Official Title: A Phase II Multi-Center, Randomized, Double-Blind, 24 Week, Parallel Group, Placebo-Controlled Study to Investigate the Efficacy and Safety of Balovaptan (RO5285119) in Children and Adolescents Age 5-17 With Autism Spectrum Disorder (ASD)

NCT Number: NCT02901431

Document Date: Protocol Version 6: 19-December-2018

PROTOCOL

TITLE: **A PHASE II MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, 24-WEEK, PARALLEL GROUP, PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF BALOVAPTAN (RO5285119) IN CHILDREN AND ADOLESCENTS AGE 5–17 WITH AUTISM SPECTRUM DISORDER (ASD)**

PROTOCOL NUMBER: BP30153

VERSION NUMBER: 6

EUDRACT NUMBER: N/A

IND NUMBER: 116,483

TEST PRODUCT: Balovaptan (RO5285119)

MEDICAL MONITOR: [redacted], *Ph.D.*

SPONSOR: F. Hoffmann-La Roche Ltd

DATE FINAL: Version 1: 23 March 2016

DATES AMENDED: Version 2: 02 Aug 2016
Version 3: 30 Jan 2017
Version 4: 19 May 2017
Version 5: 29 March 2018
Version 6: See electronic time stamp below.

PROTOCOL AMENDMENT APPROVAL

Approver's Name	Title	Date and Time (UTC)
[redacted]	Company Signatory	19-Dec-2018 02:26:35

PROTOCOL AMENDMENT, VERSION 6: RATIONALE

Protocol BP30153, Version 6 has been amended primarily to change the study design (main study) to a single balovaptan dose (10 mg age equivalent) compared with placebo study, in accordance to a randomization ratio of 1:1 of 10 mg equivalent:placebo; to increase the overall number of subjects; to reduce the screening window duration for all new subjects; to update the requirements for cardiac monitoring in response to specific requests received from the U.S. Food and Drug Administration (FDA) and removal of capillary blood draw option introduced with Version 5; to change the initial starting dose for subjects aged 8 years and above; and to define some statistical analysis details. Changes to the protocol, along with a rationale for each change, are summarized below:

- The study design has been changed to a single dose (10 mg equivalent balovaptan) compared with placebo in accordance to a randomization ratio of 1:1 of balovaptan 10 mg equivalent:placebo (Sections 3.1, 3.3, and 4.3).

  Rationale: A recent Internal Monitoring Committee (IMC) and Scientific Oversight Committee (SOC) review of pharmacokinetic (PK) data determined that subjects aged 14 years and younger received doses providing exposures lower than anticipated. Consequently, dose adjustments were implemented. However, the dose equivalencies for balovaptan 4 mg and 10 mg doses were for the majority of subjects below the respective target concentrations observed with 4 and 10 mg once daily dosing in adults. Insufficient data has been collected at the 10 mg equivalent dose whereas more data than expected has been generated in the lower concentration range. Therefore, recruitment for the 4 mg balovaptan arm has been closed to ensure that sufficient subjects can be recruited to both 10 mg and placebo.

- Adolescent subjects who have been discontinued because of lack of dose confirmation (per protocol prior to Week 8) will be replaced in the study. The total sample size of the study has been increased to 340 subjects (Sections 3.1.3 and 6.8).

  Rationale: Because the adolescent and child subjects in the 10 mg equivalent arm have been under dosed, to obtain evaluable data at the correct 10 mg balovaptan dose (exposure), the sample size has been increased.

- The initial starting dose (main study) has been changed following review of available safety and PK data from the study. A table has been added to outline updated starting doses for adolescents and children, aged 8 to 17 years (Section 4.4.2, Table 2).

  Rationale: Available PK and safety/tolerability results from this study were reviewed by the IMC and SOC and compared with the exposures measured in adults in Study BP28420. Median area under the concentration–time curve of the 5–7, 8–12, and 13–14 year old subjects were found to be considerably below the target exposures. Therefore, the initial starting dose for all adolescents and children 8 years and above has been updated and should be implemented at his or her next study drug dispensation visit.

- The screening window duration has been reduced for all new subjects (Sections 3.1.1, 4.2.1, 4.6.2.1 and Appendix 1).

  Rationale: An extended screening window was considered necessary during recruitment to the PK cohorts to try and minimize subjects being discontinued from treatment in the absence of dose confirmation. As the PK cohorts are essentially complete, the screening window duration will be decreased from 8 weeks to 4 weeks.

- [redacted]

- Capillary blood sampling (introduced in Version 5 of this protocol) has been removed (Sections 5.2.1 and 5.2.4).

  Rationale: Capillary blood sample for hematological and CPK monitoring at certain timepoints was introduced in Version 5 of the protocol 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, Troponin T has not been shown to be reliably measured from capillary blood. Thus, with the reduced CPK assessments and the increased troponin T assessments, capillary blood sampling has been removed.

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

- The study rationale has been updated to excluding data from a nonclinical study of prenatal valproic acid (VPA) in a rat model (Section 1.3).

  Rationale: A recent independent in-house analysis of the data from a nonclinical pharmacology study, the effects of balovaptan in the prenatal VPA rat model, was unable to verify the original results. It is important to note that this does not have any effect on the safety profile of balovaptan, and data in support of the mechanism of action was provided from other sources, including clinical data.

- It has been clarified that subjects who test positive for hepatitis B core antibodies (HBcAb) may still be eligible for the study if the subject has a negative hepatitis B surface antigen (HBsAg) and/or hepatitis B cDNA (HBcDNA), no medical history of hepatitis, and no indication of liver dysfunction through liver enzyme or coagulation results (Sections 3.2.3 and 4.6.1.7).

  Rationale: Few subjects screened for this study have been found positive for HBcAb in the absence of any other evidence for hepatitis including medical history. False positive HBcAb testing is a known phenomenon and has been found to occur

up to 16% [51] of cases. No liver toxicity alert for balovaptan administration has emerged in animals or in clinical trials.

- Changes in the statistical section in terms of primary treatment comparison and efficacy analysis populations (Sections 6.1, 6.3.3, and 6.6).

  Rationale: The primary efficacy analysis will compare balovaptan 10 mg equivalent dose with placebo, reflecting the change in the study objective and design. The definition of the primary efficacy analysis population has been refined in alignment with the one planned for the confirmatory Phase III balovaptan trials, which are currently ongoing/planned. The availability of both baseline and at least one postdose assessment, which was the specific of the originally planned modified intent-to-treat population, is not required for identifying the appropriate set of data according to the principle of the intention to treat. The same rationale, that is, the alignment with confirmatory Phase III framework, justifies the removal of the originally planned additional, secondary, analysis on the per-protocol population.

- Reporting of cardiovascular AESI (arrhythmia, syncope, dyspnea, palpitations, or chest pain) has been clarified (Section 5.1.3). Expedited reporting to investigators and IRBs has been removed.

  Rationale: FDA requested expedited reporting of these cardiovascular terms. This was updated in Version 5 of the protocol (Section 5.7); however, the update did not clarify that expedited reporting of these terms was only to the FDA.

- Eligibility criteria related to stability of concomitant medications for subjects who enter the OLE through the substudy have been clarified (Appendix 11).

- The Medical Monitor has been changed from [redacted], M.D. to [redacted], Ph.D., and the secondary Medical Monitor contact has been updated to [redacted], M.D. (Section 5.4.1).

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

TABLE OF CONTENTS

PROTOCOL SYNOPSIS 13

PROTOCOL ACCEPTANCE FORM 12

1. BACKGROUND AND RATIONALE 26
   - 1.1 Background on Disease 26
   - 1.2 Background on Balovaptan (RO5285119) 27
   - [redacted] 28
   - 1.2.2 Previous and Ongoing Clinical Studies 29
   - 1.2.2.1 Safety and Tolerability 29
   - 1.2.2.2 Efficacy 30
   - 1.2.2.3 Pharmacokinetics 31
   - [redacted] 32

2. OBJECTIVES 33
   - 2.1 Primary Objectives 33
   - 2.2 Secondary Objectives 33
   - 2.3 Exploratory Objectives 34

3. STUDY DESIGN 35
   - 3.1 Description of Study 35
   - 3.1.1 Overview of Study Design 35
   - 3.1.2 Internal Monitoring Committee and Scientific Oversight Committee (IMC and SOC) 37
   - [redacted] 37
   - [redacted] 37
   - 3.1.3.2 Efficacy Interim Analyses 40
   - [redacted] 40
   - 3.1.5 Open-Label Extension 40
   - 3.2 End of Study 40
   - 3.3 Rationale for Study Design 41
   - [redacted] 41

[redacted] [redacted] 42
3.3.3 Rationale for Use of Single Dose in Subjects Enrolled in Accordance to Version 6 42
3.3.4 Rationale for Study Population 43
3.3.5 Rationale for Use of Vineland™-II Adaptive Behavior Scales 2-Domain Composite Score as the Primary Efficacy Endpoint 43
3.3.6 Rationale for Open-Label Extension 44
3.3.7 Rationale for Use of Responder Definition 44
3.4 Outcome Measures 44
3.4.1 Efficacy Outcome Measures 44
3.4.2 Exploratory Outcome Measures 45
3.4.3 Safety Outcome Measures 45
3.4.4 Pharmacokinetic (PK) Outcomes 45
3.4.5 Biomarker/Genotyping Sample Collection 46
3.4.5.1 Clinical Genotyping (CG) Samples 46
3.4.5.2 Research Biosample Repository 46
4. MATERIALS AND METHODS 46
4.1 Center 46
4.2 Study Population 47
4.2.1 Recruitment Procedures 47
4.2.2 Inclusion Criteria 48
4.2.3 Exclusion Criteria 49
4.2.4 Eligibility for Open-Label Extension Phase 51
4.3 Method of Treatment Assignment and Blinding 51
4.4 Study Treatment 53
[redacted] [redacted] 53
[redacted] [redacted] 53
4.4.2 Dosage, Administration, and Compliance 53
4.4.2.1 Age-adjusted Doses 53
4.4.2.2 Administration and Compliance 55
4.4.2.3 Treatment in the Open-Label Extension, Including Age-Adjusted Doses 55
4.4.3 Investigational Medicinal Product Accountability 56

4.5 Concomitant Therapy 57
4.5.1 Permitted Therapy 57
4.5.2 Non-Pharmacological Interventions 58
4.5.3 Prohibited Therapy 58
4.5.4 Caution Regarding P-gp Substrates 59
4.6 Study Assessments 59
4.6.1 Description of Study Assessments 59
4.6.1.1 Medical History and Demographic Data 59
4.6.1.2 Physical and Neurological Examinations 60
4.6.1.3 Tanner Staging 61
4.6.1.4 Palatability Test 61
4.6.1.5 Vital Signs 61
4.6.1.6 Electrocardiograms 62
4.6.1.7 Laboratory Assessments 62
4.6.1.8 Pharmacokinetic Assessments 65
4.6.1.9 Clinical Genotyping Samples 66
4.6.1.10 Disease-Specific Assessments 66
4.6.1.11 Patient-Reported Outcomes 71
4.6.1.12 Columbia Suicide Severity Rating Scale (C-SSRS) 72
4.6.1.13 Samples for Research Biosample Repository 72
4.6.2 Timing of Study Assessments 76
4.6.2.1 Screening and Pretreatment Assessments 76
4.6.2.2 Assessments during Treatment 77
4.6.2.3 Assessments at Study Completion/Early Withdrawal Visit 78
4.6.2.4 Follow-Up Assessments 78
4.7 Subject, Study, and Site Discontinuation 79
4.7.1 Subject Discontinuation 79
4.7.1.1 Discontinuation from Study Drug 79
4.7.1.2 Withdrawal from Study 79
4.7.2 Study and Site Discontinuation 80

5. ASSESSMENT OF SAFETY 80

5.1 Safety Parameters and Definitions 80
5.1.1 Adverse Events 81
5.1.2 Serious Adverse Events (Immediately Reportable to the Sponsor) 81
5.1.3 Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor) 82
5.2 Safety Plan 82
[redacted] [redacted] 82
5.2.2 Blood Pressure and ECG Monitoring 84
[redacted] [redacted] 84
[redacted] [redacted] 85
[redacted] [redacted] 85
5.2.6 Tanner Staging 87
5.3 Methods and Timing for Capturing and Assessing Safety Parameters 87
5.3.1 Adverse Event Reporting Period 87
5.3.2 Eliciting Adverse Event Information 87
5.3.3 Assessment of Severity of Adverse Events 88
5.3.4 Assessment of Causality of Adverse Events 88
5.3.5 Procedures for Recording Adverse Events 88
5.3.5.1 Diagnosis versus Signs and Symptoms 88
5.3.5.2 Adverse Events Occurring Secondary to Other Events 89
5.3.5.3 Persistent or Recurrent Adverse Events 89
5.3.5.4 Abnormal Laboratory Values 89
5.3.5.5 Abnormal Vital Sign Values 90
5.3.5.6 Abnormal Liver Function Tests 91
5.3.5.7 Deaths 91
5.3.5.8 Preexisting Medical Conditions 91
5.3.5.9 Hospitalization or Prolonged Hospitalization 92
5.3.5.10 Patient-Reported Outcome Data 92
5.4 Immediate Reporting Requirements from Investigator to Sponsor 92
5.4.1 Emergency Medical Contacts 93

5.4.2 Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest 93
5.4.3 Reporting Requirements for Pregnancies 94
5.4.3.1 Pregnancies in Female Subjects 94
5.4.3.2 Pregnancies in Female Partners of Male Subjects 94
5.4.3.3 Abortions 94
5.4.3.4 Congenital Anomalies/Birth Defects 94
5.4.4 Reporting Requirements for Cases of Overdose, Medication Error, Drug Abuse, or Drug Misuse 95
5.5 Follow-Up of subjects after Adverse Events 96
5.5.1 Investigator Follow-Up 96
5.5.2 Sponsor Follow-Up 97
5.6 Post-Study Adverse Events 97
5.7 Expedited Reporting to Health Authorities, Investigators, Institutional Review Boards, and Ethics Committees 97
6. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN 98
6.1 Determination of Sample Size 98
6.2 Summaries of Conduct of Study 98
6.3 Analysis Populations 98
6.3.1 Safety Analysis Population 98
6.3.2 Pharmacokinetic Analysis Population 99
6.3.3 Efficacy Analysis Population 99
6.3.3.1 For All Subjects Enrolled prior to Version 6 of the Protocol: 99
6.3.3.2 For Subjects Enrolled in Accordance to Version 6 of the Protocol 99
6.4 Summaries of Treatment Group Comparability 100
6.5 Safety Analyses 100
6.5.1 Adverse Events 100
6.5.2 Clinical Laboratory Test Results 100
6.5.2.1 Standard Reference Ranges and Transformation of Data 101

6.5.2.2 Definition of Laboratory Abnormalities 101
6.5.3 Vital Signs and ECG 102
6.5.4 Concomitant Medications 102
6.6 Efficacy Analyses 102
6.7 Pharmacokinetic Analyses 103
6.8 Interim Analyses 103

7. DATA COLLECTION AND MANAGEMENT 104
7.1 Data Quality Assurance 104
7.2 Electronic Case Report Forms 104
7.3 Electronic Patient-Reported Outcome Data 105
7.4 Source Data Documentation 105
7.5 Use of Computerized Systems 106
7.6 Retention of Records 106

8. ETHICAL CONSIDERATIONS 107
8.1 Compliance with Laws and Regulations 107
8.2 Informed Consent 107
8.3 Institutional Review Board or Ethics Committee 108
8.4 Confidentiality 108
8.5 Financial Disclosure 109

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION 109
9.1 Study Documentation 109
9.2 Protocol Deviations 110
9.3 Site Inspections 110
9.4 Publication of Data and Protection of Trade Secrets 110
9.5 Protocol Amendments 111

10. REFERENCES 112

LIST OF TABLES

Table 1 Age-adjusted Starting Doses Based on PBPK Model 54
Table 2 Updated Age-Adjusted Doses Based on PK Data 54

Table 3 Management Guidelines for Cardiac Events 86
Table 4 Adverse Event Severity Grading Scale 88
[redacted] [redacted] 102

LIST OF FIGURES

Figure 1 Study Design for Subjects Enrolled prior to Version 6 36
Figure 2 Study Design for Subjects Enrolled in Accordance to Version 6 36

LIST OF APPENDICES

Appendix 1 Schedule of Assessments For all Subjects after Confirmation of Final Doses by IMC and SOC 117
Appendix 2 Schedule of Assessments For First Cohorts of 24 Adolescents and 30 Children until Confirmation of Final Doses by IMC/SOC 123
[redacted] [redacted] 128
[redacted] 129
[redacted] 130
Appendix 6 Tanner Stages 134
Appendix 7 Strong and Moderate Inhibitors and Inducers of CYP3A 135
[redacted] [redacted] 136
Appendix 9 Schedule of Assessments: Open-Label Extension for Weeks 24 to 52 138
Appendix 10 Schedule of Assessments: Open-Label Extension from Weeks 52 to 76 141
[redacted] [redacted] 144

PROTOCOL ACCEPTANCE FORM

TITLE:	**A PHASE II MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, 24-WEEK, PARALLEL GROUP, PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF BALOVAPTAN (RO5285119) IN CHILDREN AND ADOLESCENTS AGE 5-17 WITH AUTISM SPECTRUM DISORDER (ASD)**
PROTOCOL NUMBER:	BP30153
VERSION NUMBER:	6
IND NUMBER:	116,483
TEST PRODUCT:	Balovaptan (RO5285119)
MEDICAL MONITOR:	[redacted], *Ph.D.*
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 keep the signed original form in your study files, and return a copy to your local Study Monitor.

PROTOCOL SYNOPSIS

TITLE:	**A PHASE II MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, 24-WEEK, PARALLEL GROUP, PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF BALOVAPTAN (RO5285119) IN CHILDREN AND ADOLESCENTS AGE 5-17 WITH AUTISM SPECTRUM DISORDER (ASD)**
PROTOCOL NUMBER:	BP30153
VERSION:	6
EUDRACT NUMBER:	N/A
IND NUMBER:	116,483
TEST PRODUCT:	Balovaptan (RO5285119)
PHASE:	II
INDICATION:	Autism Spectrum Disorder (ASD)
SPONSOR:	F. Hoffmann-La Roche Ltd

OBJECTIVES

Primary Objectives

The primary objectives of this study are as follows:

- To evaluate the efficacy of 24-week treatment with balovaptan (RO5285119) $10\ mg\ equivalent$ compared to placebo as measured by the change from baseline on the Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II) Two Domain Composite (2DC) (average of Communication and Socialization domains)

Secondary Objectives

The secondary objectives for this study are as follows:

- To evaluate the efficacy of treatment with balovaptan $10\ mg\ equivalent$ vs. placebo on:
  - Change from baseline on the Vineland™-II Composite standard score after 12 weeks and 24 weeks of treatment
  - Change from baseline in the Vineland™-II Communication, Socialization, and Daily Living Skills domain standard scores after 12 weeks and 24 weeks of treatment
  - Proportion of subjects with ≥6-point improvement in the Vineland™-II 2DC score to evaluate clinically meaningful response
  - Change from baseline in severity of clinical impressions as measured by CGI-S (Clinical Global Impressions-Severity) and OACIS-S (Ohio Autism Clinical Impressions Scale-Severity) after 12 weeks and 24 weeks of treatment
  - Improvements in clinical impressions as measured by CGI-I (Clinical Global Impressions-Improvement) and OACIS-I (Ohio Autism Clinical Impressions Scale-Improvement) after 12 weeks and 24 weeks of treatment
  - Patient-reported Pediatric Quality of Life (PedsQL) v4.0 Generic Core Scale after 12 weeks and 24 weeks of treatment
  - Change from baseline in the Vineland™-II) Composite standard score in adolescents and children independently after 12 weeks and 24 weeks of treatment

- Change from baseline on the Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II) 2DC score after 12 weeks of treatment

- To evaluate safety and tolerability of *24 and* up to 76 weeks of treatment with balovaptan
- To evaluate the pharmacokinetics and exposure-response relationships of balovaptan and its metabolites, if appropriate

Exploratory Objectives

The exploratory objectives for this study are as follows:

- To evaluate the effect of treatment with balovaptan on:
  - *Change from baseline on the Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II) Two Domain Composite (2DC)*
  - *Change from baseline on the Vineland™-II Composite standard score after 12 weeks and 24 weeks of treatment*
  - *Change from baseline in the Vineland™-II Communication, Socialization, and Daily Living Skills domain standard scores after 12 weeks and 24 weeks of treatment*
  - Change from baseline in behaviors as measured by Aberrant Behavior Checklist (ABC) Lethargy/Social withdrawal subscale after 12 weeks and 24 weeks of treatment
  - Change from baseline in repetitive behaviors as measured by Repetitive Behavior Scale-Revised (RBS-R) after 12 weeks and 24 weeks of treatment
  - Change from baseline to Week 76 (52 weeks of open-label treatment) as measured by Vineland™-II 2DC score
  - Change from Week 24 to Week 76 (52 weeks of open-label treatment) as measured by Vineland™-II 2DC score
  - Proportion of subjects with ≥4-point improvement in Vineland™-II 2DC score
  - Proportion of subjects with ≥8-point improvement in Vineland™-II 2DC score
  - Maintenance of efficacy 6 weeks after end of study treatment as measured by Vineland™-II Composite standard score
  - Social symptoms, communication, and behavior assessed by Exit Interviews
  - Change from baseline in behaviors as measured by ABC Irritability, Stereotypic Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech subscales after 12 weeks and 24 weeks of treatment
  - Patient-reported PedsQL Cognitive Functioning Scale and parent-reported PedsQL Family Impact Scale after 12 weeks and 24 weeks of treatment
  - Caregiver-reported Global Impression of Change on communication skills, social skills, daily living skills, and overall autism symptoms (CaGI) after 12 weeks and 24 weeks of treatment
- To evaluate whether genetic variants in the AVPR1A gene affect the efficacy or safety of balovaptan
- To evaluate the palatability of study medication

STUDY DESIGN

Description of Study

For all subjects enrolled prior to Version 6 of the protocol, this is a Phase II multi--center, randomized, double-blind, 24-week, 3-arm, parallel group, placebo-controlled study to investigate the efficacy, safety, and PK of balovaptan in children and adolescents aged 5–17 years with ASD who are high functioning (IQ ≥70).

For subjects enrolled in accordance to Version 6 of the protocol, this is a Phase II multi-center, randomized, double-blind, 24-week, parallel group, placebo-controlled, 2-arm study with subjects assigned to a single dose of balovaptan (10 mg or equivalent) or placebo. All other parameters of the study design will remain as outlined.

Approximately *340* children and adolescents aged 5–17 years with ASD will be recruited to ensure a total of *160* evaluable subjects *with placebo or 10 mg eq* after 24 weeks of treatment.

All individuals who received doses of balovaptan that were not equivalent to 4 mg or 10 mg will not be included in the primary analysis.

Randomization will be stratified by age group and sex. The ratio between adolescents (aged 13–17 years) and children (aged 5–12 years) will be approximately 1:1. The children's cohort should contain up to approximately 30 subjects of whom up to approximately 16 should be aged between 5 and 7 years old. Additionally, the proportion of female subjects will be balanced in each treatment group and limited to a maximum of 20% of the entire study population.

Dose-exposure relationship in adolescents and in children will be confirmed independently. Enrollment will be staggered, starting first with adolescents (aged 13 to 17 years) and then with children (aged 5 to 12 years). Initially, a first cohort of approximately 24 adolescents will be enrolled together. Once the Internal Monitoring Committee (IMC) and the Scientific Oversight Committee (SOC) agree on acceptable safety and tolerability in the first adolescent cohort and determine the final doses, enrollment of adolescents will resume and the enrollment of a first cohort of up to approximately 30 children (aged 5 to 12 years) can commence. Once the IMC and SOC agree on acceptable safety and tolerability in the first children cohort and determine the final doses, enrollment of children for the main study will start. If IMC and SOC review of data determines the final dose and agreement on acceptable safety and tolerability in a subgroup of children (e.g., 8–12 years old) but not the full cohort aged 5–12 years, enrollment of children in this subgroup for the main study may start. Enrollment of the other subgroup for confirmation of dose-exposure relationship will continue until IMC and SOC review determines final doses and agreement on acceptable safety and tolerability in that subgroup. Children will not receive treatment for a period beyond what is supported by the non-clinical toxicology package.

Subjects who complete or have completed the double-blind 24-week treatment period will able to participate in an optional 52-week open-label extension (OLE) period where they will receive open-label balovaptan treatment.

NUMBER OF SUBJECTS

A total of approximately *340* subjects: *for 80 evaluable subjects on balovaptan 10 mg eq and 80 evaluable subjects on placebo at Week 24.*

TARGET POPULATION

High functioning (IQ ≥ 70) children and adolescents (aged 5–17 years) with ASD according to DSM-5 or ICD10 criteria.

INCLUSION CRITERIA

Subjects must meet the following criteria for study entry:

1. Males or females aged 5–17 years at randomization
2. Fluent in English
3. DSM-5 criteria for ASD or ICD10 criteria for Autism diagnosis confirmed by ADOS-2 criteria
4. SRS-2 (T-score) ≥ 66
5. CGI-S ≥ 4 (moderately ill) at screening
6. IQ ≥ 70 as assessed by WASI-II or WPPSI-IV intelligence test
7. Language, hearing, and vision compatible with the study measurements as judged by the Investigator
8. In the Investigator's opinion, the subject must be able to participate and is deemed appropriate for participation in the study, capable of following the study schedule of assessments
9. Availability of a parent or other reliable caregiver who is fluent in English and has frequent and sufficient contact with the subject. The same person must agree to accompany the subject to all clinic visits and provide information about the subject's behavior and symptoms and must agree to oversee the subject's adherence with protocol-specified procedures and study medication dosing
10. Parent or legal guardian/representative and caregiver willing and able to give written informed consent according to local requirements and subject willing and able to provide informed assent or consent according to local requirements

11. Female subjects who have experienced menarche:
    - Must have a negative urine pregnancy test at screening and are not breast feeding
    - Must agree to either remain completely sexually abstinent or use two effective contraceptive methods from screening until 28 days after the last dose of study treatment. Methods include:
        - An intrauterine device (IUD) implanted at least 2 months prior to screening
        - Established hormonal contraception (oral, vaginal, implanted, or injectable)
        - Spermicidal agent (foam, gel, cream, or suppository)
        - Barrier method, such as a condom, diaphragm, cervical/vault cap, or vaginal sponge

Notes:

The Sponsor does not require male contraception because of the minimal seminal dose transmitted via sexual intercourse.

Female subjects who experience menarche during the study must comply from this time onwards with the same requirements as outlined above, i.e., use 2 forms of effective contraception or agree to remain completely abstinent until 28 days after last dose.

EXCLUSION CRITERIA

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

Neurological/psychiatric:

1. Initiation of a major change in psychosocial intervention (including investigational) within 4 weeks prior to screening. Minor changes in ongoing treatment (e.g., missed therapy sessions due to holiday/vacation; planned break in therapy due to school holidays; changes in college/school programs) are not considered significant.
2. Unstable or uncontrolled clinically significant psychiatric and / or neurological disorder that may interfere with the safety or efficacy endpoints
3. Known personal or family history (first or second degree relatives) of cerebral aneurysm
4. Risk of suicidal behavior in the opinion of the Investigator or as evidenced by a "yes" to questions 4 and / or 5 of C-SSRS taken at screening and baseline with respect to the last 12 months, or any suicide attempt in medical history
5. Seizure within the past 6 months
6. Medical history of alcohol or substance abuse/dependence

Cardio-vascular:

7. Concurrent cardio-vascular disease not considered well controlled by the Investigator
8. Confirmed (e.g., 2 consecutive measurements) clinically significant abnormality on ECG at screening. That includes but is not limited to a QTcF of ≥ 450 milliseconds, absence of dominating sinus rhythm, AV-block II° or III°

Other organ systems:

9. Concomitant disease or condition (pulmonary, gastro-intestinal, hepatic, renal, metabolic, immunological system, or obesity (Body Mass Index [BMI] at or above the 99th percentile for the same age and sex) that could interfere with, or treatment of which might interfere with, the conduct of the study; or discontinuation of prohibited medication might pose unacceptable risks to the subject in the opinion of the Investigator
10. Evidence for current GI bleeding, e.g., active stomach ulcer disease
11. History of coagulopathies, bleeding disorders, or blood dyscrasias

12. Positive serology for hepatitis B (HBV), hepatitis C (HCV), HIV 1, or HIV 2

    Subjects who test positive for hepatitis B core antibodies may still be eligible if the subject has a combination of a negative hepatitis B surface antigen and/or hepatitis B cDNA test, no history of hepatitis, and no indication of liver dysfunction through liver enzyme or coagulation results.

13. Confirmed clinically significant abnormality in parameters of hematology, clinical chemistry, coagulation, or urinalysis (e.g., a neutrophil count of less than 1500/μL; in case of assumed "benign ethnic neutropenia", a count of less than 1300/μL constitutes exclusion)
14. Medical history of malignancy if not considered cured

Other exclusion criteria

15. Participation in an investigational drug study within 90 days or 5 times the half-life of the investigational molecule (whichever is longer) prior to randomization or participation in a study testing an investigational medical device within 90 days prior to randomization or if the device is still active.
    - Subjects from first adolescent and first children cohorts, who stopped treatment in the context of the IMC/SOC dose determination decision, are allowed to re-start originally assigned treatment for an additional 24 weeks in the main part of the study.
16. Loss of blood over 250 mL within three months prior to screening
17. Allowed medications have not been stable since 4 weeks before screening, and allowed medications for treatment of epilepsy have not been stable since 3 months before screening.
18. Use of prohibited medications within 2 weeks prior to screening visit or 5 times the half-life prior to randomization (whichever is longer)

[redacted]

ELIGIBILITY FOR OPEN-LABEL EXTENSION PHASE

Subjects who meet the following criteria may participate in the OLE:

Subjects who complete the double-blind 24-week treatment period will able to participate in an optional 52-week OLE period where they will receive open-label balovaptan treatment. All subjects that have previously completed or were required to stop dosing prior to Week 8 for non-safety reasons will need to be re-consented and meet the eligibility requirements specified in Appendix 11.

Subjects must satisfy both criteria :

1. Either completed the blinded treatment phase (Week 24) OR were required to stop dosing by the Sponsor at or before Week 8.
2. Have no relevant adverse events including laboratory abnormalities in the opinion of the investigator that are prohibitive for starting the OLE

If a subject with ASD reaches the age of 18 during the study, they will still be eligible for participation in the OLE provided that they meet all the criteria above.

Where possible, all subjects should continue directly from the blinded phase to the open-label phase. For subjects that enter the OLE, the treatment follow-up visit will be completed at the end of OLE phase.

Under specific circumstances, a subject may need to have treatment interrupted at the end of the double-blind phase before entering the OLE. These cases should be discussed with the Medical Monitor or designee.

Any subject who had treatment interruption due to lack of dose confirmation or for whom the OLE was not available will be able to enter the open label phase until the final subject recruited reaches Week 24.

LENGTH OF STUDY

The total duration of the study (from screening through to study completion) for each subject will be approximately *87* weeks divided as follows:

- Screening period (first adolescent/ children cohorts): Approximately 4 weeks
  - At least 1 week and up to *4* weeks prior to randomization
- Screening period (main study): Approximately 4 weeks
  - At least *1* week and up to *4* weeks prior to randomization
- Treatment phase*: 24 weeks
- Optional OLE: Approximately 52 weeks
- Follow-up: At least 6 weeks and up to 7 weeks after last dose

* A small group of subjects may receive blinded treatment for up to 32 weeks. These are subjects from first PK cohort who stopped treatment prior to determination of final doses by the IMC and SOC, and have the opportunity to re-start in the main study.

END OF STUDY

For primary analysis purposes, and for the clinical study report, the end of the study is defined as 31 weeks after the last subject is enrolled into the main part of the study.

For subsequent analyses and for the clinical study report addendum, the end of the study is defined as the date when the last subject last observation (LSLO) occurs. LSLO is expected to occur approximately 83 weeks after the last subject is enrolled into the main part of the study.

