the following exclusion criteria related to other organ systems will be excluded from study entry:

- Positive serology results for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, or human immunodeficiency virus (HIV) 1 or 2

  All subjects must have a negative HBsAg result and negative hepatitis C antibody screening tests prior to enrollment. If total HBcAb is positive at screening, hepatitis B virus DNA measured by polymerase chain reaction must be negative to be eligible.

- History of coagulopathies, bleeding disorders, blood dyscrasias, hematological malignancies, myelosuppression (including iatrogenic), or current major bleeding event (e.g., gastrointestinal [GI] bleeding)
- Concomitant disease or condition that could interfere with, or treatment of which might interfere with, the conduct of the study, or what would, in the opinion of the investigator, pose an unacceptable risk to the subject in this study
- Confirmed clinically significant abnormality in parameters of hematology [redacted]
- Confirmed clinically significant abnormality in parameters of clinical chemistry, coagulation, or urinalysis

  If CPK is increased above 3× ULN at screening, additional samples to measure CPK, creatinine, and potassium levels should also be obtained at Week 2 of treatment.

- Medical history of malignancy, if not considered cured

4.1.2.5 Additional Exclusion Criteria

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

- Allowed medications have not been stable for 12 weeks prior to screening
- Previous treatment with prohibited medications or herbal remedies within 2 weeks prior to randomization or 5 half-lives (whichever is longer)
- Blood donation or loss of blood >500 mL within 3 months prior to randomization
- Previous participation in an investigational drug or device study within 60 days prior to randomization or previous enrollment in investigational trials of balovaptan

4.1.3 Prohibited Medications and Food Products

Prior use of certain therapies is permitted if the individual has undergone a washout period of 2 weeks prior to randomization or 5× the half-life (whichever is longer).

The following therapies are prohibited throughout the study and include:

- Moderate and strong inhibitors of CYP3A4 (e.g., ketoconazole, clarithromycin, erythromycin, ciprofloxacin, diltiazem, and grapefruit juice)

  Moderate inhibitors of CYP3A4 (e.g., erythromycin, ciprofloxacin, diltiazem) may be allowed for a treatment duration of no more than approximately 10 days (e.g., in context of an adverse event) after discussion with the Medical Monitor or designee. The reason for and approval of such treatment is to be documented.
- Moderate and strong inducers of CYP3A4 (e.g., carbamazepine, phenytoin, St. John's Wort), with the following exceptions:

  Weak inducers are permitted.
- Concomitant oral medication that are P-gp substrates:
  - Quinidine should not be given with balovaptan.
  - Risperidone and cetirizine are allowed.
  - All other clinically relevant substrates of P-gp: An interaction cannot be ruled out and caution is advised in particular for those medications that have a narrow therapeutic window (e.g., loperamide). However, when balovaptan is administered 5 or more hours prior to such a P-gp substrate, the risk of pharmacokinetic (PK) interaction is predicted to be small.
  - Chronic adrenocorticoid or glucocorticoid use, with the following exceptions: Inhaled and topical formulations are permitted.
- Oxytocin
- Desmopression (DDAVP®)
- Bumetanide
- Agents inhibiting vasopressin receptors (e.g., tolvaptan, conivaptan)
- Use of any concomitant medication known to potentially cause peripheral neuropathy per the Warnings and Precautions section of the U.S. label or corresponding section of the local label
- Agents that have been associated with significant and/or irreversible hematologic toxicity and therefore require frequent monitoring (e.g., clozapine)
- *Use of tetrahydrocannabinol due its psychoactive effects (e.g., marijuana)*

Please refer to Section 4.4 for additional details.

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Approximately 350 subjects will be randomized to receive either placebo or 10 mg of oral balovaptan QD in a 1:1 ratio. An independent interactive voice or web-based response system (IxRS) provider will conduct randomization and hold the treatment assignment code. Randomization will be stratified by a subject's baseline Vineland-II 2DC score (<60 vs. ≥60), sex (male vs. female), region (North America vs. ROW), and age (<25 years vs. ≥25 years).

There will be no replacement of subjects should a subject's treatment be discontinued for any reason.

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

If unblinding is necessary for a medical emergency (e.g., in the case of a serious adverse event for which subject management might be affected by knowledge of treatment assignment), the investigator will be able to break the treatment code by contacting the IxRS. The investigator is not required to contact the Medical Monitor prior to breaking the treatment code; however, the treatment code should not be broken except in emergency situations. If unblinding occurs, subjects should not continue receiving study drug and should proceed to the end-of-treatment visit and subsequently safety follow-up.

If the investigator wants to know the identity of the study drug for any reason other than a medical emergency, he or she should contact the Medical Monitor directly. The investigator should document and provide an explanation for any non-emergency unblinding. The investigator will be able to break the treatment code by contacting the IxRS.

Whenever disclosure of the identity of the study drug is necessary, adequate procedures will be put in place to ensure integrity of the data. Any unblinding at the investigational site will be documented in the study report with the date, the reason for identifying the drug, and the name of all person(s) who were unblinded.

As per health authority reporting requirements, the Sponsor's Drug Safety representative will break the treatment code for all serious, unexpected suspected adverse reactions (see Section 5.7) that are considered by the investigator or Sponsor to be related to study drug. The subject may continue to receive treatment, and the investigator, subject, and Sponsor personnel, with the exception of the Drug Safety representative and personnel who must have access to subject treatment assignments to fulfill their roles (as defined above) will remain blinded to treatment assignment.

4.3 STUDY TREATMENT AND OTHER TREATMENTS RELEVANT TO THE STUDY DESIGN

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

[redacted]

4.3.2 Study Treatment Dosage, Administration, and Compliance

The treatment regimens are summarized in Section 3.1.

One tablet of study drug (balovaptan or placebo) should be taken orally QD in the morning, with or without food. The tablet should be swallowed whole with something to drink.

The first dose of the study drug will be administered on Day 1 after all predose baseline assessments have been conducted. At subsequent visits, study drug should not be taken until all protocol-mandated study assessments are completed*, unless otherwise specified on the Schedule of Activities (see Appendix 1).*

Compliance regarding administration of study drug at home will be monitored by the maintenance of adequate drug dispensing logs and return records. A study diary or application may also be provided to subjects and/or study partners.

Any overdose or incorrect administration of balovaptan or placebo should be noted on the Study Drug Administration electronic Case Report Form (eCRF). Adverse events associated with an overdose or incorrect administration of any of the study treatments should be recorded on the Adverse Event eCRF (see Section *5.3.5.11*).

Guidelines for treatment interruption or discontinuation for subjects who experience adverse events are provided in Sections 5.1.2.1 and 5.1.2.2, respectively.

4.3.3 Investigational Medicinal Product Accountability

All IMPs (balovaptan and placebo) required for completion of this study will be provided by the Sponsor where required by local health authority regulations. The study site will acknowledge receipt of IMPs supplied by the Sponsor using the IxRS to confirm the shipment condition and content. Any damaged shipments will be replaced.

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

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

The investigator is responsible for the control of drugs under investigation. Adequate records of the receipt (e.g., Drug Receipt Record) and disposition (e.g., Drug Dispensing Log) of the study drug must be maintained. The Drug Dispensing Log must be kept current and should contain the following information:

- The date(s) and quantity of study drug dispensed to the subject
- The date(s) and quantity of study drug returned by the subject

All records and drug supplies must be available for inspection by the Roche monitor (at every monitoring visit).

Written documentation of destruction must contain the following:

- Identity (batch numbers or study subject numbers) of investigational products destroyed
- Quantity of investigational products destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person (or company) who destroyed the investigational product

Unused study drug from the site that has not been stored properly should not be destroyed until the final report has been approved. If there are any issues with the drug, it should be returned to the appropriate Roche clinical trial supplies department for long-term storage and not destroyed.

4.3.4 Continued Access to Balovaptan

Currently, the Sponsor does not have any plans to provide the Roche IMP (balovaptan) or any other study treatments or interventions to subjects who have completed the study. The Sponsor may evaluate whether to continue providing balovaptan in accordance with the Roche Global Policy on Continued Access to Investigational Medicinal Product, available at the following web site:

http://www.roche.com/policy_continued_access_to_investigational_medicines.pdf

Subjects are eligible to receive balovaptan as part of the open-label extension period, provided they have completed 24 weeks of treatment, as described in Section 3.1.1.4.

4.4 CONCOMITANT THERAPY

The addition of a new medication or a change in the dose of a medication after signing the Informed Consent Form should only occur for the treatment of an adverse event.

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, approved dietary and herbal supplements, nutritional supplements, and any non-medicinal interventions, such as individual psychotherapy, cognitive behavioral therapy, smoking cessation therapy, and rehabilitative therapy) used by a subject 24 weeks prior to screening until the follow-up visit.

All concomitant medications should be reported to the Investigator and recorded on the Concomitant Medications eCRF. All therapy and/or medications administered to manage adverse events should be recorded on the Adverse Event eCRF.

4.4.1 Permitted Therapy

It is expected that subjects enrolled in the trial will be taking a variety of psychiatric medications upon entering the trial. Such standard-of-care or "background" medications may include a variety of classes acting through different mechanisms to treat psychiatric symptoms commonly associated with ASD. It is also common for individuals with ASD to be engaged in a large variety of non-medicinal therapies, including individual, group, and behavioral-based psychotherapies. All psychotherapies and medications to treat coexisting psychiatric symptoms and medical conditions should be documented on the eCRF and continued at a stable frequency and dosage from screening through the completion of the trial; any changes should be reported on the appropriate eCRF.

Examples of allowed medications include the following:

- Selective serotonin reuptake inhibitor and serotonin-norepinephrine reuptake inhibitor antidepressants, including fluoxetine
- Atypical antipsychotics *(e.g., aripiprazole, risperidone)*
- Melatonin
- Benzodiazepines, only on an as-needed basis

- Attention-deficit/hyperactivity disorder medications, including psychostimulants such as methylphenidate
- Hypnotics for insomnia and falling asleep problems (only on an as-needed basis)
- Anti-epileptic drugs used for treatment of epilepsy, including VPA and oxcarbazepine, may be used, provided they have been given at stable dosage for at least 6 months prior to screening and were well tolerated
- Concomitant oral medications that are P-gp substrates, with the exception of quinidine:
  - Risperidone and cetirizine are allowed.
  - All other clinically relevant substrates of P-gp: An interaction cannot be ruled out and caution is advised in particular for those medications that have a narrow therapeutic window (e.g., loperamide). However, when balovaptan is administered 5 or more hours prior to such a P-gp substrate, the risk of PK interaction is predicted to be small.

4.4.2 Non-Pharmacological Interventions

Non-pharmacological interventions must be stable for 6 weeks prior to screening and must remain stable throughout the study (e.g., psychotherapy, cognitive behavioral therapy, and rehabilitative therapy). Minor changes in ongoing treatment (e.g., missed therapy sessions due to holiday or vacation, planned break in therapy because of school holidays, and changes in college or school programs) are not considered significant and do not need to be discussed with the Medical Monitor or designee.

4.4.3 Prohibited Therapy

The following therapies are not allowed during the study and must be stopped 2 weeks prior to screening to ensure washout of medication. Examples of prohibited medications include the following:

- Inhibitors and inducers of CYP3A4
- Quinidine (a Pgp substrate)
- Chronic adrenocorticoid or glucocorticoid use (use of inhaled or topical formulations are allowed)
- Oxytocin
- Demopressin acetate
- Bumetanide
- Agents inhibiting vasopressin receptors (e.g., tolvaptan, conivaptan)
- Hematotoxic drugs requiring frequent hematologic monitoring of WBCs (e.g., clozapine)
- Herbal therapies and dietary supplements (unless allowed by the Medical Monitor or designee)

- Use of any concomitant medication known to potentially cause peripheral neuropathy per the Warnings and Precautions section of the U.S. label or the corresponding section of the local label
- *Use of marijuana for recreational or medical purposes*

  Must meet eligibility criteria of no substance abuse or dependency. A washout period of 3 months is required.

Medications Prohibited due to Effects Related to Cytochrome P450 Enzymes

In vitro data suggest that balovaptan is metabolized through CYP3A4 and there is a potential for drug–drug interaction with any medication that is metabolized by or strongly inhibits or induces this enzyme. Therefore, the following medications are prohibited:

- Strong inhibitors of CYP3A4 (e.g., ketoconazole, clarithromycin, grapefruit juice)
- Moderate inhibitors of CYP3A4 (e.g., erythromycin, ciprofloxacin, diltiazem)

  For treatment duration of no more than approximately 10 days in length (e.g., in the context of an adverse event), moderate inhibitors of CYP3A4 may be used after discussion with and approval by the Medical Monitor or designee. The reasons for and approval of such use must be documented.
- Strong and moderate inducers of CYP3A4 (e.g., carbamazepine, phenytoin, St. John's wort, and modafinil)

The above lists of medications are not necessarily comprehensive. The investigator should consult the prescribing information when determining whether a concomitant medication can be safely administered with study drug.

The investigator should *consult with* the Medical Monitor if questions arise regarding medications not listed above.

4.5 STUDY ASSESSMENTS

The schedule of activities to be performed during the study is provided in Appendix 1 and Appendix 3. The schedule of activities for unscheduled visits and the safety follow-up period are presented in Appendix 2. All activities must be performed and documented for each subject.

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 will maintain a screening log to

record details regarding all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

4.5.2 Medical History, Concomitant Medication, and Demographic Data

Medical history, for the previous 5 years, including clinically significant diseases, surgeries, reproductive status, smoking history, use of alcohol and drugs of abuse, will be recorded at baseline. The date of diagnosis of ASD will be collected. In addition, all medications (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by the subject within 24 weeks prior to the screening visit as well as details of psychosocial/non-pharmacological interventions used during the previous 52 weeks are to be documented.

Demographic data will include age, sex, and self-reported race/ethnicity. Subjects and study partners will also be asked about the subject's residential setting, school or employment status, level of education, participation in educational or day programs, and any non-medical hospitalizations.

4.5.3 Physical and Neurologic Examinations

A complete physical examination, performed at *the baseline visit* should include an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, musculoskeletal, respiratory, GI, and neurologic systems. Complete physical examinations will not include pelvic, rectal, or breast examinations.

Any abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF.

Limited, symptom-directed physical examinations should be performed at specified post-baseline visits and as clinically indicated. 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.

Sites will contact subjects and study partner by telephone to evaluate the presence or absence of signs and symptoms with regard to infections, as detailed in the schedule of activities (see Appendix 1 and Appendix 2).

Neurologic examinations will be performed according to schedule of activities (see Appendix 1, Appendix 2, and Appendix 3). The neurologic evaluation will be completed at all indicated visits (see Appendix 4).

4.5.4 Vital Signs

Vital signs will include measurement of respiratory rate, pulse rate, and systolic and diastolic blood pressure while the subject is in a seated position, and body temperature.

4.5.5 Temperature Measurements

Temperature will be measured at site visits as part of the vital signs assessment (see Section 4.5.4 and Appendix 1, Appendix 2, and Appendix 3).

Temperature measurements taken at home should be performed using a tympanic thermometer in an indoor environment at room temperature and recorded in a subject diary or device. Temperature measurements may be performed by the subject or the subject's study partner who will be asked to help ensure compliance and/or notify the site in case of non-compliance. Site personnel should train the subject and/or study partner on the correct use of the thermometer and the importance of compliance with the procedure.

Body temperature should be measured every 2 weeks for the double-blind period and continue into open-label extension period up until Week 52 of trial participation.

4.5.6 Laboratory, Biomarker, and Other Biological Samples

Laboratory safety tests will be collected at the timepoints specified in the schedule of activities (see Appendix 1, Appendix 2, and Appendix 3).

