Clinical Study Protocol

A Phase 1b, Double-Blind, Placebo-Controlled, First-in-Human Study to Evaluate Safety, Tolerability and Pharmacokinetics of a Two-Week Oral Treatment with STP1 in a Subgroup of Adult Patients with Autism Spectrum Disorder

TEST PRODUCT: **STP1**

STUDY NUMBER: **STP1-C004**

IND NUMBER: **139901**

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Version Number	Edition history	Date	Changes requested by:
1	Original Protocol	31 August 2020	
2	Amendment 1	23 October, 2020	FDA and STALICLA
3	Amendment 2	09 December, 2020	STALICLA
4	Amendment 3	03 May 2021	FDA and STALICLA
5	Amendment 4	13 July 2021	STALICLA
6	Amendment 5	15 November 2021	STALICLA

CONFIDENTIAL

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements including, but not limited to, ICH guidelines, the US Code of Federal Regulations, and generally accepted ethical principles for human research such as the Declaration of Helsinki.

Signed: _____ Date: Dec 4, 2021

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INVESTIGATOR, STUDY PERSONNEL AND SITE OF STUDY

This will be a single-center study.

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Protocol Amendment 5 Summary of Changes (15 November 2021)

Section # and Name	Description of Change	Brief Rationale
Title page, protocol approval page, footers, protocol amendment summary of changes.	Updated the protocol version, and date.	To reflect the new version and date of the protocol.
Cover page, Signature page, Section INVESTIGATOR, STUDY PERSONNEL AND SITE OF STUDY	Contact details of the Stalicla Chief Medical Officer added and signature page as well.	To provide information about the Medically Qualified Stalicla staff
SYNOPSIS – NUMBER OF PATIENTS	"…the objective to obtain 8 patients completing each cohort" was replaced by "the objective to obtain up to 8 patients completing each cohort". The following was added: "As a minimum, 3 patients should be exposed to the highest dose of STP1".	A sample size of at least 3 patients exposed to the highest dose will provide sufficient information to meet the primary objective (safety and tolerability).This number could be increased up to 6 patients to support secondary and exploratory objective.
SYNOPSIS – SAMPLE SIZE	«The target number of completers for this Phase 1b study is 16 ASD-Phen1 individuals.» replaced by "The target number of completers for this Phase 1b study is up to 16 ASD-Phen1 individuals.»	A sample size of at least 3 patients exposed to the highest dose will provide sufficient information to meet the primary objective (safety and tolerability).This number could be increased up to 6 patients to support secondary and exploratory objective.
Section 3.1.1. OVERVIEW OF STUDY DESIGN	"…8 additional eligible patients…» replaced by "up to 8 additional eligible patients ..."	A sample size of at least 3 patients exposed to the highest dose will provide sufficient information to meet the primary objective (safety and tolerability).This number could be increased up to 6 patients to support secondary and exploratory objective.

Section # and Name	Description of Change	Brief Rationale
Section 4.3 - METHOD OF TREATMENT ASSIGNMENT AND BLINDING	"Each dose level will include 8 patients" replaced by "Each dose level will include up to 8 patients"	A sample size of at least 3 patients exposed to the highest dose will provide sufficient information to meet the primary objective (safety and tolerability).This number could be increased up to 6 patients to support secondary and exploratory objective.
Section 6.1 - DETERMINATION OF SAMPLE SIZE	"A maximum of approximately 20 patients will be enrolled in this study, to ensure that 16 patients (8 per dose level) will have complete dataset …" replaced by "A maximum of approximately 20 patients will be enrolled in this study, to ensure that up to 16 patients (up to 8 per dose level) will have complete dataset …" AND "Six patients on active and 2 on placebo per dose level (4 on placebo in total)..." replaced by "Up to six patients on active and up to 2 on placebo per dose level (maximum 4 on placebo in total) …".	A sample size of at least 3 patients exposed to the highest dose will provide sufficient information to meet the primary objective (safety tolerability).This number could be increased up to 6 patients to support secondary and exploratory objective.
All document	Version of the investigator' brochure was removed (2020)	This change will cover the updated version(s) of the investigator' brochure.

Protocol Amendment 4 Summary of Changes (13 July 2021)

Section # and Name	Description of Change	Brief Rationale
Title page, protocol approval page, footers, protocol amendment summary of changes.	Updated the protocol version, and date.	To reflect the new version and date of the protocol.
Section SYNOPSIS – STUDY DESIGN and Section SYNOPSIS – SAFETY ASSESSMENT and Section 3.1.1 – OVERVIEW OF	PK data and PD data are removed	The Data Safety Monitoring Board (DSMB) will make the recommendations to STALICLA based on the accumulated study data for patient safety,