OUTCOME MEASURES

EFFICACY OUTCOME MEASURES

The efficacy outcome measures for this study are as follows:

- Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II)
- Clinical Global Impressions: Severity (CGI-S) and Improvement (CGI-I)
- Ohio Autism Clinical Impressions Scale: Severity (OACIS-S) and Improvement (OACIS-I)
- Pediatric Quality of Life (PedsQL) v4.0 Generic Core Scale

EXPLORATORY OUTCOME MEASURES

The exploratory outcome measures for this study are as follows:

- Pediatric Quality of Life (PedsQL) Cognitive Functioning Scale
- Pediatric Quality of Life PedsQL Family Impact Scale
- Caregiver-reported Global Impression of Change (CaGI)
- Aberrant Behavior Checklist (ABC)
- Repetitive Behavior Scale-Revised (RBS-R)
- Exit interviews
- Evaluation of safety and/ or efficacy data in the context of genetic variants in the AVPR1A gene
- Palatability test of study medication

SAFETY OUTCOME MEASURES

The safety outcome measures for this study are as follows:

- Incidence, nature, and intensity of adverse events
- Incidence and nature of laboratory abnormalities, based on hematology, clinical chemistry, [redacted], coagulation monitoring, and urinalysis testing
- Tanner staging
- Physical examination (including body height and weight) and neurological examination

- Vital signs, i.e., systolic and diastolic blood pressure, pulse rate in supine position, and orthostatic hypotension
- Frequency of body temperature exceeding 38°C (100.4°F) lasting for longer than 1 hour differentiated according to whether or not this presents with an absolute neutrophil count (ANC) below 1,000/µL (confirmed)
- [redacted]
- Suicidality evaluated by the Columbia-Suicide Severity Rating Scale (C-SSRS) or as evaluated by dedicated questions on suicidal ideation and behavior or as reported through AE

PHARMACOKINETIC OUTCOMES

The observed plasma concentrations of balovaptan and its metabolites M3 and M2 (as applicable) (and other metabolites as appropriate) will be summarized by dose and observation time, and the ratio of metabolite to parent drug concentration (molecular weight corrected) will be reported.

The key PK outcome measures for balovaptan will be:

- Apparent clearance (CL) and volume of distribution (VD)
- Covariate (e.g., age, weight) effects on CL and VD
- Exposure at steady-state ($AUC_{0\text{-}24,ss}$)

Other individual exposure estimates (e.g., C_{max}, T_{max}, AUC for a specific time interval) may also be derived as appropriate.

Apparent CL and exposure ($AUC_{0\text{-}24,ss}$) estimates for dose confirmation in the first cohort of adolescents and subgroup of children will be derived in a Bayesian feedback approach based on an adult population PK model.

BIOMARKER SAMPLE COLLECTION

Clinical Genotyping (CG) Samples

One mandatory blood sample for clinical genotyping

RESEARCH BIOSAMPLE REPOSITORY

One optional blood sample for genetic biomarker (inherited) discovery and validation from consenting subjects

INVESTIGATIONAL MEDICINAL PRODUCT(S)

Test Product

Blinded treatment phase study medication consists of balovaptan in dispersible tablets in *different* dose strengths *(including* 0.5, 2, 5, *7, and 10 mg)* as well as placebo.

Study medication (balovaptan and/or placebo) should be taken once per day in the morning with or without food. The dispersible tablets can either be swallowed whole with something to drink or with a small amount of soft food such as yogurt on a spoon, or can first be dispersed in 5 mL of water, orange or apple juice using a provided cup. Subjects will receive a total daily dose approximately equivalent to the adult dose of either 4 mg/day or 10 mg/day *equivalent* of balovaptan in terms of predicted exposure, or placebo *depending when the subject started study*.

Placebo

Matching placebo tablets will be used.

PROCEDURES (SUMMARY)

Screening and Baseline: After written informed consent is obtained, screening assessments which include general health, questionnaires, cognitive testing and feedback from caregivers will be performed. The Baseline visit will follow approximately 4 weeks (at least *1* week and up to *4* weeks) after the Screening visit.

For subjects enrolling in the first adolescent/first children cohorts, Baseline visit will take place at least 1 week and up to *4* weeks after Screening visit. The first dose of study medication will be given after completion of all pre-dose baseline assessments.

Treatment: During the 24-week treatment period, subjects will take double-blinded study medication once a day. Specific hematological and CPK safety labs will be taken at least every 4 weeks. These samples can be taken at home. During this period safety and efficacy assessments will be performed according to the schedule of assessments.

In each population of adolescents and children, a first cohort of approximately 24 subjects (adolescents) and up to approximately 30 subjects (children's cohort) will be enrolled in order to determine the final doses and to confirm acceptable safety and tolerability. At this early stage of the study the specific hematological and CPK safety labs will be taken every week.

Follow-Up: Subjects will be followed-up for at least 6 weeks after their last dose of study treatment.

STATISTICAL METHODS

PRIMARY ANALYSIS

Inferential statistical analysis will be performed on pooled data from patients taking balovaptan 10 mg eq dose compared with those from the concurrently randomized placebo in the corresponding randomization stage. The primary analysis will be performed on the modified intent-to-treat (mITT) population. The primary efficacy endpoint is absolute change from baseline after 24 weeks of treatment in Vineland™-II 2DC.

Mixed-effects model repeated measures (MMRM) methods will be used to compare treatment effects for the primary and continuous secondary efficacy endpoints on the overall population of adolescents and children. The model will include treatment and visit as main effects, individual age and baseline score (where available) as covariates, treatment-by-visit and baseline-by-visit as interaction terms, with visit week as a repeated effect. Treatment differences will be estimated together with their 90% confidence intervals.

The proportion of subjects with ≥6-point improvement in the Vineland™-II 2DC score (clinically meaningful response) will be analyzed using logistic regression. The odds ratio will be presented for descriptive purposes along with the corresponding 90% confidence interval and p-value for the comparison between balovaptan 10 mg eq and placebo after 24 weeks of treatment as well as at intermediate visits.

SAFETY ANALYSES

All safety analyses will be based on the safety population, that is, on all randomized subjects who received at least one dose of study medication. As appropriate, listings, summary tables, and graphs will be provided for safety and tolerability assessments.

PHARMACOKINETIC ANALYSES

Individual and mean plasma concentration data per time-point will be listed. Nonlinear mixed effects modeling will be used to analyze the sparse sampling dose-concentration-time data collected for balovaptan (and its metabolites as appropriate). Population and individual pharmacokinetic parameters will be estimated and the influence of various covariates on these parameters will be investigated. Additional PK, PK-PD, or dose/exposure-response analyses will be conducted as appropriate.

SAMPLE SIZE JUSTIFICATION

Prior to Version 6 of the protocol, a sample size of 240 ASD patients (80 per treatment arm) providing evaluable data at Week 24 ensure*d* the study 80% power to detect as statistically significant, at 1-sided 5% significance level, a difference between each active dose and placebo with an effect size of at least 0.4. No adjustment for multiple doses *was* performed. Considering a withdrawal rate of around 15-20%, it *was* planned to recruit approximately 300 subjects overall.

In accordance to Version 6 of the protocol, 80 subjects per treatment arm (balovaptan 10 mg eq and placebo) will be required, for a total sample size of approximately 160 subjects with ASD with evaluable data at Week 24. To maintain the number of evaluable subjects, the sample size will be increase to approximately 340 subjects overall.

Interim Analyses

Interim analyses for PK and safety in the first cohort of approximately 24 adolescents and subsequently in the first cohort of up to approximately 30 children are planned.

An efficacy and safety interim analysis is planned once approximately *80* subjects *taking either balovaptan 10 mg eq or placebo (i.e., approximately 40 subjects per treatment arm)* have completed their 12 week visit without dose *interruptions or* adjustments to allow internal decisions for the next steps of the development plan.

Given the hypothesis generating nature of this study, the Sponsor may conduct up to two interim analyses beyond what is specified elsewhere in this protocol. The decision to conduct such an interim analysis and its timing will be documented in the Sponsor's study master file prior to the conduct of the interim analysis. The clinical study report will also document that such an interim analysis occurred. Interim analyses will be performed and interpreted by the IMC and SOC members (as required), who will have full access to unblinded data. Recruitment will continue during the efficacy and safety interim analyses.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ABC	Aberrant Behavior Checklist
ADOS-2	Autism Diagnostic Observational Schedule
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
ASD	autism spectrum disorder
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{tau,ss}$	area under the concentration-time curve during the dosing interval tau at steady state
AVP	arginine vasopressin
AVPR1A	arginine vasopressin receptor 1A
AVR	microsatellite polymorphism at the AVPR1A gene
BMI	Body Mass Index
BP	blood pressure
CaGI	Caregiver-reported Global Impression
cAMP	cyclic adenosine monophosphate
CGI-I	Clinical Global Impressions of Improvement
CGI-S	Clinical Global Impressions of Severity
[redacted]	[redacted]
CL	clearance
CNTNAP2 KO	Knock out mutant for contact in associated protein-like 2 gene
C_{max}	maximum plasma concentration
CNS	central nervous system
CPK	creatine phosphokinase
CRH	corticotropin-releasing hormone
CSR	clinical study report
C-SSRS	Columbia-Suicide Severity Rating Scale
[redacted]	[redacted]
CYP2D6	cytochrome P450 2D6
CYP3A4	Cytochrome P450 3A4
DAS	Data Acquisition Specialist
DDI	drug-drug interaction

Abbreviation	Definition
DNA	deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSUR	Development Safety Update Report
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture
ePRO	electronic patient-reported outcome
ESF	Eligibility Screening Form
EU	European Commission
FE	food effect
FDA	U.S. Food and Drug Administration
GI	gastrointestinal
GLP	Good Laboratory Practice
HbA1c	glycated hemoglobin
HBcAb	total hepatitis B core antibody
HBsAg	hepatitis B surface
HBV	hepatitis B virus
HCV	hepatitis C virus
HDL	high-density lipoprotein
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
[redacted]	[redacted]
IB	Investigator's Brochure
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
IC_{20}	concentration required for 20% inhibition
ICF	informed consent form
ICH	International Council for Harmonisation
IQ	intelligence quotient
IUD	intrauterine device
LDH	Lactate dehydrogenase
IEC	independent Ethics Committee
IMC	Internal Monitoring Committee
IMP	investigational medicinal product
IND	Investigational New Drug (application)

Abbreviation	Definition
IRB	Institutional Review Board
ITT	intent to treat
mITT	modified intent to treat
IxRS	Interactive voice or web-based response system
Ki	inhibitory constant
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LLN	lower limit of normal
MAD	multiple ascending doses (study)
MedDRA	Medical Dictionary for Regulatory Activities
mg eq	age adjusted dose equivalent to adult dose
MMRM	mixed-effect model for repeated measures
NOAE-AUC	no-observed adverse effect—area under the curve
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
[redacted]	[redacted]
OACIS –I or S	Ohio Autism Clinical Impressions Scale Improvement or Severity
OCD	obsessive-compulsive disorder
PBPK	physiologically based pharmacokinetic
PD	pharmacodynamic
PedsQL	Pediatric Quality of Life
P-gp	P-glycoprotein
PK	pharmacokinetic
PNS	peripheral nervous system
POC	proof-of-concept
POM	proof of mechanism
PopPK	population pharmacokinetic
PP	per protocol
PR	pulse rate
PRO	patient-reported outcome
PT	prothrombin time
Q2W	every 2 weeks
Q4W	every 4 weeks
QRS	QRS interval on an electrocardiogram (ECG)
QT	QT interval on ECG
QTcB	QT interval on ECG with Bazett correction
QTcF	QT interval on ECG with Fridericia correction

Abbreviation	Definition
RBS-R	Repetitive Behavior Scale-Revised
RNA	ribonucleic acid
RR	RR interval
RS1	microsatellite polymorphism at the AVPR1A gene
RS3	microsatellite polymorphism at the AVPR1A gene
SAD	single-ascending dose (study)
SAE	serious adverse event
SI	International System of Units
SoA	Schedule of Assessments
SOC	Scientific Oversight Committee
SMT	Study Management Team
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
SRS-2	Social Responsiveness Scale, Second Edition
T_{max}	time to maximum plasma concentration
TSH	thyroid stimulating hormone
T4	thyroxine
ULN	upper limit of normal
VABS-II	Vineland™-II Adaptive Behavior Scales, Second Edition
Vineland™-II	Vineland™-II Adaptive Behavior Scales, Second Edition
VPA	valproate
V_{ss}	volume of distribution at steady state
V1a	vasopressin receptor type 1a
V1b	vasopressin receptor type 1b
V2	vasopressin receptor 2
WASI-II	Wechsler Abbreviated Scale of Intelligence Scale, Second Edition
WBC	white blood cell
WGS	whole genome sequencing
WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence, Fourth Edition

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND ON DISEASE

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 ASDs is between 1 in 50 to 1 in 88 children [1, 2]. Core symptoms of ASD are usually observed by three years of age, although typical language development might delay identification of symptoms. Moreover, although initial symptoms may be identified between 12 and 24 months, typically this does not manifest as a formal diagnosis and even if a diagnosis is made, this may not be stable [3, 4]. 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 may include a delay in verbal language development, impaired expressive language, deficient language pragmatics, as well as stereotyped, repetitive, or idiosyncratic use of language. Stereotyped and repetitive behavior manifests as a preoccupation with stereotyped or restricted interests, adherence to routines, rigidity, perseveration, motor mannerisms, and preoccupation or fascination with parts of items and unusual visual exploration. In addition to these core deficits, individuals with ASD may suffer from a range of co-morbid conditions and associated behavioral problems, including irritability, depression or anxiety, attention deficits, obsessive compulsive symptoms, seizures and sleep disruption. 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, making ASD the neuropsychiatric disorder most affected by genetic factors [2].

At present, no pharmacological treatment has been approved by Health Authorities to treat the core deficits of ASD, and currently available drugs address only associated behavioral problems [5]. Non-pharmacological treatments have been developed to address the core symptoms; however, clear efficacy has been difficult to demonstrate in large controlled clinical trials [6]. Accordingly, there is a high unmet medical need for pharmacological treatments of these core symptoms of the disorder.

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 (V1a, V1b and 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 (cAMP) production. V1a receptors are the primary subtype found in the central nervous system (CNS), expressed in several areas of the limbic system (hypothalamus, septum, hippocampus, amygdala) but are also

present in several tissues (vascular smooth muscle, liver, kidney, platelets, spleen) [7, 8, 9]. V1b receptors are also present in several brain regions, but appear to be most important for the increase in corticotropin-releasing hormone (CRH)-induced adrenocorticotropic hormone (ACTH) 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 [10]. Central administration of a V1 peptide antagonist has shown anxiolytic effects in the elevated plus-maze, a standard animal model of anxiety [11] and antidepressant-like effects in the Forced Swim Test, a model of depressive behavior. Similarly, V1a receptor knock-out mice also show reduced anxiety in open-field, light-dark box and elevated plus-maze tests [12, 13]. In addition, central injection of arginine vasopressin (AVP) in rodents (voles and hamsters), has been shown to induce offensive aggressive behavior [14, 15], which can be prevented by a V1a receptor antagonist [16]. Scratching and grooming, reminiscent of obsessive-compulsive behavior, can also be observed in mice after central injection of vasopressin [17].

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 [18]. Consistent with behavioral studies in animals (see above), these risk alleles have been found to modulate activation of the amygdala during emotional face processing [19] and to be associated with specific personality traits in healthy volunteers [21]. 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 subgenual cingulate regions [22].

Further 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 (OCD) and aggressive behavior [22]. Also, increased levels of AVP in plasma of subjects with ASD have been reported [23], although other researchers failed to replicate this observation.

1.2 BACKGROUND ON BALOVAPTAN (RO5285119)

Balovaptan is a potent and highly selective human vasopressin 1a (V1a) receptor antagonist that blocks the activation of the V1a G protein-coupled receptor.

[redacted]

[redacted]

[redacted]

[redacted]

1.2.2 Previous and Ongoing Clinical Studies

1.2.2.1 Safety and Tolerability

To date, balovaptan has been investigated in healthy subjects in *five* completed clinical pharmacology studies (Studies BP25694, BP28318, BP28977, BP29279, *and WP40038*) and one completed proof of mechanism study (Study BP29412). *Clinical pharmacology studies (WP40608 and WP40609) are clinically complete.*

Additionally, balovaptan has been investigated n a completed Phase II study in adult male patients with ASD (Study BP28420 [VANILLA]). Recruitment for this Phase II study in 5 to 17 year-old children and adolescent patients with ASD was initiated in November 2016 and is ongoing (Study BP30153 [aV1ation]). Approximately *340* patients are planned to be randomized. Treatment duration is 24 weeks with an optional open label extension of 52 weeks.

In addition, balovaptan is currently being investigated in a global Phase III study in adult patients with ASD (Study WN39434, V1aduct). Recruitment was initiated in August 2018 and is ongoing.

To date, no drug-related safety concerns have emerged from Adverse Events (AE) reporting, vital signs monitoring or assessment of hematology, clinical chemistry, and urinalysis. The maximum tolerated dose was not reached in the SAD (highest dose: 76 mg) or in the MAD (highest dose: 52 mg/day for 2 weeks). When balovaptan (12 mg single dose) was co-administered with multiple doses of fluoxetine (20 mg/day), an increased number of euphoric and asthenia AEs was reported; however, this has not been reported in patients taking SSRIs in Study BP28420 (see below).

Study BP28420 (VANILLA) is a completed Phase II study in 223 adult ASD patients. The scheduled treatment duration of 12 weeks was completed by 25 subjects randomized to a once daily dose of 1.5 mg, by 69 subjects treated once daily with 4 mg, by 29 subjects treated with 10 mg once daily, and by 64 subjects randomized to placebo. Analyses of AEs reported, including those considered serious or a non-serious *adverse events of special interest (*AESIs*)* and those that resulted in premature withdrawal from treatment, as well as analyses of laboratory results and physical and neurological examinations did not reveal any safety signal thought to be associated with the administration of balovaptan.

[redacted]

[redacted]

1.2.2.2 Efficacy

Efficacy of balovaptan has been investigated in a Phase II study in adult male patients with ASD (Study BP28420 [VANILLA]).

No significant treatment effects were observed on the SRS-2, the primary efficacy endpoint, in individuals with ASD after 12 weeks of treatment with balovaptan compared with placebo, and a large placebo effect was observed on the Total t-score and the five sub-scales.

The evaluation of change from baseline in adaptive functioning and skills was measured by the Vineland™-II. Improvement from baseline after 12 weeks of treatment was observed in adaptive behaviors as assessed by the Vineland™-II Composite Standard Score (see below). Greater improvement was observed with increasing doses of balovaptan as assessed by the estimated treatment difference on the Vineland-II Adaptive Behavior Composite Score (±90% confidence intervals) between balovaptan treatment and placebo as follows:

- 1.5 mg: 1.96 (−2.00, 5.92)
- 4 mg: 3.95 (1.66, 6.24)
- 10 mg: 4.87 (0.40, 9.33)

Greater improvement with increasing doses of balovaptan has also been observed in the Socialization and Communication domains. Among exploratory analyses, results suggested an improvement in the Pediatric Quality of Life (PedsQL)™ generic core scales, cognitive functioning scale, and family impact total score in 10 mg/day balovaptan group compared with placebo.

For further details regarding Study BP28420, please see the IB [45].

1.2.2.3 Pharmacokinetics

Plasma exposure of balovaptan increased in a greater than dose-proportional manner following single doses of 0.5 to 76 mg, whereas an approximately linear increase in exposure was observed after repeated dosing with 12 to 52 mg/day for 14 days. balovaptan was rapidly absorbed with a median Tmax between 1 and 4.5 hours after administration of single doses, and between 3 to 4 hours after multiple dosing. Steady state was achieved after approximately 7 days of daily dosing. Food had no relevant 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. A further 30% of the administered dose was recovered in feces.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2. OBJECTIVES

2.1 PRIMARY OBJECTIVES

The primary objectives of this study are as follows:

- To evaluate the efficacy of 24-week treatment with balovaptan (RO5285119) $10\ mg\ equivalent$ compared to placebo as measured by the change from baseline on the Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II) Two Domain Composite (2DC) (average of Communication and Socialization domains)

2.2 SECONDARY OBJECTIVES

The secondary objectives for this study are as follows:

- To evaluate the efficacy of treatment with balovaptan $10\ mg\ equivalent$ vs. placebo on:
    - Change from baseline on the Vineland™-II Composite standard score after 12 weeks and 24 weeks of treatment
    - Change from baseline in the Vineland™-II Communication, Socialization, and Daily Living Skills domain standard scores after 12 weeks and 24 weeks of treatment
    - Proportion of subjects with ≥6-point improvement in the Vineland™-II 2DC score to evaluate clinically meaningful response
    - Change from baseline in severity of clinical impressions as measured by CGI-S (Clinical Global Impressions-Severity) and OACIS-S (Ohio Autism Clinical Impressions Scale-Severity) after 12 weeks and 24 weeks of treatment
    - Improvements in clinical impressions as measured by CGI-I (Clinical Global Impressions-Improvement) and OACIS-I (Ohio Autism Clinical Impressions Scale-Improvement) after 12 weeks and 24 weeks of treatment
    - Patient-reported Pediatric Quality of Life (PedsQL) v4.0 Generic Core Scale after 12 weeks and 24 weeks of treatment

- Change from baseline in the Vineland™-II Composite standard score in adolescents and children independently after 12 weeks and 24 weeks of treatment
- Change from baseline on the Vineland™-II 2DC score after 12 weeks of treatment

- To evaluate safety and tolerability of *24 and* up to 76 weeks of treatment with balovaptan
- To evaluate the pharmacokinetics and exposure-response relationships of balovaptan and its metabolites, if appropriate

2.3 EXPLORATORY OBJECTIVES

The exploratory objectives for this study are as follows:

- To evaluate the effect of treatment with balovaptan on:
  - *Change from baseline on the Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II) Two Domain Composite (2DC)*
  - *Change from baseline on the Vineland™-II Composite standard score after 12 weeks and 24 weeks of treatment*
  - *Change from baseline in the Vineland™-II Communication, Socialization, and Daily Living Skills domain standard scores after 12 weeks and 24 weeks of treatment*
  - Change from baseline in behaviors as measured by Aberrant Behavior Checklist (ABC) Lethargy/Social withdrawal subscale after 12 weeks and 24 weeks of treatment
  - Change from baseline in repetitive behaviors as measured by Repetitive Behavior Scale-Revised (RBS-R) after 12 weeks and 24 weeks of treatment
  - Change from baseline to Week 76 (52 weeks of open-label treatment) as measured by Vineland™-II 2DC score
  - Change from Week 24 to Week 76 (52 weeks of open-label treatment) as measured by Vineland™-II 2DC score
  - Proportion of subjects with ≥4-point improvement in Vineland™-II 2DC score
  - Proportion of subjects with ≥8-point improvement in Vineland™-II 2DC score
  - Maintenance of efficacy 6 weeks after end of study treatment as measured by Vineland™-II Composite standard score
  - Social symptoms, communication, and behavior assessed by Exit Interviews
  - Change from baseline in behaviors as measured by ABC Irritability, Stereotypic Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech subscales after 12 weeks and 24 weeks of treatment

- Patient-reported PedsQL Cognitive Functioning Scale and parent-reported PedsQL Family Impact Scale after 12 weeks and 24 weeks of treatment
- Caregiver-reported Global Impression of Change on communication skills, social skills, daily living skills, and overall autism symptoms (CaGI) after 12 weeks and 24 weeks of treatment

- To evaluate whether genetic variants in the AVPR1A gene affect the efficacy or safety of balovaptan
- To evaluate the palatability of study medication

3. STUDY DESIGN

3.1 DESCRIPTION OF STUDY

For all subjects enrolled prior to Version 6 of the protocol, this is a Phase II multi--center, randomized, double-blind, 24-week, 3-arm, parallel group, placebo-controlled study to investigate the efficacy, safety, and PK of balovaptan in children and adolescents aged 5–17 years with ASD who are high functioning (IQ ≥70).

For subjects enrolled in accordance to Version 6 of the protocol, this is a Phase II multi-center, randomized, double-blind, 24-week, parallel group, placebo-controlled, 2-arm study with subjects assigned to a single dose of balovaptan (10 mg or equivalent) or placebo. All other parameters of the study design will remain as outlined.

3.1.1 Overview of Study Design

The total duration of the study (from screening through to study completion) for each subject will be approximately *87* weeks divided as follows:

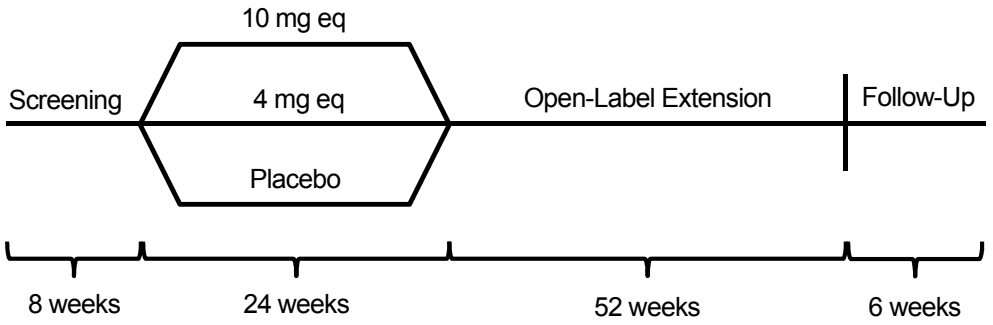
- Screening period (first adolescent/children cohorts): Approximately 4 weeks
  - at least 1 week and up to *4* weeks prior to randomization
- Screening period (main study): Approximately 4 weeks
  - at least *1* week and up to *4* weeks prior to randomization
- Treatment phase*: 24 weeks
- Optional OLE: Approximately 52 weeks
- Follow-up: At least 6 weeks and up to 7 weeks after last dose

* A small group of subjects may receive blinded treatment for up to 32 weeks. These are subjects from the first PK cohort who stopped treatment prior to determination of final doses by the IMC and SOC, and have the opportunity to re-start in the main study (see protocol Section 3.1.3.1).

Approximately *340* children and adolescents aged 5–17 years with ASD will be recruited to ensure a total of *160* evaluable subjects *with placebo or 10 mg eq* after 24 weeks of treatment (*see* Figure 1*and* Figure 2). *All individuals who received doses of balovaptan that were not equivalent to 4 mg or 10 mg will not be included in the primary analysis.*

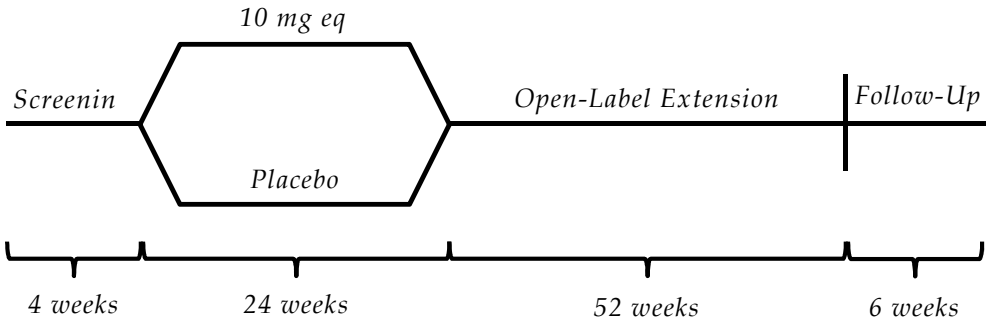
Randomization will be stratified by age group and sex. The ratio between adolescents (aged 13–17 years) and children (aged 5–12 years) will be approximately 1:1. The children's cohort should contain up to approximately 30 subjects of whom up to approximately 16 should be aged between 5 and 7 years old. Additionally, the proportion of female subjects will be balanced in each treatment group and limited to a maximum of 20% of the entire study population.

Figure 1 Study Design ***for Subjects Enrolled prior to Version 6***



Subjects will be randomized in a ratio of 1:1:1 and receive doses providing an exposure approximately equivalent to the 4 mg/day and 10 mg/day or placebo exposures observed in adults (see Table 1 for predicted doses). Age-adjusted doses were derived using a physiologically-based pharmacokinetic (PBPK) model.

Figure 2 Study Design for Subjects Enrolled in Accordance to Version 6



eq = equivalent.

Subjects will be randomized in a ratio of 1:1 and receive doses equivalent to the 10 mg/day or placebo exposures observed in adults (see Table 2 for predicted doses).

Dose-exposure relationship in adolescents and in children will be confirmed independently. Enrollment will be staggered, starting first with adolescents (aged 13 to 17 years) and then with children (aged 5 to 12 years). Initially, a first cohort of

approximately 24 adolescents will be enrolled together. Once the Internal Monitoring Committee (IMC) and the Scientific Oversight Committee (SOC) agree on acceptable safety and tolerability in the first adolescent cohort and determine the final doses, enrollment of adolescents will resume and the enrollment of a first cohort of up to approximately 30 children (aged 5 to 12 years) can commence. Once the IMC and SOC agree on acceptable safety and tolerability in the first children cohort and determine the final doses, enrollment of children for the main study will start (see Sections 3.1.3 and 4.4.2 for more details). If IMC and SOC review of data determines the final dose and agreement on acceptable safety and tolerability in a subgroup of children (e.g., 8–12 years old) but not the full cohort aged 5–12 years, enrollment of children in this subgroup for the main study may start. Enrollment of the other subgroup for confirmation of dose-exposure relationship will continue until IMC and SOC review determines final doses and agreement on acceptable safety and tolerability in that subgroup (Section 3.1.4).

3.1.2 Internal Monitoring Committee and Scientific Oversight Committee (IMC and SOC)

An IMC together with a SOC will review all data available at the time-points specified in the section below and in the IMC and SOC agreement or on an ad-hoc basis, if deemed necessary.

The IMC consists of a selected subset of **internal** Roche representatives while the SOC members are independent experts **external** to Roche. The IMC and SOC members will be unblinded to individual subject treatment allocation.

The IMC and SOC agreement will provide further details about the roles and responsibility of the IMC and SOC, identifying members, defining the timing of meetings, and communication plans for interim results.

[redacted]

[redacted]

3.1.3.2 Efficacy Interim Analyses

In accordance to Version 6 of the protocol, an efficacy and safety interim analysis is planned once approximately *80* subjects *have taken either 10 mg eq or placebo (i.e., approximately 40 subjects per treatment arm)* have completed the 12-week visit without interruptions or dose adjustments to allow internal decisions for the next steps of the development plan (see also Section 6.8).

[redacted]

3.1.5 Open-Label Extension

Subjects who complete the double-blind 24-week treatment period will able to participate in an optional 52-week OLE period where they will receive open-label balovaptan treatment.

Participation in the OLE will also be permitted for all subjects who have completed treatment to Week 24, prior to implementation of the OLE. Furthermore, individuals who participated in the PK cohort and were required to stop dosing before week 8, and then did not participate further in BP30153, will be allowed to participate in the OLE (see *Section* 4.2.4 for detailed eligibility criteria). All subjects meeting these requirements will need to be re-consented for participation in the OLE after meeting requirements specified in Appendix 11.

Subjects who do not want to participate in the OLE will complete the follow-up visits as detailed in the Schedule of Assessments (see Appendix 1). Subject treatment allocation during the blinded treatment period will not be unblinded until all patients have completed the double-blind period.

During the OLE portion of the study, all subjects will be provided with 10 mg *eq* balovaptan irrespective of previous treatment allocation.

3.2 END OF STUDY

For primary analysis purposes, and for the clinical study report, the end of the study is defined as 31 weeks after the last subject is enrolled into the main part of the study; see also Section 3.1.1.

For subsequent analyses and for the clinical study report addendum, the end of the study is defined as the date when the last subject last observation (LSLO) occurs. LSLO

is expected to occur approximately 83 weeks after the last subject is enrolled into the main part of the study; see also Section 3.1.1.

3.3 RATIONALE FOR STUDY DESIGN

The trajectory of a potential pharmacologically-induced improvement of social and communication deficits in children and adolescents with ASD is unknown. There are no published large multicenter 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 reported a systematic review and meta-analysis of the effect of the TEACCH program [26]. 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 ASD subjects as measured by the Vineland™-II is in between these numbers. As a consequence, treatment duration of 24 weeks should allow sufficient exposure to adequately and efficiently improve clinically meaningful responses in both social communication and functional social outcomes as well as characterize the safety and tolerability profile of balovaptan.