Additional blood or urine samples may be obtained at the discretion of the investigator if the results of any test fall outside the reference ranges or clinical symptoms necessitate additional testing to monitor subject safety. When the clinical significance of abnormal laboratory results is considered uncertain, screening laboratory tests may be repeated before randomization to confirm eligibility. If there is an alternative explanation for a positive urine or blood test for drugs of abuse (e.g., previous occasional intake of food or a medication containing, such as, codeine, benzodiazepines, or opiates), the test may be repeated to confirm washout.

In the event of unexplained abnormal clinically significant laboratory test values, the tests should be repeated immediately and sent to the central laboratory for testing. Abnormal results should be followed until they have returned to the normal range and/or an adequate explanation of the abnormality is found. [redacted] Results of clinical laboratory testing will be recorded on the eCRF or be received as electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

If clinically indicated, laboratory and PK samples may be obtained at an unscheduled visit (see Appendix 2).

Samples for the following laboratory tests will be sent to the relevant central laboratory for analysis, unless noted otherwise:

- Hematology: WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, and other cells)

- Serum chemistry panel: sodium, potassium, chloride, bicarbonate, glucose, BUN or urea, creatinine, CPK, total protein, albumin, phosphorus, calcium, total and direct bilirubin, ALP, ALT, AST, uric acid, and LDH
- [redacted]
- Coagulation: INR, aPTT, and PT
- Lipids: cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides
- Quantitative immunoglobulins: IgA, IgG, IgM, and IgE
- Viral serology: HIV, HBsAg, total hepatitis B core antibody (HBcAb), and HCV antibody

  All subjects must have a negative HBsAg result and negative hepatitis C antibody screening tests prior to enrollment. If total HBcAb is positive at screening, hepatitis B virus DNA measured by polymerase chain reaction must be negative to be eligible.
- Urinalysis, including dipstick: pH, specific gravity, glucose, protein, ketones, and blood will be performed at the study site

  If there is a clinically significant positive blood or protein result, the urine sample will be sent to the central laboratory for microscopy and culture.
- Urine drug screen and alcohol test: Urine samples will be analyzed for the presence of the following drugs: alcohol, cannabinoids, opiates, methadone, cocaine, barbiturates, and phencyclidine (PCP)

  The drug screen and alcohol test will be mandatory for all subjects at screening and may be performed for any subject at any visit at the discretion of the investigator.
- Plasma samples for PK analysis
- Pregnancy test: All females with menarche (including those who have had a tubal ligation) will have a urine pregnancy test at screening and specified subsequent visits. A positive urine pregnancy test should be confirmed with a serum test through the central laboratory prior to any additional dosing with study drug. Study drug will be withheld until pregnancy test is negative.
- Unscheduled visit laboratory assessments: In the event that additional laboratory parameters are required to interpret any adverse events or abnormal hematologic or chemistry finding, the following assessments may be included *(however, additional tests may be performed at the investigator's discretion):*

  Hematology: leukocytes, erythrocytes, hemoglobin, hematocrit, platelets, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, and other cells)

  Complete serum chemistry panel: sodium, potassium, chloride, bicarbonate, glucose, urea, creatinine, phosphate, calcium, total and direct bilirubin, ALP, ALT, AST, protein, albumin, LDH, CPK, total cholesterol, LDL cholesterol

4.5.6.2 Clinical Genotyping Samples

A mandatory predose whole blood sample will be obtained for DNA extraction from every participant on Day 1. The DNA will be used to evaluate whether genetic variants of the *AVPR1A* or *CNTNAP2* genes affect the efficacy, safety, and/or pharmacokinetics of balovaptan. Clinical genotyping samples and derived analytical materials will be destroyed no later than 2 years after the date of the final Clinical Study Report.

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.

For sampling procedures, storage conditions, and shipment instructions, see the laboratory manual.

4.5.7 Electrocardiograms

At baseline, 12-lead ECGs will be recorded in triplicate. Single 12-lead ECG recordings will be obtained at the other timepoints as outlined in the schedule of activities (see Appendix 1, Appendix 2, and Appendix 3).

All ECG recordings must be performed using a standard high-quality, high-fidelity digital electrocardiograph machine equipped with computer-based interval measurements.

Lead placement should be as consistent as possible. ECG recordings must be performed after the subject has been resting in a supine position for at least 5 minutes. All ECGs are to be obtained prior to other procedures scheduled at the same time (e.g., vital sign measurements, blood draws) and should not be obtained within 3 hours after any meal. Circumstances that may induce changes in heart rate, including environmental distractions (e.g., television, radio, conversation), should be avoided during the pre-ECG resting period and during the ECG recording.

For safety monitoring purposes, the investigator must review, sign, and date all ECG tracings. Paper copies of ECG tracings will be kept as part of the subject's permanent study file at the site. Digital recordings will be stored at a central ECG laboratory. The following parameters will be provided as non-eCRF data: heart rate, RR interval, QRS interval, PR duration, uncorrected QT interval, and QT interval corrected through use of Fridericia's formula (QTcF) based on the machine readings of the individual ECG tracings or, if considered appropriate by the Sponsor, ECGs may be analyzed at a central laboratory.

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

4.5.8 Clinical Outcome Assessments

Subjects, study partners, and clinicians will use an electronic device to complete electronic clinical outcome assessments (eCOAs) with the exception of the ADOS-2 and WASI®-II (IQ) scales. All item-level, domain-level, and relevant total score data will be transmitted electronically to a centralized database at the eCOA vendor. The ADOS-2 and WASI®-II data will be transferred to the electronic device by site staff and then transmitted as described for all other scales. The data can be accessed by appropriate study personnel securely via the worldwide web. Entries should be reviewed for completeness by the site staff during the visit. Sites should only use paper forms for the scales specified. A backup device will be provided to each site in the event of device issues.

Rater qualification for specific outcomes assessments, including, but not limited to, the Vineland-II, ADOS-2, and Hamilton Anxiety Rating Scale (HAM-A) scales will be reviewed per the criteria established by the eCOA vendor. Only raters confirmed by the vendor to be qualified on those assessments should rate in this study.

If the same qualified rater is not available within the visit window, delaying the visit is preferred to changing the rater. The same rater or clinician will perform the Vineland-II together with the same study partner throughout the study as established at the baseline visit. *Also, the same clinician should complete the Clinical Global Impression scales for a specific subject for the duration of the study.*

Demonstrating good reliability and validity, the Vineland-II is recognized as an appropriate outcome measure in clinical ASD trials and will be used as the primary outcome measure in future ASD trials conducted by Roche. The Vineland-II assessment will be administered by qualified raters as a semi-structured interview performed with a reliable study partner. The Rater Academy will provide raters the opportunity to improve their skills in interviewing participants so that they can qualify as eligible raters for the this study (refer to Appendix 7 for more details).

4.5.8.1 Vineland-II Adaptive Behavior Scales, Second Edition

The Vineland-II is an instrument that measures communication, daily living skills, socialization, maladaptive behavior (not assessed in this study), and motor skills (not administered in adults and will not be assessed in this study) (Sparrow et al. 2005). The Survey Interview Form (i.e., a semi-structured interview) will be administered to a subject's reliable study partner in this study, during which the rater will ask the study partner open-ended questions relating to the subject's activities and behavior. Standardized domain scores will be obtained for the individual domains of Socialization, Communication, and Daily Living Skills. Standardized scores on the domains and composite score range from 20 to 160, with higher scores indicating better functioning.

For the purpose of this study, the Vineland-II 2DC score will be derived as the primary efficacy outcome measure.

The interview will take approximately 45–60 minutes to complete and will be audio recorded. At a clinic visit, the Vineland-II should be performed first prior to all informant-based assessments with the study partner (see Section 3.1.1.3). The Vineland-II should not be administered by telephone interview.

4.5.8.2 Wechsler Abbreviated Scale of Intelligence®, Second Edition

The WASI®-II is a tool used to evaluate an individual's cognitive functioning and generate IQ scores (Wechsler 2011) and will be administered by a certified rater at screening only. Scores range from 40 to 160, with higher scores indicating higher IQ.

The assessment will take approximately 30 minutes to complete.

4.5.8.3 Autism Diagnostic Observation Schedule, Second Edition

The ADOS-2 is a diagnostic tool used to document the presence of ASD (Lord et al. 2000; Gotham et al. 2007). During a semi-structured evaluation, the individual is

observed in a naturalistic social situation and assessed across areas of social communication, imagination, and restricted and/or repetitive behaviors.

The ADOS-2 includes four modules for use with different age groups and language levels; Modules 2, 3, and 4 will be used in this study. The appropriate module of the ADOS-2 will be administered to subjects by a certified rater at screening. Scores range from 1 to 10 once the algorithm is applied, with higher scores indicating greater severity of ASD symptoms.

The assessment will take approximately 30–45 minutes to complete.

4.5.8.4 Social Responsiveness Scale, Second Edition, Adult (Relative/Other Report)

The SRS-2 is a 65-item, informant-based rating scale designed to measure an individual's ability to engage in emotionally appropriate reciprocal social behavior in a naturalistic social setting (Constantino and Gruber 2005).

Each item on the scale assesses an observed aspect of reciprocal social behavior that is rated on a scale from 1 (not true) to 4 (almost always true). Social skill levels are assessed over five domains: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Autistic Mannerisms. The total score generated serves as an index of severity of social deficits in the autism spectrum; scores range from 35 to ≥90, with higher scores indicative of more severe deficits. A total proxy (study partner–rated) *t*-score of 66 indicates moderate severity of symptoms and is the threshold for inclusion in this study.

The assessment will take approximately 15–20 minutes to complete.

4.5.8.5 Clinical Global Impression–Improvement

The CGI-I is a single-item, clinician-rated measure, assessing the clinician's impression about changes in the subject's condition, in this case, ASD (not other conditions or co-morbidities), compared with baseline.

The CGI-I utilizes a 7-point response scale, ranging from "very much improved" (1) to "very much worse" (7).

Clinicians should make a judgment on any changes from baseline based on the totality of information available to them (e.g., insights from subject and/or study partner, information captured during the completion of other trial assessments).

The assessment will take up to 5 minutes to complete.

4.5.8.6 Clinical Global Impression–Severity

The CGI-S is a single-item, clinician-rated measure, assessing the clinician's impression of the severity of a subject's condition, in this case, ASD (not other conditions/co-morbidities).

The CGI-S utilizes a 7-point response scale, ranging from "normal, not at all ill" (1) to "among the most extremely ill subjects" (7).

Clinicians should make a judgment on a subject's severity based on the totality of their experience of the population.

The assessment will take up to 5 minutes to complete.

4.5.8.7 Caregiver Global Impression–Change

The Caregiver Global Impression–Change (CaGI-C) is a 4-item, informant-based measure assessing the study partner's impression about changes in subject's communication skills, social skills, daily living skills, and overall ASD compared with baseline.

The CaGI-C items utilize a 7-point response scale, ranging from "very much improved" (1) to "very much worse" (7). Each item is scored independently.

The assessment will take up to 5 minutes to complete.

4.5.8.8 Caregiver Global Impression–Severity

The Caregiver Global Impression–Severity (CaGI-S) is a 4-item, informant-based measure, assessing the study partner's impression about the severity of impairment of the subject's communication skills, social skills, daily living skills, and overall ASD during the preceding 7 days.

The CaGI-S items utilize a 5-point response scale, ranging from "no difficulty" (1) to "extreme difficulty" (5). Each item is scored independently.

The assessment will take up to 5 minutes to complete.

4.5.8.9 Repetitive Behavior Scale–Revised

The RBS-R is a 44-item informant-based questionnaire, assessing the variety of restricted and repetitive behaviors observed in individuals with ASD (Bodfish et al. 2000). The scale is grouped into six subscales: Stereotyped, Self-Injurious, Compulsive, Ritualistic, Sameness, and Restricted Behaviors.

Each item utilizes a 4-point response scale assessing the presence and severity of a restricted or repetitive behavior. Response options range from "Behavior does not occur" (0) to "Behavior occurs and is a severe problem" (3). On the last question, participants are asked to "lump together" all of the behaviors described in the questionnaire and

provide a rating for how much of a problem these repetitive behaviors are overall, on a scale from 1 to 100: "not a problem at all" (1) to "as bad as you can imagine" (100).

A total score is generated for each subscale and an overall total score encompasses all the subscales. The overall total score ranges from 0 to 129; higher scores are indicative of more severe restricted and repetitive behaviors.

The assessment will take approximately 20–30 minutes to complete.

4.5.8.10 Pediatric Quality of Life Inventory Family Impact Module

The PedsQL Family Impact Module, Version 2 (Varni et al. 2004) is a 36-item, informant-based measure that will be completed by the study partner. The instrument was developed to measure parent and family functioning. It encompasses six scales covering Physical Functioning (6 items), Emotional Functioning (5 items), Social Functioning (4 items), Cognitive Functioning (5 items), Communication (3 items), and Worry (5 items) and two scales measuring parent-reported family functioning, Daily Activities (3 items) and Family Relationships (5 items). The acute form, using a recall period of 7 days, will be employed in this trial.

Each item utilizes a 5-point response scale, ranging from "never (a problem)" (0) to "almost always (a problem)" (4). Items are then reverse-scored and linearly transformed to a 0–100 scale (0=100, 1=75, 2=50, 3=25, 4=0), such that higher scores indicate better functioning (less negative impact).

In addition to the eight scale scores, a total score, a parent HRQoL summary score, and a family summary score can also be computed by averaging across the relevant domains.

The assessment will take approximately 15–20 minutes to complete.

4.5.8.11 Hamilton Anxiety Rating Scale

The HAM-A is a 14-item, clinician-administered interview, assessing the severity of anxiety symptoms during the past 7 days (Hamilton 1959). Seven items assess psychic anxiety and the remaining seven items assess somatic anxiety.

Each item utilizes a 5-point symptom severity response scale, ranging from "none" (0) to "very severe" (4). A total score is calculated that ranges from 0 to 56; higher scores are indicative of more severe anxiety.

The assessment will take approximately 10–15 minutes to complete.

4.5.8.12 EuroQol Five-Dimensions Questionnaire, Five-Levels Version

The EuroQoL Five Dimensions Questionnaire–Five Levels (EQ-5D-5L) is a validated, self-reported health status questionnaire that is used to calculate a health status utility score for use in health economic analyses (EuroQol Group 1990; Brooks 1996;

Herdman et al. 2011; Janssen et al. 2013). There are two components to the EQ-5D-5L: a 5-item health state profile that assesses mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, as well as a visual analog scale that measures health state. Published weighting systems allow for creation of a single composite score of a subject's health status.

Subjects and study partners will complete the measure (reporting on their own health status) to generate health status data to support pharmacoeconomic evaluations.

The assessment will take approximately 5 minutes to complete.

4.5.8.15 Pediatric Quality of Life Inventory Generic Core Scales

The PedsQL 4.0 Core is a 23-item, self-reported assessment encompassing four core scale domains (Varni et al. 2004): Physical Functioning (8 items), Emotional Functioning (5 items), Social Functioning (5 items), and School Functioning (5 items).

The acute form, using a recall period of 7 days, will be employed in this study. Different age-appropriate versions will be utilized: the Young Adult version (subjects, ages 18–25 years) and the Adult version (subjects, ages ≥26 years).

Each item utilizes a 5-point response scale ranging from "never (a problem)" (0) to "almost always (a problem)" (4). Items are then reverse-scored and linearly transformed

to a 0–100 scale (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0), such that higher scores indicate better HRQoL.

In addition to the four scale scores (Physical Functioning, Emotional Functioning, Social Functioning, School/Work Functioning), a Psychosocial Health Summary score, a Physical Health Summary score, and a total score can also be computed by averaging across the relevant domains.