[redacted]

3.3.3 *Rationale for Use of Single Dose in Subjects Enrolled in Accordance to Version 6*

Study BP30153 was initiated as a 3-arm study, two doses of balovaptan (10 mg eq and 4 mg eq) and placebo. These doses were adjusted per age (see Section 3.1.3 and Table 1). Following the IMC and SOC review in July 2018, data showed that subjects received doses that produced exposures lower than those targeted from Study BP28420. To ensure that 10 mg is adequately characterized, the 4 mg dose arm will be discontinued, and all subjects who enroll after Version 6 approval will receive either 10 mg eq or placebo.

Balovaptan 10 mg eq/day (age adjusted dose equivalent to adult dose) has been selected as the single dose to be studied. Results from Study BP28420 suggested that 10 mg

balovaptan once daily showed consistent efficacy across multiple domains of the Vineland-II and other instruments explored. The safety profile of balovaptan across doses was similar. The 10-mg balovaptan once daily 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 the Socialization and Communication domains of the Vineland-II scales.

3.3.4 Rationale for Study Population

Young subjects with ASD (age 5–17) will be enrolled in this study to assess efficacy in this pediatric population and to generate a safety and tolerability database in this population. The study population includes cognitively high functioning (IQ ≥ 70) children and adolescents (aged 5–17 years) with clinical diagnosis of ASD confirmed by Autism Diagnostic Observational Schedule (ADOS-2).

The lower age limit (5 years) will allow building a significant safety database from older pediatric population before exposing preschool children and toddlers to balovaptan. Furthermore, although initial symptoms may be identified very early, typically this does not lead to a formal diagnosis and even if a diagnosis is made, this may not be stable over time [4]. In conclusion, although data suggest that the diagnosis of ASD is possible below the age of 5, the diagnosis is less reliable and may not be stable, and as a consequence, younger subjects may be included later in future trials in the development program.

Cognitively high functioning (IQ ≥ 70) individuals define a more homogeneous population that will facilitate signal detection and it is expected that this population may benefit more from a therapy improving social and communication aspects of ASD. Subjects with significant social and communication deficits will be recruited to ensure room for potential treatment-mediated improvements. To this aim, only subjects with SRS-2 T-scores ≥ 66 and CGI-S ≥ 4 (at least moderately-ill) at screening will be enrolled. Individuals with prominent social and communication deficits will be recruited since this group has more need for improvement in social and communication deficits.

3.3.5 Rationale for Use of Vineland™-II Adaptive Behavior Scales 2-Domain Composite Score as the Primary Efficacy Endpoint

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 parent [50]. 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 [33] and have been used independently as endpoints in clinical trials of ASD [49]. 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 support the Sponsor's proposal to combine the two domains into a single endpoint. The use of the 2DC has been discussed with both FDA and EMA.

3.3.6 Rationale for Open-Label Extension

At the time that study BP30153 was initiated, there was no evidence supporting the efficacy of balovaptan in individuals with ASD. However, study BP28420 has provided preliminary evidence that balovaptan may have efficacy on the social and communication deficits observed in adults with ASD (see Section 1.2.2.2). Consequently, the Sponsor now proposes to include a 52-week OLE to evaluate effects of balovaptan in children over a longer time period. Therefore, the treatment duration will be extended to 76 weeks with the following treatment periods: double-blind from baseline to Week 24; and open-label from Week 24 to Week 76.

All children will receive 10 mg (age-equivalent) balovaptan during the OLE. The dose selection was based on preliminary evidence from Study BP28420 in adult ASD patients, which indicated consistent and potentially clinically relevant changes in the Socialization and Communication domains of the Vineland™-II Scales without having revealed relevant safety liabilities. To date, there is no indication from this ongoing BP30153 study that the safety profile would be different in children and adolescents, respectively.

3.3.7 Rationale for Use of Responder Definition

A clinically meaningful response (or "responder") is defined as ≥6 point improvement on the Vineland™-II 2DC at Week 24. This threshold was determined through anchor- and distribution-based analyses conducted on Study BP28420 data. 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 x Standard Error of Measurement (SEM) 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.

3.4 OUTCOME MEASURES

3.4.1 Efficacy Outcome Measures

The efficacy outcome measures for this study are as follows:

- Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II)
- Clinical Global Impressions: Severity (CGI-S) and Improvement (CGI-I)

- Ohio Autism Clinical Impressions Scale: Severity (OACIS-S) and Improvement (OACIS-I)
- Pediatric Quality of Life (PedsQL) v4.0 Generic Core Scale

3.4.2 Exploratory Outcome Measures

The exploratory outcome measures for this study are as follows:

- Pediatric Quality of Life (PedsQL) Cognitive Functioning Scale.
- Pediatric Quality of Life PedsQL Family Impact Scale
- Caregiver-reported Global Impression of Change (CaGI)
- Aberrant Behavior Checklist (ABC)
- Repetitive Behavior Scale-Revised (RBS-R)
- Exit interviews
- Evaluation of safety and/ or efficacy data in the context of genetic variants in the AVPR1A gene
- Palatability test of study medication

3.4.3 Safety Outcome Measures

The safety outcome measures for this study are as follows:

- Incidence, nature, and intensity of adverse events
- Incidence and nature of laboratory abnormalities, based on hematology, clinical chemistry, [redacted], coagulation monitoring, and urinalysis testing
- Tanner staging (see Appendix 6)
- Physical examination (including body height and weight) and neurological examination (see Appendix 5)
- Vital signs, i.e., systolic and diastolic blood pressure, pulse rate in supine position, and orthostatic hypotension
- Frequency of body temperature exceeding 38°C (100.4°F) lasting for longer than 1 hour differentiated according to whether or not this presents with an absolute neutrophil count (ANC) below 1,000/µL (confirmed)
- [redacted]
- Suicidality evaluated by the Columbia-Suicide Severity Rating Scale (C-SSRS) or as evaluated by dedicated questions on suicidal ideation and behavior or as reported through AE

3.4.4 Pharmacokinetic (PK) Outcomes

The observed plasma concentrations of balovaptan and its metabolites M3 and M2 (as applicable) (and other metabolites as appropriate) will be summarized by dose and observation time, and the ratio of metabolite to parent drug concentration (molecular weight corrected) will be reported.

A population PK modeling approach will be used to characterize the pharmacokinetics and variability of balovaptan (and its metabolites, if warranted) in the pediatric population. The key PK outcome measures for balovaptan will be:

- Apparent clearance (CL) and volume of distribution (VD)
- Covariate (e.g., age, weight) effects on CL and VD
- Exposure at steady-state ($AUC_{0\text{-}24,ss}$)

Other individual exposure estimates (e.g., C_{max}, T_{max}, AUC for a specific time interval) may also be derived as appropriate.

Apparent CL and exposure ($AUC_{0\text{-}24,ss}$) estimates for dose confirmation in the first cohort of adolescents and a subgroup of children will be derived in a Bayesian feedback approach based on an adult PopPK model.

The results of population PK model derived outcome measures may be reported in a document separate from the clinical study report.

3.4.5 Biomarker/Genotyping Sample Collection

3.4.5.1 Clinical Genotyping (CG) Samples

A mandatory pre-dose whole blood sample will be taken for DNA extraction from every participant on Day 1. The DNA will be used to evaluate if genetic variants of the AVPR1A gene affect the efficacy/safety and/or PK of balovaptan. CG samples and derived analytical materials will be destroyed no later than 2 years after the date of the final clinical study report.

3.4.5.2 Research Biosample Repository

Subjects enrolled in the study will be asked to donate one optional blood sample for the Research Biosample Repository (Section 4.6.1.13). Specimens will be used to promote, facilitate, and improve individualized healthcare by better understanding/predicting drug efficacy, dose response, safety, mode of actions, disease biology, and progression.

Samples will be collected from consenting subjects at the time points indicated in the Schedule of Assessments. Specimens will be stored and used until no longer needed or until they are exhausted.

4. MATERIALS AND METHODS

4.1 CENTER

This is a multi-center study to be conducted in the United States.

Administrative and Contact Information and List of Investigators are provided separately.

4.2 STUDY POPULATION

The study will include children and adolescents between 5 to 17 years of age with a diagnosis of ASD according to the DSM-5 or ICH 10 criteria who are high functioning (IQ ≥ 70).

4.2.1 Recruitment Procedures

A total of approximately *340* children and adolescents with ASD will be stratified by age and sex and randomized in order to obtain evaluable data after 24 weeks of treatment from approximately *160* subjects, considering a withdrawal rate of approximately 15%–20%. *Individuals who received doses lower than intended will not be evaluable for the primary objective.*

Subjects in first adolescent/first children cohorts with dose adjustments, or who stopped and re-started treatment in the context of the interim PK and safety reviews (see Section 3.1.3), will not contribute to the approximately *160* evaluable subjects *(80 subjects balovaptan 10 mg eq and 80 subjects placebo) for* the primary *efficacy* analysis. Additional subjects will be recruited until approximately *160* evaluable subjects is reached (Section 6.3.3).

Subjects who have been discontinued for reasons of dose confirmation, prior to Week 8, will be eligible to re-screen for the OLE without having to complete the 24 weeks of double-blind treatment.

Subjects must meet all of the inclusion criteria and none of the exclusion criteria to qualify for the study. Unless otherwise stated, inclusion and exclusion criteria refer to screening.

Abbreviated re-screening (written informed consent/assent, medical history, physical examination, substance use, alcohol test, fasted laboratory safety, coagulation, serology, urinalysis, inclusion / exclusion criteria) may be allowed under circumstances in which the subject is screen-passed but could not be randomized within the *4*-week screening window due to a study halt, logistical, personal, or technical reason. At no time shall the duration between the original screening visit and the abbreviated re-screening visit exceed 3 months. Abbreviated re-screening will only be permitted by the Sponsor (Medical Monitor or designee) in cases where this poses no safety risk to the subject.

Screen-failed subjects can be re-screened with Sponsor permission (Medical Monitor or designee) if there is a substantial change in the subject's general condition (e.g., prohibited medication was stopped or weight loss) and if recruitment for the study is still ongoing. Re-screening will not be allowed if the subject failed earlier to meet the disease specific inclusion criteria (e.g., SRS-2 T-score).

Safety laboratory tests which would exclude the subject at screening may be repeated once (as unscheduled labs) if it is suspected that the abnormal result is transient and likely to be normal at repeat testing.

Individuals are not permitted to be re-randomized to receive a second course of treatment. However, subjects from first adolescent and first children cohorts, who stopped treatment in the context of the interim safety and PK decisions, (see Section 3.1.3) will be allowed to re-start in the same treatment arm, according to their original randomization, for 24 weeks in the main study. Subjects may only re-start if the Investigator can exclude relevant changes in the subject's general condition, i.e., no change in risk-benefit for treatment of the individual subject in study BP30153. In case of uncertainty the Investigator should discuss the case with the Sponsor (Medical Monitor or designee). Re-starting subjects will have a new, abbreviated baseline visit, and will continue with all subsequent visits according to the schedule of assessments (Appendix 1).

Study subjects may be identified for potential recruitment using pre-screening enrollment logs, IRB approved newspaper/radio advertisements and mailing lists prior to consenting to take place on this study.

4.2.2 Inclusion Criteria

Subjects must meet the following criteria for study entry:

1. Males or females aged 5–17 years at randomization
2. Fluent in English
3. DSM-5 criteria for ASD or ICD10 criteria for Autism diagnosis confirmed by ADOS-2 criteria
4. SRS-2 (T-score) $\geq$ 66
5. CGI-S $\geq$ 4 (moderately ill) at screening
6. IQ $\geq$ 70 as assessed by WASI-II or WPPSI-IV intelligence test
7. Language, hearing, and vision compatible with the study measurements as judged by the Investigator
8. In the Investigator's opinion, the subject must be able to participate and is deemed appropriate for participation in the study, capable of following the study schedule of assessments
9. Availability of a parent or other reliable caregiver who is fluent in English and has frequent and sufficient contact with the subject. The same person must agree to accompany the subject to all clinic visits and provide information about the subject's behavior and symptoms and must agree to oversee the subject's adherence with protocol-specified procedures and study medication dosing

10. Parent or legal guardian/representative and caregiver willing and able to give written informed consent according to local requirements and subject willing and able to provide informed assent or consent according to local requirements
11. Female subjects who have experienced menarche:
    - Must have a negative urine pregnancy test at screening and are not breast feeding
    - Must agree to either remain completely sexually abstinent or use two effective contraceptive methods from screening until 28 days after the last dose of study treatment. Methods include:
        - An intrauterine device (IUD) implanted at least 2 months prior to screening
        - Established hormonal contraception (oral, vaginal, implanted, or injectable)
        - Spermicidal agent (foam, gel, cream, or suppository)
        - Barrier method, such as a condom, diaphragm, cervical/vault cap, or vaginal sponge

Notes:

- The Sponsor does not require male contraception because of the minimal seminal dose transmitted via sexual intercourse [46, 47 and see also Section 1.2.1].
- Female subjects who experience menarche during the study must comply from this time onwards with the same requirements as outlined above, i.e., use 2 forms of effective contraception or agree to remain completely abstinent until 28 days after last dose.

4.2.3 Exclusion Criteria

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

Neurological/psychiatric:

1. Initiation of a major change in psychosocial intervention (including investigational) within 4 weeks prior to screening. Minor changes in ongoing treatment (e.g., missed therapy sessions due to holiday/vacation; planned break in therapy due to school holidays; changes in college/school programs) are not considered significant.
2. Unstable or uncontrolled clinically significant psychiatric and / or neurological disorder that may interfere with the safety or efficacy endpoints
3. Known personal or family history (first or second degree relatives) of cerebral aneurysm
4. Risk of suicidal behavior in the opinion of the Investigator or as evidenced by a "yes" to questions 4 and / or 5 of C-SSRS taken at screening and baseline with respect to the last 12 months, or any suicide attempt in medical history
5. Seizure within the past 6 months
6. Medical history of alcohol or substance abuse/dependence

Cardio-vascular:

7. Concurrent cardio-vascular disease not considered well controlled by the Investigator
8. Confirmed (e.g., 2 consecutive measurements) clinically significant abnormality on ECG at screening. That includes but is not limited to a QTcF of ≥450 milliseconds, absence of dominating sinus rhythm, AV-block II° or III

Other organ systems:

9. Concomitant disease or condition (pulmonary, gastro-intestinal, hepatic, renal, metabolic, immunological system, or obesity (Body Mass Index [BMI] at or above the 99th percentile for the same age and sex [see Section 4.6.1.2]) that could interfere with, or treatment of which might interfere with, the conduct of the study; or discontinuation of prohibited medication might pose unacceptable risks to the subject in the opinion of the Investigator
10. Evidence for current GI bleeding, e.g., active stomach ulcer disease
11. History of coagulopathies, bleeding disorders, or blood dyscrasias
12. Positive serology for hepatitis B (HBV), hepatitis C (HCV), HIV 1, or HIV 2.

    Subjects who test positive for hepatitis B core antibodies may still be eligible if the subject has a combination of a negative hepatitis B surface antigen and/or hepatitis B cDNA test, no history of hepatitis, and no indication of liver dysfunction through liver enzyme or coagulation results.

13. Confirmed clinically significant abnormality in parameters of hematology, clinical chemistry, coagulation, or urinalysis [redacted]
14. Medical history of malignancy if not considered cured

Other exclusion criteria

15. Participation in an investigational drug study within 90 days or 5 times the half-life of the investigational molecule (whichever is longer) prior to randomization or participation in a study testing an investigational medical device within 90 days prior to randomization or if the device is still active.
    a) Subjects from first adolescent and first children cohorts, who stopped treatment in the context of the IMC/SOC dose determination decision (see Section 3.1.3.1), are allowed to re-start originally assigned treatment for an additional 24 weeks in the main part of the study.
16. Loss of blood over 250 mL within three months prior to screening
17. Allowed medications have not been stable since 4 weeks before screening, and allowed medications for treatment of epilepsy have not been stable since 3 months before screening.

18. Use of prohibited medications within 2 weeks prior to screening visit or 5 times the half-life prior to randomization (whichever is longer)
19. [redacted]

4.2.4 Eligibility for Open-Label Extension Phase

Subjects who meet the following criteria may participate in the OLE:

Subjects who complete the double-blind 24-week treatment period will able to participate in an optional 52-week OLE period where they will receive open-label balovaptan treatment. All subjects that have previously completed or were required to stop dosing prior to Week 8 for non-safety reasons will need to be re-consented and meet the eligibility requirements specified in Appendix 11.

Subjects must satisfy both criteria:

1. Either completed the blinded treatment phase (Week 24) OR were required to stop dosing by the Sponsor at or before Week 8.
2. Have no relevant adverse events including laboratory abnormalities in the opinion of the investigator that are prohibitive for starting the OLE

If a subject with ASD reaches the age of 18 during the study, they will still be eligible for participation in the OLE provided that they meet all the criteria above.

Where possible, all subjects should continue directly from the blinded phase to the open-label phase. For subjects that enter the OLE, the treatment follow-up visit will be completed at the end of OLE phase.

Under specific circumstances, a subject may need to have treatment interrupted at the end of the double-blind phase before entering the OLE. These cases should be discussed with the Medical Monitor or designee.

Any subject who had treatment interruption due to lack of dose confirmation or for whom the OLE was not available will be able to enter the open label phase until the final subject recruited reaches Week 24.

4.3 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Subjects who meet all of the inclusion criteria and none of the exclusion criteria will be stratified by age group and sex and randomized in a *1:1* ratio *(10 mg eq:placebo)* to receive *of* 10 mg/day *(age adjusted dose equivalent to adult dose)* 24 weeks as double-blinded study medication or placebo. The allocation to individual treatment will be implemented using an Interactive (voice/web) Response System (IxRS).

Approximately equal numbers of children (aged 5–12 years) and adolescents (aged 13−17 years) will be enrolled. Additionally, the proportion of female subjects will be limited to a maximum of 20% of the entire study population. Furthermore, stratification will guarantee (for exposure checks purpose, see Section 3.1.3.1) that, in the first 30 children cohort and first 24 adolescent cohort, 10 of the youngest subjects 13-14 years old in adolescent group and approximately 10 of the 5–7 years old in children group will take active treatment. However, if final doses cannot be determined for the subgroup or cohort, and additional subjects are required for dose confirmation to occur, the size of the cohort will increase (see Section 3.1.3.1 for further details). The children's cohort should contain up to approximately 16 subjects aged between 5 and 7 years old. Subjects from first adolescent and first children cohorts, who stopped treatment in the context of the interim safety and PK decisions, (see Section 3.1.3) will be allowed to re-start in the same treatment arm, according to their original randomization, for an additional 24 weeks in the main study, provided the Investigator can exclude relevant changes in the subject's general condition (see Section 4.2.1). Unblinding should not occur except in the case of emergency situations. Unblinding will be performed by means of the IxRS.

As per regulatory reporting requirements, Roche will unblind the identity of the study medication for all unexpected serious adverse events that are considered by the Investigator to be related to study drug as per safety reference document(s), i.e., Investigator Brochure [45]. Details of subjects who are unblinded during the study will be included in the Clinical Study Report.

Whenever disclosure of the identity of the study medication 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 date, reason for identifying the drug, and the name of all person(s) who had to be unblinded.

With the exceptions described below and in the IMC+SOC agreement (see also Section 3.1.2), the randomization list will not be available to the Roche project and study team or any personnel at the study centers. Access to potentially unblinding data (e.g., PK data) will be restricted as described below.

PK/PD data can be received and cleaned on an ongoing basis by an unblinded Data Acquisition Specialist (DAS). In addition, a pharmacometrician may be unblinded to enable the frontloading of modeling activities. These activities are solely for building the pharmacometric models and frontloading the analyses.

Subjects, Investigators, and all other individuals directly involved in this study will remain blinded until the end of the study when the final analysis becomes available.

4.4 STUDY TREATMENT

[redacted]

4.4.2 Dosage, Administration, and Compliance

4.4.2.1 Age-adjusted Doses

Subjects *enrolled before Version 6* will receive age-adjusted total daily dose approximately equivalent to the adult doses of either 4 mg/day or 10 mg/day of balovaptan or placebo:

The median exposures achieved in adults at doses of 4 mg/day and 10 mg/day, respectively, will be targeted in the adolescents and children enrolled in this study. The following age-adjusted doses are therefore planned as starting doses based on PBPK model predictions, to achieve exposures similar to those in adults at 4 mg/day and at 10 mg/day:

Subjects enrolled under Version 6 will receive age-adjusted total daily dose approximately equivalent to the adult doses of 10 mg/day of balovaptan or placebo.

Table 1 Age-adjusted Starting Doses Based on PBPK Model

Age (years)	4 mg eq* Dose (mg)	10 mg eq* Dose (mg)
5–7	1.5	3
8–11	2	5
12–14	3	7
15–17	4	10

*eq = dose predicted to achieve exposures *equivalent* to 4 mg/day or 10 mg/day, respectively, in adults.

Following IMC and SOC reviews, the starting doses were shown to not achieve the target exposure in children aged between 5 and 14 years. Therefore, new doses were identified to produce exposures equivalent to the 4 mg and 10 mg doses used in adults (Study BP28420). See Table 2 for details.

Table 2 Updated Age-Adjusted Doses Based on PK Data

Age (years)	*4 mg eq Dose (mg)*	*10 mg eq Dose (mg)*
5–7 [a]	*3*	*7*
8–17	*4*	*10*

eq = age adjusted dose equivalent to adult dose; PK = pharmacokinetic.

[a] *Dose predicted to achieve exposures equivalent to 4 mg/day or 10 mg/day, respectively, in adults. Note: Dose is not confirmed – the IMC and SOC will recommend the final dose based on review of data (see Section 3.1.3.1) to achieve equivalent adult exposures. Any changes will be communicated to all parties.*

The starting doses *have been* taken by the first cohort of 24 adolescents and the first cohort of approximately 30 children. Enrollment will be staggered by age, starting first with adolescents. The exposures will be monitored closely by the IMC and SOC *throughout* the study. *The* age at inclusion in the double-blind portion or the age at inclusion in the OLE will be relevant for selection of doses.

The total daily doses of the blinded treatment phase will be administered as *tablets of* balovaptan or placebo once daily.

Following an ad-hoc interim assessment of the PK data by the IMC and SOC, adolescents and children 8 years and above will receive higher starting doses as outlined in Table 2. Subjects already enrolled should receive the adjusted doses (4 or 10 mg eq) as soon as practicable, per his or her randomized assignment.

Subjects enrolled in accordance with earlier protocol versions will continue with his or her assigned dose until Week 24. All newly enrolled subjects (in accordance to Version 6) will be given either 10 mg eq or placebo.

In the OLE phase, subjects will receive 10 mg *eg*/day of balovaptan (see *Table 2* for further details). Details of tablet administration will be provided in the Instructions for Use.

Guidelines for treatment interruption or discontinuation are provided in Section 5.2.

4.4.2.2 Administration and Compliance

Study medication (balovaptan or placebo) should be taken once per day in the morning with or without food. The dispersible tablets can either be swallowed whole with something to drink or with a small amount of soft food such as yogurt on a spoon, or can first be dispersed in 5 mL of water, orange or apple juice using a provided cup (for more details please see document 'Instructions for pediatric use'). Administration with larger amounts of food should be avoided as the tablets should be taken as whole tablets in one mouthful avoiding chewing. Tablets taken with soft food must be swallowed immediately after adding the tablets to the soft food on a spoon and must not be stored for future use. At inclusion of each subject to the blinded portion of the study, a decision will be made for the entire study whether the subject will swallow the study medication as a whole or after dispersion in a liquid and that decision will be recorded on the eCRF and drug diary.

The first dose of the study medication will be administered on Day 1, after all pre-dose baseline assessments have been conducted. Compliance regarding administration of study medication at home will be monitored by the completion of a diary card by the subject or caregiver. Diary cards will be reviewed at each clinic and home visit.

If, during the first adolescent/first children cohorts, it is not possible to collect a pre-dose PK sample during home visits in Week 1, Week 2, and Week 4, the dose must be taken as normal and a post-dose PK sample should be taken at a convenient time. It is important that the exact time of dosing is recorded (see also Section 4.6.1.8 and Appendix 2).

The qualified individual responsible for dispensing the study drug will prepare the correct dose according to the randomization schedule. This individual will write the date dispensed, subject number, and initials on the study drug vial label and on the Drug Accountability Record. This individual will also record the study drug batch or lot number received by each subject during the study.

4.4.2.3 Treatment in the Open-Label Extension, Including Age-Adjusted Doses

Subjects will receive 10 mg/day *equivalent* of balovaptan (see Table 1 *and Table 2* for further details).

Study medication (balovaptan) should be taken once per day in the morning with or without food (for more details, please see document “Instructions for pediatric use”). At inclusion of each subject, a decision will be made for the entire OLE whether the subject will swallow the study medication as a whole or after dispersion in a liquid, and that decision will be recorded on the eCRF and drug diary.

The first dose of the study medication in the OLE will be administered at the Week 24 visit for those that are continuing directly into the OLE and following completion of eligibility assessments in the OLE for those that are returning to the study (see Appendix 11). Administration will take place after all assessments have been conducted on the visit. Compliance regarding administration of study medication at home will be monitored by the completion of a diary card by the subject or caregiver. Diary cards will be reviewed at each clinic and home visit.

4.4.3 Investigational Medicinal Product Accountability

All investigational medicinal products (IMPs) required for completion of this study (balovaptan and placebo) will be provided by the Sponsor. The investigational site will acknowledge receipt of IMPs, to confirm the shipment condition and content. Any damaged shipments will be replaced.

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 identification of the subject to whom the study drug was dispensed (for example subject initials and date of birth)
- The date(s), quantity of the study drug dispensed to the subject
- The date(s) and quantity of the study drug returned by the subject
- All records and drug supplies must be available for inspection by the Roche Monitor [at every monitoring visit]

IMPs will either be disposed of at the study site according to the study site’s institutional standard operating procedure or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed upon by the Sponsor. Local or institutional regulations may require immediate destruction of used IMP for safety reasons. In these cases, it may be acceptable for investigational study site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned, and destroyed, and provided that adequate storage and integrity of drug has been confirmed.

The site must obtain written authorization from the Sponsor before any IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Written documentation of destruction must contain the following:

- Identity [batch numbers or study subject numbers] of investigational product[s] destroyed
- Quantity of investigational product[s] destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person [or company] who destroyed investigational product[s]

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.

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.

4.5 CONCOMITANT THERAPY

Adding a new medication or changing the dose of a medication after signing the Informed Consent Form (ICF) 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-medication interventions (e.g. individual psychotherapy, cognitive behavioral therapy, smoking cessation therapy, and rehabilitative therapy) used by a subject from 12 weeks prior to screening until the follow-up visit.

All concomitant medications should be reported to the Investigator and recorded on the Concomitant Medications electronic Case Report Form (eCRF). All therapy and/or medication administered to manage adverse events should be recorded on the Adverse Event eCRF.

4.5.1 Permitted Therapy

As a general rule, no concomitant medication will be permitted, with the exception of medications listed in this section, unless the rationale for exception is discussed and clearly documented between the Investigator and the Sponsor (Medical Monitor or designee). Subjects must be on a stable prescribed medication regimen for 4 weeks before screening, with the exception of antiepileptic treatments in which subjects need to be on a stable regimen for at least 3 months prior to screening. Any stable prescribed medication at screening should remain stable throughout the study. However, dose changes due to adjustments for change in body weight / age and or triggered by therapeutic monitoring will be allowed. Other changes in prescribed medication need to be approved by the Sponsor (Medical Monitor or designee).

Examples of allowed medications:

- Selective Serotonin Reuptake Inhibitor / Serotonin-Norepinephrine Reuptake Inhibitor (SSRI/SNRI), including fluoxetine
- Melatonin
- Benzodiazepines when used to treat anxiety
- Aripiprazole
- Risperidone
- ADHD medications including psychostimulants like methylphenidate
- Hypnotics for insomnia, falling asleep problems (only on an as needed basis, limited to 3 times per week)
- Antiepileptic drugs (AED) used for treatment of epilepsy including valproic acid and oxcarbazepine may be used provided they have been given at stable dosage for at least 3 months prior to screening and were well tolerated
- Occasional use of paracetamol/acetaminophen
- Topical application of local anesthetic is permitted if necessary as pain relief for all blood samples, including hematology and PK

Any other medications and cases of polypharmacy require the approval from the investigator and the Medical Monitor or designee.

4.5.2 Non-Pharmacological Interventions

Non-pharmacological interventions must be stable for 4 weeks prior to screening and must remain stable throughout the ongoing study (e.g., subject psychotherapy, cognitive behavioral therapy, and rehabilitative therapy). Minor changes in ongoing treatment (e.g., missed therapy sessions due to holiday/vacation; planned break in therapy due to school holidays; changes in college/school programs) are not considered significant and need not to be discussed with the Sponsor (Medical Monitor or designee).

4.5.3 Prohibited Therapy

These therapies are not allowed during the study and must be stopped 2 weeks prior to screening to ensure a washout of at least 4 weeks prior to Baseline assessment or must be stopped earlier to ensure a washout of 5 times the medication half-life before randomization (whichever is longer).

Examples of prohibited medications:

- 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 (e.g. in the context of an Adverse Event), moderate inhibitors of CYP3A4 may be used after discussion with, and agreement by, the Sponsor (Medical Monitor or designee). The reasons for and approval of such use must be documented.
- Strong inducers of CYP3A4 (e.g., carbamazepine, phenytoin, St. John's Wort
- Quinidine
- Chronic adrenocorticoid or glucocorticoid use (use of inhaled or topical formulations are allowed)
- Oxytocin
- Agents inhibiting vasopressin receptors (e.g., tolvaptan, conivaptan)
- Desmopressin (DDAVP)
- Hematotoxic drugs requiring frequent hematological monitoring of white blood cells (e.g., clozapine)
- Neurotoxic drugs that per warning/precaution section of U.S. label are considered to have a risk of peripheral neuropathy
- Herbal therapies and dietary supplements (unless allowed by the medical monitor or designee)

4.5.4 Caution Regarding P-gp Substrates

Based on in vitro experiments, quinidine should not be given with balovaptan, but no systemic interactions are predicted with cetirizine, dabigatran etexilate, or digoxin. Caution is advised when using balovaptan together with other medications known to be clinically-relevant substrates of P-gp, in particular for those medications that have a narrow therapeutic window (e.g., loperamide). However, when such medications are dosed 5 or more hours after administration of balovaptan, the risk of pharmacokinetic interaction is predicted to be small.

4.6 STUDY ASSESSMENTS

4.6.1 Description of Study Assessments

All examinations listed below will be performed according to the schedule of assessments outlined in Appendix 1, Appendix 2, Appendix 9, and Appendix 10.

4.6.1.1 Medical History and Demographic Data

Medical history includes clinically significant diseases experienced up to Screening plus for the previous 5 years: developmental history, date of diagnosis with ASD, smoking history, use of alcohol and drugs of abuse, medical interventions (e.g., immunizations/vaccines and surgeries).

All medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the subject within 12 weeks prior to the screening visit as well as details of psychosocial/non-pharmacological interventions used over the past year are to be documented.

Demographic data will include age, sex, and self‑reported race/ethnicity as well as applicable information about residential setting, school or employment status, day care facilities, level of education, participation in educational or day programs, and any non-medical hospitalizations.

General information will also be collected on the caregiver who will oversee the subject's adherence with protocol-specified procedures and study medication, and report on the subject's status. This will include, for example, their relationship to the subject, time spent with the subject, and whether they live in the same residence.

4.6.1.2 Physical and Neurological Examinations

At the time-points listed in the Schedule of Assessments (Appendix 1, Appendix 2, Appendix 9, and Appendix 10) a complete physical examination will be performed at screening and Week 24 or early withdrawal visit and will include an evaluation of the head, eyes, ears, nose, throat, neck, and lymph nodes and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. At other clinic visits listed in Appendix 1, Appendix 2, Appendix 9, and Appendix 10 or as clinically indicated, limited, symptom-directed physical examinations should be performed. Changes from baseline abnormalities should be recorded in subject's notes. The physical exam will not include pelvic, rectal, or breast exams.