The assessment will take approximately 15–20 minutes to complete.

4.5.8.16 Pediatric Quality of Life Cognitive Functioning Module

The PedsQL Cognitive Functioning Module is a 6-item, self-reported assessment of cognitive functioning (Varni et al. 2004).

The acute form, with a recall period of 7 days, will be employed in this study. Different age-appropriate versions will be utilized: the Young Adult version (subjects, ages 18–25 years) and the Adult version (subjects, ages ≥26 years).

Each item utilizes a 5-point response scale, ranging from "never (a problem)" (0) to "almost always (a problem)" (4). Items are then reverse-scored and linearly transformed to a 0–100 scale (0=100, 1=75, 2=50, 3=25, 4=0) and summed to generate a total score. Higher scores indicate better functioning.

The assessment will take approximately 5 minutes to complete.

4.5.8.17 Pittsburg Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) assesses sleep quality during the previous month (Buysse et al. 1989). It consists of 19 self-rated questions. A wide variety of factors relating to sleep quality are assessed, including estimates of sleep duration, latency, and the frequency and severity of specific sleep-related problems. The 19 items are grouped into seven component scores, each weighted equally on a 0–3 scale. The global PSQI score has a range from 0 to 21, with higher scores indicating worse sleep quality.

The entire index will take approximately 5–10 minutes to complete.

4.5.8.18 Patient Global Impression–Change

The Patient Global Impression–Change (PGI-C) is a 4-item, self-reported measure assessing a subject's impression about changes in his or her communication skills, social skills, daily living skills, and overall autism compared with baseline.

The PGI-C items utilize a 7-point response scale, ranging from "very much improved" (1) to "very much worse" (7). Each item is scored independently.

The assessment will take up to 5 minutes to complete.

4.5.8.19 Patient Global Impression–Severity

The Patient Global Impression–Severity (PGI-S) is a 4-item, self-reported measure, assessing the subject's impression about the severity of impairment of his or her communication skills, social skills, daily living skills, and overall autism during the preceding 7 days.

The PGI-S items utilize a 5-point response scale, ranging from "no difficulty" (1) to "extreme difficulty" (5). Each item is scored independently.

The assessment will take up to 5 minutes to complete.

4.5.8.20 Columbia-Suicide Severity Rating Scale

The assessment for suicidality in clinical trials is a requirement for CNS-active molecules requested by health authorities.

The Columbia-Suicide Severity Rating Scale (C-SSRS; http://www.cssrs.columbia.edu) is a clinician-rated tool recommended by health authorities, including the U.S. FDA, to assess previous suicidality of a subject (C-SSRS Baseline version) as well as any new instances of suicidality during this study (C-SSRS Since Last Visit version to be used at subsequent visits). The C-SSRS incorporates a structured interview to prompt recollection of suicidal ideation, including the intensity of the ideation, behavior, and attempts with actual/potential lethality.

If the investigator concludes there is a risk of suicidality for a subject, the investigator must further evaluate the risk, which may involve local experts in the field of suicidality.

4.5.9 Optional Samples for Research Biosample Repository

4.5.9.1 Overview of the Research Biosample Repository

The RBR is a centrally administered group of facilities used for the long-term storage of human biologic specimens, including body fluids, solid tissues, and derivatives thereof (e.g., DNA, RNA, proteins, peptides). The collection, storage, and analysis of RBR specimens will facilitate the rational design of new pharmaceutical agents and the development of diagnostic tests, which may allow for individualized drug therapy for subjects in the future.

Specimens for the RBR will be collected from subjects who give specific consent to participate in this optional research. RBR specimens will be used to achieve the following objectives:

- To study the association of biomarkers with efficacy, adverse events, or disease progression
- To increase knowledge and understanding of disease biology
- To study drug response, including drug effects and the processes of drug absorption and disposition

- To develop biomarker or diagnostic assays and establish the performance characteristics of these assays

4.5.9.2 Approval by the Institutional Review Board or Ethics Committee

Collection and submission of biological samples to the RBR is contingent upon the review and approval of the exploratory research and the RBR portion of the Informed Consent Form by each site's Institutional Review Board or Ethics Committee (IRB/EC) and, if applicable, an appropriate regulatory body. If a site has not been granted approval for RBR sampling, this section of the protocol (Section 4.5.9) will not be applicable at that site.

4.5.9.3 Sample Collection

One optional sample for DNA extraction will be collected per the schedule of activities in Appendix 1; however, if it is not collected during the scheduled visit, it may be collected at any time (after subject randomization) during the conduct of the clinical study.

The sample collected for DNA extraction may be sent to one or more laboratories for analysis of germline or somatic mutations via whole genome sequencing (WGS) or other genomic analysis methods.

Genomics is increasingly informing researcher's understanding of disease pathobiology. WGS provides a comprehensive characterization of the genome and exome, respectively, and, along with clinical data collected in this study, may increase the opportunity for developing new therapeutic approaches. Data will be analyzed in the context of this study but will also be explored in aggregate with data from other studies. The availability of a larger dataset will assist in identification of important pathways, guiding the development of new targeted agents.

For sampling procedures, storage conditions, and shipment instructions, see the laboratory manual.

RBR specimens are to be stored until they are no longer needed or until they are exhausted. However, the RBR storage period will be in accordance with the IRB/EC-approved Informed Consent Form and applicable laws (e.g., health authority requirements).

4.5.9.4 Confidentiality

Specimens and associated data will be labeled with a unique subject identification number.

Subject medical information associated with RBR specimens 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.

Given the complexity and exploratory nature of the analyses of RBR specimens, 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.

Data generated from RBR specimens must be available for inspection upon request by representatives of national and local health authorities, and Sponsor monitors, representatives, and collaborators, as appropriate.

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

4.5.9.5 Consent to Participate in the Research Biosample Repository

The Informed Consent Form will contain a separate section that addresses participation in the RBR. The investigator or authorized designee will explain to each subject the objectives, methods, and potential hazards of participation in the RBR. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate, specific signature will be required to document a subject's agreement to provide optional RBR specimens. Subjects who decline to participate will not provide a separate signature.

The investigator should document whether or not the subject has given consent to participate and (if applicable) the date(s) of consent, by completing the RBR Research Sample Informed Consent eCRF.

In the event of an RBR participant's death or loss of competence, the participant's specimens and data will continue to be used as part of the RBR research.

4.5.9.6 Withdrawal from the Research Biosample Repository

Subjects who give consent to provide RBR specimens have the right to withdraw their consent at any time for any reason. *After withdrawal of consent, any remaining samples will be destroyed or will no longer be linked to the subject.* However, if RBR specimens have been tested prior to withdrawal of consent, results from those tests will remain as part of the overall research data. If a subject wishes to withdraw consent to the testing of his or her specimens, the investigator must inform the Medical Monitor in writing of the subject's wishes through use of the appropriate RBR Subject Withdrawal Form and, if the trial is ongoing, must enter the date of withdrawal on the RBR Research Sample Withdrawal of Informed Consent eCRF.

If a patient wishes to withdraw consent to the testing of his or her RBR samples after closure of the site, the investigator must inform the Sponsor by emailing the study number and patient number to the following email address:

global_rcr-withdrawal@roche.com

A subject's withdrawal from Study WN39434 does not, by itself, constitute withdrawal of specimens from the RBR. Likewise, a subject's withdrawal from the RBR does not constitute withdrawal from Study WN39434.

4.5.9.7 Monitoring and Oversight

RBR specimens will be tracked in a manner consistent with Good Clinical Practice by a quality-controlled, auditable, and appropriately validated laboratory information management system, to ensure compliance with data confidentiality as well as adherence to authorized use of specimens as specified in this protocol and in the Informed Consent Form. Sponsor monitors and auditors will have direct access to appropriate parts of records relating to subject participation in the RBR for the purposes of verifying the data provided to the Sponsor. The site will permit monitoring, audits, IRB/EC review, and health authority inspections by providing direct access to source data and documents related to the RBR samples.

4.5.10 Optional Exploratory Substudies

Subjects who are randomized into the main study WN39434 may have the option to participate in exploratory substudies provided in separate protocols and upon consent and fulfillment of additional exploratory protocol criteria. [redacted]

All other exploratory substudies will be described in separate substudy protocols. [redacted]

[redacted]

4.6 TREATMENT, SUBJECT, STUDY, AND SITE DISCONTINUATION

An excessive rate of withdrawals (either because of subjects discontinuing study drug or withdrawal from the study) can render the study non-interpretable. Therefore, unnecessary withdrawal of subjects should be avoided and all efforts should be taken to motivate subjects to comply with all the study specific procedures and to be followed until the end of the 24-week placebo controlled treatment period.

Every attempt should be made to keep subjects on study drug throughout the duration of the trial.

Before permanently discontinuing study drug (either initiated by the subject or the investigator), an interruption should be considered. Subjects, who temporarily discontinue study drug for any reason, should restart as soon as medically justified in the opinion of the investigator.

The investigator should show due diligence and explore all possible options to reach a subject who fails to return to a visit. The site must document all attempts to try to contact the subject in the subject's medical records and source documents.

In order to avoid loss-to-follow-up, the investigator should ask the subject at the study start for the contact details of a relative or friend who can be contacted in case the subject cannot be reached.

Subjects should not be withdrawn from follow-up unless a subject explicitly withdraws consent to be contacted. All efforts should therefore be made to minimize the number of subjects who withdraw consent.

If premature withdrawal from the study occurs for any reason, the investigator must determine the primary reason for a subject's premature withdrawal from the study and record this information in the subject's medical records and on the eCRF.

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 or Sponsor determines may jeopardize the subject’s safety if he or she continues to receive study treatment

- Investigator or Sponsor determines it is in the best interest of the subject
- *P*regnancy
- [redacted]
- Subject unable to continue to comply with study requirements

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF. Subjects who discontinue study treatment prematurely will not be replaced.

All subjects who withdraw or discontinue from the study treatment early will be asked to return as soon as possible (and within 1 week after the last dose of study drug) for an early termination visit, when the primary and secondary efficacy assessments will be performed. Subjects will also be asked to return for assessments of the primary and secondary efficacy outcomes at the end of the 24-week planned visit, regardless of their adherence to treatment. If the time between the early termination visit and the 24-week planned visit is fewer than 6 weeks, then subjects are exempted from the 24-week planned visit. If the time between the early termination visit and the previous visit is fewer than 6 weeks, then subjects are exempted from completing again all the primary and secondary assessments at the early termination visit.

4.6.2 Subject Discontinuation from Study

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

- Subject withdrawal of consent
- Study termination or site closure
- Any medical condition that may jeopardize the subject's safety if he or she continues in the study as determined by the investigator or Sponsor
- In the best interest of the subject as determined by the investigator or Sponsor
- Subject non-compliance, defined as failure to comply with protocol requirements as determined by the investigator or Sponsor

Every effort should be made to obtain information on subjects who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. If a subject requests to be withdrawn from the study, this request must be documented in the source documents and signed by the investigator. Subjects who withdraw from the study will not be replaced.

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 adverse events in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory

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

4.6.4 Site Discontinuation

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

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

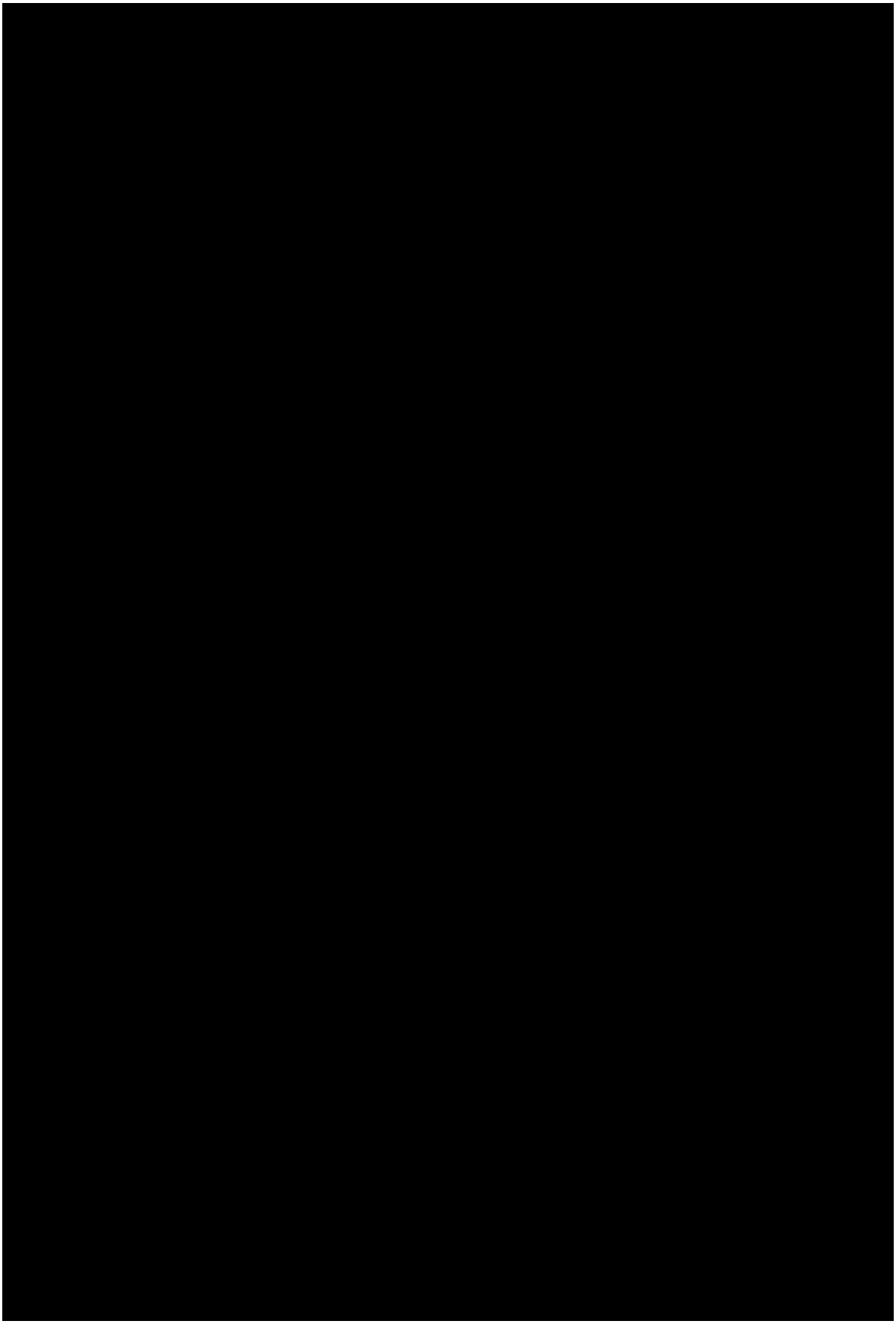
5. ASSESSMENT OF SAFETY

5.1 SAFETY PLAN

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

Several measures will be taken to ensure the safety of subjects participating in this study. Eligibility criteria have been designed to exclude subjects at potential 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 interruption or discontinuation, are provided in the following sections.

[redacted]



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5.1.2 Management of Subjects Who Experience Adverse Events

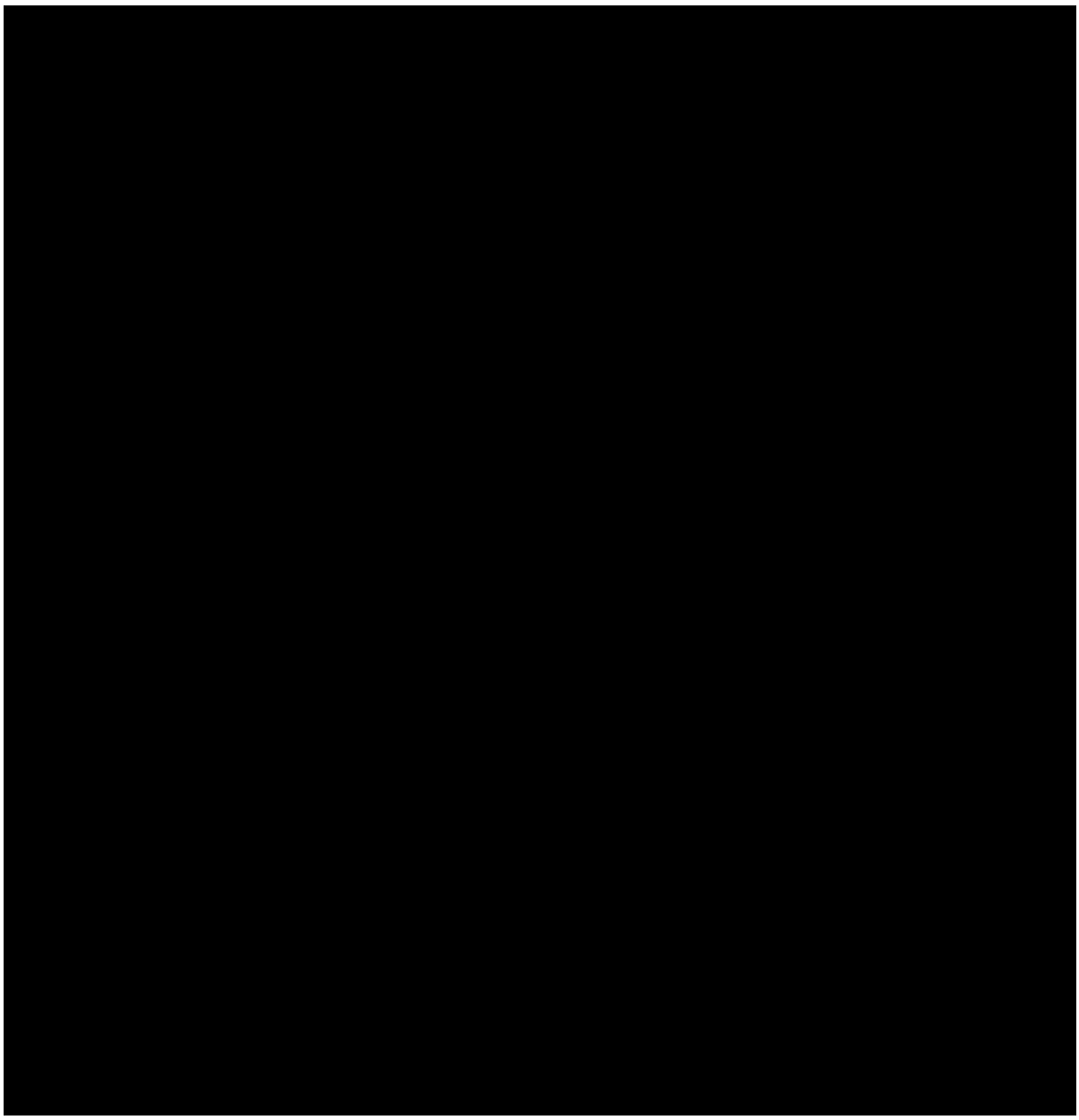
5.1.2.1 Dose Modifications

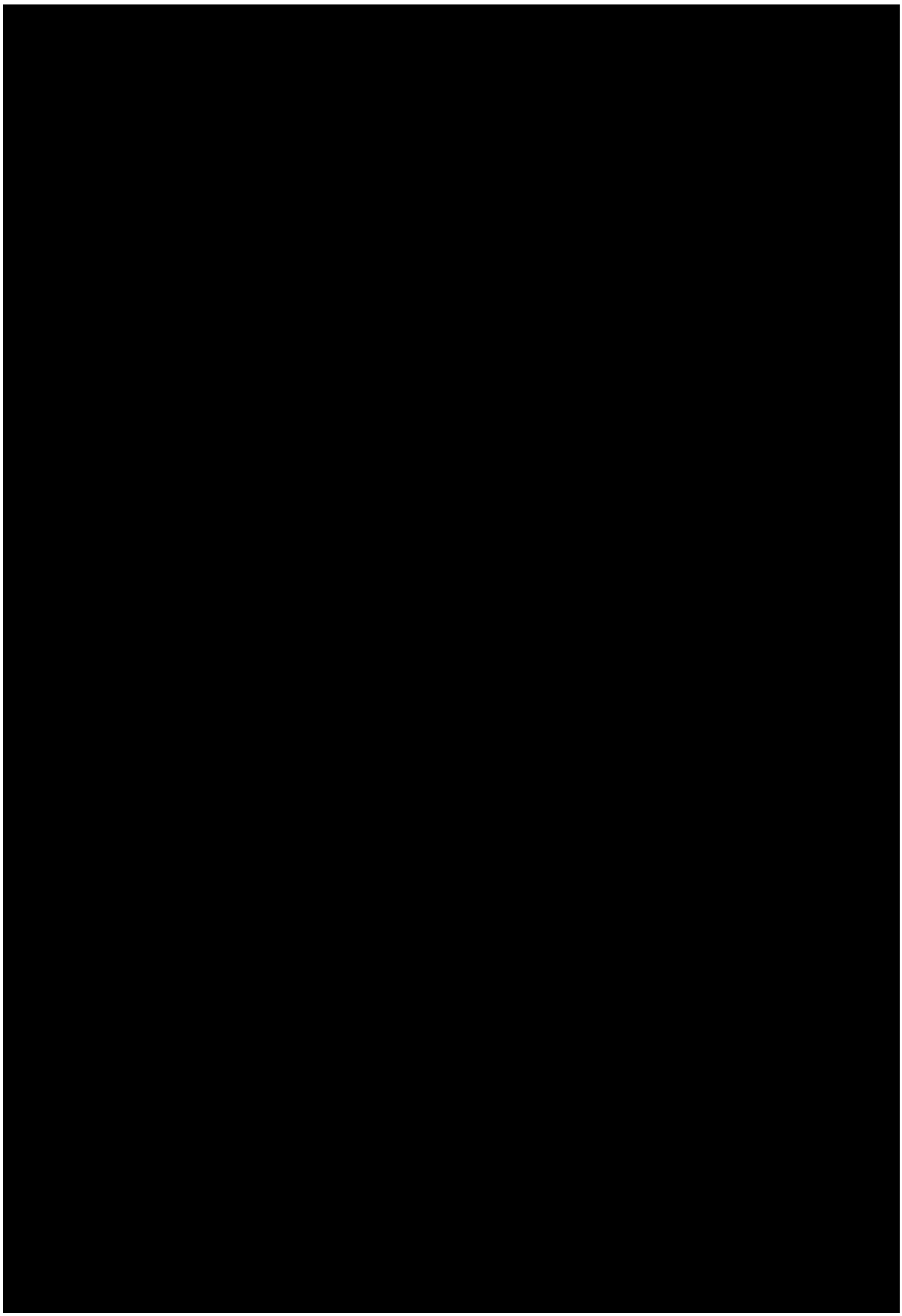
No dose modifications will be allowed in this study.

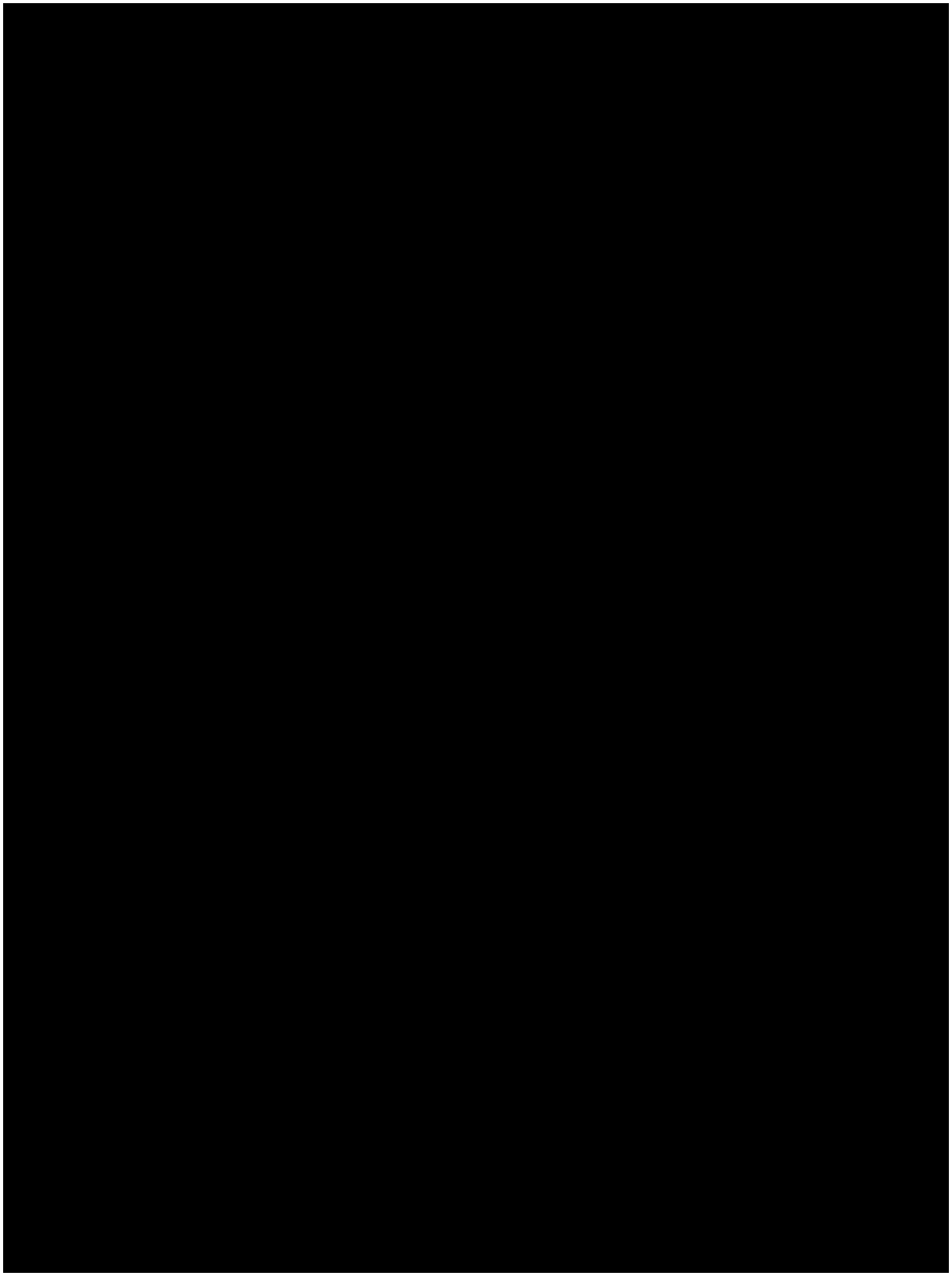
5.1.2.2 Treatment Interruption

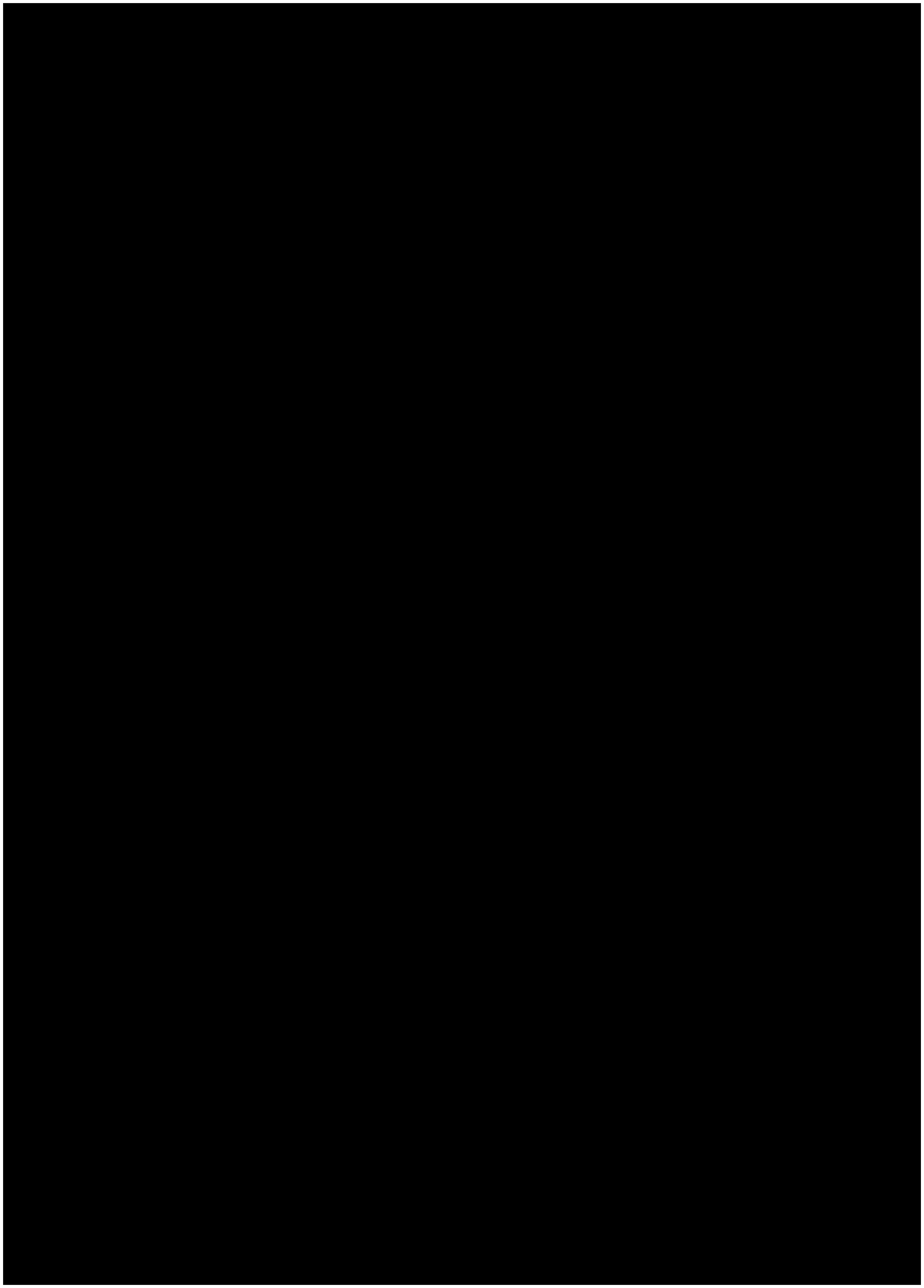
Balovaptan treatment may be temporarily or permanently suspended in subjects who experience toxicity considered to be related to study drug. Balovaptan treatment may be suspended for reasons other than toxicity (e.g., surgical procedures) with Medical Monitor approval. The investigator and the Medical Monitor will determine the acceptable length of treatment interruption.