At all time-points listed in the Schedule of Assessments (Appendix 1, Appendix 2, Appendix 9, and Appendix 10), a complete and neurological examination including an examination for symptoms of peripheral neuropathy will be performed, see Appendix 5 and Section 5.2.3).

Any abnormality identified at screening should be recorded on the General Medical History and Baseline Conditions eCRF section and carefully evaluated in terms of eligibility of subject for enrollment into this study.

Body height and weight will be recorded as specified in Appendix 1, Appendix 2, Appendix 9, and Appendix 10. Body Mass Index (BMI) including age and sex specific BMI Percentiles relevant for inclusion will be derived at screening. These anthropometric parameters will be determined according to https://nccd.cdc.gov/dnpabmi/Calculator.aspx. Screenshots of this derivation will be kept in the source data.

Results from these examinations will be documented in the eCRF forms and new or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

[redacted]

4.6.1.3 Tanner Staging

Tanner stages will be determined in subjects aged at least 7 years, but not after menarche in female and not after voice break in male subjects, and based upon agreement/approval by the Principle Investigator, legally authorized representative, patient and IRB as outlined in Appendix 6 and at the time points specified in the Schedule of Assessments (Appendix 1, Appendix 2, Appendix 9, and Appendix 10).

4.6.1.4 Palatability Test

Palatability (taste and acceptability) testing of the dispersible tablet formulation of the study medication will include questions directed to the subjects to capture subjective experiences with the dispersible tablets. The input of the care-giver will also be sought to aid interpretation of the experience or reaction of the subjects.

Palatability assessments will be performed at the time points specified in the Schedule of Assessments (Appendix 1) after administration of study medication.

4.6.1.5 Vital Signs

Vital signs will include measurements of temperature, respiratory rate, pulse rate, and systolic and diastolic blood pressure after the subject has been in supine position for at least 5 minutes. Additionally, orthostatic function will be assessed post dose at selected visits comparing the blood pressure and the pulse rate after at least 5 minutes in supine position and again 3 minutes after having rapidly changed into standing position.

Vital signs will be recorded at the time points specified in the Schedule of Assessments, (Appendix 1, Appendix 2, Appendix 9, and Appendix 10).

When vital signs and a blood draw are scheduled at the same time, the vital signs should be measured prior to blood draw or at least 10 minutes after the last blood draw. When possible, the same arm should be used for all blood pressure measurements.

Blood pressure, pulse rate, and respiratory rate should be obtained in a quiet room at a comfortable temperature, with the subject's arm unconstrained by clothing or other material. All measurements will be obtained from the same arm and using a well-calibrated automatic instrument with a digital readout, throughout the study. Cuff size should be selected as appropriate for age/arm circumference thus that cuff bladder width

is about 40% and cuff bladder length is about 80% of arm circumference. As the subjects grow during study participation, the Investigator should check for appropriate cuff size according to this guide.

Temperature assessments (home): body temperature is to be measured using a tympanic thermometer in a closed room at room temperature. Body temperature should be recorded in a patient diary provided for that purpose.

4.6.1.6 Electrocardiograms

Triplicate ECG recordings (i.e., three useful ECGs without artifacts) will be obtained at the Baseline visit before first dosing with approximately 2–5 minutes between each recording. For the triplicate recordings at baseline, the average of the three readings will be used to determine ECG intervals (e.g., PR, QRS, QT).

Single ECG recording will be performed at screening and the other time-points listed in the Schedule of Assessments (Appendix 1, Appendix 2, Appendix 9, and Appendix 10).

To minimize variability, it is important that subjects be in a resting position for at least 5 minutes prior to each ECG evaluation. Body position should be consistently maintained for each ECG evaluation to prevent changes in heart rate. Environmental distractions (e.g., television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording. ECGs should be performed prior to any scheduled vital sign measurements and blood draws. In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality.

For safety monitoring purposes, the Investigator or designee must review, sign, and date all ECG tracings. Paper or electronic copies will be kept as part of the subject's permanent study file at the site. If considered appropriate by Roche, ECGs may be analyzed retrospectively at a central laboratory.

The recordings will be electronically transferred to a central ECG analysis vendor. The following parameters will be obtained from the digital recordings: heart rate, QRS duration, PQ (PR), RR and QT intervals (QTcB (Bazett's correction) and QTcF (Fridericia's correction)) as well as information on T-wave and U-wave and overall ECG interpretation.

4.6.1.7 Laboratory Assessments

Normal ranges for the study laboratory parameters, by age and sex where appropriate, must be supplied to the Sponsor before relevant assessments are completed. Laboratory safety tests shall be collected at time-points specified in the Schedule of Assessments (Appendix 1, Appendix 2, Appendix 9, and Appendix 10).

Additional blood or urine samples may be taken 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. Where the clinical significance of abnormal lab results is considered uncertain, screening lab 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 a medication or food containing for example codeine, benzodiazepines or opiates, the test could be repeated to confirm washout.

In the event of unexplained abnormal clinically significant laboratory test values, the tests should be repeated immediately and followed up 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 local or central laboratory, if applicable.

- Hematology: leukocytes, erythrocytes, hemoglobin, hematocrit, platelets, differential count [neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells]. Additional hematology monitoring will be performed at so called “home visits” (Appendix 1, Appendix 2, Appendix 9, Appendix 10, and Section 5.2.1).
- Serum chemistry:
  As specified in the schedule of assessments (see Appendix 1, Appendix 2, Appendix 9, and Appendix 10), different serum chemistry assessments will be performed at specific visits:
  - Full Serum chemistry panel: sodium, potassium, chloride, bicarbonate, glucose (fasting for at least 4 hours at screening only), urea, creatinine, phosphate, calcium, total and direct bilirubin, alkaline phosphatase, ALT, AST, protein, albumin, urate, LDH, CPK, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides.
    Additionally, HbA1c, TSH, and free T4 will be assessed at screening, Week 24/76 early withdrawal, and 6-week follow-up visits.
  - [redacted]
    - [redacted]
    - [redacted]
    - [redacted]
- Coagulation: INR, aPTT, PT

- Viral serology:
  - HIV (specific tests HIV-1 Antibody, HIV-1/2 Antibody, HIV-2 Antibody)
  - Hepatitis B surface antigen (HBsAg) *or hepatitis B cDNA*
  - Total hepatitis B core antibody (HBcAb)
    - *Any positive HBV serology will be followed by confirmatory assays.*
  - Hepatitis C virus (HCV) antibody
- Urinalysis, including dipstick: pH, specific gravity, glucose, protein, ketones, blood will be performed at the study center. If there is a clinically significant positive blood or protein result, urine 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, and PCP. Drug screen and alcohol test will be mandatory for adolescents at screening and may be performed in any subject at any visit at the discretion of the study physician.
- Pregnancy test: All females with menarche (including those who have had a tubal ligation) will have a urine pregnancy test at screening and specified sub-sequent visits.
- Unscheduled Visit: In the event that additional laboratory parameters are required to interpret any adverse events or abnormal hematology/chemistry, the following assessments may be included:
  - Hematology: leukocytes, erythrocytes, hemoglobin, hematocrit, platelets, differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells)
  - Full serum chemistry panel: sodium, potassium, chloride, bicarbonate, glucose (fasting for at least 4 hours at screening only), urea, creatinine, phosphate, calcium, total and direct bilirubin, alkaline phosphatase, ALT, AST, protein, albumin, urate, LDH, CPK, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides

[redacted]

The procedures for the collection, handling, and shipping of samples will be provided in the Central Laboratory Manual.

4.6.1.8 Pharmacokinetic Assessments

Blood samples for pharmacokinetic determination of plasma concentrations of balovaptan and its metabolite M3 and M2 (as applicable) (and other metabolite(s) as relevant) will be collected as specified in the Schedule of Assessments and the PK sampling schedule detailed below by qualified personal. Samples scheduled at least 4 hours post dose on a clinic visit day can be taken by a nurse at home.

Topical application of local anesthetic is permitted if necessary. See also lab manual for further details.

PK sampling for first cohorts of 24 adolescents and 30 children

Two first cohorts of 24 adolescents and approximately 30 children will undergo over the first 4 weeks a more intense PK sampling until the IMC and SOC confirms the final doses for each age group (see also Appendix 2 and see Section 3.1.3.1):

- Week 1, one sample: pre-dose at home together with
- Week 2, four samples: pre-dose, 2 (± 0.5) hours post-dose, 4 (± 0.5) hours post-dose, and at least 6 hours post-dose; PK samples need to be at least 2 hours apart.
- Week 4, one sample: pre-dose at home
- End of Treatment visit (if needed), one sample anytime during the visit

PK sampling for second cohorts of adolescents and children after confirmation of final doses by IMC and SOC:

All subjects in this study will follow at least a minimal PK sampling scheme (to obtain adequate estimates of key PK parameters, i.e., apparent clearance and volume of distribution). PK samples will be taken from all subjects on the following visits (see also Appendix 1, Appendix 9, and Appendix 10):

- Week 8, 1 sample: post-dose in the evening at home together with safety labs (the dose will be taken in the morning)
- Week 12 and 24, three samples: pre-dose, 2 hours post-dose, and at the end of the clinic visit but at least 4 hour post-dose
- Early withdrawal visit: one sample anytime during the visit

[redacted]

The actual time of the PK blood sampling, together with the exact time for dosing on day before and on day of PK sampling, needs to be precisely entered into the corresponding eCRF section.

Plasma concentrations of balovaptan and its metabolite M3 and M2 (as applicable) will be measured by a specific and validated LC-MS/MS method. Other balovaptan-derived metabolites in plasma may be investigated as appropriate by an exploratory method.

PK samples will be destroyed no later than 2 years after the date of the final clinical study report.

4.6.1.9 Clinical Genotyping Samples

A mandatory whole blood sample will be taken for DNA extraction from every subject on Day 1. The DNA will be used to evaluate if genetic variants in the AVPR1A gene affect the efficacy or safety of balovaptan. The sample may be used also to assess if genetic variants (e.g., in CYP3A4 or CYP2D6) can be related to the PK and safety of balovaptan. Data arising from all biosamples including samples for analyses of inherited DNA will be subject to the confidentiality standards described in Section 8.4. CG samples and derived analytical materials will be destroyed no later than 2 years after the date of the final clinical study report.

4.6.1.10 Disease-Specific Assessments

Caregivers and clinicians will use an electronic device to capture disease specific questionnaires. The data will be transmitted electronically to a centralized database at the electronic PRO (ePRO) vendor. 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 and the caregiver should be requested to complete any blank items. Changes to the form should not be made once the caregiver has left the site for that visit.

4.6.1.10.1 Requirements for Caregivers

All disease specific assessments will take place on-site at the clinic. The same caregiver will provide feedback on all informant based assessments throughout the study *as established at baseline*. If a caregiver visit cannot be completed as arranged—e.g., the caregiver is delayed in transit—visits should be rescheduled as soon as possible after the original appointment. Caregiver completed assessments cannot be conducted over the telephone. Caregiver initials will be reported on eCRF at all assessments.

The reliable caregiver or parent must live with the subject or have substantial and sufficient periods of contact with the subject and is willing and able to attend the on-site visits when required. The reliable caregiver or parent must oversee the subject's adherence with protocol-specified procedures and study medication dosing, and report on the subject's status via completion of study assessments.

If the caregiver is not living with the subject, the Investigator has to be satisfied that the subject can contact the caregiver readily during the times when the caregiver is not with the subject. If in doubt about whether a subject's care arrangements are suitable for

inclusion, the Investigator should discuss this with Sponsor (Medical Monitor or designee). A non-cohabitating caregiver must spend sufficient time with the subject so that, in the opinion of the Investigator, the caregiver can reliably assess the subject's mental status, activities and behavior, and report on the subject's adherence and health. This would normally be possible when the caregiver spends a few hours each day with the subject.

4.6.1.10.2 Wechsler Abbreviated Scale of Intelligence Scale–Second Edition (WASI-II)

The WASI-II is a tool used to evaluate an individual's cognitive functioning and generate IQ scores [29] and will be administered by a certified rater at screening only.

For subjects older than 7:7 years the WASI-II will be the only choice at screening. For children from 6 to 7:7 years the WASI-II will be the first choice to determine eligibility at screening and for children aged 5 years only the WPPSI-IV can be used (see Section 4.6.1.10.3).

If the WASI or an equivalent assessment (e.g., the Wechsler Intelligence Scale for Children (WISC) or the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) has been performed by a certified rater and documented within 12 months of the screening visit, there will be no requirement to repeat it.

The assessment will take approximately 30 minutes to complete.

4.6.1.10.3 Wechsler Preschool and Primary Scale of Intelligence–Fourth Edition (WPPSI-IV)

The WPPSI-IV is a tool used to evaluate cognitive functioning and generate IQ scores in preschoolers and young children [30] and will be administered by a certified rater at screening only.

If the WPPSI has been performed by a certified rater and documented within 12 months of the screening visit, there will be no requirement to repeat it.

The assessment will take approximately 45-60 minutes to complete.

4.6.1.10.4 Autism Diagnostic Observation Schedule (ADOS-2)

The ADOS second edition is a diagnostic tool used to document the presence of ASD [31, 32]. 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 includes four modules for use with different age groups and language levels; module 2, 3, and 4 will be used in this study. The appropriate module of the ADOS will be administered by a certified rater at screening.

If the ADOS assessment has been performed by a certified rater and documented within 12 months of the screening visit, there will be no requirement to repeat it.

The assessment will take approximately 35 to 40 minutes to complete.

4.6.1.10.5 Social Responsiveness Scale Second Edition (SRS-2)

The SRS-2 is a 65-item informant-based rating scale designed specifically for use in ASD to quantitatively measure an individual's ability to engage in emotionally appropriate reciprocal social behavior in a naturalistic social setting [35]. Each item on the scale inquires about 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.

The reliable caregiver will complete the rating scale at screening. A clinician will be available to assist the caregiver during completion of the questionnaire.

This assessment will take approximately 15-20 minutes.

4.6.1.10.6 Vineland-II Adaptive Behavior Scales, Second Edition (Vineland™-II)

The Vineland™-II is an instrument that measures communication, daily living skills, socialization, motor skills (only in children up to 6 years) and maladaptive (not assessed in this study) behavior of individuals with developmental disabilities [33]. The Survey Interview Form (i.e., semi -structured interview) will be administered to a subject's reliable caregiver in this study, during which the rater or clinician will ask to the caregiver open ended questions relating to the subject's activities and behavior. Domain scores will be obtained for the individual domains of Socialization, Communication, Daily Living Skills, and motor skills (up to 6 years only) and used to calculate the Vineland™-II Adaptive Behavior Composite score. Standardized scores on the Adaptive behavior composite range from 20-160 with higher scores indicating better functioning.

The same rater or clinician will perform the Vineland™-II together with the same caregiver throughout the study as established at baseline visit. The initials from caregiver and rater will be reported on eCRF at all assessments.

This interview will take approximately 45-60 minutes to complete. At a clinic visit the Vineland™-II should be performed as the first of all informant based assessments with the caregiver (see Section 4.6.2.2).

4.6.1.10.7 Aberrant Behavior Checklist (ABC)

The ABC is an informant-based questionnaire consisting of 58 items subdivided amongst 5 scales: irritability, lethargy and social withdrawal, stereotypic behavior, hyperactivity/non-compliance, and inappropriate speech [34]. A score for each item

ranges from 0 indicating “no problem” to 3 indicating “severe problem”. Scale scores are calculated by summing the items within that scale. Higher scores indicate greater impairment.

The reliable caregiver identified upon subject’s screening visit will complete the rating scale. A clinician will be available to assist the caregiver during completion of the questionnaire.

This assessment will take approximately 10-20 minutes to complete.

4.6.1.10.8 Ohio Autism Clinical Impressions—Severity (OACIS-S) and Improvement (OACIS-I)

The OACIS-S and -I are 10-item, clinician-completed measures based upon interview and/or observation [38].

The OACIS-S and OACIS-I assess severity and improvement, respectively, in social interaction, aberrant behavior, repetitive or ritualistic behavior, verbal communication, nonverbal communication skills, hyperactivity and inattention, anxiety and fearfulness, sensory sensitivities, restricted and narrow interests, and a global rating of autism.

Each item of the OACIS-S is rated on a 7-point scale ranging from no symptoms (1) to very severe symptoms (7). The OACIS-I is used to assess the clinical change as compared to symptoms at baseline using a 7-point scale, ranging from very much improved (1) to very much worse (7).

4.6.1.10.9 Clinical Global Impression—Severity (CGI-S) and Improvement (CGI-I)

The CGI rating scales are tools used to evaluate both the severity of illness and change from baseline [39]. The CGI-S reflects the rater’s impression of the subject’s current autism severity on a 7-point scale ranging from no symptoms (1) to very severe symptoms (7). The CGI-I is used to assess the clinical change as compared to symptoms at baseline using a 7-point scale, ranging from very much improved (1) to very much worse (7). For this study modified versions will be used [40, 41].

Whenever possible, for any individual, these assessments should be done by the same rater throughout the study.

4.6.1.10.10 Caregiver-reported Global Impression (CaGI)

The Caregiver Global Impression (CaGI) is a four item informant-based questionnaire assessing the caregiver's impression about changes in subject’s communications skills, social skills, daily living skills and overall symptoms associated with autism as compared to Baseline. The CaGI items utilize a 7-point scale, ranging from very much improved (1) to very much worse (7) to report the changes as compared to Baseline. For this study an adapted version for Autism will be used (see Appendix 4 and [42]).

This scale will take up to 5 minutes to complete.

4.6.1.10.11 Repetitive Behavior Scale—Revised (RBS-R)

The RBS-R is a 43-item informant-based questionnaire assessing the variety of restricted and repetitive behaviors observed in individuals with ASD [43]. The scale is grouped into six subscales: Stereotyped, Self-injurious, Compulsive, Ritualistic, Sameness, and Restricted Behaviors.

This scale will take approximately 20–30 minutes to complete.

4.6.1.10.12 PedsQL Family Impact Scale

The Pediatric Quality of Life Inventory (PedsQL™) Family Impact Module version 2 [44] is a 36-item questionnaire which is completed by the caregiver and was developed to measure parent and family functioning. It encompasses six scales covering 1) Physical Functioning (6 items), 2) Emotional Functioning (5 items), 3) Social Functioning (4 items), 4) Cognitive Functioning (5 items), 5) Communication (3 items), 6) Worry (5 items), and 2 scales measuring parent reported family functioning; 7) Daily Activities (3 items) and 8) Family Relationships (5 items). For each item a 5-point response scale is utilized (0 = never a problem; 4 = always a problem). Items are then reverse-scored and linearly transformed to a 0–100 scale (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0), so that higher scores indicate better functioning (less negative impact).

Scale Scores are computed as the sum of the items divided by the number of items answered (this accounts for missing data). If more than 50% of the items in the scale are missing, the Scale Score is not computed. A Total Score, a Parent HRQOL Summary Score, and a Family Summary Score can also be computed by averaging across the relevant domains (Total Score by averaging across all items, Parent HRQOL Summary Score by averaging the 20 items in Physical, Emotional, Social, and Cognitive Functioning, and the Family Summary Score by averaging 8 items in Daily Activities and Family Relationships). The acute form with a recall period of 7 days will be employed in this trial.

[redacted]

[redacted]

[redacted]

4.6.1.11 Patient-Reported Outcomes

Subjects will use an electronic device to capture PRO questionnaire data. The data will be transmitted electronically to a centralized database at the electronic PRO (ePRO) vendor. 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 and the patient should be requested to complete any blank items. Changes to the form should not be made once the subject has left the site for that visit.

4.6.1.11.1 Pediatric Quality of Life Inventory (PedsQL™) Generic Core & Cognitive Functioning Scales

The Pediatric Quality of Life Inventory PedsQL™4.0 Generic Core Scale assessment consists of a 23 item questionnaire encompassing 4 core scale domains: Physical Functioning (8 items); Emotional Functioning (5 items); Social Functioning (5 items); and School Functioning (5 items). Additionally, the PedsQL Cognitive Functioning Scale which contains 6 items will also be completed. The acute self-completed forms with a recall period of 7 days will be employed in this trial [44]. Different versions exist for 5–7, 8–12, and 13–18 year age ranges.

For children aged 8 years and above, the PedsQL items are scored on a 5 point Likert-type response scale (0 = never a problem; 1 = almost never a problem; 2 = sometimes a problem; 3 = often a problem; and 4 = almost always a problem). For children aged 5-7 years, scoring is based on a three-point scale (0 = Not at all, 2 = Sometimes, 4 = A lot). Once scored, items will be reverse scored and linearly transformed to a 0-100 scale (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0), so that higher scores indicate better health-related quality of life.

For children aged 5–7 years, an administrator will read out the questions and the child will respond by pointing to one of three smiley faces. Children aged 8 years and above complete the questionnaire themselves. Subjects should complete the same version of the questionnaire throughout the study even if they have moved up an age group.

After completion of the PedsQL at Week 24, Week 76, or Early Withdrawal visit (see Schedule of Assessments, Appendix 1 or Appendix 10), subjects will be asked up to two additional open-ended questions on their perception of change from baseline. [redacted]

The questions are as follows:

- "Have you noticed any changes since starting the study medication?"
- If yes, “Can you tell me about these changes?”

This information will be recorded verbatim from the subject on an electronic device and transcribed.

4.6.1.12 Columbia Suicide Severity Rating Scale (C-SSRS)

The assessment for suicidality in clinical trials is a requirement for CNS active molecules requested by Health Authorities.

The C-SSRS (http://www.cssrs.columbia.edu) is a clinician-rated tool recommended by Health Authorities including the FDA to assess previous suicidality of a subject at screening (C-SSRS screening to be used at screening) as well as any new instances of suicidality during the clinical study (C-SSRS since last visit, 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.

Age specific versions of the C-SSRS will be used.

The pediatric version will be used for children 5–11 years old. After completion of the pediatric questionnaire, the Investigator will ask both the caregiver and the child the following additional questions relating to the areas of suicidal ideation, suicidal behavior, and self-injurious behavior:

- Suicidal Ideation:
  Has the child wished he/she were dead or wished they could go to sleep and not wake up?
- Suicidal Behavior:
  Has the child made a suicide attempt?
  Has he or she done anything to harm him or herself?
- Self-injurious Behavior:
  Has the child engaged in nonsuicidal self-injurious behavior?

If the Investigator concludes on a suicidality risk of the subject, the Investigator must take care for further evaluation of the risk which may involve local experts in the field of suicidality.

4.6.1.13 Samples for Research Biosample Repository

Overview of the Research Biosample Repository

The Roche Research Biosample Repository is a centrally administered group of facilities 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 these 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 will be collected from subjects who give specific consent to participate in this optional Research Biosample Repository. Collected 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

These samples may be used to explore the associations of variants of genes implicated in susceptibility and pathogenesis of ASD. The pharmacogenetic information gathered through the analysis of specimens in the Research Biosample Repository is hoped to improve subject's outcomes by predicting which subjects are more likely to respond to specific drug therapies, predicting which subjects are susceptible to developing adverse side-effects, and/or predicting which subjects are likely to progress to more severe disease states.

The results of specimen analysis from the Research Biosample Repository may 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.

Approval by the Institutional Review Board or Ethics Committee

Sampling for the Research Biosample Repository is contingent upon the review and approval of the exploratory research and the Research Biosample Repository portion of the Informed Consent and assent 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 Research Biosample Repository sampling, this section of the protocol will not be applicable at that site.

Sample Collection

One optional sample for DNA extraction will be collected per Appendix 1 and Appendix 2, 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 used for whole genome sequencing (WGS) and other genetic analysis and may be sent to one or more laboratories for analysis.

Genomics is increasingly informing researcher's understanding of disease pathobiology. WGS provides a comprehensive characterization of the genome 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 all samples, dates of consent and specimen collection should be recorded on the associated Research Biosample Repository page of the eCRF. For sampling procedures, storage conditions, and shipment instructions, see the separate laboratory manual.

Research Biosample Repository specimens will be stored and used until no longer needed or until they are exhausted. The Research Biosample Repository storage period will be in accordance with the IRB/EC-approved Informed Consent Form and applicable laws (e.g., Health Authority requirements).

The repository specimens will be subject to the confidentiality standards described in Section 8.4.

Confidentiality

Data generated from Research Biosample Repository specimens must be available for inspection upon request by representatives of national and local Health Authorities, and Roche monitors, representatives, and collaborators, as appropriate.

Subject medical information associated with Research Biosample Repository specimens is confidential and may only be disclosed to third parties 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.

Data derived from Research Biosample Repository specimen analysis on individual subjects will generally not be provided to study Investigators unless a request for research use is granted. The aggregate results of any conducted research will be available in accordance with the effective Roche policy on study data publication.

Genetic research data and associated clinical data may be shared with researchers who are not participating in the study or submitted to government or other health research databases for broad sharing with other researchers. Patients will not be identified by name or any other personally identifying information. Given the complexity and exploratory nature of these analyses, genetic data and analyses will not be shared with Investigators or patients unless required by law.

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

Consent to Participate in the Research Biosample Repository

The Informed Consent and assent Form will contain a separate section that addresses participation in the Research Biosample Repository. The Investigator or authorized designee will explain to each subject the objectives, methods, and potential hazards of participation in the Research Biosample Repository. Subject will be told that they are free to refuse to participate and may withdraw their specimens 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 Research Biosample Repository 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 by completing the Research Biosample Repository Sample Informed Consent eCRF.

In the event of death or loss of competence of a subject who is participating in the research, the participant's specimens and data will continue to be used as part of the Research Biosample Repository research.

Withdrawal from the Research Biosample Repository

Subjects who give consent to provide specimens for the Research Biosample Repository have the right to withdraw their specimens at any time for any reason. If a subject wishes to withdraw consent to the testing of his or her specimens, the Investigator must inform the Monitor in writing of the subject's wishes using the Research Biosample Repository Withdrawal Form and, if the trial is ongoing, must enter the date of withdrawal on the Research Biosample Repository Withdrawal of Informed Consent eCRF. The subject will be provided with instructions on how to withdraw consent after the trial is closed.
A Study Subject's withdrawal from Study BP30153 does not, by itself, constitute withdrawal of specimens from the Research Biosample Repository. Likewise, a Study Subject's withdrawal from the Research Biosample Repository does not constitute withdrawal from Study BP30153. Data already generated before time of withdrawal of consent to Research Biosample Repository will still be used.

Monitoring and Oversight

Specimens collected for the Research Biosample Repository 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. Roche monitors and auditors will have direct access to appropriate parts of records relating to Study Subject participation in the Research Biosample Repository for the purposes of verifying the data provided to Roche. 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 samples.

4.6.2 Timing of Study Assessments

The Schedules of Assessments can be found in Appendix 1, Appendix 2, Appendix 9, and Appendix 10.

The first cohorts of 24 adolescents and up to approximately 30 children will follow at study start a specific Schedule of Assessments (see Appendix 2) with additional assessments until the IMC and SOC determines the final doses for each age group (see protocol Section 3.1.3.1). After determination of the final doses for the cohort these subjects will continue following the Schedule of Assessments for the entire study (Appendix 1). If the dose is judged acceptable on an individual level, the subject may continue on that dose and follow the Schedule of Assessments for the entire study. During the early interim Pharmacokinetic and safety reviews the IMC and SOC may decide to stop treatment (see Section 3.1.3.1).

If necessary, site visits (Week 6, Week 12, Week 18, Week 24, Week 48, Week 64, Week 76, Early Withdrawal Visit, and Follow-Up Visit) can be performed over two consecutive days. Assessments at screening can be performed at any time during the screening period. Baseline assessments can be performed up to 1 week prior to first dose (see Appendix 1, Appendix 2, Appendix 9, and Appendix 10).

4.6.2.1 Screening and Pretreatment Assessments

Written informed consent and Assent for participation in the study must be obtained before performing any study-specific screening tests or evaluations. Informed Consent Forms and Assent Forms for enrolled subjects and for subjects who are not subsequently enrolled will be maintained at the study site.

All screening and pre-treatment assessments must be completed and reviewed to confirm that subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure.

An Eligibility Screening Form (ESF) documenting the Investigator's assessment of each screened subject with regard to the protocol's inclusion and exclusion criteria is to be completed by the Investigator and kept at the investigational site.

Abbreviated re-screening (written informed consent, medical history, physical examination, substance use, alcohol test, fasted laboratory safety, coagulation, serology, urinalysis, inclusion/exclusion criteria) may be allowed under circumstances where the subject is screen-passed but could not be randomized within the 4-week screening window due to a study halt, logistical, personal, or technical reasons. At no time should the duration between the original screening visit and the abbreviated re-screening visit exceed 3 months. Abbreviated re-screening will only be permitted in cases where this poses no safety risk to the subject.

Screen-failed subjects can be re-screened if there is a substantial change in the subject's general condition (e.g., prohibited medication was stopped or weight loss) and if recruitment for the study is still ongoing. Re-screening will not be allowed if the subject failed earlier to meet the disease specific inclusion criteria (e.g., SRS-2 T-score).

Safety laboratory tests which would exclude the subject at screening may be repeated once (as unscheduled labs) if it is suspected that the abnormal result is transient and likely to be normal at repeat.

Subjects from first adolescent and first children cohorts, who stopped treatment in the context of the interim safety and PK decisions, (see Section 3.1.3) will be allowed to re-start in the same treatment arm, according to their original randomization, for 24 weeks in the main study, provided the Investigator can exclude relevant changes in the subject's general condition (see Section 4.2.1). These subjects will have a new, abbreviated Baseline visit, and will continue with all subsequent visits according to the schedule of assessments until completion of their time on the study (Appendix 1).

In addition, re-starting subjects will be assigned a new subject number. Some data from their original screening visit will be required to be re-entered in the eCRF under the new subject number.

A screening examination will be performed approximately 4 weeks (at least 3 weeks and up to 4 weeks) before the first drug administration (Day 1) per the Schedule of Assessments, (see Appendix 1). Subjects must fulfill all entry criteria to be accepted into the study.

For subjects enrolling in first adolescent/first children cohorts (see Section 3.1 and Section 3.1.3.1), a screening examination will be performed approximately 4 weeks (at least 1 week and up to 4 weeks) before the first drug administration (Day 1) per the Schedule of Assessments (Appendix 2).

The Investigator, or designee, will use the eCRF with the assigned subject number and enter the corresponding number for allocation to the treatment group in the appropriate place on each subject's eCRF.

The randomization numbers are to be allocated sequentially in the order in which the subjects are enrolled. A Subject Enrollment and Identification Code List must be maintained by the Investigator.

4.6.2.2 Assessments during Treatment

All assessments must be performed as per Schedule of assessments (see Appendix 1, Appendix 2, Appendix 9, and Appendix 10).

Subjects will arrive at each study visit without having taken their daily dose of study medication. Following collection of the pre-dose PK blood sample, and the safety labs

subjects will take their next dose of study medication. Site staff will record the actual date and time of the dose intake. The last dose of study medication will be administered at the Week 76 visit.

When blood samples are scheduled for the same nominal time as other assessments the following order should be followed:

- 12-lead ECGs
- Vital signs
- Blood samples

As a general rule, the test sequence for all disease specific assessments should remain the same for a given subject as established at screening and Baseline. Additionally, testing should occur around the same time of the day at each visit.

For the informant based assessments the following order will be followed:

- Always the Vineland™-II will be the first informant based assessment with the caregiver.
- For all other informant based assessments there is no restriction in the order, however a sequence established at screening and Baseline should remain the same for a given subject.

The patient reported outcome measures can be performed in parallel with the informant based assessments. The following sequence is proposed: first the C-SSRS followed by the PedsQL. Ideally these assessments should be performed before administration of study medication or within 1 hour after administration.

The remaining clinician-completed measures will be completed after all other disease specific assessment to minimize any possible bias on caregiver reported assessments.

4.6.2.3 Assessments at Study Completion/Early Withdrawal Visit

Subjects who complete the study treatment period (defined as completion of 24 weeks of study treatment) or discontinue from the study early, will perform with the caregiver all assessments for the Week 24 or the Early Withdrawal visit with the caregiver, as per the schedule of assessments (see Appendix 1 and Appendix 10).

4.6.2.4 Follow-Up Assessments

As per the schedule of assessments, all subjects will have safety hematology/CPK blood samples taken 2 weeks after last dose and a clinic visit 6 weeks after last dose (see Appendix 1 or Appendix 10). After the study completion/Early Withdrawal visit, adverse events should be followed as outlined in Sections 5.5 and 5.6.