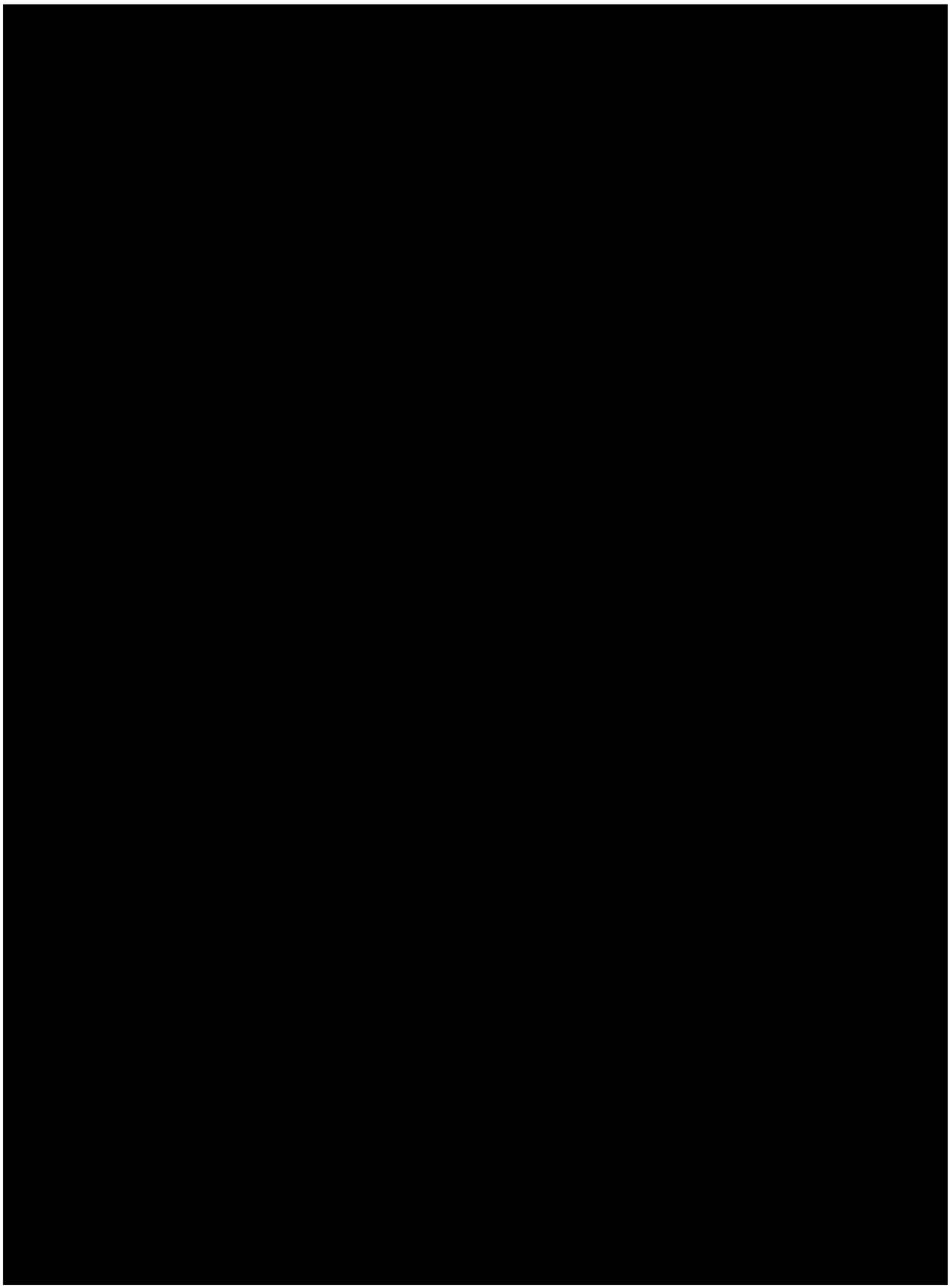
Temporary interruptions of study drug may occur and should be considered before deciding to permanently stop study drug. Reinstatement of study medication should be considered as soon as medically justified. In general, temporary study drug interruptions should be kept as short as possible. In the event of a subject experiencing intolerable adverse events considered by the investigator to be related to study drug the subjects should permanently discontinue study drug.











[redacted]

5.2 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording adverse events, including serious adverse events and adverse events of special interest, performing protocol-specified safety laboratory assessments, measuring protocol-specified vital signs, ECGs, and conducting other protocol-specified tests that are deemed critical to the safety evaluation of the study.

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

5.2.1 Adverse Events

According to the ICH guideline for Good Clinical Practice, an adverse event is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An adverse event 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) (see Sections 5.3.5.8 and 5.3.5.10 for more information)
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

5.2.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

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

- Is fatal (i.e., the adverse event actually causes or leads to death)
- Is life threatening (i.e., the adverse event, in the view of the investigator, places the subject at immediate risk of death)

  This does not include any adverse event that, had it occurred in a more severe form or was allowed to continue, might have caused death.
- Requires or prolongs inpatient hospitalization (see Section 5.3.5.9)
- Results in persistent or significant disability/incapacity (i.e., the adverse event 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 drug
- Is a significant medical event in the investigator's judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (e.g., rated as mild, moderate, or severe, or according to Adverse Events Severity Grading Scale; 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 adverse event recorded on the eCRF.

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

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

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

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

  Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is

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

- [redacted]

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The investigator is responsible for ensuring that all adverse events (see Section 5.2.1 for definition) are recorded on the Adverse Event eCRF and reported to the Sponsor in accordance with instructions provided in this section and in Sections 5.4–5.6.

For each adverse event recorded on the Adverse Event eCRF, the investigator will make an assessment of seriousness (see Section 5.2.2 for seriousness criteria), severity (see Section 5.3.3), and causality (see Section 5.3.4).

5.3.1 Adverse Event Reporting Period

Investigators will seek information on adverse events at each subject contact. All adverse events, 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.

After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention (e.g., invasive procedures such as biopsies, discontinuation of medications) should be reported (see Section 5.4.2 for instructions for reporting serious adverse events). Any other adverse event should not be reported.

After initiation of study drug, all adverse events, regardless of the relationship to study drug, will be reported until 6 weeks after the last dose of study drug.

After a period of 6 weeks from the last dose of study drug, investigators should only report any deaths, serious adverse events, or other adverse events of concern that are believed to be related to prior treatment with study drug (see Section 5.6).

5.3.2 Eliciting Adverse Event Information

A consistent methodology of non-directive questioning should be adopted for eliciting adverse event information at all subject evaluation timepoints. 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 4 provides guidance for assessing adverse event severity.

Table 4 Adverse Event Severity Grading Scale

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

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

5.3.4 Assessment of Causality of Adverse Events

Investigators should use their knowledge of the subject, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether an adverse event is considered to be related to the study drug, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration:

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

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

5.3.5 Procedures for Recording Adverse Events

Investigators should use correct medical terminology/concepts when recording adverse events on the Adverse Event eCRF. Avoid colloquialisms and abbreviations.

Only one adverse event term should be recorded in the event field on the Adverse Event eCRF.

5.3.5.1 Diagnosis versus Signs and Symptoms

For adverse events, a diagnosis (if known) should be recorded on the Adverse Event eCRF 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. If a diagnosis is subsequently established, all previously reported adverse events based on signs and symptoms should be nullified and replaced by one adverse event 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, adverse events 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 adverse event that is separated in time from the initiating event should be recorded as an independent event on the Adverse Event eCRF. 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 GI hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and consequent fracture, all three events should be reported separately on the eCRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the eCRF.

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

5.3.5.3 Persistent or Recurrent Adverse Events

A persistent adverse event is one that extends continuously, without resolution, between subject evaluation timepoints. Such events should only be recorded once on the Adverse Event eCRF. The initial severity (intensity or grade) of the event will be recorded at the time the event is first reported. If a persistent adverse event becomes

more severe, the most extreme severity should also be recorded on the Adverse Event eCRF. Details regarding any increases or decreases in severity will be captured on the Adverse Event Intensity or Grade Changes eCRF. 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 should be updated by changing the event from "non-serious" to "serious," providing the date that the event became serious and completing all data fields related to serious adverse events.

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

5.3.5.4 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an adverse event. A laboratory test result must be reported as an adverse event 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's judgment

It is the investigator'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 adverse event.

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

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, 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 adverse event. 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 (see Section 5.3.5.3 for details on recording persistent adverse events).

For NT-proBNP, ULN and diagnostic cutoffs with regard to heart failure in adults from published analyses are defined for the study as provided in Table 5.

Table 5 NT-proBNP ULN and Diagnostic Cutoff Values

ULN [a]		*Diagnostic Cutoff* [b]	
Age	*pg/mL*	*Age*	*pg/mL*
≥18	*125*	*≥18*	*300*

ULN = upper limit of normal.

[a] *Nir et al. 2009.*

[b] *Lin et al. 2014.*

5.3.5.5 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an adverse event. A vital sign result must be reported as an adverse event 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's judgment

It is the investigator'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 adverse event.

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.

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

5.3.5.6 Abnormal Liver Function Tests

The finding of an elevated ALT or AST (>3× ULN) in combination with either an elevated total bilirubin (>2× 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, investigators must report as an adverse event the occurrence of either of the following:

- Treatment-emergent ALT or AST >3× ULN in combination with total bilirubin >2× ULN

- Treatment-emergent ALT or AST >3× 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 (see Section 5.3.5.2) and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event), either as a serious adverse event or an adverse event of special interest (see Section 5.4.2).

5.3.5.7 Deaths

All deaths that occur during the protocol-specified adverse event reporting period (see Section 5.3.1), regardless of relationship to study drug, must be recorded on the Adverse Event eCRF and 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. 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. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death. The term **"sudden death"** should not be used unless combined with the presumed cause of death (e.g., "sudden cardiac death").

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

5.3.5.8 Preexisting Medical Conditions

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

A preexisting medical condition should be recorded as an adverse event only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF, 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 Lack of Efficacy or Worsening of Autism Spectrum Disorder

Medical occurrences or symptoms of deterioration that are anticipated as part of ASD should be recorded as an adverse event if judged by the investigator to have unexpectedly worsened in severity or frequency or changed in nature at any time during the study. When recording an unanticipated worsening of symptoms related to ASD on the Adverse Event eCRF, it is important to convey the concept that the condition has changed by including applicable descriptors.

5.3.5.10 Hospitalization or Prolonged Hospitalization

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

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

- Hospitalization for respite care
- 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 adverse event

5.3.5.11 *Reporting Requirements for Cases of Overdose, Medication Error, Drug Abuse, or Drug Misuse*

Overdose (accidental or intentional), medication error, drug abuse, and drug misuse (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*
- *Intentional overdose: intentional 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.
- *Drug abuse: intentional excessive use of a drug that may lead to addiction or dependence, physical harm, and/or psychological harm*
- *Drug misuse: intentional deviation in the administration of a drug that does not qualify as drug abuse*

  In cases where drug is to be self administered by the subject, drug misuse could involve the drug being administered to someone other than the subject.

Special situations are not in themselves adverse events, but may result in adverse events. All special situations associated with balovaptan regardless of whether they result in an adverse event, 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.*

- *Intentional overdose: Enter the drug name and "intentional overdose" as the event term. Check the "Intentional overdose" box. If drug abuse is suspected, check the "Drug abuse" box. If drug abuse is not suspected, check the "Drug misuse" box.*
- *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.*
- *Drug abuse that does not qualify as an overdose: Enter the drug name and "drug abuse" as the event term. Check the "Drug abuse" box.*
- *Drug abuse that qualifies as an overdose: Enter the drug name and "intentional overdose" as the event term. Check the "Intentional overdose" and "Drug abuse" boxes.*
- *Drug misuse that does not qualify as an overdose: Enter the drug name and "drug misuse" as the event term. Check the "Drug misuse" box.*
- *Drug misuse that qualifies as an overdose: Enter the drug name and "intentional overdose" as the event term. Check the "Intentional overdose" and "Drug misuse" boxes.*
- *Drug administered to someone other than the subject: Enter the drug name and "subject supplied drug to third party" as the event term. Check the "Drug misuse" box.*

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

- *Accidental overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.*
- *Enter the adverse event term. Check the "Intentional overdose" box. If drug abuse is suspected, check the "Drug abuse" box. If drug abuse is not suspected, check the "Drug misuse" box.*
- *Medication error that does not qualify as an overdose: Enter the adverse event term. Check the "Medication error" box.*
- *Medication error that qualifies as an overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.*

- *Drug abuse that does not qualify as an overdose: Enter the adverse event term. Check the "Drug abuse" box.*
- *Drug abuse that qualifies as an overdose: Enter the adverse event term. Check the "Intentional overdose" and "Drug abuse" boxes.*
- *Drug misuse that does not qualify as an overdose: Enter the adverse event term. Check the "Drug misuse" box.*
- *Drug misuse that qualifies as an overdose: Enter the adverse event term. Check the "Intentional overdose" and "Drug misuse" boxes.*

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

5.3.5.12 Patient-Reported or Observer-Reported Outcome Data

Adverse event reports will not be derived from PRO or observer-reported outcome (ObsRO) data by the Sponsor, and safety analyses will not be performed using PRO or ObsRO data. Sites are not expected to review the PRO or ObsRO data for adverse events.

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 must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

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

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

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

- Additional narrative information on the clinical course of the event

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

5.4.1 Emergency Medical Contacts

Medical Monitor Contact Information for All Sites

Medical Monitor: [redacted], M.D. (Primary)

Mobile Telephone No.: [redacted]

Medical Monitor: [redacted], O.T.

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 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.

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

5.4.2.1 Events That Occur prior to Study Drug Initiation

After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. The paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators 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 investigators.

5.4.2.2 Events That Occur after Study Drug Initiation

After initiation of study drug, serious adverse events and adverse events of special interest will be reported until 6 weeks after the last dose of study drug. Investigators should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the Adverse Event eCRF and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to Roche Safety Risk Management by the EDC system.

In the event that the EDC system is unavailable, the paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators 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 investigators. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

Instructions for reporting serious adverse events that occur *beyond* 6 weeks 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 should use the contraception method defined by the protocol for the duration of the trial and for 28 days after receiving their last dose of balovaptan and should take all appropriate precautions to avoid becoming pregnant. Regular pregnancy tests will be performed during the study.

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

In the event of pregnancy, the investigator must counsel the subject as to the risks of continuing with the pregnancy and the possible effects on the fetus. Given there are insufficient, well-controlled data from studies testing the use of balovaptan in pregnant or breastfeeding women, all dosing of balovaptan must be suspended until the end of pregnancy and breastfeeding.

Monitoring of the subject should continue until conclusion of the pregnancy. Any serious adverse events 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. In addition, the investigator 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 Abortions

A *spontaneous* abortion should be classified as a serious adverse event (as the Sponsor considers abortions to be medically significant), 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).

If a therapeutic or elective abortion was performed because of an underlying maternal or embryofetal toxicity, the toxicity should be classified as a serious adverse event, 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 adverse event.

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

5.4.3.3 Congenital Anomalies/Birth Defects

Any congenital anomaly/birth defect in a child born to a female subject or a female partner of a male subject exposed to study drug should be classified as a serious adverse event, 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).

5.5 FOLLOW-UP OF SUBJECTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the subject is lost to follow-up, or the subject withdraws consent. Every effort should be made to follow all serious adverse events 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 in the subject's medical record to facilitate source data verification. If, after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded on the Adverse Event eCRF.

All pregnancies reported during the study should be followed until pregnancy outcome and reported according to the instructions provided in Section 5.4.3.

5.5.2 Sponsor Follow-Up

For serious adverse events, adverse events of special interest, 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 investigator is not required to actively monitor subjects for adverse events after the end of the adverse event reporting period (defined as 6 weeks after the last dose of study drug). However, the Sponsor should be notified if the investigator becomes aware

of any serious adverse event that occurs after the end of the adverse event reporting period if the event is believed to be related to prior study drug treatment.

The investigator 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 serious adverse events and adverse events of special interest against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, IRBs, ECs, and applicable health authorities based on applicable legislation.

Independent from the causality assessment, the following serious adverse events and non-serious adverse events 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 adverse event cases, the Sponsor will assess the expectedness of these events to balovaptan using the following reference document:

- Balovaptan Investigator's Brochure

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's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed. An iDMC will monitor the incidence of these events during the study. 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

Database lock to enable the analysis of the 24-week, placebo-controlled phase of the study will occur once all subjects have either completed the 24-week assessment or withdrawn from the study early, and all data required for analysis have been cleaned and verified. After database lock, treatment assignments will be unblinded to the Sponsor.

The final analysis of the 24-week placebo-controlled period will be performed after database lock. The final analysis of all data collected in the study, including the open-label extension, will occur after all subjects have completed the study.

Details of the planned statistical analyses mentioned below in this section will be fully specified in the Statistical Analysis Plan, which will be finalized prior to the locking the study database and unblinding the study.

6.1 ANALYSIS POPULATIONS

6.1.1 Safety Population

The safety population will consist of all subjects who receive any study treatment. Randomized subjects who receive incorrect therapy from that intended will be summarized in the group according to their planned randomized treatment.

The safety population will be the primary population for all analyses of safety data.

6.1.2 Intent-to-Treat Population

The ITT population will consist of all subjects who receive any study treatment. Randomized subjects who receive incorrect therapy from that intended will be summarized in the group according to their planned randomized treatment.

The ITT population will be the primary population for all analyses of primary and secondary efficacy variables.

6.2 DETERMINATION OF SAMPLE SIZE

The sample size of 350 randomized subjects (175 subjects per arm) provides 85% power to detect a difference in means between treatments in the change from baseline at Week 24 on the Vineland-II 2DC score of at least 4.0 points, assuming a two-sided 5% significance level and a standard deviation of about 12.5 points. A blinded interim analysis of the change in Vineland-II 2DC score from baseline may be performed by the Sponsor to allow an increase in the sample size if necessary to achieve appropriate study power.

6.3 SUMMARIES OF CONDUCT OF STUDY

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

6.4 SUMMARIES OF TREATMENT GROUP COMPARABILITY

Demographic and baseline characteristics (including age, sex, WASI®-II IQ score, SRS-2 total *t*-score) will be summarized by treatment group 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.

6.5 EFFICACY ANALYSES

6.5.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline at Week 24 on the Vineland-II 2DC score, defined as the mean of the Communication domain standard score and the Socialization domain standard score.

6.5.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are identified in Section 2, Table 1.

6.5.3 Exploratory Efficacy Endpoints

The exploratory efficacy endpoints are identified in Section 2, Table 1.

6.5.4 Estimands

The primary efficacy study estimand is the difference in means between balovaptan and placebo in the change from baseline at Week 24 in Vineland-II 2DC score in the targeted ASD adult population, as identified by the study inclusion and exclusion criteria.

Intercurrent events that occur after treatment initiation and either preclude observation of the variable or affect its interpretation might occur. The appropriate strategy to treat intercurrent events will be described in the Statistical Analysis Plan. As a general principle, if treatment discontinuation is due to:

- Study drug–related reasons (e.g., treatment-related adverse event, or lack of efficacy) then the actual “off-treatment” values will be used in the analysis
- Not study drug–related reasons (e.g., lost to follow-up), then “imputed hypothetical” values, as if subjects had continued receiving study drug, will be used in the analysis

The primary statistical analysis will be performed according to a combination of "treatment-policy strategy" and "hypothetical strategy" in order to account for the different types of intercurrent events.

The Sponsor will put mechanisms in place to ensure that, as much as possible, all subjects are followed and their data are collected, up to and including the Week 24 visit, regardless of their adherence to treatment ("retrieved dropout" strategy). In case missing data are still present, imputation techniques may be considered separately for each treatment arm by using values while the subject was "on-treatment." The primary estimator will be derived by the statistical model described in Section 6.5.5 on the dataset completed after multiple imputation. Details of each imputation statistical approach (e.g., the number of imputed datasets created, imputation methodology) will be described in the Statistical Analysis Plan.

6.5.5 Primary Efficacy Analysis

The primary efficacy analysis for this trial will compare balovaptan with placebo at Week 24. The following null (H_0) and alternative (H_a) hypotheses will be tested at a two-sided α=0.05 level:

- H_0: $MEAN_{balovaptan} = MEAN_{placebo}$ versus
- H_a: $MEAN_{balovaptan} \neq MEAN_{placebo}$

for which the $MEAN_{RO}$ and $MEAN_{placebo}$ refer to the mean change from baseline for balovaptan and placebo, respectively.

The analysis of the primary endpoint, the change from baseline at Week 24 on the Vineland-II 2DC score, will be performed by means of analysis of covariance. The model will include the corresponding endpoint baseline score as covariate and treatment, age group, sex, and geographical region as fixed effects. Based on this analysis, least square means, standard errors, treatment difference, and corresponding 95% confidence intervals will be reported.

The effects on continuous secondary endpoints, as well as the short-term effect at 12 weeks will be similarly analyzed.

As a supplementary analysis, a mixed-model repeated measurement (MMRM) analysis, will be performed. The MMRM model will include the corresponding endpoint baseline score as a covariate, with visit, treatment, age group, sex, and geographical region as fixed effects, and treatment by visit interaction; visit will be fitted as a repeated effect with unstructured correlation across visits within each subject.

6.5.6 Secondary Efficacy Analyses

Similar hypotheses will also be tested for the secondary continuous efficacy parameters. Methods for controlling the type I error in the testing of secondary endpoints as well as their hierarchical order will be described in the Statistical Analysis Plan.

Categorical endpoints, such as the CGI-I and change from baseline of CGI-S, will be analyzed by means of logistic regression after dichotomizing the results into two categories (improved and not improved), considering the CGI-S baseline value as a covariate, and treatment, age group, sex and geographical region as factors. Logistic regression will be performed also for the responder endpoint, considering the Vineland-II 2DC score at baseline as a covariate, and treatment, age group, sex, and geographical region as factors.

6.5.7 Subgroup Analyses

The results of selected efficacy variables will be summarized within subgroups using descriptive statistics. The following variables will be used to define the subgroups:

- Baseline 2DC score of the Vineland-II ($<$60 vs. $\geq$60)

- Sex (male vs. female)
- Geographical region (North America vs. ROW)
- Age (<25 years vs. ≥25 years)

Additional exploratory analyses of efficacy results may be performed using an MMRM model, with subgroup, treatment-by-subgroup interaction, and subgroup-by-time interaction terms included along the independent effects described above. Such exploratory analyses will be performed if the descriptive statistics results indicate substantial differences between the subgroups.

6.5.8 Exploratory Analyses

The exploratory endpoints will be summarized using tables, listings, and graphs as appropriate.

6.5.9 Futility Analyses

One or more interim futility analyses *will* be performed during the study. The analysis will be performed by the external independent Data Coordinating Center that generates the unblinded results reviewed by the iDMC on a regular basis, as described in Section 3.1.1.6.

The iDMC will review the unblinded results for the primary efficacy endpoint (change from baseline at Week 24 in the Vineland-II 2DC score) and for the Adaptive Behavior Composite and the three individual Vineland-II domains standard scores. *The iDMC will be given clear criteria, detailed in the Statistical Analysis Plan, regarding what would constitute futility, and it is expected to recommend stopping the trial if the futility analysis results meet those criteria.* If the analysis of the primary endpoint results at the time of this interim analysis indicates that the conditional probability of success for the trial is less than a prespecified threshold, then the committee may recommend that the trial be terminated for futility. The iDMC will communicate to the Roche Data Review Board (DRB) only this recommendation (whether or not to stop the study for futility); detailed results of the futility analysis will not be shared with the Sponsor unless specifically requested by the DRB. *Roche DRB Chair (or designate) will make the final decision regarding stopping the trial.*

No adjustment for multiple comparisons will be made to the α-level for this analysis, as the decision rules for the futility analysis will not allow for the opportunity to stop the study early for overwhelming efficacy.

The specifics of the interim futility analysis will be documented prior to its conduct in the Statistical Analysis Plan.

6.6 SAFETY DATA ANALYSIS

All verbatim adverse event terms will be mapped to MedDRA thesaurus terms, and adverse event severity will be graded according to Table 4. All safety data will be reported in individual listings and summarized by treatment for each assessment time using descriptive statistics. Data collected during the follow-up period (i.e., after discontinuation of study treatment) will be summarized separately from the data collected during the treatment period.

The incidence of adverse events will be summarized on the basis of body systems and dictionary preferred terms. The incidence of adverse events by severity and relationship to study drug and incidence of marked abnormal laboratory test results will be provided. Laboratory tests, ECG, and vital signs data will be summarized by descriptive statistics both as original value as well as the change from baseline.

The iDMC will review safety data throughout the study. Analyses required for the iDMC data review will be performed as described in the iDMC Charter.

6.7 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

A population-PK analysis will be performed to describe the pharmacokinetics of balovaptan in subjects with ASD and to assess the influence of covariates of interest on the PK parameters. Non-linear mixed effects modeling (using the software, NONMEM) will be used to analyze the concentration–time data of balovaptan. The starting model will be a model developed based on data from previous Phase I/II studies (Roche Report No. 1081347). The data collected in this study may be pooled with data collected in previous studies as appropriate to inform the population-PK model. Population and individual primary PK parameters (e.g., clearances and volumes) will be estimated. Secondary PK parameters such as AUC and maximum plasma concentration will be derived from the individual post-hoc predictions. In addition, measured plasma concentrations of M2 (as applicable) and M3 metabolites will be summarized by timepoint and the relationship of parent and metabolite, as well as the influence of covariates of interest may be explored graphically. Plasma concentrations of balovaptan, its metabolites M2 (RO7045402) as applicable, and M3 (RO5273004) will be measured using a specific and validated liquid chromatography tandem mass spectrometry method.

Graphical exploration of the relationship between balovaptan exposure and the Vineland-II 2DC score and other selected clinical efficacy and safety endpoints will be performed. If indicated by such exploration, more formal analyses of the exposure–response relationship will be performed using pharmacometric modeling methods.

Details of the PK and pharmacodynamic analyses will be described in a Modeling and Simulation Analysis Plan. The results will be reported in a document separate from the Clinical Study Report.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

The Sponsor will be responsible for data management of this study, including quality checking of the data. Data entered manually will be collected via EDC through use of eCRFs. Sites will be responsible for data entry into the EDC system. In the event of discrepant data, the Sponsor will request data clarification from the sites, which the sites will resolve electronically in the EDC system. Discrepancies in vendor data (e.g., central laboratory and eCOA) will be managed outside of the EDC system.

The Sponsor will produce an EDC Study Specification document (Data Quality Review Plan and Data Management Plan) that describes the quality checking to be performed on the data. Central laboratory data and eCOA data will be sent directly to the Sponsor, using the Sponsor's standard procedures to handle and process the electronic transfer of these data.

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

eCOA data will be collected through the use of an electronic device provided by a vendor (see Section 7.3 for details).

7.2 ELECTRONIC CASE REPORT FORMS

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

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

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

7.3 ELECTRONIC CLINICAL OUTCOME DATA

Subjects, study partners, and clinicians will use an electronic device to capture eCOA data. The device is designed for entry of data in a way that is attributable, secure, and accurate, in compliance with U.S. FDA regulations for electronic records (21 CFR,

Part 11). The data will be transmitted to a centralized database maintained by the electronic device vendor.

The electronic data will be available for view access only, via a secure vendor web portal. Only identified and trained users may view the data, and their actions will become part of the audit trail. The Sponsor will have view access only. System backups for data stored by the Sponsor and records retention for the study data will be consistent with the Sponsor's standard procedures.

Once the study is complete, the data, audit trail, and trial and system documentation will be archived. The investigator will receive subject data for the site in both human- and machine-readable formats on an archival-quality compact disc that must be kept with the study records as source data. Acknowledgement of receipt of the compact disc is required. In addition, the Sponsor will receive all data in a machine-readable format on a compact disc.

7.4 SOURCE DATA DOCUMENTATION

Study monitors will perform ongoing source data verification and review to confirm that critical protocol data (i.e., source data) entered on 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, PROs, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, the types of source documents that are to be generated will be clearly defined in the Trial Monitoring Plan. This includes any protocol data to be entered directly on 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 on the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 7.6.

To facilitate source data verification and review, the investigators 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.

7.5 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.

7.6 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, electronic or paper PRO and ObsRO data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 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 Clinical Study Report has been completed 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 Good Clinical Practice 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 United States or under a U.S. Investigational New Drug (IND) Application will comply with U.S. FDA regulations and applicable local, state, and federal laws. Studies conducted in the European Union or European Economic Area will comply with the E.U. Clinical Trial Directive (2001/20/EC) *and applicable local, regional, and national laws.*

8.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form will be provided to each site. If applicable, it will be provided in a certified translation of the local language. The

Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC submission. The final IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

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

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

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

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

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

For sites in the United States, each Consent Form may also include subject authorization to allow use and disclosure of personal health information in compliance with the U.S. 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. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments (see Section 9.6).

In addition to the requirements for reporting all adverse events to the Sponsor, investigators must comply with requirements for reporting serious adverse events to the local health authority and IRB/EC. Investigators may receive written IND safety reports or other safety-related communications from the Sponsor. Investigators 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 Roche policy on study data publication (see Section 9.5).

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

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 and governmental 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.

The Sponsor shall also submit as a periodic safety report once a year, a Development Safety Update Report, to the independent EC and regulatory authorities according to local regulatory requirements and timelines of each country participating in the study.

It is the understanding of the Sponsor that this protocol (and any modifications), as well as appropriate consent procedures and advertisements, will be reviewed and approved by an IRB. This board must operate in accordance with the current federal regulations. The Sponsor will be sent a letter or certificate of approval prior to initiation of the study and whenever subsequent amendments modifications are made to the protocol.

A Clinical Study Report will be written and submitted to relevant IRBs/ECs and regulatory authorities in accordance with local requirements. To fulfill the requirements for the E.U. Directive No. 75/318/EEC, the Clinical Study Report will be signed by a coordinating investigator who will be designated at a later stage.

9.2 PROTOCOL DEVIATIONS

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

9.3 SITE INSPECTIONS

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

9.4 ADMINISTRATIVE STRUCTURE

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

Approximately 50 sites globally will participate to enroll approximately 350 subjects. Enrollment will be managed through an IxRS.

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 4.5.5. Accredited local laboratories will be used for routine monitoring; local laboratory ranges will be collected.

An iDMC will be employed to monitor and evaluate subject safety throughout the study.

9.5 PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, 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, provided the requirements of Roche's global policy on data sharing have been met.* For more information, refer to the Roche Global Policy on Sharing of Clinical Trials Data at the following web site:

www.roche.com/roche_global_policy_on_sharing_of_clinical_study_information.pdf

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

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

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

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

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

9.6 PROTOCOL AMENDMENTS

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

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

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Appendix 1
Schedule of Activities: Double-Blind Period to Week 24

	Screening	Treatment								
Week(s)	–4 to –1	BL	1	2	4	8	12	16	20	24 or ET [a, b]
Day(s) (±3 days)	–28 to –1	1 [c]	8 ☎	15	29	57	85 [c]	113	141	169 [c]
Informed consent	x									
[redacted]										
Medical history, demographics, and baseline conditions [d]	x									
Weight and height (height to be measured at screening only)	x						x			x
Vital signs [e, f]	x	x		x	x	x	x	x	x	x
PK blood sample [g]				x		x	x		x	x
Clinical genotyping sample		x								
RBR blood sample (optional) [h]		x								
Complete physical examination [i]		x								
Limited physical examination [i]							x			x
Neurologic evaluation [j]		x			x	x	x	x	x	x
Clinical signs and symptoms of infection check [k]		x	Every 2 weeks							
12-Lead ECG [l]	x	x			x		x			x
Pregnancy test [m]	x	x				x		x		x
Serology (HIV, hepatitis B, and hepatitis C) [n]	x									

Appendix 1
Schedule of Activities: Double-Blind Period to Week 24 (cont.)