4.7 SUBJECT, STUDY, AND SITE DISCONTINUATION

4.7.1 Subject Discontinuation

The Investigator has the right to discontinue a subject from balovaptan or withdraw a subject from the study at any time. In addition, subjects have the right to voluntarily discontinue study drug or withdraw from the study at any time for any reason. Reasons for discontinuation of study drug or withdrawal from the study may include, but are not limited to, the following:

- Subject withdrawal of consent at any time
- Any medical condition that the Investigator or Sponsor determines may jeopardize the subject's safety if he or she continues in the study
- Investigator or Sponsor determines it is in the best interest of the subject
- Subject non-compliance

4.7.1.1 Discontinuation from Study Drug

The Investigator must diligently review incoming safety data. In particular hematological or neurological abnormalities should be considered as a potential reason to withdraw a patient from further trial participation.

Subjects must be discontinued from study drug in case of:

[redacted]

- Subject unable to continue to comply with study requirements

Subjects who discontinue study drug prematurely will be asked to return to the clinic for a study completion/Early Withdrawal visit as soon as possible (see Section 4.6.2.3) including detailed safety and efficacy assessments (see Schedule of Assessments, Appendix 1 and Appendix 2) and may undergo follow-up assessments (see Section 4.6.2.4). The primary reason for premature study drug discontinuation should be documented on the appropriate eCRF.

4.7.1.2 Withdrawal from Study

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 5.1.3 documented on the appropriate eCRF.

Subjects will not be followed for any reason after full consent has been withdrawn.

When a subject voluntarily withdraws from the study, or is withdrawn by the Investigator, samples collected until the date of withdrawal will be analyzed, unless the subject specifically requests for these to be discarded, or local laws require their immediate

destruction. A subject's withdrawal from Study BP30153 does not, by itself, constitute withdrawal of specimens donated to the Research Biosample Repository.

4.7.2 Study and Site 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
- Three or more subjects (delta between active and placebo arm) are pre-maturely withdrawn from the study because of serious adverse events or adverse events of special interest that are of similar nature and considered related to the intake of balovaptan (see also Appendix 3).

The Sponsor will notify the Investigator and Health Authorities if the study is placed on hold, or if the Sponsor decides to discontinue the study or development program.

The Sponsor has the right to close or replace a site at any time. Reasons for replacing or 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

5. ASSESSMENT OF SAFETY

5.1 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording adverse events, including serious adverse events, and non-serious adverse events of special interest; measurement of protocol‑specified safety laboratory assessments; measurement of protocol-specified vital signs, ECGs; and 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.

5.1.1 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), except as described in Section 5.3.5.8.
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline.
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug.
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies).

5.1.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

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

- Fatal (i.e., the adverse event actually causes or leads to death)
- 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)
- Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug
- 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 (rated as mild, moderate, or severe (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).

5.1.3 Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

Non-serious 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 include the following:

[redacted]

- Cases of an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined in 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 patient exposed to a medicinal product.
    This term applies only when a contamination of the study drug is suspected.

5.2 SAFETY PLAN

[redacted]

[redacted]

[redacted]

5.2.2 Blood Pressure and ECG Monitoring

While the overview of available respective clinical experience does not indicate an effect of balovaptan on heart rate and blood pressure, hypothetically antagonism of V1a could result in a decrease in blood pressure and impairment of orthostatic function.

- Blood pressure and pulse rate in supine position and in context of orthostatic challenge testing will be monitored in this study in pediatric patients.

As a standard policy of clinical trials sponsored by Roche, and not because of any specific concern of balovaptan, the following stopping rule is implemented with respect to QTc:

- A confirmed value for QTcF above 500 ms or a change from baseline by more than 60 ms will result in drug discontinuation (to confirm the initial abnormal value the ECG should be repeated within 30 minutes).

[redacted]

[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]

5.2.6 Tanner Staging

Animal studies do not point to any adverse effects of balovaptan with respect to sexual maturation. As a matter of routine monitoring, Tanner stages will be determined in subjects aged at least 7 years, but no longer after menarche in female and no longer after voice break in male subjects, and based upon agreement/approval by the site Principle Investigator, legally authorized representative, patient, and IRB as outlined in Appendix 6.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The Investigator is responsible for ensuring that all adverse events (see Section 5.1.1 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 to 5.6. For each adverse event recorded on the Adverse Event eCRF, the Investigator will make an assessment of seriousness (see Section 5.1.2 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. Adverse events will then be reported on the Adverse Event eCRF as follows:

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 (e.g., serious adverse events related to invasive procedures such as biopsies). Any other adverse event should not be reported.

After initiation of study drug, all adverse events, regardless of 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 time-points. 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 5.1.2).

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 or not 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, considering especially the effects of dose reduction, discontinuation of study drug, or re-introduction of study drug (where 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 subject 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 Occurring Secondary to Other Events

In general, adverse events occurring 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. However, medically significant adverse events occurring secondary to an initiating event that are separated in time should be recorded as independent events 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 gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and subsequent fracture, all three 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 time-points. Such events should only be recorded once on the Adverse Event eCRF. The initial severity of the event should be recorded, and the severity should be updated to reflect the most extreme severity any time the event worsens. If the event becomes serious, the Adverse Event eCRF should be updated to reflect this.

A recurrent adverse event is one that resolves between subject evaluation time-points and subsequently recurs. Each recurrence of an adverse event should be recorded separately 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 should be reported as an adverse event if it meets any of the following criteria:

- 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
- 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., alkaline phosphatase and bilirubin 5 times the upper limit of normal (ULN) associated with cholecystitis), only the diagnosis (i.e., cholecystitis) 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 if 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 not be repeatedly recorded on the Adverse Event eCRF, unless the etiology changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.5.5 Abnormal Vital Sign Values

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

- 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
- 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 not be repeatedly recorded on the Adverse Event eCRF, unless the etiology changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.5.6 Abnormal Liver Function Tests

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

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

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

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 Hospitalization or Prolonged Hospitalization

Any adverse event that results in hospitalization (i.e., in-patient 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 5.3.5.9), 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 suffered an adverse event
- Prolonged hospitalization due to lack of home care facilities, caregiver issues, transport issues, etc.

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

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

5.3.5.10 Patient-Reported Outcome Data

Adverse event reports will not be derived from PRO data by the Sponsor, and safety analyses will not be performed using PRO data. Sites are not expected to review the PRO 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 5.1.2; see Section 5.4.2 for details on reporting requirements)

- Non-serious adverse events of special interest (defined in Section 5.1.3; see Section 5.4.2 for details on reporting requirements)
- Pregnancies (see Section 5.4.3)
- Overdoses, medication errors, drug abuse, or drug misuse (see 5.4.4 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], *Ph.D.* (Primary)
Telephone No.:	[redacted]
Mobile Telephone No.:	[redacted]
Medical Monitor:	[redacted], *M.D.* (Secondary)
Telephone No.:	[redacted]
Mobile Telephone No.:	[redacted]

To ensure the safety of study patients, 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 Non-Serious Adverse Events of Special Interest

For reports of serious adverse events and non-serious adverse events of special interest (see Sections 5.1.2 and 5.1.3), Investigators should record all case details that can be

gathered on the Serious Adverse Reporting Form and forward this form to the SAE Responsible within 24 hours.

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Subjects

Female subjects of childbearing potential will be instructed to immediately inform the Investigator if they become pregnant during the study or within 90 days after the last dose of study drug. A Clinical Trial Pregnancy Reporting Form should be completed by the Investigator and submitted to the Sponsor within 24 hours after learning of the pregnancy. Pregnancy should not be recorded on the Adverse Event eCRF. The Investigator should discontinue study drug and counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

5.4.3.2 Pregnancies in Female Partners of Male Subjects

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

5.4.3.3 Abortions

Any spontaneous abortion in a pregnant female subject or a female partner of a male subject should be classified as a serious adverse event (as the Sponsor considers spontaneous abortions to be medically significant events), 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).

5.4.3.4 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).

5.4.4 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 patient, drug misuse could involve the drug being administered to someone other than the patient.

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 and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event). Special situations should be recorded as described below:

- Accidental overdose: Enter the drug name and "accidental overdose" as the event term. Check the "Accidental overdose" and "Medication error" boxes.
- 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 patient: Enter the drug name and "patient supplied drug to third party" as the event term. Check the "Drug misuse" box.

For balovaptan, 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). For balovaptan, 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.
- 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.

5.5 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 patient

is lost to follow-up, or the patient 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 patient'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, non-serious adverse events of special interest, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, 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 POST-STUDY ADVERSE EVENTS

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 death or any other serious adverse event occurring 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 Serious Adverse Event/Adverse Event of Special Interest Reporting Form using the fax number or email address provided to Investigators (see "Protocol Administrative and Contact Information & List of 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 non-serious adverse events of special interest against cumulative product experience to identify and expeditiously communicate possible new safety findings to Investigators, IRBs, and applicable health authorities based on applicable legislation.

To determine reporting requirements for single adverse event cases, the Sponsor will assess the expectedness of these events to balovaptan, which is the only IMP studied in this trial, using the following reference document as "Reference Safety Information":

- Section 6.4 of the IB. [45]

Independent from the causality assessment, the following SAEs and non-serious AESIs will be reported to FDA on an expedited basis:

Death, arrhythmia, syncope, dyspnea, palpitations, and chest pain

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.

The IMC and the independent SOC will monitor the incidence of these expected 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

6.1 DETERMINATION OF SAMPLE SIZE

Prior to Version 6 of the protocol, a sample size of 240 ASD patients (80 per treatment arm) providing evaluable data at Week 24 ensure*d* the study 80% power to detect as statistically significant, at 1-sided 5% significance level, a difference between each active dose and placebo with an effect size of at least 0.4. No adjustment for multiple doses *was* performed. Considering a withdrawal rate of around 15-20%, it *was* planned to recruit approximately 300 subjects overall.

In accordance to Version 6 of the protocol, 80 subjects per treatment arm (balovaptan 10 mg eq and placebo) will be required, for a total sample size of approximately 160 subjects with ASD with evaluable data at Week 24. To maintain the number of evaluable subjects, the sample size will be increase to approximately 340 subjects overall.

6.2 SUMMARIES OF CONDUCT OF STUDY

Inclusion/exclusion criteria violations and the use of prohibited co-medication during the study will be summarized descriptively to check the quality and integrity of the conduct of the study.

6.3 ANALYSIS POPULATIONS

6.3.1 Safety Analysis Population

All subjects who have received at least one dose of the study medication, whether prematurely withdrawn from the study or not, will be included in the safety analysis population. Data will be analyzed according to the treatment as randomized. Any dosing error will be reviewed and the implications of such errors on safety interpretation will be assessed.

6.3.2 Pharmacokinetic Analysis Population

All subjects providing pharmacokinetic data will be included in the PK Population. Subjects will be excluded if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol or if data are unavailable or incomplete which may influence the pharmacokinetic analysis. Excluded cases will be documented together with the reason for exclusion.

6.3.3 Efficacy Analysis Population

6.3.3.1 *For All Subjects Enrolled prior to Version 6 of the Protocol:*

Modified Intent-To-Treat Population

The modified intent-to-treat (mITT) population is defined as consisting of all subjects who give informed consent, are randomized, received at least one dose of double-blind medication, and for whom a baseline and at least one post-dose assessment is available. Subjects in first adolescent/first children cohorts with dose adjustments, or who were on a different dose than the final dose for their age group, will be excluded from the primary efficacy analysis, and will not contribute to the approximately *160* evaluable subjects expected in the mITT population (see Section 4.2.1). Data will be analyzed according to the treatment as randomized.

The mITT population is the primary population of interest.

Per-Protocol Population

The Per-Protocol (PP) population will be precisely defined in the statistical analysis plan, which will be finalized before database closure, as the subset of the mITT population without major protocol deviations.

The PP population will not be analyzed if this population comprises more than 90% or less than 50% of the mITT population. Only the key efficacy outcome measures (see Section 3.4.1) will be analyzed using the PP population.

6.3.3.2 *For Subjects Enrolled in Accordance to Version 6 of the Protocol:*

Intent-To-Treat Population

The intent-to-treat (ITT) population is defined as consisting of all subjects who give informed consent, are randomized, and receive at least one dose of double-blind medication. Subjects with dose adjustments or interruptions, or who were on a different dose than the final dose for their age group, will be excluded from the primary efficacy analysis and will not contribute to the approximately 160 evaluable subjects expected after 24 weeks. Data will be analyzed according to the treatment as randomized.

The ITT population is the primary and the population assessed for efficacy.

6.4 SUMMARIES OF TREATMENT GROUP COMPARABILITY

Demographics, baseline characteristics (including subject disposition and medical history) and all baseline laboratory values will be summarized descriptively by treatment using frequency tables and summary statistics providing means, medians, standard deviations, and extreme values.

6.5 SAFETY ANALYSES

All safety analyses will be based on the safety analysis population.

As appropriate, listings, summary tables, and graphs by treatment will be provided for safety and tolerability assessments, including

- Incidence of adverse events (overall, by intensity, and by relationship to study medication).
- Serious adverse events and adverse events of special interest will be reported separately.
- [redacted]
- Vital signs (systolic blood pressure, diastolic blood pressure, pulse rate, and orthostatic effects)
- [redacted]
- Physical examination (height, weight, neurological examination)
- Safety laboratory values (including hematology, blood chemistry, [redacted], coagulation, and urinalysis parameters)
- Incidence of marked laboratory abnormalities
- Clinical assessment of suicidality (C-SSRS)

6.5.1 Adverse Events

Verbatim descriptions of adverse events recorded on the eCRF by the Investigator during the study period will be mapped to the Medical Dictionary for Regulatory Activities (MedDRA) thesaurus terms. All adverse events will be tabulated by body system and preferred term for individual events within each body system. Adverse events will also be tabulated by severity and relationship to the study medication. Serious adverse events will be summarized separately.

6.5.2 Clinical Laboratory Test Results

All clinical laboratory data will be stored on the database in the units in which they were reported. Subjects' listings and summary statistics at each assessment time will be presented using the International System of Units (SI units; Système International

d'Unités). Laboratory data not reported in SI units will be converted to SI units before processing.

Laboratory test values will be presented by individual listings with flagging of values outside the normal ranges.

6.5.2.1 Standard Reference Ranges and Transformation of Data

Roche standard reference ranges, rather than the reference ranges of the Investigator, will be used for all parameters. For most parameters the measured laboratory test result will be assessed directly using the Roche standard reference range. A transformation will be performed on some laboratory tests that lack sufficiently common procedures and have a wide range of Investigator ranges, e.g., enzyme tests that include AST, ALT, and alkaline phosphatase and total bilirubin. If the standard reference ranges for these parameters have a lower limit of zero only the upper limits of the ranges will be used in transforming the data.

6.5.2.2 Definition of Laboratory Abnormalities

For all laboratory parameters included, there exists a Roche predefined standard reference range. [redacted] Laboratory values falling outside this standard reference range will be labeled "H" for high or "L" for low in study subject listings of laboratory data.

In addition to the standard reference range, a marked reference range has been predefined by Roche for each laboratory parameter. The marked reference range is broader than the standard reference range. Values falling outside the marked reference range that also represent a defined change from baseline will be considered marked laboratory abnormalities (i.e., potentially clinically relevant). If a baseline value is not available for a subject, the midpoint of the standard reference range will be used as the subject's baseline value for the purposes of determining marked laboratory abnormalities. Marked laboratory abnormalities will be labeled in the subject listings as "HH" for very high or "LL" for very low.

[redacted]

6.5.3 Vital Signs and ECG

Vital signs and ECG will be presented by individual listings and summary tables of raw values and change from baseline.

6.5.4 Concomitant Medications

The original terms recorded on the study subjects' eCRF by the Investigator for concomitant medications will be standardized by the Sponsor by assigning preferred terms. Concomitant medications will be presented in summary tables and listings.

6.6 EFFICACY ANALYSES

All efficacy data will be summarized by treatment and time (visit). Listings, summary tables of descriptive statistics, frequency tables of raw values and of change from baseline (if available) and corresponding graphs will be provided as appropriate.

Inferential statistical analysis will be performed on pooled data from patients taking balovaptan 10 mg eq dose compared with those from the concurrently randomized placebo in the corresponding randomization stage. The primary efficacy endpoint, absolute change from baseline of Vineland™-II 2DC, as well as Vineland™-II Composite Standard score and domains standard scores (Communication, Socialization, and Daily Living Skills) and the factors obtained from ABC*,* RBS-R*, and PedsQL* will be analyzed using MMRM (Mixed Model Repeat Measurements) on the overall population of adolescents and children. The MMRM model will include treatment and visit (week) as main effects, individual age and baseline score (where available) as covariates, and treatment-by-visit and baseline-by-visit as interaction terms. An unstructured correlation will be employed to allow for correlation among repeated observations within a patient. In the event that the model fails to reliably fit, alternative models may be considered. All main effects and interactions will be retained in the final model regardless of their statistical significance. The results of the analysis will be presented as point estimates, 90% confidence intervals and associated p-values for the adjusted mean differences

between balovaptan $10\ mg\ eq$ and placebo after 24 weeks of treatment as well as at intermediate visits.

[redacted]

Further details on the data analysis will be provided in the Statistical Analysis Plan.

6.7 PHARMACOKINETIC ANALYSES

The observed plasma concentrations of balovaptan and its metabolite M3 and M2 (as applicable) (and other metabolites as appropriate) will be summarized by dose and observation time, and the ratio of metabolite to parent drug concentration (molecular weight corrected) will be calculated.

A population PK modeling approach (nonlinear mixed-effect modeling) will be used to characterize the pharmacokinetics and variability of balovaptan (and its metabolites, if warranted) in the pediatric population. The structural PK model will be built based on prior information in adults. Key pharmacokinetic parameters, (i.e. apparent clearance and volume of distribution) and variability will be reported and the influence of covariates (e.g., age, body size, genetic variability of CYP3A4) on the population PK parameters will also be investigated, as appropriate. The pediatric data in this study (BP30153) may be combined with balovaptan exposure data in adults as needed to adequately characterize the PK in the pediatric population. Individual exposure at steady-state (AUC_{ss}) will be derived from the model. Other individual exposure estimates (e.g. C_{max}, T_{max}, AUC for a specific time interval) may also be derived from the model or from the observed data, if possible.

Apparent CL and exposure (AUC_{ss}) estimates for dose confirmation in the first cohort of adolescents and children will be derived in a Bayesian feedback approach based on an adult PopPK model.

Additional PK, PK-PD, or dose/exposure response analyses will be conducted as appropriate.

The results of population PK and PK-PD modeling may be reported separately.

6.8 INTERIM ANALYSES

Interim analyses for PK and safety in the first cohort of approximately 24 adolescents and subsequently in the first cohort of up to approximately 30 children are planned (see Section 3.1.3.1).

An efficacy and safety interim analysis is planned once approximately *80* subjects *taking either balovaptan 10 mg eq or placebo (i.e., approximately 40 subjects per treatment arm)* have completed their 12 week visit without dose *interruptions or* adjustments to allow internal decisions for the next steps of the development plan.

Given the hypothesis generating nature of this study, the Sponsor may conduct up to two interim analyses beyond what is specified elsewhere in this protocol. The decision to conduct such an interim analysis and its timing will be documented in the Sponsor's study master file prior to the conduct of the interim analysis. The clinical study report will also document that such an interim analysis occurred. Interim analyses will be performed and interpreted by the IMC and SOC members (as required), who will have full access to unblinded data. Recruitment will continue during the efficacy and safety interim analyses.

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. Sites will be responsible for data entry into the EDC system.

A comprehensive validation check program will verify the data. Discrepancies will be generated automatically in the system at the point of entry or added manually for resolution by the Investigator.

The Sponsor will produce a Data Handling Manual and a Data Management Plan that describes the quality checking to be performed on the data. Central laboratory data or Other electronic data will be sent directly to the Sponsor, using the Sponsor's standard procedures to handle and process the electronic transfer of these data.

System backups for data stored by the Sponsor and records retention for the study data will be consistent with the Sponsor's standard procedures.

7.2 ELECTRONIC CASE REPORT FORMS

Data for this study will be captured via an on line Electronic Data Capture (EDC) system. The data collected in the source documents is entered onto the study eCRF. An audit trail will maintain a record of initial entries and changes made; reasons for change; time and date of entry; and user name of person authorizing entry or change. For each subject enrolled, an eCRF must be completed and electronically signed by the principal Investigator or authorized delegate from the study staff. If a subject withdraws from the study, the reason must be noted on the eCRF. If a subject is withdrawn from the study because of a treatment‑limiting adverse event, thorough efforts should be made to clearly document the outcome.

The Investigator should ensure the accuracy, completeness and timeliness of the data reported to the Sponsor/CRO in the eCRFs and in all required reports.

eCRFs will be submitted electronically to the Sponsor/CRO and should be handled in accordance with instructions from the Sponsor/CRO.

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 PATIENT-REPORTED OUTCOME DATA

Subjects will use an ePRO device to capture PRO data. The data will be transmitted electronically to a centralized database at the ePRO vendor. The data can be reviewed by site staff via secure access.

Once the study is complete, the ePRO data, audit trail, and trial and system documentation will be archived. The Investigator will receive patient 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 patient data in a machine-readable format on a compact disc.

ePRO data will be collected using an electronic device provided by an ePRO vendor. The device is designed for entry of data in a way that is attributable, secure, and accurate, in compliance with U.S. Food and Drug Administration (FDA) regulations for electronic records (21 CFR Part 11). The ePRO device data are available for view access only via secure access. Only identified and trained users may view the data, and their actions 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.

7.4 SOURCE DATA DOCUMENTATION

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

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

Before study initiation, data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data must be defined in the Trial Monitoring Plan.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 7.6.

To facilitate source data verification, 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 investigational site must also allow inspection by applicable health authorities.

7.5 USE OF COMPUTERIZED SYSTEMS

When clinical observations are entered directly into an investigational 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, ePRO data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 15 years after completion or discontinuation of the study, or for the length of time required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations. No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

If audio recordings have been used, they will be maintained on a secure server throughout the duration of the study and will be destroyed one year after study completion on approval by the Sponsor.

For countries where ethics committees or the Ministry of Health will not approve audio recording of subject interviews, review of the scale worksheets, submitted as part of the assessment source information, will be performed to verify accuracy of scoring and adherence to study conventions.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. 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.

8.2 INFORMED CONSENT

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

In this study, caregiver specific information will be collected to evaluate caregiver burden requiring that a separate written informed consent be obtained from the caregiver.

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 of 1996 (HIPAA). 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 9.5).

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 only be disclosed to third parties 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.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA and other 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 one year after completion of the study (i.e., Last Subject Last Visit).

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, which includes an audit trail containing a complete record of all changes to data.

Roche shall also submit as a periodic safety report once a year a Development Safety Update Report (DSUR) to the IEC and regulatory authorities according to local regulatory requirements and timelines of each country participating in the study.

Sampling for the Research Biosample Repository is contingent on review and approval for the exploratory biomarker assessments and written informed consent by an appropriate regulatory body (depending on the country where the study is performed) and a site's Institutional Review Board (IRB) / Ethics Committee (EC). If a regulatory or site's IRB/ EC does not approve the sampling for the exploratory assessments the section on biomarker sampling will not be applicable.

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 Institutional Review Board (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 also whenever subsequent amendments /modifications are made to the protocol.

A Clinical Study Report (CSR) will be written and submitted to relevant Institutional Review Board (IRB) / Ethics Committee (EC) and regulatory authorities in accordance

with local requirements. To fulfill the requirements for the EU Directive No 75/318/EEC the CSR 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 patient 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 PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

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

www.roche.com/roche_global_policy_on_sharing_of_clinical_study_information.pdf

The results of this study may be published or presented at scientific congresses. For all clinical trials in patients 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 patients 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.

9.5 PROTOCOL AMENDMENTS

Any substantial protocol amendments will be prepared by the Sponsor. Substantial 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 any non-substantial changes, as defined by regulatory requirements.

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Appendix 1
Schedule of Assessments
For all Subjects after Confirmation of Final Doses by IMC and SOC

Week	Screening [2] (~4 weeks)	Baseline	Abbr. Baseline [19] (for re-start patients only)	☎ [22]	Home Visit [11]	Site	Home Visit [11]	☎ [22]	Site	☎ [22]	Home Visit [11]	Site	Home Visit [11]	☎ [22]	Site	Site	FU (2 W)	FU (6 W)
				2	4	6	8	10	12 [2]	14	16	18 [2]	20	22	24 [2]	EW [2]	26	30 [2]
Day	–28	1	1	15	29	43	57	71	85	99	113	127	141	155	169	NA	183	211
Time window (days)	*–28* to *–7*	NA	NA	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	NA	±2	+7
Study site visits	x	x	x			x			x			x			x	x		x
Informed consent	x																	
IQ test [8]	x																	
ADOS 2 [8]	x																	
SRS-2	x																	
Inclusion/Exclusion criteria	x																	
Randomization [17]		P																
Demography [4]	x																	
Medical history (including psychiatry)	x																	
Physical and neurological examination	x		x			x			x			x			x	x		x
Tanner staging [12]		x 1 week [1]	x 1 week [1]						x						x	x		x
Anthropometric measurements	x	x	x						x						x	x		x

Appendix 1
Schedule of Assessments
For all Subjects after Confirmation of Final Doses by IMC and SOC (cont.)

Week	Screening [2] (~4 weeks)	Baseline	Abbr. Baseline [19] (for restart patients only)	☎ [22] 2	Home Visit [11] 4	Site 6	Home Visit [11] 8	☎ [22] 10	Site 12 [2]	☎ [22] 14	Home Visit [11] 16	Site 18 [2]	Home Visit [11] 20	☎ [22] 22	Site 24 [2]	Site EW [2]	FU (2 W) 26	FU (6 W) 30 [2]
Day	–28	1	1	15	29	43	57	71	85	99	113	127	141	155	169	NA	183	211
Time window (days)	*–28* to *–7*	NA	NA	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	NA	±2	+7
Vital signs	x	P, x [10]	P, x [10]			P, x [10]			P, x [10]			P, x [10]			P, x [10]	x		x
ECG-12 lead	x	P [3]	P [19]			x [7]									x [7]	x		x
Fasting	x																	
Hematology [23]	x	P	P	(x [22])	x	(P [22])	x	(x [22])	P	(x [22])	x	(P [22])	x	(x [22])	*P*	x	x	x
Full serum chemistry [5]	x [6]	P *6*	P *6*						P*6*						*P* [6]	x [6]		x [6]
Safety questions [21]				x	x	x	x	x	x	x	x	x	x	x	x		x	
Coagulation	x														P	x		
Urinalysis	x	P	P			x			x			x			x	x		x
Pregnancy test [13]	x	P	P						x						x	x		x
Serology	x																	
Substance use [9]	x								x						x	x		x
Alcohol test [9]	x								x						x	x		x
Dispensing of study medication		x	x			x			x			x			x [20]			
Administration of study medication		x [P]	x [P]	x	x	x	x	x	x	x	x	x	x	x	x			
Palatability									x [15]									

Appendix 1
Schedule of Assessments
For all Subjects after Confirmation of Final Doses by IMC and SOC (cont.)

Week	Screening [2] (~4 weeks)	Baseline	Abbr. Baseline [19] (for restart patients only)	☎ [22] 2	Home Visit [11] 4	Site 6	Home Visit [11] 8	☎ [22] 10	Site 12 [2]	☎ [22] 14	Home Visit [11] 16	Site 18 [2]	Home Visit [11] 20	☎ [22] 22	Site 24 [2]	Site EW [2]	FU (2 W) 26	FU (6 W) 30 [2]
Day	–28	1	1	15	29	43	57	71	85	99	113	127	141	155	169	NA	183	211
Time window (days)	*–28 to –7*	NA	NA	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	NA	±2	+7
PK blood samples [14]							x		P, x						P, x	x		
Clinical genotyping [17]		x																
Research Biosample Repository (optional)		x																
Vineland™–II (survey)		x 1 week [1]	x 1 week [1]						x						x	x		x
ABC		x 1 week [1]	x 1 week [1]			x			x			x			x	x		x
RBS-R		x 1 week [1]	x 1 week [1]			x			x			x			x	x		x
CaGI									x						x	x		
PedsQL (Family Impact Scale)		x 1 week [1]	x 1 week [1]						x						x	x		
Exit interviews															x	x		
C-SSRS [18]	x	x 1 week [1]	x 1 week [1]			x			x			x			x	x		x
PedsQL (Core and Cognitive Scale)		P 1 week [1]	P 1 week [1]						x						x [16]	x [16]		
OACIS-S		P 1 week [1]	P 1 week [1]			x			x			x			x	x		x

Appendix 1
Schedule of Assessments
For all Subjects after Confirmation of Final Doses by IMC and SOC (cont.)

Week	Screening [2] (~4 weeks)	Baseline	Abbr. Baseline [19] (for restart patients only)	☎ [22] 2	Home Visit [11] 4	Site 6	Home Visit [11] 8	☎ [22] 10	Site 12 [2]	☎ [22] 14	Home Visit [11] 16	Site 18 [2]	Home Visit [11] 20	☎ [22] 22	Site 24 [2]	Site EW [2]	FU (2 W) 26	FU (6 W) 30 [2]
Day	–28	1	1	15	29	43	57	71	85	99	113	127	141	155	169	NA	183	211
Time window (days)	*–28* to *–7*	NA	NA	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	NA	±2	+7
OACIS-I						x			x			x			x	x		x
CGI-S	x	P 1 week [1]	P 1 week [1]			x			x			x			x	x		x
CGI-I						x			x			x			x	x		x
Body temperature monitoring				←				Temperature monitoring every 2 weeks										→
Adverse events	←																	→
Previous and concomitant treatments	←																	→

Abbr. = Abbreviated; ABC = Aberrant Behavior Checklist; ADOS 2 = Autism Diagnostic Observational Schedule; CaGI = Caregiver-reported Global Impression; CGI-I = Clinical Global Impressions of Improvement; CGI-S = Internal Monitoring Committee; CPK = creatinine phosphokinase; [redacted] *T*; IQ = intelligence quotient; OACIS-I or -S = Ohio Autism Clinical Impressions Scale Improvement or Severity; PedsQL = Pediatric Quality of Life; PK = pharmacokinetic; RBS-R = Repetitive Behavior Scale-Revised; SOC = Scientific Oversight Committee; SRS-2 = Social Responsiveness Scale, Second Edition; W = Week(s).

☎ Phone call interview

P Pre-dose. The first dose of study medication has to be taken after all pre-dose assessments.

1. These assessments can be performed at baseline/abbreviated baseline or within 1 week prior to first dose.
2. If necessary these clinic visits (Week 6, Week 12, Week 18, Week 24, Early Withdrawal Visit, and 6-week Follow-Up Visit) can be performed over two consecutive days, assessments at screening can be performed over the entire screening time window. After premature discontinuation, an Early Withdrawal Visit should be arranged as soon as possible (see also Section 4.7.1.1).
3. Triplicate ECG at Baseline only.
4. Demography includes Educational Level, Diagnosis of ASD, Caregiver Information, Employment and/or school Status, and Living Arrangements.

Appendix 1
Schedule of Assessments
For all Subjects after Confirmation of Final Doses by IMC and SOC (cont.)