	Screening	Treatment								
Week(s)	–4 to –1	BL	1	2	4	8	12	16	20	24 or ET [a, b]
Day(s) (±3 days)	–28 to –1	1 [c]	8 ☎	15	29	57	85 [c]	113	141	169 [c]
Hematology [o]	x	x			x	x	x	x	x	x
Coagulation [p]	x									x
Chemistry [q]	x	x		(x)			x			x
[redacted]										
Quantitative immunoglobulins		x								x
Urinalysis [r]	x	x								x
Urine analysis for substance use [s]	x									x
ADOS-2 (if not completed within 12 months by a certified rater)	x									
SRS™-2 (study partner)	x									
IQ test (if not completed within 12 months by a certified rater) [t]	x									
Vineland™-II (clinician and study partner)		x					x			x
PedsQL™–4.0 Core (subject)		x					x			x
PedsQL–Cognitive Functioning (subject)		x					x			x
PSQI (subject)		x					x			x
EQ-5D-5L (subject)		x					x			x
PGI-S (subject)		x					x			x
PGI-C (subject)							x			x

Appendix 1
Schedule of Activities: Double-Blind Period to Week 24 (cont.)

	Screening	Treatment								
Week(s)	–4 to –1	BL	1	2	4	8	12	16	20	24 or ET [a, b]
Day(s) (±3 days)	–28 to –1	1 [c]	8 ☎	15	29	57	85 [c]	113	141	169 [c]
HAM-A (clinician and subject)		x					x			x
C-SSRS BL/SLV [u]		x			x		x		x	x
PedsQL Family Impact (study partner)		x					x			x
RBS-R (study partner)		x					x			x
EQ-5D-5L (study partner)		x					x			x
CaGI-S (study partner)		x					x			x
CaGI-C (study partner)							x			x
CGI-S (clinician)	x	x			x		x		x	x
CGI-I (clinician)					x		x		x	x
[redacted]										
Previous and concomitant medications [w]	x	x	x	x	x	x	x	x	x	x
Adverse events [x]	x	x	x	x	x	x	x	x	x	x
Study drug dispensation [y]		x			x	x	x	x	x	(OLE)

Appendix 1
Schedule of Activities: Double-Blind Period to Week 24 (cont.)

ADOS-2=Autism Diagnostic Observation Schedule, Second Edition; BL=baseline; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impression–Improvement; CGI-S=Clinical Global Impression–Severity; C-SSRS =Colombia-Suicide Severity Rating Scale; *cTnI=troponin I;* eCRF= electronic Case Report Form; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; ET=early termination; HAM-A=Hamilton Anxiety Rating Scale; HBcAb = hepatitis B core antibody; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; [redacted] IQ=intelligence quotient; OLE=open-label extension; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBR=Research Biosample Repository; RBS-R=Repetitive Behavior Scale–Revised; SLV=since last visit; SRS™-2=Social Responsiveness Scale™, Second Edition; ULN=upper limit of normal; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition; *WAIS®-IV=Wechsler Adult Intelligence Scale®, Fourth Edition;* WASI®-II=Wechsler Abbreviated Scale of Intelligence®, Second Edition.

Notes: The visit window is ±3 days, and all assessments should be completed within the visit window. Subjects should return to the initial planned schedule per randomization for subsequent visits. For subjects who terminate early, assessments listed under the ET visit should be completed. For the schedule of activities to be performed at unscheduled visits and during the safety follow-up period, see Appendix 2.

[a] To be conducted if a subject discontinues from the study or study drug treatment prematurely.

[b] If a subject continues into the OLE period, the follow-up visit will be conducted 1 week, 2 weeks, and 12 weeks after the last dose of study drug in OLE period. If the subject does not enter the OLE period, the follow-up visit will be conducted 1 week, 2 weeks, and 12 weeks after completion of the double-blind phase (see Appendix 2).

[c] The visit may be split over 2 days, as long as the visit remains within visit window. If the baseline visit is split over 2 days, then dosing should occur on the second day. If the Week 12 or 24 visit is split over 2 days, then dosing should occur at the end of the each visit, i.e., no dosing should be skipped.

[d] Medical history includes clinically significant diseases, surgeries, reproductive status, smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by the subject within 24 weeks prior to the screening visit as well as details of psychosocial or non-pharmacological interventions used during the previous 52 weeks. Demographic data will include age, sex, and self-reported race/ethnicity. Subjects and study partners will also be asked about the subject's residential setting, school, or employment status, level of education, participation in educational or day programs, and any non-medical hospitalizations. Demographics of the subject's study partner will also be recorded, including age, relationship to subject, education level attained, and employment status.

Appendix 1
Schedule of Activities: Double-Blind Period to Week 24 (cont.)

[e] Vital signs include measurements of respiratory rate, pulse rate, and systolic and diastolic blood pressure while the subject is in a seated position, and body temperature (measured using a tympanic thermometer). Vital sign assessments should be performed just prior to study drug administration. All vital signs should be recorded on the eCRF.

[f] Temperature will be recorded (measured using a tympanic thermometer) every 2 weeks for the double-blind period and continue into OLE period up until Week 52 of trial participation by the subject or study partner and recorded in a subject diary or device.

[g] For Weeks 2, 12, and 24, PK samples should be obtained just prior to study drug administration, if possible. For Weeks 8 and 20, the subject should take the study drug approximately 2 hours prior to the study visit. The PK sample should be obtained at least 3 hours after study drug administration. Accurate recording of the time of study drug administration and sample collection is critical.

[h] Not applicable for a site that has not been granted approval for RBR sampling. Perform only for subjects at participating sites who have provided written informed consent to participate.

[i] Includes evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, and neurologic systems. Complete physical examinations will not include pelvic, rectal, or breast examinations. Record abnormalities observed at baseline on the General Medical History and Baseline Conditions eCRF. At subsequent visits, record new or worsened clinically significant abnormalities on the Adverse Event eCRF. Limited, symptom-directed physical examinations should be performed at Weeks 12 and 24, and as clinically indicated. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

[j] Neurologic evaluations will be completed at the baseline and at the Week 4, 8, 12, 16, 20, and 24 visits (see Appendix 4).

[k] Site personnel will contact the subject and/or study partner every 2 weeks to ask about signs and symptoms of infections (see Appendix 5 for details). In the event of such reports, the investigator should use clinical judgement as to whether to call a subject in for further evaluations.

[l] Twelve-lead ECGs are to be performed after the subject has been in a supine position for 5 minutes. ECGs for each subject should be obtained from the same machine whenever possible and performed prior to any blood draws. At Week 4, perform predose and 4 hours postdose. ECGs obtained at baseline will be in triplicate. All others will be single recordings. In the event of prolongation of the QTc interval, an unscheduled PK sample should be obtained.

[m] Females of childbearing potential (including those who have had a tubal ligation) must have a urine pregnancy test performed at the site prior to each dose administration *at the specified visits*. If a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test at the central laboratory. Results are to be recorded only in the source documents.

Appendix 1
Schedule of Activities: Double-Blind Period to Week 24 (cont.)

[n] Viral serology includes HIV (specific tests for HIV-1 antibody, HIV-1 and -2 antibodies, and HIV-2 antibody), HBsAg, HBcAb, and HCV antibody.

[o] Hematology includes WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, and other cells).

[p] Coagulation assessments include INR, aPTT, and PT.

[q] Serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, BUN or urea, creatinine, CPK, total protein, albumin, phosphorus, calcium, total and direct bilirubin, ALP, ALT, AST, uric acid, LDH, cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, [redacted]

[r] Urinalysis will be performed at the site by dipstick for blood, protein, glucose, ketones, specific gravity, and pH. Microscopic examination performed at the central laboratory if blood and/or protein results are positive or strongly positive. If there is an explanation for the positive dipstick results (e.g., menses), it should be recorded and there is no need to perform microscopy and culture. Results do not need to be recorded on the eCRF.

[s] Urine samples will be analyzed for the presence of the following drugs: alcohol, cannabinoids, opiates, cocaine, barbiturates, methadone, and phencyclidine (PCP). Results will be used to verify subject eligibility pertaining to drugs of abuse. Inconclusive results may be repeated once during the screening period. Investigators should use their best clinical judgment in cases where results may be erroneous (e.g., permitted use of opiates or ingestion of food or food supplements).

[t] IQ tests may include *WAIS*®-IV or WASI®-II as long as full scale IQ scores and subtest domain scores are available and the test was completed within 12 months prior to screening,

[u] The C-SSRS Baseline version should be used on the baseline visit; subsequent visits should use the C-SSRS Since Last Visit version.

[v] [redacted]

[w] Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, approved dietary and herbal supplements, nutritional supplements and any non-medicinal interventions, such as individual psychotherapy, cognitive behavioral therapy, smoking cessation therapy, and rehabilitative therapy) used by a subject 24 weeks prior to screening until the follow-up visit.

Appendix 1
Schedule of Activities: Double-Blind Period to Week 24 (cont.)

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[x] After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. The investigator is not required to actively monitor subjects for adverse events after the end of the adverse event reporting period (defined as 6 weeks after the last dose of study drug). However, the Sponsor should be notified if the investigator becomes aware of any serious adverse event that occurs after the end of the adverse event reporting period if the event is believed to be related to prior study drug treatment. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

[y] Study drug administration should occur only after all assessments and rating scales for the subject are completed. Subjects should be instructed to take their medication at the same time each day, with the exception of study visits when they should take only their study drug once all assessments are completed. Study drug dispensation can take place at any time during the visit. For the Week 8 and 20 study visits, the subject should take his or her medication approximately 2 hours prior to the study visit.

Appendix 2
Unscheduled Visit and Follow-Up Assessments: Double-Blind and OLE Periods

	Unscheduled Visit [a]	Follow-Up Period [b] (±3 days)		
Week	NA	1	2	12
Day	NA	176	183	253
Study site visit	NA	☎	x	x [c]
Vital signs [d]	x		x	x
PK blood sample [e]	x			
Physical examination				x
Neurologic evaluation [f]	x			x
Temperature [g]				x
Clinical signs and symptoms of infection check [h]	x			x
12-Lead ECG [i]	x			x
Pregnancy test [j]				x
Hematology [k]	x		x	x
Chemistry [l]	x		x	x
[redacted]				
Coagulation [m]				x
Urinalysis [n]	x			x
Urine analysis for substance use [o]				x
Vineland™-II (clinician and study partner)				x
PedsQL™–4.0 Core (subject)				x
PedsQL–Cognitive Functioning (subject)				x
PSQI (subject)				x
EQ-5D-5L (subject)				x
PGI-S (subject)				x
PGI-C (subject)				x
HAM-A (clinician and subject)				x
C-SSRS BL/SLV	x			x
PedsQL Family Impact (study partner)				x
RBS-R (study partner)				x
EQ-5D-5L (study partner)				x
CaGI-S (study partner)				x
CaGI-C (study partner)				x

Appendix 2
Unscheduled Visit and Follow-Up Assessments: Double-Blind and OLE Periods (cont.)

	Unscheduled Visit [a]	Follow-Up Period [b] (±3 days)		
Week	NA	1	2	12
Day	NA	176	183	253
Study site visit	NA	☎	x	x [c]
CGI-S (clinician)			x	x
CGI-I (clinician)			x	x
Previous and concomitant medications [p]	x	x	x	x
Adverse events [q]	x	x	x	x

BL=baseline; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impression–Improvement; CGI-S=Clinical Global Impression–Severity; C-SSRS =Colombia-Suicide Severity Rating Scale; eCRF=electronic Case Report Form; EQ-5D-5L=EuroQoL Five Dimensions Questionnaire–Five Levels; HAM-A=Hamilton Anxiety Rating Scale; HBcAb=hepatitis B core antibody; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; NA=not applicable; OLE=open-label extension; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; SLV=since last visit; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

[a] Assessments performed at unscheduled visits will depend on the clinical needs of the subject. This applies to both the double-blind period and the OLE period.

[b] If a subject continues into the OLE period, the follow-up visit will be conducted 1 week, 2 weeks, and 12 weeks after the last dose of study drug in OLE period. If the subject does not enter the OLE period, the follow-up visit will be conducted 1 week, 2 weeks, and 12 weeks after completion of the double-blind phase.

[c] Visit may be split over 2 days as long as visit remains within the visit window. All assessments for that particular day should be completed prior to dosing.

[d] Vital signs include measurements of respiratory rate, pulse rate, and systolic and diastolic blood pressure while the subject is in a seated position, and body temperature (measured using an oral or tympanic thermometer). Vital sign assessments should be performed just prior to study drug administration. All vital signs should be recorded on the eCRF.

[e] Accurate recording of the time of sample collection is critical.

[f] Neurologic evaluation as presented in Appendix 4 should be used.

[g] Temperature will be recorded (measured using a tympanic thermometer) every 2 weeks by the subject or study partner and values recorded on a patient diary or device that will be provided to them for the first 52 week of a subject's study participation.

[h] Site personnel will contact the subject and/or study partner during the first 52 weeks of the study to ask about signs and symptoms of infections (see Appendix 5 for details). In the event of such reports, the site should use clinical judgement as to whether to call subject in for further evaluations.

Appendix 2
Unscheduled Visit and Follow-Up Assessments: Double-Blind and OLE Periods (cont.)

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[i] Twelve-lead ECGs are to be performed after the subject has been in a supine position for 5 minutes. ECGs for each subject should be obtained from the same machine whenever possible and performed prior to any blood draws. In the event of prolongation of the QTc interval, an unscheduled PK sample should be obtained.

[j] Females of childbearing potential (including those who have had a tubal ligation) must have a urine pregnancy test performed at the site prior to each dose administration. If a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test at the central laboratory. Results are to be recorded only in the source documents.

[k] Hematology includes WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, and other cells).

[l] Serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, BUN or urea, creatinine, CPK, total protein, albumin, phosphorus, calcium, total and direct bilirubin, ALP, ALT, AST, uric acid, LDH, cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, [redacted]

[m] Coagulation assessments include INR, aPTT, and PT.

[n] Urinalysis will be performed at the site by dipstick for blood, protein, glucose, ketones, specific gravity, and pH. Microscopic examination performed at the central laboratory if blood and/or protein results are positive or strongly positive. If there is an explanation for the positive dipstick results (e.g., menses), it should be recorded and there is no need to perform microscopy and culture. Results do not need to be recorded on the eCRF.

[o] Urine samples will be analyzed for the presence of the following drugs: cannabinoids, opiates, cocaine, barbiturates, methadone, and phencyclidine (PCP). Results will be used to verify subject eligibility pertaining to drugs of abuse. Inconclusive results may be repeated once during the screening period. Investigators should use their best clinical judgment in cases where results may be erroneous (e.g., permitted use of opiates or ingestion of food or food supplements).

[p] Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, approved dietary and herbal supplements, nutritional supplements and any non-medicinal interventions, such as individual psychotherapy, cognitive behavioral therapy, smoking cessation therapy, and rehabilitative therapy) used by a subject since the last visit until the follow-up visit.