5. See protocol Section 4.6.1.7 and lab manual for more details about full serum chemistry.
6. At screening, Week 24/Early Withdrawal Visit and Follow-Up Visit (6 weeks), glycated hemoglobin (HbA1c), thyroid stimulating hormone (TSH), and free thyroxine (T4) will be assessed. [redacted]
7. ECG at Week 6 and Week 24 will be performed 3 hours post-dose.
8. ADOS-2 and IQ test up to 12 months old can be accepted at screening; Wechsler Abbreviated Scale of Intelligence Scale, Second Edition (WASI-II) or Wechsler Preschool and Primary Scale of Intelligence, Fourth Edition (WPPSI-IV) intelligence test will be used at screening.
9. Alcohol test and substance use tests are mandatory at screening for adolescents. At other visits and for children, only to be performed if deemed necessary by investigator.
10. Vital signs will be done pre-dose and 3 hours post-dose. Additionally, orthostatic function will be assessed pre-dose and 3 hours post-dose at baseline/abbreviated baseline, and 3 hours post-dose at Week 6 and Week 24 or Early Withdrawal Visit.
11. Planned as off-site/home visit and can be done by a nurse, however can also be performed as clinic visits.
12. Tanner staging will be done in patients 7 years and older, but not after menarche in female patients and not after voice break in male patients.
13. Pregnancy tests will be performed in female patients after menarche only.
14. PK samples: Week 8: in the evening post-dose together with safety labs; Week 12 and Week 24: pre + 2 hours post-dose + end of visit but at least 4 hours post-dose; Early Withdrawal Visit: one at any time. Samples scheduled at least 4 hours post-dose on a clinic visit day can be taken by a nurse at home.
15. Subject and caregiver will provide feedback on palatability of study medication.
16. Subjects will be asked additional questions after completion of the PedsQL.
17. RBR and CG samples should be taken at baseline upon consent, but if missed, may be taken at any visit after.
18. For children 5–11 years old, additional questions will be asked in context of Columbia-Suicide Severity Rating Scale (C-SSRS; see Section 4.6.1.12).
19. Subjects from first adolescent/first children cohorts who stopped treatment on or before Week 8 in the context of the IMC/SOC dosing decision (see Appendix 2, Section 3.1.3.1) will be allowed to restart for an additional 24 weeks in the main study in the same treatment arm without new randomization, provided the investigator can exclude relevant changes in patient's general condition (see Section 4.2.1). These patients will have a new, abbreviated baseline visit, and will continue with all subsequent visits according to the schedule of assessments. In this abbreviated baseline visit, a single ECG should be taken.
20. For those subjects who consent to the OLE and are able to proceed directly into the OLE, medication will be administered after all assessments are complete. This will be defined as Day 1 of the OLE.

Appendix 1
Schedule of Assessments
For all Subjects after Confirmation of Final Doses by IMC and SOC (cont.)

21. Safety monitoring will follow semi-structured interview as listed in Appendix 8. This will include a check on compliance with body temperature monitoring.
22. If these hematology samples are required, visits must be conducted either at the site or by a home nurse if appropriate.
23. *Blood samples indicated in parentheses are applicable only in the event that temperature monitoring could be implemented or individuals are not compliant.*

Appendix 2
Schedule of Assessments
For First Cohorts of 24 Adolescents and 30 Children until Confirmation of Final Doses by IMC/SOC

	Screening [2] (~4 weeks)	Baseline	Home Visit at W1	W2	W4 [2]	W6 [2]	Home Visit [11] at W8	For Discontinuation and Stopping Treatment Only [16]		
								EoT [2]	FU (2 W)	FU (6 W) [2]
Day	–28	1	8	15	29	43	57		14	42
Time window (days)	*–28* to *–7*	NA	±2	±2	±2	±2	±2	NA	±2	+7
Study site visits	x	x		x	x	x	For details about Week 8 visit see Appendix 1 and continue according to Appendix 1.	x		x
Informed consent	x									
IQ test [8]	x									
ADOS 2 [8]	x									
SRS-2	x									
Inclusion/Exclusion criteria	x									
Randomization [17]		P								
Demography [4]	x									
Medical history (incl. psychiatry)	x									
Physical and neurological examination	x					x		x		x
Tanner staging [12]		x 1 week [1]								x

Appendix 2
Schedule of Assessments
For First Cohorts of 24 Adolescents and 30 Children until Confirmation of Final Doses by IMC/SOC (cont.)

	Screening [2] (~4 weeks)	Baseline	Home Visit at W1	W2	W4 [2]	W6 [2]	Home Visit [11] at W8	For Discontinuation and Stopping Treatment Only [16]		
								EoT [2]	FU (2 W)	FU (6 W) [2]
Day	–28	1	8	15	29	43	57		14	42
Time window (days)	*–28* to *–7*	NA	±2	±2	±2	±2	±2	NA	±2	+7
Anthropometric measurements	x	x					For details about week 8 visit see Appendix 1 and continue according to Appendix 1			x
Vital signs	x	P, x [10]		P, x [10]	P	P, x [10]		x		x
ECG-12 lead	x	P [3]		x [7]	P	x [7]		x		x
Fasting	x									
Hematology	x	P		P	P	P		x	x	x
Full serum chemistry [5]	x [6]	P *[6]*			P			x [6]		x [6]
Coagulation	x									
Urinalysis	x	P				x		x		x
Pregnancy test [13]	x	P								x
Serology	x									
Substance use [9]	x									x
Alcohol test [9]	x									x
Dispensing of study medication		x				x				
Administration of study medication		x [P]	x	x	x	x				
PK blood samples [14]			P	P, x	P			x		
Clinical genotyping [17]		x								

Appendix 2
Schedule of Assessments
For First Cohorts of 24 Adolescents and 30 Children until Confirmation of Final Doses by IMC/SOC (cont.)

	Screening [2] (~4 weeks)	Baseline	Home Visit at W1	W2	W4 [2]	W6 [2]	Home Visit [11] at W8	For Discontinuation and Stopping Treatment Only [16]		
								EoT [2]	FU (2 W)	FU (6 W) [2]
Day	–28	1	8	15	29	43	57		14	42
Time window (days)	*–28* to *–7*	NA	±2	±2	±2	±2	±2	NA	±2	+7
Research Biosample Repository (optional)		x					For details about week 8 visit see Appendix 1 and continue according to Appendix 1			
Vineland™–II (survey)		x 1 week [1]								
ABC		x 1 week [1]				x		x		x
RBS-R		x 1 week [1]				x		x		x
PedsQL (Family Impact Scale)		x 1 week [1]								
C-SSRS [18]	x	x 1 week [1]		x	x	x		x		x
PedsQL (Core and Cognitive Scale)		P 1 week [1]								
OACIS-S		P 1 week [1]				x		x		x
OACIS-I						x		x		x

Appendix 2
Schedule of Assessments
For First Cohorts of 24 Adolescents and 30 Children until Confirmation of Final Doses by IMC/SOC (cont.)

	Screening [2] (~4 weeks)	Baseline	Home Visit at W1	W2	W4 [2]	W6 [2]	Home Visit [11] at W8	For Discontinuation and Stopping Treatment Only [16]		
								EoT [2]	FU (2 W)	FU (6 W) [2]
Day	–28	1	8	15	29	43	57		14	42
Time window (days)	*–28* to *–7*	NA	±2	±2	±2	±2	±2	NA	±2	+7
CGI-S	x	P 1 week [1]				x	For details about week 8 visit see Appendix 1 and continue according to Appendix 1	x		x
CGI-I						x		x		x
Body temperature monitoring		←	—	—	—	→				
Adverse events	←	—	—	—	—	→		←	—	→
Previous and concomitant treatments	←	—	—	—	—	→		←	—	→

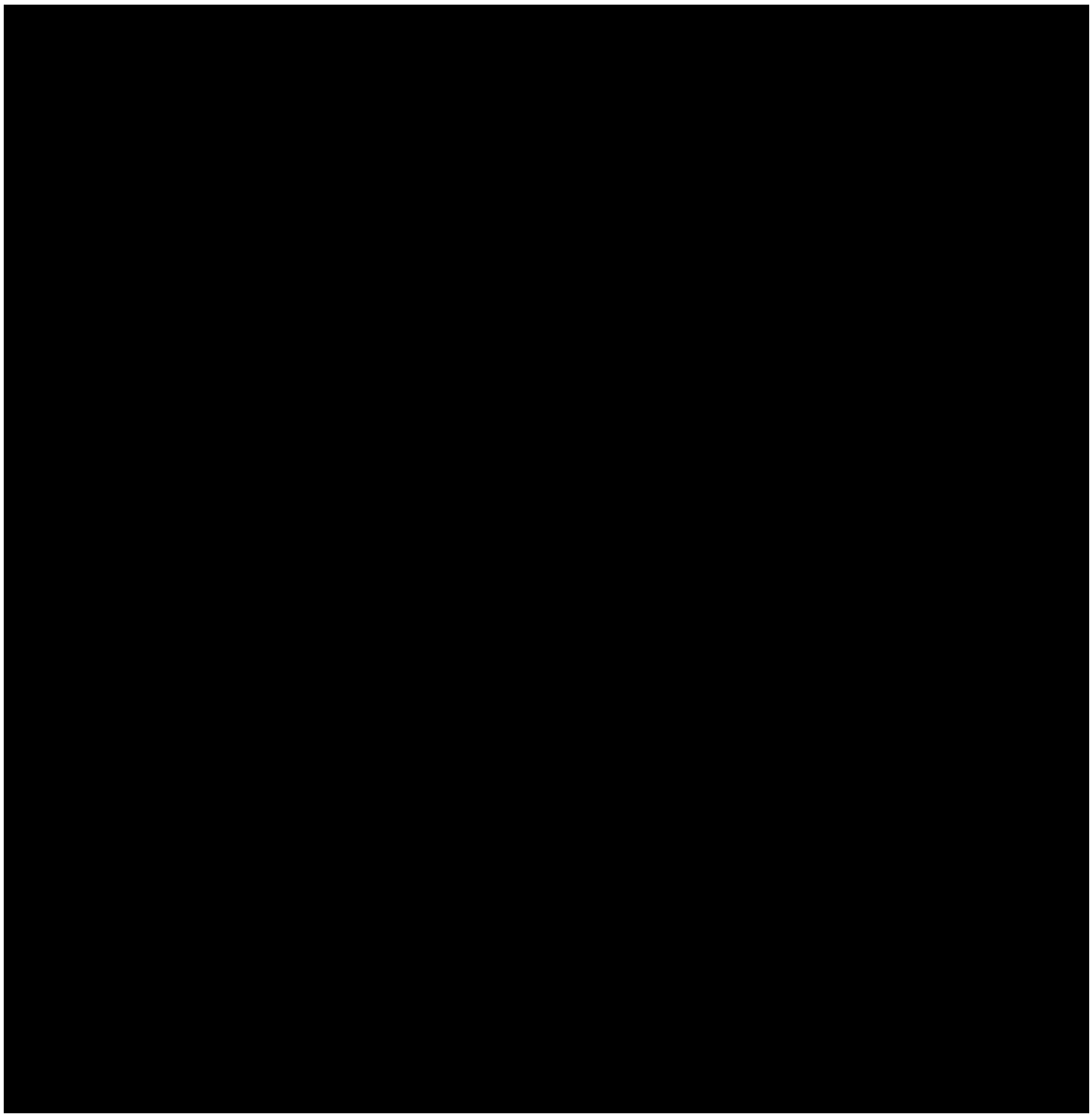
ABC = Aberrant Behavior Checklist; ADOS 2 = Autism Diagnostic Observational Schedule; CaGI = Caregiver-reported Global Impression; CGI-I = Clinical Global Impressions of Improvement; CGI-S = Clinical Global Impressions of Severity; CPK = creatinine phosphokinase; ECG = electrocardiogram; EoT = End of Treatment; FU = Follow-Up; IMC = independent Ethics Committee; IQ = intelligence quotient; OACIS-I or -S = Ohio Autism Clinical Impressions Scale Improvement or Severity; PedsQL = Pediatric Quality of Life; PK = pharmacokinetic; RBS-R = Repetitive Behavior Scale-Revised; SOC = Scientific Oversight Committee ; SRS-2 = Social Responsiveness Scale, Second Edition; W = Week(s).

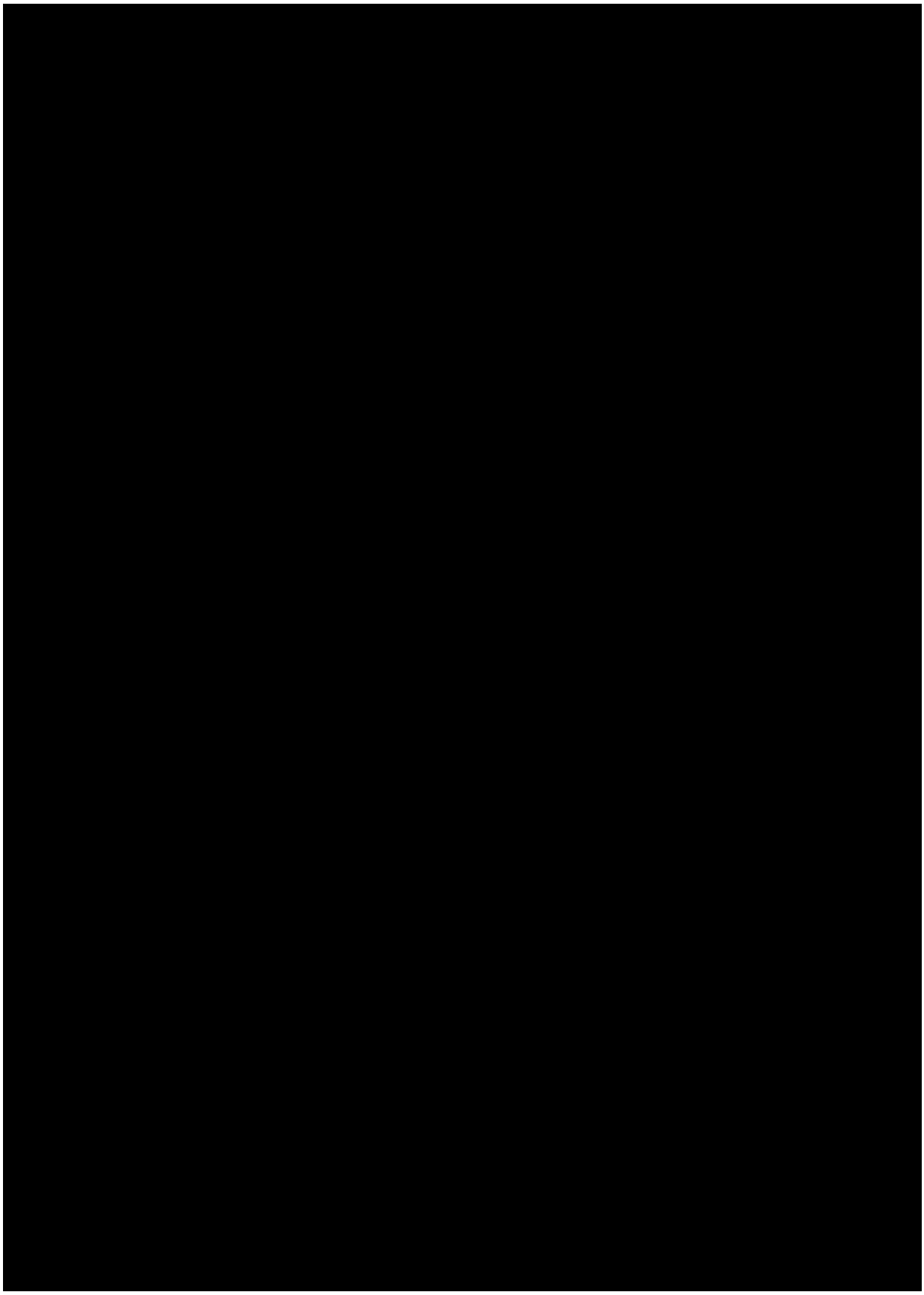
P Pre-dose. The first dose of study medication has to be taken after all pre-dose assessments.

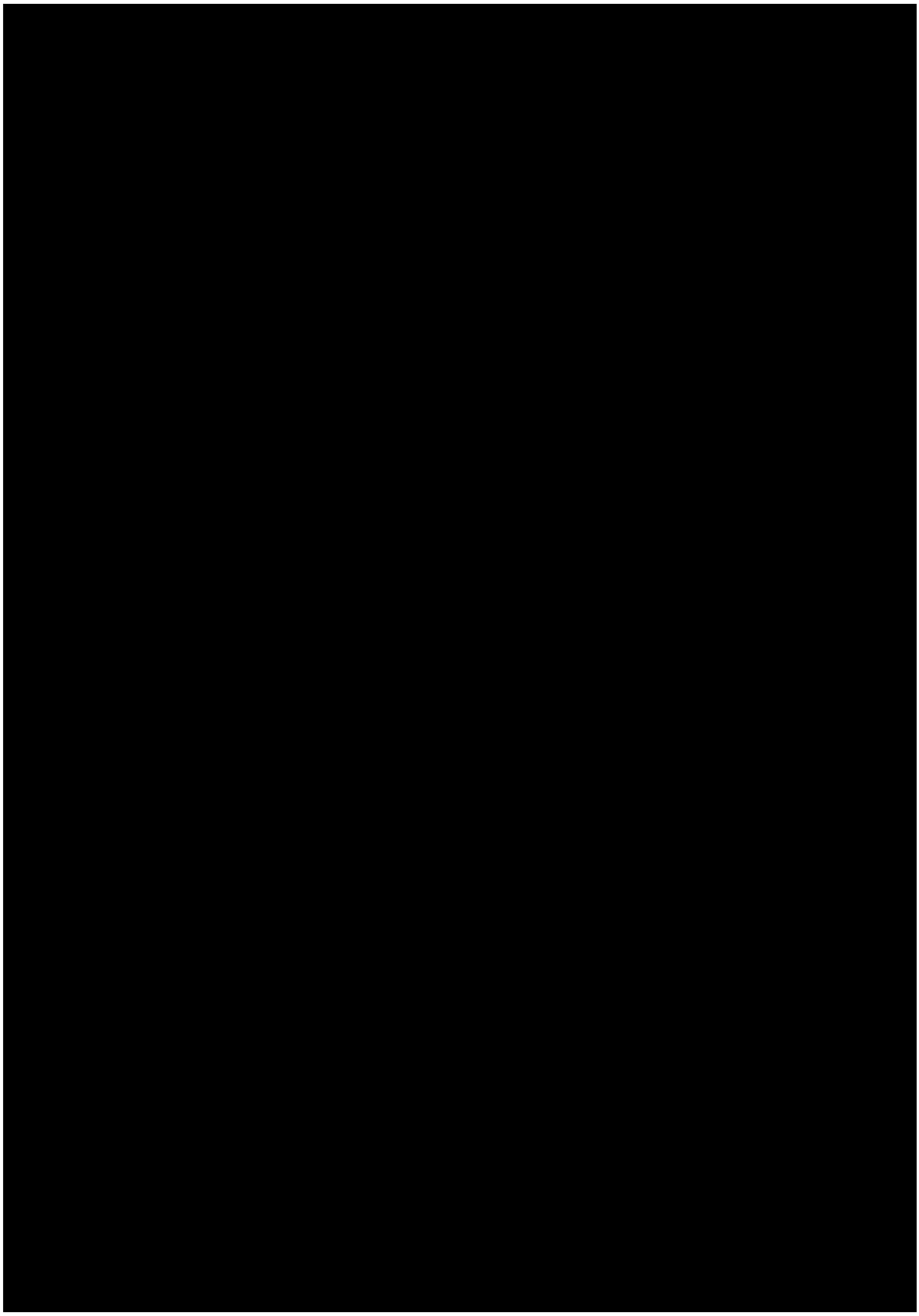
1. These assessments can be performed at baseline or within 1 week prior to first dose.
2. If necessary these clinic visits (Week 6, End of Treatment Visit, Follow-Up Visit after 6 weeks) can be performed over two consecutive days, assessments at screening can be performed over the entire screening time window.

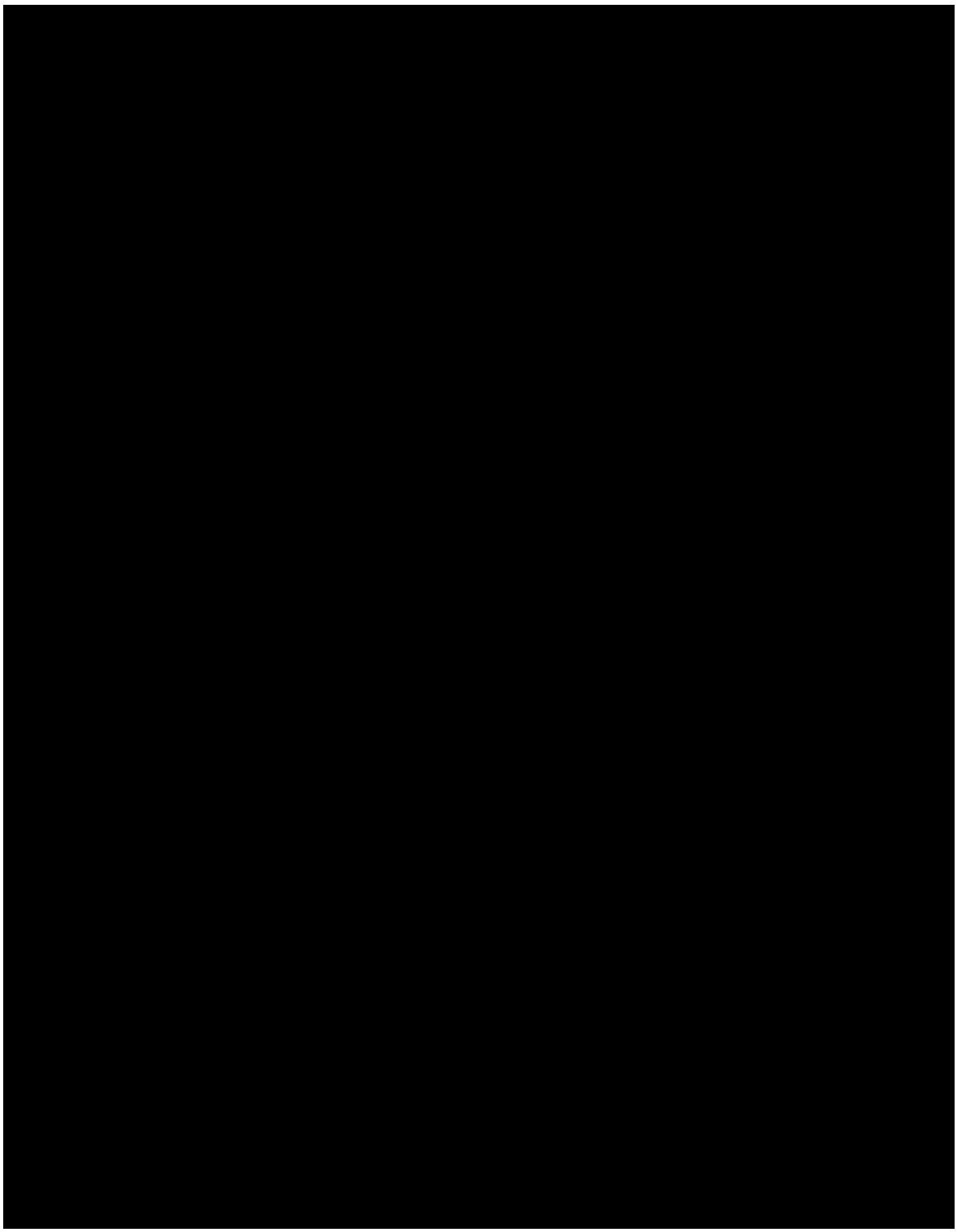
Appendix 2
Schedule of Assessments
For First Cohorts of 24 Adolescents and 30 Children until Confirmation of Final Doses by IMC/SOC (cont.)

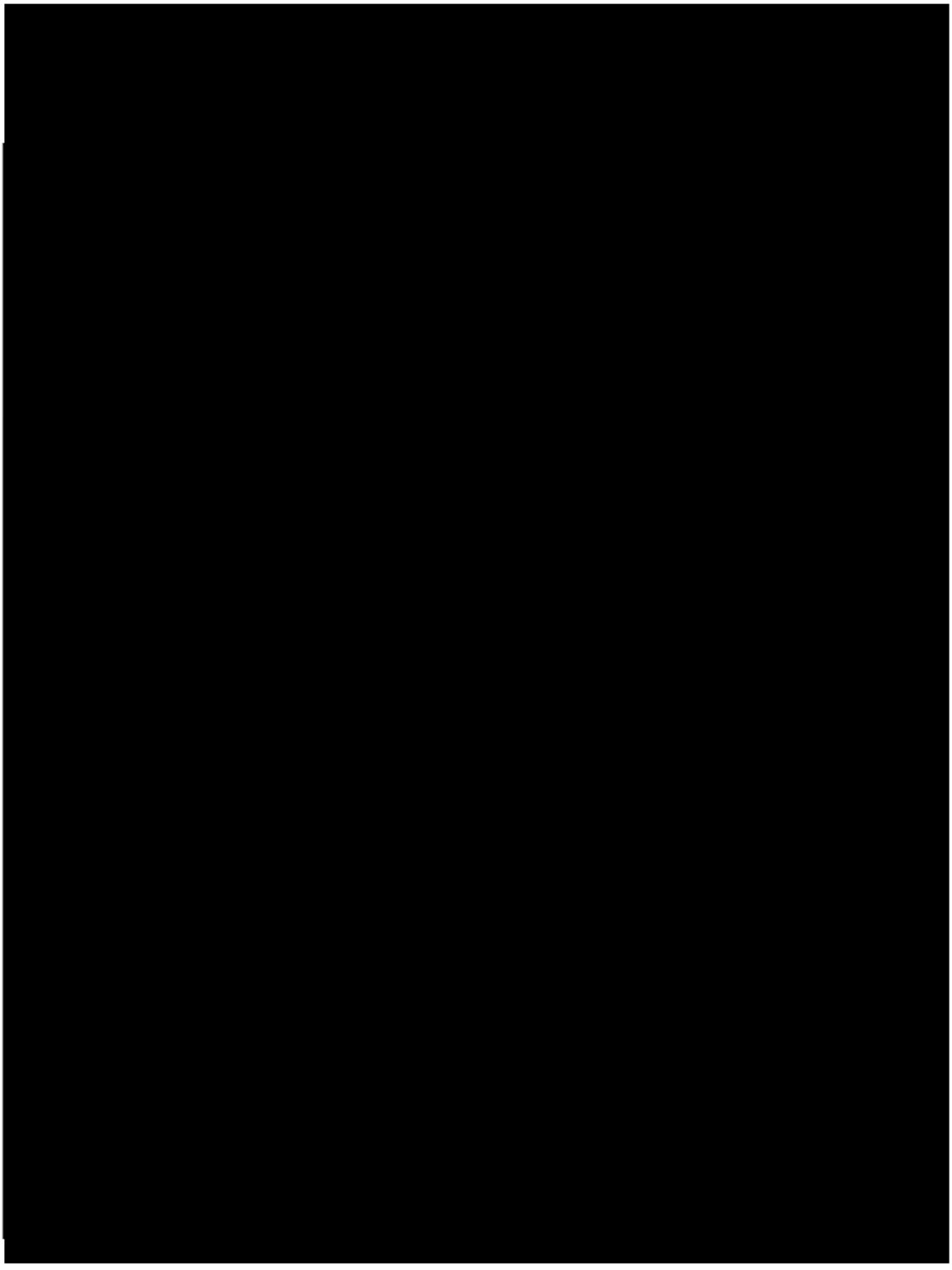
3. Triplicate ECG at baseline only.
4. Demography includes Educational Level, Diagnosis of ASD, Caregiver Information, Employment and/or school Status and Living Arrangements.
5. See protocol Section 4.6.1.7 and lab manual for more details about full and abbreviated serum chemistry.
6. At screening and Follow-Up Visit (6 weeks) glycated hemoglobin (HbA1c), thyroid stimulating hormone (TSH), and free thyroxine (T4) will be assessed. [redacted]
7. At Week 2 and Week 6, a single ECG 3 hours post-dose; at Week 4 a single ECG preferably pre-dose.
8. ADOS-2 and IQ test up to 12 months old can be accepted at screening; Wechsler Abbreviated Scale of Intelligence Scale, Second Edition (WASI-II) or Wechsler Preschool and Primary Scale of Intelligence, Fourth Edition (WPPSI-IV) intelligence test will be used at screening.
9. Alcohol test and substance use tests are mandatory at screening for adolescents. At other visits and for children only to be performed if deemed necessary by investigator.
10. Vital signs will be done pre-dose and additionally 3 hours post-dose at baseline, Week 2 and Week 6. Orthostatic function will be assessed 3 hours post-dose at baseline and Week 6.
11. Planned as off-site/home visit and can be done by a nurse, however can also be performed as clinic visits.
12. Tanner staging will be done in patients 7 years and older, but not after menarche in female patients and not after voice break in male patients.
13. Pregnancy tests will be performed in female patients after menarche only.
14. PK samples: Week 1: pre-dose; Week 2: pre; + 2 (+/- 0.5); hour + 4 (+/- 0.5) hour + at least 6 hours post-dose; PK samples need to be at least 2 hours apart; Week 4: pre-dose; End of Treatment Visit: one at any time. Samples scheduled at least 4 hours post-dose on a clinic visit day can be taken by a nurse at home.
15. If treatment continues without halt and with final doses determined, ongoing patients will have their next visit (e.g., visit Week 8) according to Appendix 1 and continue from thereafter according to Appendix 1.
16. *Subjects can continue in the study beyond 8 weeks, even if dose confirmation has not occurred, as long as individual review of PK has been completed and exposure is within agreed limits.*
17. RBR and clinical genotyping (CG) samples should be taken at baseline upon consent, but if missed, may be taken at any visit after.
18. For children 5–11 years old additional questions will be asked in context of Columbia-Suicide Severity Rating Scale (C-SSRS; see Section 4.6.1.12).











Appendix 6
Tanner Stages [48]

Stage	Male Genitalia	Pubic Hair	Female Breasts
1	Preadolescent testes, scrotum, and penis are childlike in size	None; may be vellus hair, as over abdomen	Preadolescent elevation of papilla only
2	Slight enlargement of scrotum with reddening of skin; little or no enlargement of penis	Sparse growth of long, slightly pigmented, downy hair, straight or slightly curled, primarily at base of penis or along labia	Breast bud stage; breast and papilla form a small mound; areolar diameter enlarges
3	Further enlargement of scrotum; penis enlarges, mainly in length	Hair considerably darker, coarser, and more curled; spreads sparsely over junction of pubes	Further enlargement of breast and areola with no separation of their contours
4	Further enlargement and darkening of scrotum; penis enlarges, especially in breadth; glans develops	Adult-type hair that does not extend onto thighs, covering a smaller area than in adult	Areola and papilla project to form a secondary mound above the contour of the breast; stage 4 development of the areolar mound does not occur in 10% of girls and is slight in 20%; when present, it may persist well into adulthood
5	Adult in size and shape	Adult in quantity and type with extension onto thighs but not up linea alba	Mature female; papilla projects and areola recesses to general contour of breast

In boys, if different scores are obtained for pubic hair and genitalia, the score for genitalia should be used.

In girls, if different scores are obtained for pubic hair and breast development, the score for breast development should be used.

In this study Tanner staging will be done in subjects 7 years and older, but not after menarche in female and not after voice break in male subjects

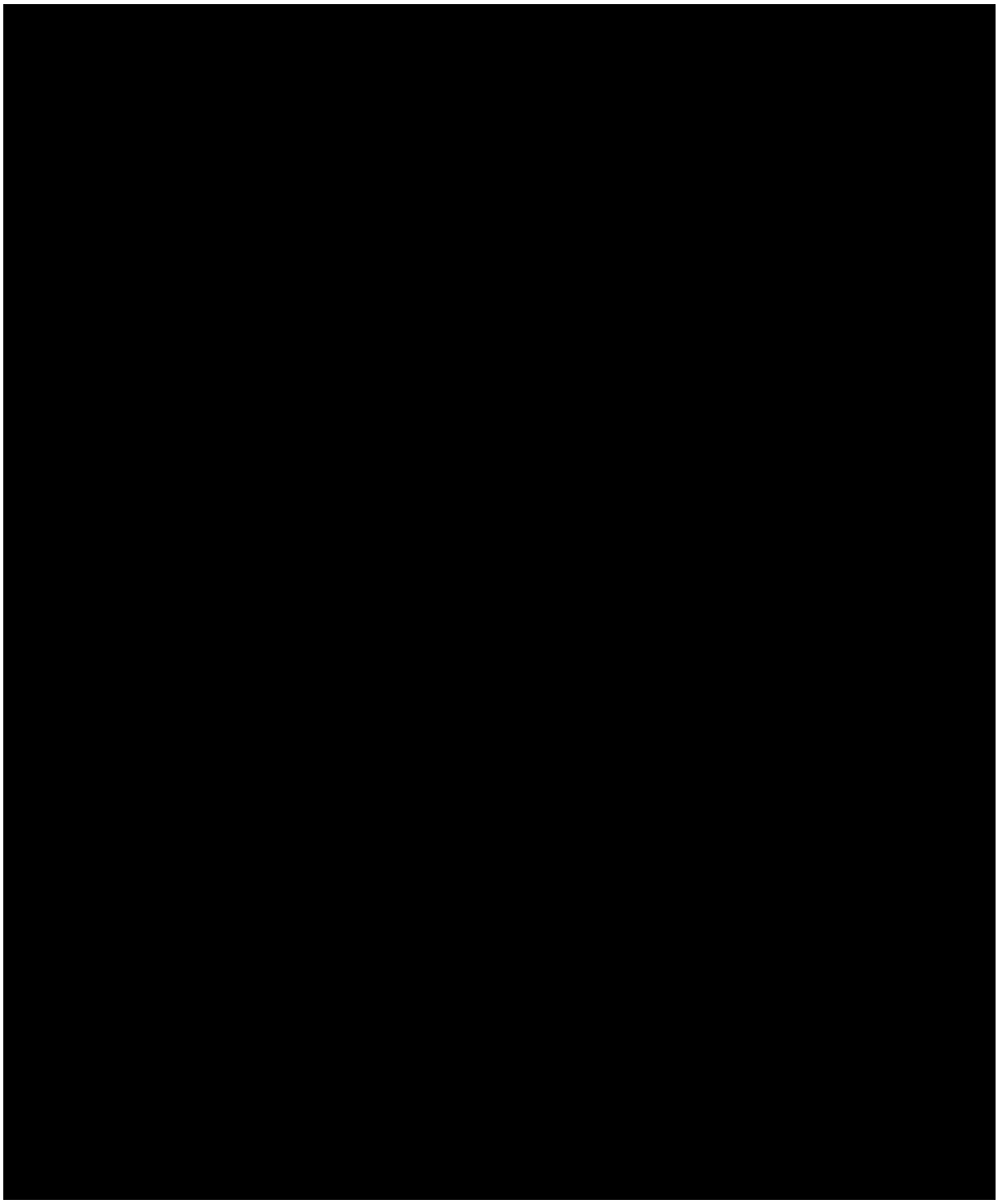
Appendix 7
Strong and Moderate Inhibitors and Inducers of CYP3A [37]

Concomitant administration of strong Cytochrome P450 3A4 (CYP3A) inducers or inhibitors is prohibited. Moderate CYP3A inhibitors are also prohibited, but for treatment duration of shorter than approximately 10 days (e.g., in the context of an adverse event) possible exceptions can be discussed with the Sponsor (Medical Monitor or designee).

Based on in vitro experiments, quinidine should not be given with balovaptan, but no systemic interactions are predicted with cetirizine, dabigatran etexilate, or digoxin. Caution is advised when using balovaptan together with other medications known to be clinically-relevant substrates of P-gp, in particular for those medications that have a narrow therapeutic window (e.g., loperamide). However, when such medications are dosed 5 or more hours after administration of balovaptan, the risk of pharmacokinetic interaction is predicted to be small.

CYP3A Inhibitor*	CYP3A Inducer*
Itraconazole	St John's wort
Clarithromycin	Dexamethasone
Ketoconazole	Retinoic acid
Grapefruit juice	Carbamazepine
Erythromycin	Phenytoin
Diltiazem	Rifampin
Verapamil	
Ciprofloxacin	
Clotrimazole	
Fluconazole	
Tofisopam	

* This table is not exhaustive.



Appendix 9
Schedule of Assessments: Open-Label Extension for Weeks 24 to 52

Week	Unique to substudy (see Appendix 11)		☎ [19]	Home Visit [11]	Site	Home Visit [11]	☎ [19]	Site	☎ [19]	Home Visit [11]	Site	Home Visit [11]	☎ [19]	Home Visit [11]	☎ [19]	Site
			26	28	30 [2]	32	34	36 [2]	38	40	42 [2]	44	46	48	50	52 [2]
Day	-14 to -1	1	183	197	211	225	239	253	267	281	295	309	323,	337	351	365
Time window (days)	± 7	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2
Informed consent	x															
Medical history	x															
Demography [4]	x															
Physical and neurological examination	x				x			x			x					x
Tanner staging [12]	x							-								x
Anthropometric measurements	x							x								x
Vital signs	x				P, x [10]			P, x			P, x					P, x
ECG-12 lead	x [3]				x [7]											x [7]
Hematology	x		(x [17])	x	(P)	x	(x [17])	P	(x [17])	x	(P)	x	(x[17])	x	(x[17])	*P*
Full serum chemistry [5]	x [6]							P								*P* [6]
Safety questions [16]			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Coagulation	x															P
Urinalysis	x				x			x			x					x
Pregnancy test [9]	x				x			x			x					x

Appendix 9
Schedule of Assessments: Open-Label Extension to Week 52 (cont.)

Week	Unique to substudy (see Appendix 11)		☎ [19]	Home Visit [11]	Site	Home Visit [11]	☎ [19]	Site	☎ [19]	Home Visit [11]	Site	Home Visit [11]	☎ [19]	Home Visit [11]	☎ [19]	Site
			26	28	30 [2]	32	34	36 [2]	38	40	42 [2]	44	46	48	50	52 [2]
Day	-14 to -1	1	183	197	211	225	239	253	267	281	295	309	323,	337	351	365
Time window (days)	± 7	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2
Alcohol / Substance use [8]	x															x
Dispensing of study medication		x			x			x			x					x
Administration of study medication		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PK blood samples [13]								P								x
Vineland™–II (survey)		x [1]						x								x
ABC		x [1]						x								x
RBS-R		x [1]						x								x
CaGI								x								x
PedsQL (Family Impact Scale) [15]		x [1]						x								x
C-SSRS [14]		x			x			x			x					x
PedsQL (Core and Cognitive Scale)		x [1]						x								x
CGI-S		x			x			x			x					x
CGI-I					x			x			x					x
Body temperature monitoring			←					Temperature monitoring every 2 weeks								→
Adverse events	SAE only		←													→
Previous and concomitant treatments	x	x	←													→

Appendix 9
Schedule of Assessments: Open-Label Extension to Week 52 (cont.)

ABC = Aberrant Behavior Checklist; CaGI = Caregiver-reported Global Impression; CGI-I = Clinical Global Impressions of Improvement; CGI-S = Clinical Global Impressions of Severity; CPK = creatinine phosphokinase; ECG = electrocardiogram; EW = Early Withdrawal; FU = Follow-Up; [redacted] *T*; PedsQL = Pediatric Quality of Life; PK = pharmacokinetic; RBS-R = Repetitive Behavior Scale-Revised; SAE=serious adverse event; W = Week(s).

☎	Phone call interview
P	Pre-dose. The first dose of study medication has to be taken after all pre-dose assessments.
1	These assessments can be performed at or within 1 week prior to first dose.
2	If necessary, these clinic visits (Week 30, Week 36, Week 42, Week 52, Early Withdrawal Visit, and 6-week Follow-Up Visit) can be performed over 2 consecutive days; assessments at screening can be performed over the entire screening time window. After premature discontinuation, an Early Withdrawal Visit should be arranged as soon as possible (see also Section 4.7.1.1).
3	Triplicate ECG at rescreening (substudy) only.
4	Demography includes Educational Level, Diagnosis of ASD, Caregiver Information, Employment and/or school Status, and Living Arrangements
5	See Section 4.6.1.7 and lab manual for more details about full serum chemistry [redacted].
6	At rescreening (substudy only), Week 76/Early Withdrawal Visit and Follow-Up Visit (6 weeks), glycated hemoglobin (HbA1c), thyroid stimulating hormone (TSH), and free thyroxine (T4) will be assessed. [redacted]
7	ECG at Week 30 will be performed 3 hours post-dose.
8	Alcohol test and substance use tests are mandatory at screening for adolescents. At other visits and for children, only to be performed if deemed necessary by investigator.
9	Pregnancy tests will be performed in female patients after menarche only.
10	Vital signs will be done pre-dose *and 3 hours post--dose*. Additionally, orthostatic function will be assessed pre-dose and 3 hours post-dose at Week 30.
11	Planned as off-site/home visit and can be done by a nurse, however can also be performed as clinic visits.
12	Tanner staging will be done in patients 7 years and older, but not after menarche in female patients and not after voice break in male patients.
13	PK samples should be collected in those that withdraw from the study early as long as ≤7 days after last dose.
14	For children 5–11 years old, additional questions will be asked in context of Columbia-Suicide Severity Rating Scale (C-SSRS; see Section 4.6.1.12).
15	Patients will be asked additional questions after completion of the PedsQL.
16	Safety interview will occur either at site or during telephone visit and will follow semi-structured interview as listed in Appendix 8. It will include a check on compliance with body temperature monitoring.
17	If these hematology samples are required, visits must be conducted either at the site or by a home nurse if appropriate.

Appendix 10
Schedule of Assessments: Open-Label Extension from Weeks 52 to 76

	Site	Site	Site	FU (2 W)	FU (6 W)
Week	64 [2]	76 [2]	EW [2]	78	88 [2]
Day	449	533	NA		
Time window (days)	±2	±2	NA	±2	7
Study site visits	x	x	x		x
Informed consent					
Physical and neurological examination	x	x	x		x
Tanner staging [3]					x
Anthropometric measurements	x	x	x		x
Vital signs		x	x		x
ECG-12 lead		x	x		x
Hematology	P	x	x	x	x
Full serum chemistry [5]	P	x [6]	x [6]		x [6]
Safety questions [1]	x	x	x	x	
Coagulation		x	x		
Urinalysis		x	x		x
Pregnancy test [4]	x	x	x		x
Dispensing of study medication	x				
Administration of study medication	x	x	x		
PK blood samples		x	x [7]		

Appendix 10
Schedule of Assessments: Open-Label Extension to Week 76 (cont.)

	Site	Site	Site	FU (2 W)	FU (6 W)
Week	64 [2]	76 [2]	EW [2]	78	88 [2]
Day	449	533	NA		
Time window (days)	±2	±2	NA	±2	7
Vineland™–II (survey)	x	x	x		x
ABC	x	x	x		x
RBS-R	x	x	x		x
CaGI	x	x	x		
PedsQL (Family Impact Scale)	x	x	x		
C-SSRS [8]	x	x	x		x
PedsQL (Core and Cognitive Scale) [9]	x	x [9]	x [9]		
CGI-S	x	x	x		x
CGI-I	x	x	x		x
Adverse events	←	—	—	—	→
Previous and concomitant treatments	←	—	—	—	→

ABC = Aberrant Behavior Checklist; CaGI = Caregiver-reported Global Impression; CGI-I = Clinical Global Impressions of Improvement; CGI-S = Clinical Global Impressions of Severity; ECG = electrocardiogram; EW = Early Withdrawal; FU = Follow-Up; PedsQL = Pediatric Quality of Life; PK = pharmacokinetic; RBS-R = Repetitive Behavior Scale-Revised; W = Week(s).

☎ Phone call interview

P Pre-dose. The first dose of study medication has to be taken after all pre-dose assessments.

1 Safety interview will occur either at site or during telephone visit and will follow semi-structured interview as listed in Appendix 8.

Appendix 10
Schedule of Assessments: Open-Label Extension to Week 76 (cont.)

2 If necessary these clinic visits (Week 64, Week 76, Early Withdrawal Visit, and 6-week Follow-Up Visit) can be performed over 2 consecutive days; assessments at screening can be performed over the entire screening time window. After premature discontinuation, an Early Withdrawal Visit should be arranged as soon as possible (see also Section 4.7.1.1)

3 Tanner staging will be done in patients 7 years and older, but not after menarche in female patients and not after voice break in male patients.

4 Pregnancy tests will be performed in female patients after menarche only.

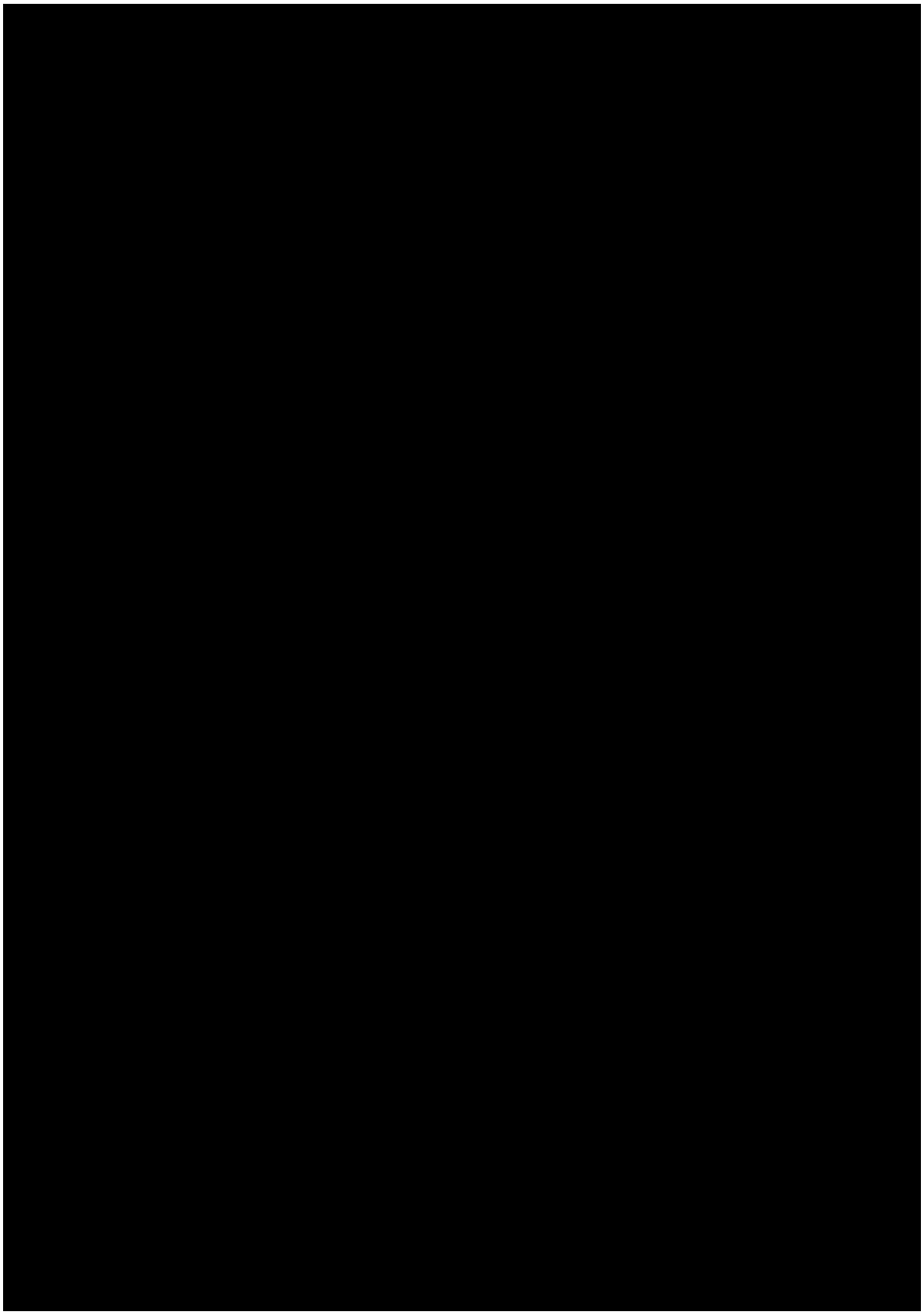
5 See Section 4.6.1.7 and lab manual for more details about full serum chemistry.

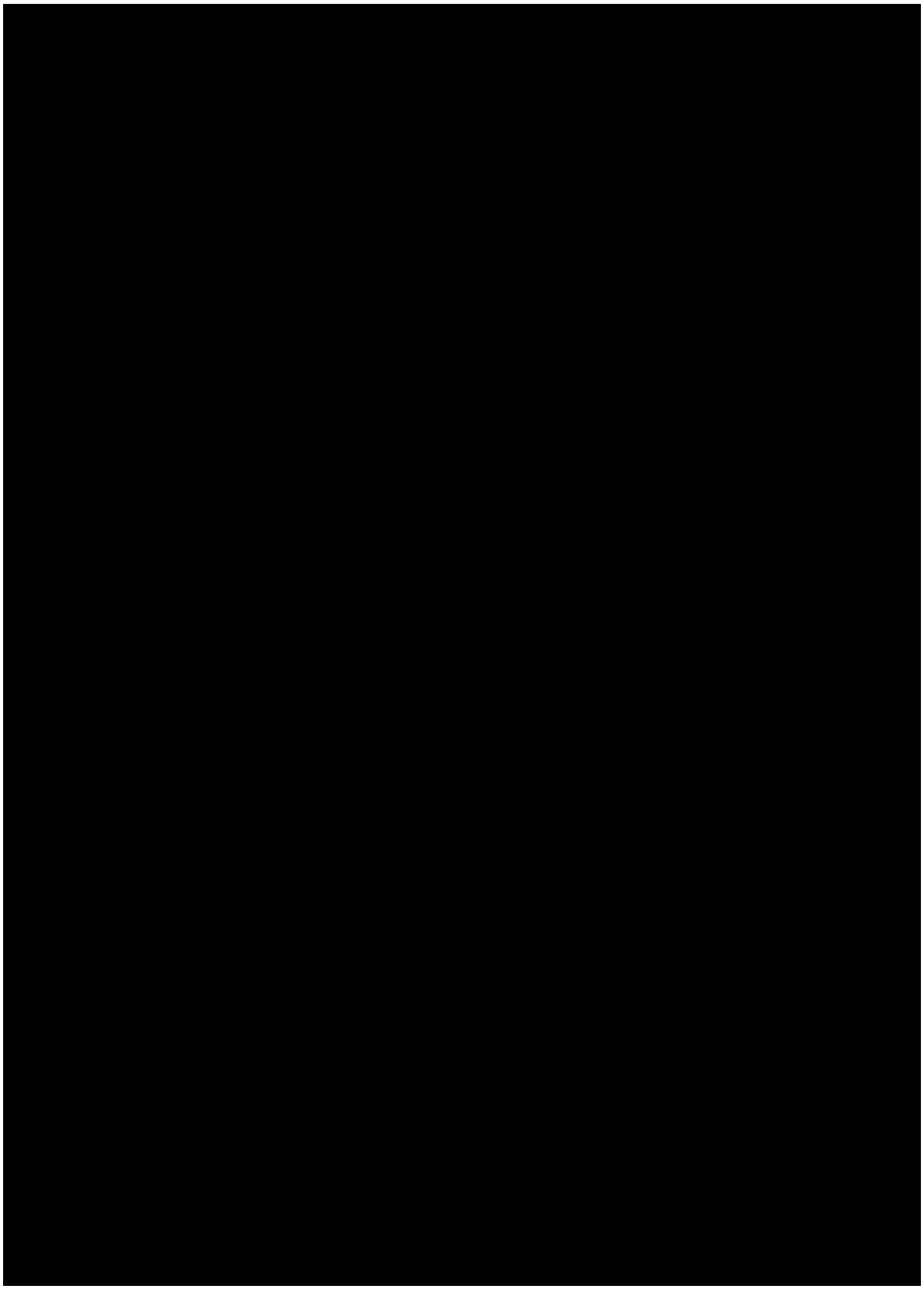
6 At rescreening (substudy only), Week 76/Early Withdrawal Visit, and Follow-Up Visit (6 weeks), glycated hemoglobin (HbA1c), thyroid stimulating hormone (TSH), and free thyroxine (T4) will be assessed.

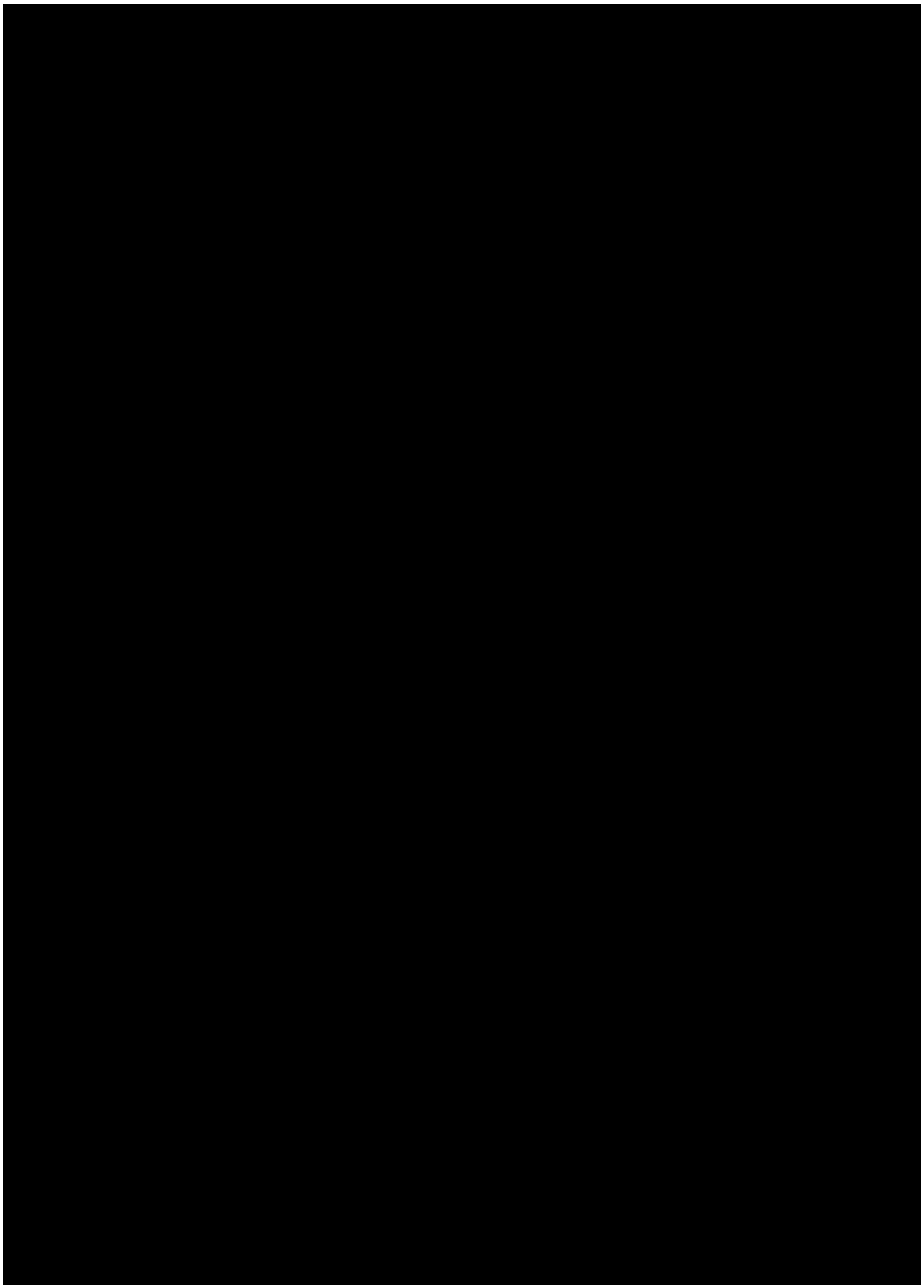
7 PK samples should be collected in those that withdraw from the study early.

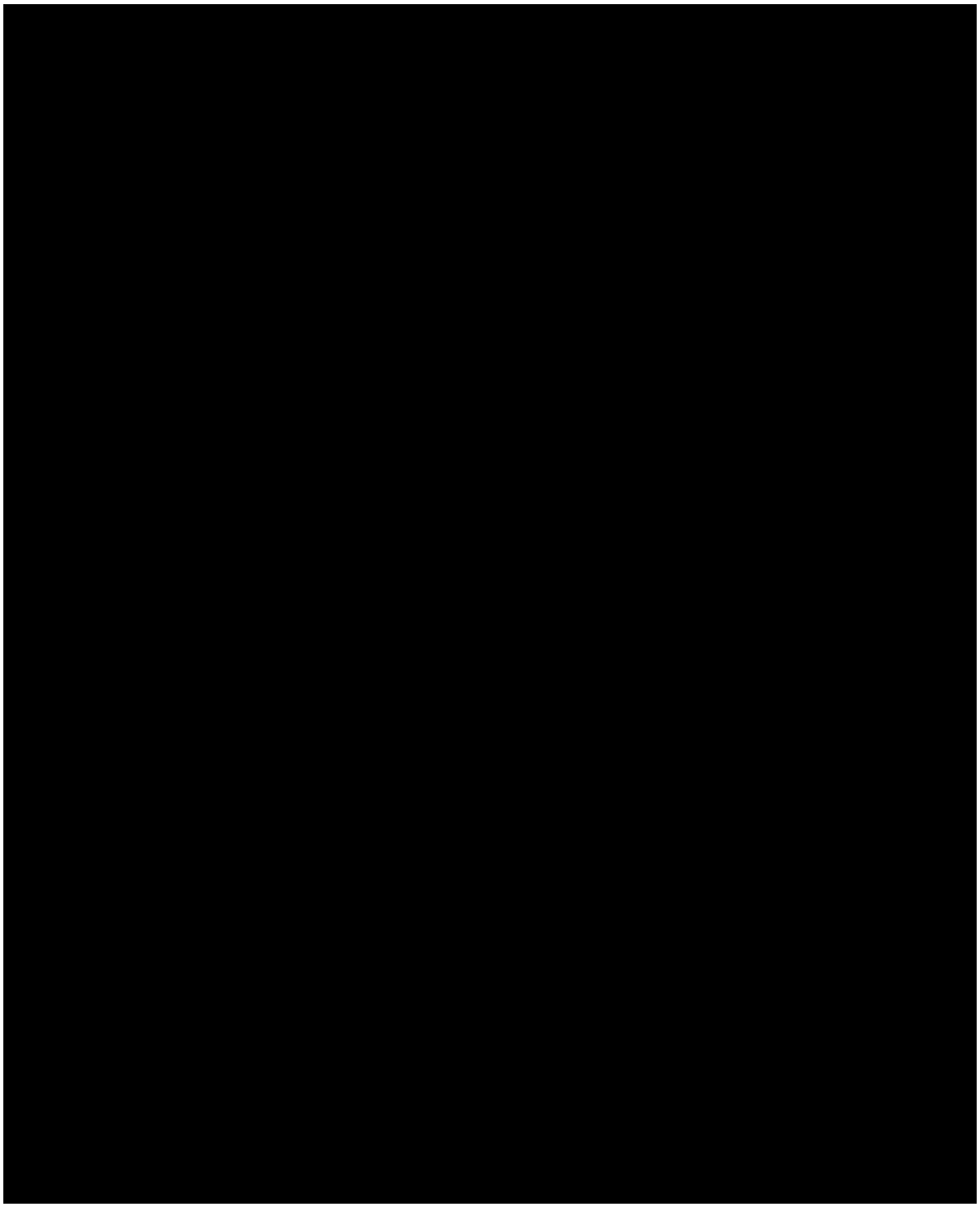
8 For children 5–11 years old, additional questions will be asked in context of Columbia-Suicide Severity Rating Scale (C-SSRS; see Section 4.6.1.12).

9 Patients will be asked additional questions after completion of the PedsQL.









Official Title: A Phase II Multi-Center, Randomized, Double-Blind, 24 Week, Parallel Group, Placebo-Controlled Study to Investigate the Efficacy and Safety of Balovaptan (RO5285119) in Children and Adolescents Age 5-17 With Autism Spectrum Disorder (ASD)

NCT Number: NCT02901431

Document Date: SAP Version 2: 14-May-2019

Technical Document detailing the Statistical Analysis Plan of Study BP30153 (aV1ation)

Title: A PHASE II MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, 24-WEEK, PARALLEL GROUP, PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF BALOVAPTAN (RO5285119) IN CHILDREN AND ADOLESCENTS AGE 5-17 WITH AUTISM SPECTRUM DISORDER (ASD)

Protocol Number: BP30153 (aV1ation)

Study Drug: Balovaptan

[redacted]

14 May 2019
Date

[redacted]

21 May 2019
Date

[redacted]

5 June 2019
Date

Version: 2.0

Date: 14-MAY-2019

Table of Contents

1 BACKGROUND 4

2 STUDY DESIGN 4

Figure 1. Study Design for subjects enrolled up to Version 5 5

Table 1. Age-adjusted Starting Doses Based on PBPK Model 5

Table 2. Updated Age-adjusted Doses Based on PK Data 5

Figure 2. Study Design for subjects enrolled in accordance to Version 6 6

Table 3. Study Treatments in the 24-weeks Main Part (Study Part 2) 7

2.1 OBJECTIVES 8

2.1.1 Primary Objective 8

2.1.2 Secondary Objectives 8

2.2 OUTCOME MEASURES 8

2.2.1 Primary Efficacy Endpoint 8

2.2.2 Secondary Efficacy Endpoints 9

2.2.3 Exploratory Efficacy Endpoints 9

2.2.4 Pharmacokinetic Endpoints 9

2.2.5 Safety Endpoints 9

2.3 DETERMINATION OF SAMPLE SIZE 10

2.4 ANALYSIS TIMING 10

3 STUDY CONDUCT 10

3.1 RANDOMIZATION CONSIDERATIONS 10

3.2 TREATMENT GROUPING 11

3.3 TIME-WINDOWS 12

4 STATISTICAL METHODS 12

4.1 ANALYSIS DATASETS 12

Table 4. Patterns of subjects' disposition 13

4.2 ANALYSIS POPULATIONS 13

4.2.1 Safety Population 14

4.2.2 Efficacy Population 14

Table 5. Patterns of subjects' disposition identifying Safety and Efficacy Analysis Population/Dataset 15

4.2.3 PK/PD Population 15

4.3 ANALYSIS OF STUDY CONDUCT 15

4.4 ANALYSIS OF TREATMENT GROUP COMPARABILITY 15

4.5 EFFICACY ANALYSES 15

4.5.1 Subgroup Analyses 16

4.5.2 Interim Analysis 17
4.5.3 PK/PD Analyses 17
4.6 SAFETY ANALYSES 17
5 DERIVED ENDPOINTS 19

1 BACKGROUND

This document describes the methods of summarizing and analyzing the data collected in Study BP30153. The main purpose of this document is to describe the data handling rules, derivation rules and statistical analysis methods for the final analysis of the double-blind, 24-week, placebo-controlled period that will be performed by Biostatistics and Statistical Programming and Analysis and that will be reported in the Clinical Study Report. An independent document will be prepared to describe the methods that will be used to summarize the open-label extension (OLE) data. Rules and methods here described will be applied, as appropriate, for any efficacy and safety interim analysis allowed by the study protocol.

Details on the PK and PK/PD analyses performed by the Pharmacometrics group within Clinical Pharmacology may be reported in a document separate from the Clinical Study Report.

This version 2.0 has been developed based on version 1.0, issued on 6-Aug-2018, on study protocol BP30153 version 6, issued on 19-Dec-2018, and on Study File Note "Dose Adjustment Following Review of PK Data by the IMC SOC" issued on 19-Jan-2019.

2 STUDY DESIGN

Study protocol BP30153 version 1-5 were conceived as "A PHASE II MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, 24-WEEK, **3-ARM, PARALLEL GROUP**, PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF *BALOVAPTAN (*RO5285119*)* IN CHILDREN AND ADOLESCENTS AGE 5-17 WITH AUTISM SPECTRUM DISORDER (ASD)", and version 6 as "A PHASE II MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, 24-WEEK, **PARALLEL GROUP**, PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE EFFICACY AND SAFETY OF BALOVAPTAN (RO5285119) IN CHILDREN AND ADOLESCENTS AGE 5-17 WITH AUTISM SPECTRUM DISORDER (ASD)" with recruitment to the balovaptan 4mg equivalent treatment arm closed.