[q] The investigator is not required to actively monitor subjects for adverse events after the end of the adverse event reporting period (defined as 6 weeks after the last dose of study drug). However, the Sponsor should be notified if the investigator becomes aware of any serious adverse event that occurs after the end of the adverse event reporting period if the event is believed to be related to prior study drug treatment. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

Appendix 3
Schedule of Activities: Open-Label Extension Period, Week 28 and Onward

	Open-Label Treatment													
Week	28	32	36	40	44	48	52	64	76	88	104	116	128	
Day	197	225	253 [a]	281	309	337	365 [a]	449	533 [a]	617	729 [a]	813	897 [a]	ET [a, b]
Vital signs [c]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Weight							x				x		x	x
PK blood sample [d]							x		x		x		x	x
Complete physical examination [e]														x
Limited physical examination [e]			x				x		x		x		x	
Neurologic evaluation [f]	x	x	x	x	x	x	x	x	x		x		x	x
Temperature [g]	x	x	x	x	x	x	x							x
12-Lead ECG [h]			x				x		x		x		x	x
Pregnancy test [i]			x			x		x	x	x	x	x	x	x
Hematology [j]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Chemistry [k]			x				x	x	x	x	x	x	x	x
[redacted]														
Urinalysis [l]							x		x		x		x	x
Urine analysis for substance use [m]							x		x		x		x	x
Vineland™-II (clinician and study partner)			x				x		x		x		x	x
PedsQL™–4.0 Core (subject)			x				x		x		x		x	x

Appendix 3
Schedule of Activities: Open-Label Extension Period, Week 28 and Onward (cont.)

	Open-Label Treatment													
Week	28	32	36	40	44	48	52	64	76	88	104	116	128	
Day	197	225	253 [a]	281	309	337	365 [a]	449	533 [a]	617	729 [a]	813	897 [a]	ET [a, b]
PedsQL–Cognitive Functioning (subject)			x				x		x		x		x	x
PSQI (subject)			x				x		x		x		x	x
EQ-5D-5L (subject)			x				x		x		x		x	x
PGI-S (subject)			x				x		x		x		x	x
PGI-C (subject)			x				x		x		x		x	x
HAM-A (clinician and subject)			x				x		x		x		x	x
C-SSRS BL/SLV		x	x	x		x	x	x	x	x	x	x	x	x
PedsQL Family Impact (study partner)			x				x		x		x		x	x
RBS-R (study partner)			x				x		x		x		x	x
EQ-5D-5L (study partner)			x				x		x		x		x	x
CaGI-S (study partner)			x				x		x		x		x	x
CaGI-C (study partner)			x				x		x		x		x	x
CGI-S (clinician)			x				x	x	x	x	x	x	x	x
CGI-I (clinician)			x				x	x	x	x	x	x	x	x
Study drug dispensation [n]	x	x	x	x	x	x	x	x	x	x	x	x		
Previous and concomitant medications [o]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Adverse events [p]	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Appendix 3
Schedule of Activities: Open-Label Extension Period, Week 28 and Onward (cont.)

BL=baseline; CaGI-C=Caregiver Global Impression–Change; CaGI-S=Caregiver Global Impression–Severity; CGI-I=Clinical Global Impression–Improvement; CGI-S=Clinical Global Impression–Severity; C-SSRS =Colombia-Suicide Severity Rating Scale; eCRF=electronic Case Report Form; EQ-5D-5=EuroQoL Five Dimensions Questionnaire–Five Levels; ET = early termination; HAM-A=Hamilton Anxiety Rating Scale; PedsQL™=Pediatric Quality of Life Inventory™; PedsQL™ 4.0 Core=Pediatric Quality of Life Inventory™ Generic Core Scales, Version 4.0; PGI-C=Patient Global Impression–Change; PGI-S=Patient Global Impression–Severity; PK=pharmacokinetic; PSQI=Pittsburgh Sleep Quality Index; RBS-R=Repetitive Behavior Scale–Revised; SLV = since last visit; Vineland™-II=Vineland™ Adaptive Behavior Scales, Second Edition.

Notes: The visit window is ±3 days and all assessments should be completed within the visit window. Subjects should return to initial planned schedule per randomization for subsequent visits. For subjects who terminate early, assessments listed under the ET visit should be completed. For the schedule of activities to be performed at unscheduled visits and during the safety follow-up period, see Appendix 2.

[a] The Week 36, 52, 76, 104, 128, and ET visits may be split over 2 days, as long as the visit remains within the visit window. All assessments for that particular day should be completed prior to dosing.

[b] To be conducted if a subject discontinues prematurely for any reason.

[c] Vital signs include measurements of respiratory rate, pulse rate, and systolic and diastolic blood pressure while the subject is in a seated position, and body temperature (measured using an oral or tympanic thermometer). Vital sign assessments should be performed just prior to study drug administration. All vitals should be recorded on the eCRF.

[d] All scheduled PK samples should be obtained just prior to study drug administration, if possible. Accurate recording of the time of study drug administration and sample collection is critical.

[e] Includes evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, and neurologic systems. Complete physical examinations will not include pelvic, rectal, or breast examinations. Record abnormalities observed at baseline on the General Medical History and Baseline Conditions eCRF. At subsequent visits, record new or worsened clinically significant abnormalities on the Adverse Event eCRF. Limited, symptom-directed physical examinations should be performed at Weeks 36, 52, 76, 104, and 128, and as clinically indicated. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

[f] The neurologic evaluation will be completed at the Week 36, 48, 52, 64, 76, 104, and 128 visits.

Appendix 3
Schedule of Activities: Open-Label Extension Period, Week 28 and Onward (cont.)

[g] Temperature will be recorded (measured using a tympanic thermometer) every 2 weeks by the subject or study partner, and the values should be recorded in a subject diary or device that will be provided. Site personnel will contact the subject and/or study partner every 2 weeks up until Week 52 to ask about signs and symptoms of infections (see Appendix 5 for details). In the event of such reports, the site should use clinical judgement as to whether to call the subject in for further evaluations.

[h] Twelve-lead ECGs are to be performed after the subject has been in a supine position for 5 minutes. ECGs for each subject should be obtained from the same machine whenever possible and performed prior to any blood draws. In the event of prolongation of the QTc interval, an unscheduled PK sample should be obtained.

[i] Females of childbearing potential (including those who have had a tubal ligation) must have a urine pregnancy test performed at the site prior to each dose administration. If a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test at the central laboratory. Results are to be recorded only in the source documents.

[j] Hematology includes WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, and other cells).

[k] Serum chemistry includes sodium, potassium, chloride, bicarbonate, glucose, BUN or urea, creatinine, CPK, total protein, albumin, phosphorus, calcium, total and direct bilirubin, ALP, ALT, AST, uric acid, LDH, cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, [redacted]

[l] Urinalysis will be performed at the site by dipstick for blood, protein, glucose, ketones, specific gravity, and pH. Microscopic examination performed at the central laboratory if blood and/or protein results are positive or strongly positive. If there is an explanation for the positive dipstick results (e.g., menses), it should be recorded and there is no need to perform microscopy and culture. Results do not need to be recorded on the eCRF.

[m] Urine samples will be analyzed for the presence of the following drugs: cannabinoids, opiates, cocaine, barbiturates, methadone, and phencyclidine (PCP). Results will be used to verify subject eligibility pertaining to drugs of abuse. Inconclusive results may be repeated once during the screening period. Investigators should use their best clinical judgment in cases where results may be erroneous (e.g., permitted use of opiates or ingestion of food or food supplements).

[n] Study drug administration should occur only after all assessments and rating scales for the subject are completed. Subjects should be instructed to take their medication at the same time each day, with the exception of study visits at which they should take only their study drug once all assessments are completed. Study drug dispensation can take place at any time during the visit.

Appendix 3
Schedule of Activities: Open-Label Extension Period, Week 28 and Onward (cont.)

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[o] Concomitant therapy includes any medication, e.g., prescription drugs, over-the-counter drugs, approved dietary and herbal supplements, nutritional supplements and any non-medicinal interventions (e.g., individual psychotherapy, cognitive behavioral therapy, smoking cessation therapy, and rehabilitative therapy) used by a subject since the last visit until the follow-up visit.

[p] The investigator is not required to actively monitor subjects for adverse events after the end of the adverse event reporting period (defined as 6 weeks after the last dose of study drug). However, the Sponsor should be notified if the investigator becomes aware of any serious adverse event that occurs after the end of the adverse event reporting period if the event is believed to be related to prior study drug treatment. Every effort should be made to follow all serious adverse events considered to be related to study drug or trial-related procedures until a final outcome can be reported.

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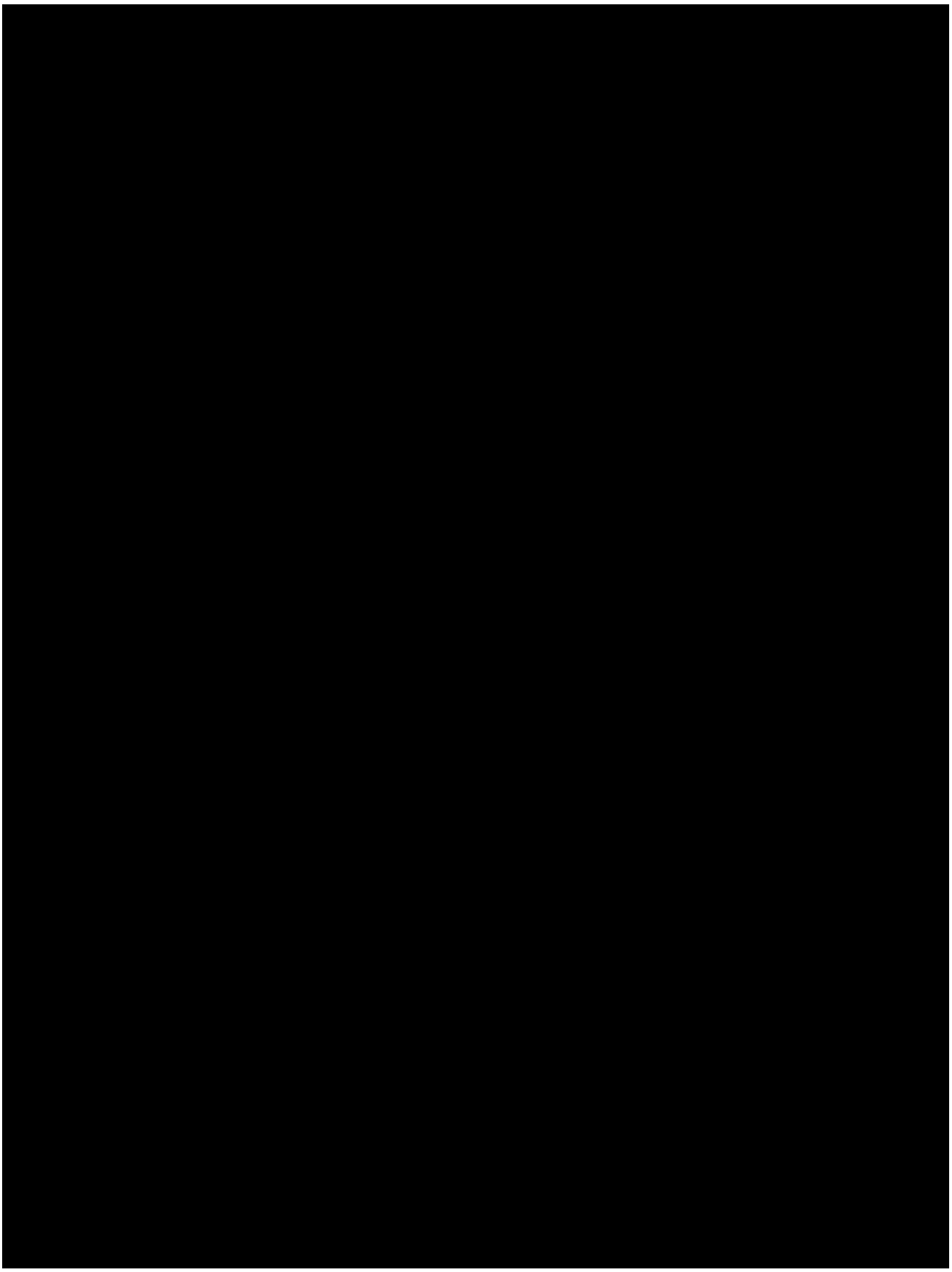

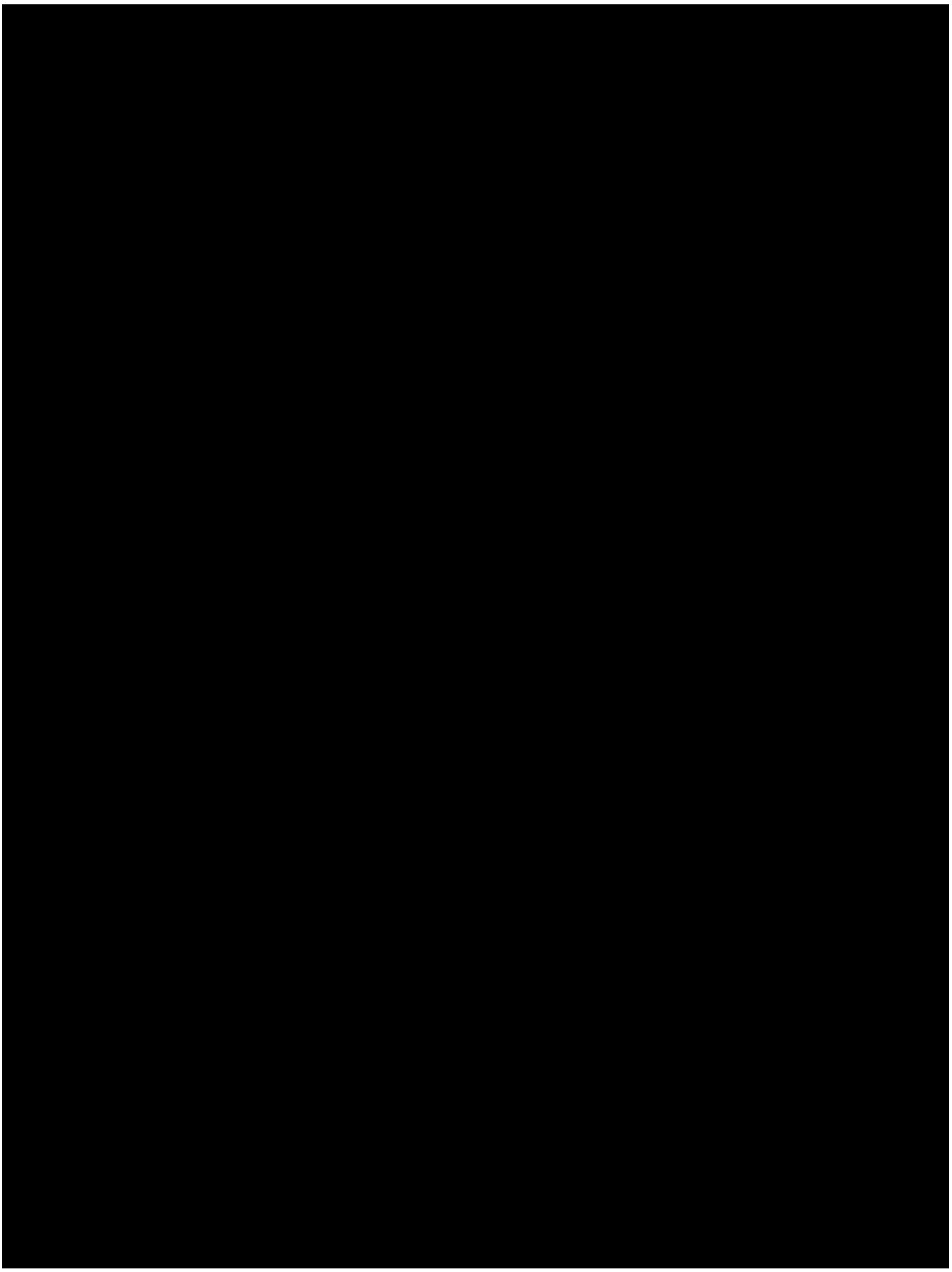

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