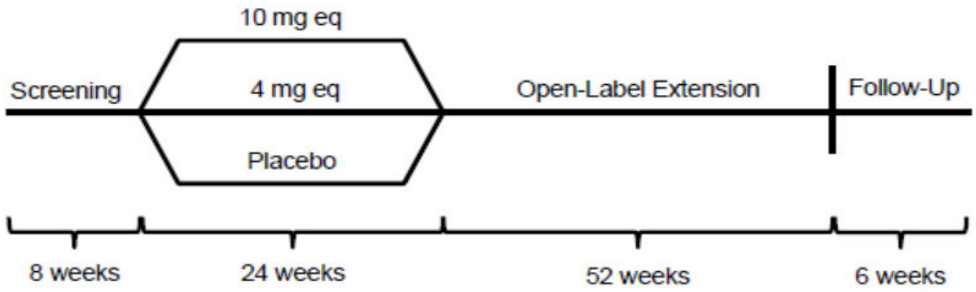
The overall study design was built by staggering enrollment by subject's age, starting first with adolescents (aged 13 to 17 years) and then with children (aged 5 to 12 years). Once the Internal Monitoring Committee (IMC) and the Scientific Oversight Committee (SOC) agreed on acceptable safety and tolerability in the first adolescent cohort (based on data from 8 weeks of treatment, First Study part) and determined the final doses, enrollment of adolescents resumed (for 24 weeks of treatment, Main Study part) and enrollment of a first cohort of younger subjects commenced. Similar process occurred for the first children cohort. See the Study Protocol for more details.

Approximately 340 children and adolescents aged 5 – 17 years with ASD are expected to be recruited to ensure a total of 160 evaluable subjects with placebo or 10 mg equivalent after 24 weeks of treatment.

Subjects who complete the double-blind 24-week treatment period are allowed to participate in an optional 52-week Open-Label Extension (OLE) period where they receive open-label balovaptan treatment (from Week 24 to Week 76).

For all subjects enrolled prior to protocol version 6, study design can be visualized as follows:

Figure 1. Study Design for subjects enrolled up to Version 5



Doses were initially administered as follows:

Table 1. Age-adjusted Starting Doses Based on PBPK Model

Age (years)	4 mg eq* Dose (mg)	10 mg eq* Dose (mg)
5 - 7	1.5	3
8 - 11	2	5
12 -14	3	7
15- 17	4	10

*e.q., dose predicted to achieve exposures to 4 mg/day or 10 mg/day, respectively, in adults

During the study, at planned IMC and SOC reviews of accrued data, the starting doses in Table 1 were shown to not achieve the target exposure in children aged between 5 and 14 years. Therefore, new doses were identified to produce exposures equivalent to the 4 mg and 10 mg doses used in adults (Study BP28420) as described by the following Table:

Table 2. Updated Age-adjusted Doses Based on PK Data

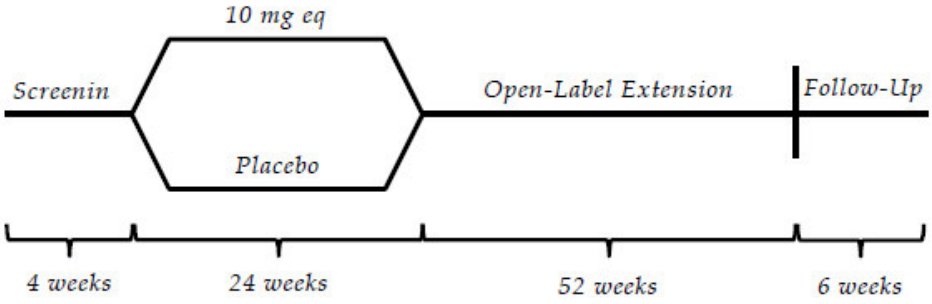
Age (years)	*4 mg eq Dose (mg)*	*10 mg eq Dose (mg)*
5–7 [a]	*3*	*7*
8–17	*4*	*10*

eq = age adjusted dose equivalent to adult dose; PK = pharmacokinetic.

[a] *Dose predicted to achieve exposures equivalent to 4 mg/day or 10 mg/day, respectively, in adults*

For subjects enrolled in accordance to protocol version 6, study design can be visualized as follows:

Figure 2. Study Design for subjects enrolled in accordance to Version 6



eq = equivalent.

For subjects randomized in accordance to protocol version 5 but after the 1st of October 2018 the dose is given, once the local IRB approved the plan described in the "Dose Adjustment Following Review of PK Data by the IMC SOC", at the age-adjusted dose level as described in Table 2.

For subjects still on-treatment in accordance to protocol version 5 after the 1st of October 2018 the dose is increased, once the local IRB approved the plan described in the "Dose Adjustment Following Review of PK Data by the IMC SOC", to the age-adjusted dose level as described in Table 2.

The following Table describes the disposition of subjects in relationship with study treatment in the Main study part, 24-weeks period.

Table 3. Study Treatments in the 24-weeks Main Part (Study Part 2)

Age (yrs)	Treatments randomized (1:1:1) according to Protocol Version 1-5			Treatments randomized (1:1:1) according to Protocol Version 5 and since 1st Oct 2018 allowed to start as (*) or to increase to (→) the Adult dose after local IRB approval				Treatments randomized (1:1) according to Protocol Version 6	
	Plc	4 mg eq	10 mg eq		Plc	4 mg eq	10 mg eq	Plc	10 mg eq
5-7	Plc	1.5 mg	3 mg	Treatment started as:	Plc*	3 mg*	7 mg*	Plc	7 mg
				Treatment increased to:	Plc → Plc	1.5 mg → 3 mg	3 mg → 7 mg		
8-12	Plc	2 mg	5 mg	Treatment started as:	Plc*	4 mg*	10 mg*	Plc	10 mg
				Treatment increased to:	Plc → Plc	2 mg → 4 mg	5 mg → 10 mg		
13-14	Plc	3 mg	7 mg	Treatment started as:	Plc*	4 mg*	10 mg*	Plc	10 mg
				Treatment increased to:	Plc → Plc	3 mg → 4 mg	7 mg → 10 mg		
15-17	Plc	4 mg	10 mg		Plc*	4 mg	10 mg	Plc	10 mg

The primary study objective (balovaptan 10 mg equivalent compared to placebo) will be addressed by analyzing the dataset obtained by pooling data from subjects that took placebo or balovaptan 10 mg equivalent, as highlighted in orange in Table 3, and belonging to patterns c) or d), as highlighted in yellow in Table 4 (see Section 4. Statistical Methods for more details).

2.1 OBJECTIVES

2.1.1 Primary Objective

The primary objectives of this study is:

- To evaluate the efficacy of 24-week treatment with balovaptan (RO5285119) 10 mg equivalent compared to placebo as measured by the change from baseline on the Vineland™-II Adaptive Behavior Scales, second edition (Vineland™-II) Two Domain Composite (2DC) (average of Communication and Socialization domains)

2.1.2 Secondary Objectives

The secondary objective of this study are as follows:

- To evaluate the efficacy of treatment with balovaptan 10 mg equivalent vs. placebo on:
  - Change from baseline on the Vineland™-II Composite standard score after 12 weeks and 24 weeks of treatment
  - Change from baseline in the Vineland™-II Communication, Socialization and Daily Living Skills domain standard scores after 12 weeks and 24 weeks of treatment
  - Proportion of subjects with ≥6-point improvement in the Vineland™-II 2DC score (clinically meaningful response)
  - Change from baseline in severity of clinical impressions as measured by CGI-S (Clinical Global Impressions-Severity) and OACIS-S (Ohio Autism Clinical Impressions Scale-Severity) after 12 weeks and 24 weeks of treatment
  - Improvements in clinical impressions as measured by CGI-I (Clinical Global Impressions-Improvement) and OACIS-I (Ohio Autism Clinical Impressions Scale-Improvement) after 12 weeks and 24 weeks of Treatment
  - Change from baseline in patient- or parent-reported Pediatric Quality of Life (PedsQL) v4.0 Generic Core Scale after 12 weeks and 24 weeks of treatment
  - Change from baseline in the Vineland™-II Composite standard score in adolescents and children independently after 12 weeks and 24 weeks of treatment
  - Change from baseline on the Vineland™-II 2DC score after 12 weeks of treatment
- To evaluate safety and tolerability of 24 and up to 76 weeks of treatment with balovaptan
- To evaluate the pharmacokinetics and exposure-response relationships of balovaptan and its metabolites, if appropriate

2.2 OUTCOME MEASURES

2.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline at Week 24 on the Vineland™-II Adaptive Behavior Scales 2-Domain Composite (2DC) Score, defined as the mean of the Communication domain standard score and the Socialization domain standard score.

2.2.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are as identified in the list of secondary objectives (see Section 2.1.2)

2.2.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are as follows:

- Change from baseline in behaviors as measured by Aberrant Behavior Checklist (ABC) Lethargy/Social withdrawal subscale after 12 weeks and 24 weeks of treatment
- Change from baseline in repetitive behaviors as measured by Repetitive Behavior Scale-Revised (RBS-R) after 12 weeks and 24 weeks of treatment
- Change from baseline to Week 76 (52 weeks of open-label treatment) as measured by Vineland™-II 2DC score
- Change from Week 24 to Week 76 (52 weeks of open-label treatment) as measured by Vineland™-II 2DC score
- Proportion of subjects with ≥4-point improvement in Vineland™-II 2DC score
- Proportion of subjects with ≥8-point improvement in Vineland™-II 2DC score
- Data (social symptoms, communication, and behavior) from Exit Interviews
- Change from baseline in behaviors as measured by ABC Irritability, Stereotypic Behavior, Hyperactivity/Noncompliance, and Inappropriate Speech subscales after 12 weeks and 24 weeks of treatment
- Change from baseline in patient- or parent-reported PedsQL Cognitive Functioning Scale and parent-reported PedsQL Family Impact Scale after 12 weeks and 24 weeks of treatment
- Change from baseline in caregiver-reported Global Impression (CaGI) severity on communication skills, social skills, daily living skills and overall autism symptoms after 12 weeks and 24 weeks of treatment
- Improvements in caregiver-reported Global Impression (CaGI) impression on communication skills, social skills, daily living skills and overall autism symptoms after 12 weeks and 24 weeks of treatment
- Palatability assessments (taste and acceptability)

2.2.4 Pharmacokinetic Endpoints

The pharmacokinetic endpoints are as follows:

- Apparent clearance (CL) and volume of distribution (VD)
- Exposure at steady-state (AUC0-24,ss)
- Other individual exposure estimates (e.g., Cmax, Tmax, AUC for a specific time interval) may also be derived as appropriate

2.2.5 Safety Endpoints

Safety will be assessed through:

- 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, ECGs and C-SSRS

2.3 DETERMINATION OF SAMPLE SIZE

Prior to protocol version 6, a sample size of 240 subjects with ASD (80 per treatment arm) providing evaluable data at Week 24 ensured the study 80% power to detect as statistically significant, at 1-sided 5% significance level, a difference between each active dose and placebo with an effect size of at least 0.4. For the Vineland™-II 2DC score, assuming a standard deviation of about 12.5 points, then the effect size of 0.4 corresponds to 5 points. No adjustment for multiple doses was performed. Considering a withdrawal rate of around 15-20%, it was planned to recruit approximately 300 subjects overall.

In accordance to protocol version 6, 80 subjects per treatment arm (balovaptan 10 mg equivalent and placebo) are required, for a total sample size of approximately 160 subjects with ASD with evaluable data at Week 24. To achieve this number of evaluable subjects, the overall sample size in the study since it started is expected to increase to approximately 340 subjects.

2.4 ANALYSIS TIMING

The primary analysis will be conducted when the double-blind 24-week treatment period ends. Database lock 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.

An efficacy and safety interim analysis is planned once approximately 80 subjects taking either balovaptan 10 mg equivalent or placebo (i.e., approximately 40 subjects per treatment arm) have completed their 12-week visit without dose interruptions or adjustments to allow internal decisions for the next steps of the development plan.

Given the hypothesis generating nature of this study, the Sponsor may conduct up to two interim analyses beyond what is specified elsewhere in this protocol. The decision to conduct such an interim analysis and its timing will be documented in the Sponsor's study master file prior to the conduct of the interim analysis. The clinical study report will also document that such an interim analysis occurred. Interim analyses will be performed and interpreted by the IMC and SOC members (as appropriate), who will have full access to unblinded data.

3 STUDY CONDUCT

3.1 RANDOMIZATION CONSIDERATIONS

After all subjects have been randomized, the data entered into the IxRS system will be reviewed for consistency with the data entered into the Case Report Form (CRF). In

particular, the randomization dates and stratification factors (sex and age) will be checked and a summary of the discrepancies between the IxRS and CRF will be reviewed.

3.2 TREATMENT GROUPING

As the starting doses in children aged between 5 and 14 years were shown to not achieve the target adult exposure, the treatment grouping originally expected - Placebo, balovaptan 4 mg equivalent and balovaptan 10 mg equivalent - is no longer tenable.

Given the numerous different dose regimens occurred in the study, as described in Table 3, the individual treatment level will be ultimately identified based on individual subject's PK exposure, estimated as the average plasma concentration (C_{ave}) since treatment start.

C_{ave} was not specifically listed in the Pharmacokinetic endpoints section of the protocol, however, in the situation of possible dose adaptations within an individual during the course of the study, C_{ave} is the PK exposure measure that reflects most realistically the individual exposure. Moreover, C_{ave} also takes into account potential dose interruptions during treatment. For subjects without dose changes or dose interruptions, Cave is approximately equal to $AUC_{0\text{-}24,ss}$ divided by 24 hrs. Individual C_{ave} will be estimated by the pharmacometrics group in Clinical Pharmacology for all subjects with at least one adequately documented PK measurement at steady-state, using a population PK modeling approach. C_{ave} at week 12 will be used as a classification factor to split the subjects receiving balovaptan into three equally sized exposure sub-groups: Low Tertile, Medium Tertile and High Tertile.

If insufficient exposure data are available for a subject and C_{ave} cannot be derived (this can happen, for example, to withdrawals or in case of issues with PK sample analyses), then his/her data will contribute to the same treatment group of the majority of his/her coetaneous taking the same regimen as per randomisation. For example, if a 6 years-old initially randomized to 1.5mg switched to 3mg and no PK data are available for him/her, then his/her data will be attributed to the Low Tertile if most of the 5-7 years-old subjects taking the same regimen are allocated, based on their available exposure, to the Low Tertile. In case no other coetaneous providing exposure data went through the same regimen pattern (as in the example, increasing dose from 1.5mg to 3mg) then data will be attributed to the same exposure sub-group of the majority of his/her coetaneous taking the same final dose (in the example, 3mg).

This treatment grouping will be used for descriptive (ie, not inferential) statistical reporting purposes of efficacy and safety data.

For pharmacometric exploratory graphical analyses, missing PK information will not be inputed. In case of missing PK information for subjects assigned to a starting dose other than Placebo, the subject with missing PK information will be excluded from exposure-efficacy analyses.

3.3 TIME-WINDOWS

Efficacy and safety analyses will be performed according to the data collected at the originally scheduled visit.

In case of deviations from the visit date scheduled as per protocol the following boundaries will be implemented to identify time-windows:

Site Visit	Time-window (days)	Range (days)
Baseline	Before or soon after Start Dosing	Up to the 1st day of dosing
Week 6	43 ± 21	23 – 64
Week 12	85 ± 21	65 – 106
Week 18	127 ± 21	107 – 148
Week 24	169 ± 21	149 – at most 21 days after last double-blind Dose and prior to OLE Screening

For Screening, the scheduled visit will be used regardless of the actual assessment days relative to the first day of study drug.

Baseline Definition

Unless otherwise stated, baseline is defined as the last non-missing value recorded before the first study drug administration of each repeated study drug period (ie. 8 weeks for the First Part, and 24 weeks for the Main Part) or soon after the start of the dosing (see Table above). The last observation can be an unscheduled / repeated measurement.

Baseline for Electrocardiograms (ECGs) is derived as the mean of the triplicate measurements of pre-dose assessments.

Unscheduled Measurements

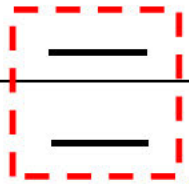
Unscheduled measurements will be included in the listings. Except for unscheduled measurements used for baseline, unscheduled measurements will be excluded from the descriptive statistics and statistical analysis.

4 STATISTICAL METHODS

4.1 ANALYSIS DATASETS

According to the protocol, the disposition of subjects in relationship with each Study Part may be as follows:

Table 4. Patterns of subjects' disposition

Pattern	**Description**: Subjects …	**First Part** (Study part 1): up to 8 weeks of Treatment	**Main Part** (Study part 2): 24 weeks of Treatment
a)	stopped treatment prior to determination of final doses by IMC/SOC		
b)	stopped treatment prior to determination of final doses by the IMC/SOC and took the opportunity to restart in the Main Part		
c)	allowed by the IMC/SOC to continue and complete the 24 weeks of treatment without dose interruption/adjustment		
d)	enrolled in the Main Part		

The primary **Efficacy** treatment comparison (balovaptan 10 mg equivalent dose vs. placebo) will be performed on the dataset obtained by pooling data from patients that were expected to take treatment for 24 weeks, as described by patterns c) and d) [highlighted in yellow].

Safety analysis will be performed separately on two datasets, according to the duration of the seamless treatment period that subjects have been exposed to. Therefore, two safety analysis datasets are identified:

- **First Part (8 weeks)** - Data from subjects that took treatment up to a maximum of 8 weeks, as described in pattern a) and 1st part of b) [framed with dotted red line]
- **Main Part (24 weeks)** - Data from subjects that took treatment up to a maximum of 24 weeks, as described in pattern c), d), and 2nd part of b) [framed with dotted blue line]

4.2 ANALYSIS POPULATIONS

Given the impact of the early findings from the starting doses on the actual doses regimens implemented in the trial, on the final treatment classification required for summary purposes and on the identification of the appropriate dataset to estimate the treatment comparison, the following populations are considered in order to provide an exhaustive reporting of this study data.

4.2.1 Safety Population

- *Safety Global*

The “Safety Global” population consists of all subjects who gave informed consent and received at least one dose of study medication, whether prematurely withdrawn from the study or not.

Data will be summarized according to treatment classified as derived from PK exposure: Placebo, Low Tertile, Medium Tertile, High Tertile and All Treated with Active.

- *Safety Inferential*

The “Safety Inferential” population consists of the subset of the “Safety Global” population that is identified to address the primary efficacy study objective.

Data will be summarized according to treatment classified as: Placebo, 10mg equivalent.

4.2.2 Efficacy Population

- *Efficacy ITT Global*

The “Efficacy Global” population consists of all subjects who gave informed consent and received at least one dose of study medication, whether prematurely withdrawn from the study or not.

Data will be summarized according to treatment classified as derived from PK exposure: Placebo, Low Tertile, Medium Tertile, High Tertile.

- *Efficacy ITT Inferential*

The “Efficacy ITT Inferential” population is the primary population of interest. It consistsof the subset of the “Efficacy Global” population obtained by pooling data from subjects taking balovaptan 10 mg equivalent or the concurrently randomized placebo in the corresponding age band and in the same randomization stage, as highlighted in orange in Table 3, and belonging to patterns c) or d), as highlighted in yellow in Table 4. Subjects with dose adjustments or interruptions, or on a different dose than the final dose for their age group, are excluded and do not contribute to this analysis population.

Data will be summarized according to treatment classified as: Placebo, 10mg equivalent.

The following Table shows the combination of subjects' patterns (described in Table 4) to identify the Safety and Efficacy analyses population/dataset:

Table 5. Patterns of subjects' disposition identifying Safety and Efficacy Analysis Population/Dataset

Analysis Population:	Analysis dataset:	
	First Part (8 weeks)	**Main Part (24 weeks)**
Safety Global	a + 1st part of b	2nd part of b + c + d
Safety Inferential	-	c + d
Efficacy ITT Global	-	2nd part of b + c + d
Efficacy ITT Inferential	-	c + d

4.2.3 PK/PD Population

The PK/PD population will be used for exploratory PK/PD analyses. The PK/PD population consists of a subset of the Efficacy Global population and includes all subjects with at least one post-baseline efficacy measurement and an exposure estimate. For subjects assigned to Placebo, exposure will be assumed to be 0. In case of missing PK information for subjects assigned to a starting dose other than Placebo, the subject with missing PK information will be excluded from exposure-efficacy analyses.

4.3 ANALYSIS OF STUDY CONDUCT

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

4.4 ANALYSIS OF TREATMENT GROUP COMPARABILITY

Demographic and baseline characteristics (including age, sex, WASI-II IQ score, SRS-2 total t-score, Vineland™-II 2DC score) and clinically relevant disease characteristics (including background antipsychotics) will be summarized for the Safety population/dataset by treatment group using means, standard deviations, medians and ranges for continuous variables and proportions for categorical variables, as appropriate.

4.5 EFFICACY ANALYSES

Summary tables and graphs will be provided both for the "Efficacy ITT Global" and for the "Efficacy ITT Inferential" populations, while listings of individual data will be provided for the "Efficacy ITT Global" population only, separately by subjects' patterns (b, c, d).

Inferential statistical analysis will be performed on pooled data from subjects taking balovaptan 10 mg equivalent dose compared with those from the concurrently randomized placebo in the corresponding randomization stage, as identified by the "Efficacy ITT Inferential" population. Multiple endpoints will be analyzed however, due to the exploratory nature of the study, multiplicity will not be statistically adjusted for and the risk of false positive results will be taken into consideration during the interpretation of the results.

The primary efficacy endpoint, the change from baseline of Vineland™-II 2-DC standard score, as well as the secondary endpoints expressed in terms of change from baseline on a continuous scale (namely, Vineland™-II standard scores, PedsQL Scale factors, ABC factors and RBS-R factors) will be analyzed using Mixed Model Repeat Measurements (MMRM) on the overall population of adolescents and children. The MMRM model will include treatment, sex and visit (week) as main effects, individual age and baseline score as covariates, and treatment-by-visit and baseline-by-visit as interaction terms. Visit will be fitted as a repeated effect with an unstructured correlation structure across visits within each subject. All main effects and interactions will be retained in the final model regardless of their statistical significance.

The results of the analysis will be presented as point estimates, 90% confidence intervals and associated p-values for the adjusted mean differences between 10 mg eq balovaptan and placebo after 24 weeks of treatment as well as at intermediate visits.

The main analysis outlined above will be done using a mixed effect model, which can handle missing data without any need to recur to imputation or discarding of data.

The proportion of subjects with ≥6-point improvement in the Vineland™-II 2DC score (clinically meaningful response) will be analyzed using logistic regression. A Generalised Estimating Equations (GEE) model will be fitted including treatment, sex and visit (week) as main effects, age as covariate, baseline-by-visit and treatment-by-visit interactions, with subject fitted as a repeated effect. It will be based on the binomial distribution and thus the link function will be the 'logit'.

The odds ratio will be presented for descriptive purposes along with the corresponding 90% confidence interval and p-value for the comparisons between 10 mg eq balovaptan and placebo after 24 weeks of treatment as well as at intermediate visits.

Change from baseline in severity of clinical impressions as measured by CGI-S
The proportion of subjects with at least 1-point decrease (i.e. improvement) from baseline on CGI-S will be analyzed using logistic regression. A GEE model will be fitted including treatment, sex and visit (week) as main effects, age and baseline CGI-S as covariates, baseline-by-visit and treatment-by-visit interactions, with subject fitted as a repeated effect. It will be based on the binomial distribution and thus the link function will be the 'logit'.

The odds ratio will be presented for descriptive purposes along with the corresponding 90% confidence interval and p-value for the comparisons between 10 mg eq balovaptan and placebo after 24 weeks of treatment as well as at intermediate visits.

Improvements in clinical impressions, as measured by CGI-I scores
The proportion of subjects with a value of at least 2 (i.e., "Much improved" or better) on CGI-I will be analyzed using logistic regression applying the same model above described for CGI-S.

4.5.1 Subgroup Analyses

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

- Sex (male, female)
- Age (adolescents 13-17yrs, children 5-12yrs)

The statistical model for subgroup analyses of efficacy data will be performed using MMRM with subgroup, treatment-by-subgroup, subgroup-by-time and treatment-by-subgroup-by-time interaction terms included along the independent effects described above.

Estimates of treatment effect and associated 90% CIs will be presented in forest plots. Unadjusted p-values will also be presented for these analyses.

4.5.2 Interim Analysis

The populations assessed at the Interim Analysis will be the "Safety Inferential" and the "Efficacy ITT Inferential" with all subjects randomized prior to 21FEB2019, which is the date of the last subject expected to provide 12 week data for the 10mg-Placebo comparison by 16MAY19.

The efficacy interim analysis will focus on the outcome measures derived from Vineland™-II and, only descriptively, from PedsQL v4.0 Generic Core Scale, CGI-I and CGI-S.

Summary statistics will be provided for the overall population and for the adolescents/ children subgroups separately.

4.5.3 PK/PD Analyses

An exploratory graphical analysis of the relationship between pharmacokinetic exposure and efficacy will be performed by Clinical Pharmacology. For the exploratory graphical exposure-efficacy analyses, C_{ave} will be used either as a continuous covariate or as grouping variable. Graphical displays will include (but are not limited to) efficacy outcome measure vs. exposure by visit, and efficacy outcome measure vs. time, with trend lines split by exposure category (e.g. placebo, Low, Medium and High Tertile of C_{ave}). The effect of subpopulations will also be investigated.

If warranted after review of initial graphical displays, a model based approach may be considered to quantify the exposure-effect relationship for Vineland™-II 2-DC. A model based exposure-efficacy analysis of other efficacy outcome measures might be considered upon agreement with the project team.

Results from pharmacometric analyses may be reported in a document separate from the clinical study report.

4.6 SAFETY ANALYSES

Summary tables and graphs will be provided both for the "Safety Global" and for the "Safety Inferential" populations, while listings of individual data will be provided for the "Safety Global" population only, separately by subjects' patterns (a, b, c, d).

As appropriate, listings, summary tables and graphs by treatment group will be provided for safety and tolerability assessments, including:

- Incidence of adverse events (overall, by intensity, and by relationship to study medication).

- Serious adverse events and adverse events of special interest will be reported separately.
- [redacted]
- Vital signs (systolic blood pressure, diastolic blood pressure, pulse rate, and orthostatic effects)
- [redacted]
- Physical examination (height, weight, neurological examination)
- Safety laboratory values (including hematology, blood chemistry, coagulation, and urinalysis parameters)
- Incidence of marked laboratory abnormalities
- Clinical assessment of suicidality (C-SSRS)

Adverse Events

Verbatim descriptions of adverse events recorded on the eCRF by the Investigator during the study period will be mapped to the Medical Dictionary for Regulatory Activities (MedDRA) thesaurus terms. All adverse events will be tabulated by body system and preferred term for individual events within each body system. Adverse events will also be tabulated by severity and relationship to the study medication.

Serious adverse events will be summarized separately.

Clinical Laboratory

Subjects' listings and summary statistics by treatment group at each assessment time will be presented using the International System of Units (SI units; Système International d'Unités). Laboratory data not reported in SI units will be converted to SI units before processing.

Laboratory test values will be presented by individual listings with flagging of values outside the normal ranges Roche standard reference ranges, rather than the reference ranges of the Investigator, will be used for all parameters. For most parameters the measured laboratory test result will be assessed directly using the Roche standard reference range. A transformation will be performed on some laboratory tests that lack sufficiently common procedures and have a wide range of Investigator ranges, e.g., enzyme tests that include AST, ALT, and alkaline phosphatase and total bilirubin. If the standard reference ranges for these parameters have a lower limit of zero only the upper limits of the ranges will be used in transforming the data.

For all laboratory parameters included, there exists a Roche predefined standard reference range. Laboratory values falling outside this standard reference range will be labeled "H" for high or "L" for low in study subject listings of laboratory data.

In addition to the standard reference range, a marked reference range has been predefined by Roche for each laboratory parameter. The marked reference range is broader than the standard reference range. Values falling outside the marked reference range that also

represent a defined change from baseline will be considered marked laboratory abnormalities (i.e., potentially clinically relevant). If a baseline value is not available for a subject, the midpoint of the standard reference range will be used as the subject's baseline value for the purposes of determining marked laboratory abnormalities. Marked laboratory abnormalities will be labeled in the subject listings as "HH" for very high or "LL" for very low.

Vital Signs and ECG

Vital signs and ECG will be presented by individual listings and summary tables by treatment group and time of raw values and change from baseline.

Concomitant Medications

Concomitant medications will be presented in summary tables and listings.

5 DERIVED ENDPOINTS

- **Aberrant Behavior Checklist (ABC)**

  The ABC consists of 58 items subdivided in 5 subscales: Irritability, Lethargy and Social Withdrawal, Stereotypic Behavior, Hyperactivity/Non-Compliance and Inappropriate Speech.

  The total scores for each of the 5 subscales (but not the values of all 58 items) will be electronically transferred to Roche.

- **Pediatric Quality of Life Inventory (PedsQL ™ 4.0) Generic Core**

  The Pediatric Quality of Life Inventory PedsQL™4.0 Generic Core Scale assessment consists of 23 items encompassing 4 core scale domains:

  — Physical Functioning (8 items)

  — Emotional Functioning (5 items)

  — Social Functioning (5 items)

  — School Functioning (5 items)

  summarised into:

  — Total Score (23 items)

  To create the scores for the above described 4 Functioning Scales and the Total Score a mean is computed as the sum of the items divided by the number of items answered (this accounts for missing data). If more than 50% of the items in the scale are missing, the Scale Score is not computed [Viecili and Weiss, 2015].

  Additional Summary Scores will be derived as follows:

  — Psychosocial Health Summary Score, as the sum of the items divided by the number of items (15, in case of no missing values) answered in the Emotional, Social, and School Functioning Scales.

- Physical Health Summary Score, which is the same as the Physical Functioning Scale Score.
- PedsQL Total Score, computed as the sum of all the items divided by the number of items (23, in case of no missing values) answered on all the Scales.

If more than 50% of the items are missing, the Summary Score is not computed [Viecili and Weiss, 2015].

The above described Functioning Scales and Summary Scores will be derived by Roche Statistical Programmer.

- **Pediatric Quality of Life Cognitive Functioning Scale**

  The PedsQL Cognitive Functioning Scale consists of 6 items.

  To create the Cognitive Functioning Total Score a mean is computed as the sum of the items divided by the number of items answered (this accounts for missing data). If more than 50% of the items in the scale are missing, the Scale Score is not computed [Viecili and Weiss, 2015].

- **Pediatric Quality of Life Family Impact Scale**

  The Pediatric Quality of Life Inventory (PedsQL™) Family Impact Module version 2 consists of 36 items encompassing the following 6 scales

  - Physical Functioning (6 items)
  - Emotional Functioning (5 items)
  - Social Functioning (4 items)
  - Cognitive Functioning (5 items)
  - Communication (3 items)
  - Worry (5 items)

  and the 2 following scales measuring parent-reported family functioning

  - Daily Activities (3 items)
  - Family Relationships (5 items)

  The above described 6+2 scales are summarised by mean scores computed as the sum of the items scores divided by the number of items answered (this accounts for missing data). If more than 50% of the items in the scale are missing, the Scale Score is not computed [Varni et al., 2004].

  Additional Summary Scores will be derived as follows:

  - Total Score, by averaging across all 36 items
  - Parent HRQoL Summary Score, by averaging the 20 items in Physical, Emotional, Social and Cognitive Functioning
  - Family Summary Score, by averaging the 8 items in Daily Activities and Family Relationships

The above described Functioning Scale and Summary Scores will be derived by Roche Statistical Programmer.

- **Repetitive Behavior Scale - Revised (RBS-R)**

  The RBS-R is a 43-item informant-based questionnaire assessing the variety of restricted and repetitive behaviors observed in individuals with ASD. The scale is grouped into 6 subscales: Stereotyped, Self-injurious, Compulsive, Ritualistic, Sameness, and Restricted Behaviors.

  The subscales score will be electronically transferred to Roche.

- **Vineland™-II Adaptive Behaviour Scale (VABS)**

  Values of Vineland™-II Adaptive Behavior Composite Score (as Sum and Standard score), domains (as v-scale and standard score for each of the 3 domains, Communication, Daily Living Skills, Socialization), subdomains (as raw and v-scale for each of the subdomains) and age-equivalent values (years:months, for each subdomain) will be derived via computerized system and electronically transferred to Roche.

  The value for the primary endpoint, Vineland™-II Adaptive Behavior Scales 2-Domain Composite (2DC) Score, defined as the mean of the Communication domain standard score and the Socialization domain standard score, will be derived by Roche Statistical Programmer. If any of the two individual domain standard scores is missing the 2DC score is not computed.