Section # and Name	Description of Change	Brief Rationale
STUDY DESIGN and Section 3.1.3 - DATA SAFETY MONITORING BOARD (DSMB)		study conduct and progress and not based on the PK or PD data.
Section - SYNOPSIS – INCLUSION CRITERIA and Section 1.2.1 - CLINICAL SIGNS AND SYMPTOMS OF ASD-PHEN 1 and Section 4.2.2 - INCLUSION CRITERIA and Section 4.6.1.1 – MEDICAL / FAMILY HISTORY AND DEMOGRAPHIC DATA	"Patients must have a documented [redacted] (according to the Centers for Disease Control and Prevention [CDC] growth charts [CDC, 2019])" replaced by "Patients must have a documented [redacted] (according to the Centers for Disease Control and Prevention [CDC] growth charts [CDC, 2019]) or being diagnosed with [redacted]"	Using systems biology computerized modeling, STALICLA has identified a subgroup of patients with ASD. This population referred to as ASD-Phen1, that is defined by the presence of two specific clinical signs and symptoms: an [redacted]
Section 4.2.1 – RECRUITMENT PROCEDURES	"receive" removed	Typo: "receive" was included twice in the previous version
Section 4.6.2 - TIMING OF STUDY ASSESSMENTS – VISIT 4	Visit 4: Urine sampling for PK to be removed	For consistency: timing of PK sampling was modified in a previous amendment, the flow chart was updated accordingly

Section # and Name	Description of Change	Brief Rationale
		but not the section 4.6.2.
Section 4.6.2 - TIMING OF STUDY ASSESSMENTS – END OF STUDY (EOS) FOLLOW-UP VISIT	Blood sampling for PK at any time	For consistency: timing of PK sampling was modified in a previous amendment, the flow chart was updated accordingly but not the section 4.6.2.
Section 4.7.1.1 - DISCONTINUATION FROM STP1	"… 24 patients" replaced by "…16 patients"	The number of patients was modified and approved in the protocol amendment #3 but by mistake the change was not implemented in this section 4.7.1.1
Section 4.7.1.2 – WITHDRAWAL FROM STUDY	"…a total of 24 patients." was replaced by "…a total of 16 patients."	The number of patients was modified and approved in the protocol amendment #3 but by mistake the change was not implemented in this section 4.7.1.2
Section 9.5 – PROTOCOL AMENDMENTS	"chief of translational development and study biostatistician" replaced by "Chief Medical Officer"	New organization in place associated with a new title
Section 11 - APPENDIX 1 SCHEDULE OF ASSESSMENTS – SRS-2	SRS-2 assessment at D28 removed	Correction of the inconsistency between the flowchart (Appendix 1) and the section 4.6.1.8. The assessment is only conducted at the screening and D14 visits as described and the section 4.6.1.8.

Protocol Amendment 3 Summary of Changes (03 May 2021)

Section # and Name	Description of Change	Brief Rationale
Title page, protocol approval page, footers, protocol amendment summary of changes.	Updated the protocol version, and date.	To reflect the new title, version and date of the protocol.
Contact details Primary Sponsor Contact	Update the primary sponsor contact details.	To reflect sponsor's update on personnel.
Protocol synopsis and throughout the protocol	Change the sample size from 12 patients per cohort to 8 patients per cohort	A sample size of eight patients is sufficient to meet the primary objective (safety tolerability)

Section # and Name	Description of Change	Brief Rationale
Protocol synopsis and Section 4.6.2.1 SCREENING AND PRE-TREATMENT ASSESSMENTS.	Glucose measurement has been included in the screening assessment.	As per FDA request.
Protocol synopsis and Section 4.6.1.6 PHARMACOKINETICS ASSESSMENTS and Section 4.6.2 TIMING OF STUDY ASSESSMENT and Section 11 APPENDIX 1 SCHEDULE OF ASSESSMENT	Changes in blood and urine sampling for PK to cover at least 3 half-lives of the IMP.	As per FDA request.
Protocol synopsis, Section 2.3 EXPLORATORY OBJECTIVES and Section 3.3.3 EXPLORATORY OUTCOME MEASURE and Section 4.6.1.8 STUDY-SPECIFIC ASSESSMENTS and Section 4.6.2 TIMING OF STUDY ASSESSMENT and Section 11 APPENDIX 1 SCHEDULE OF ASSESSMENTS	The CGI-S has been included within exploratory assessments.	As per FDA request.
Protocol synopsis, Section 3.3.1 SAFETY OUTCOME MEASURES, Section 4.6.2 TIMING OF STUDY ASSESSMENTS, Section 5.1.4 OTHER SAFETY ASSESSMENTS and Section 11 APPENDIX 1 SCHEDULE OF ASSESSMENTS	Given the risk of ototoxicity with [redacted], hearing assessments will be performed multiple times.	As per FDA request.
Section 1.5.2 BENEFIT-RISK ASSESSMENT and Section 4.5.1.3 MEDICATIONS TO BE AVOIDED WITH STP1.	The concomitant treatment with CYP3A4 moderate or strong inhibitors is excluded.	As per FDA request.
Protocol synopsis and Section 3.1.2 STOPPING RULES and Section 5.1.3 ADVERSE EVENTS OF SPECIAL INTEREST (IMMEDIATELY REPORTABLE TO THE SPONSOR)	Dehydration and orthostasis have been included within the adverse events of special interest.	As per FDA request.

Section # and Name	Description of Change	Brief Rationale
Section 6.7.2 OTHER EXPLORATORY ENDPOINTS.	All exploratory endpoints are now reorganized and also included in the screening process.	For clarity and comprehension. Exploratory endpoints also performed before starting treatment (in the screening period).
Protocol synopsis and Section 4.2.2 INCLUSION CRITERIA	Abstinence is not any more mentioned as an appropriate method of birth control for this study.	As per FDA request
Protocol synopsis and Section 4.2.2. INCLUSION CRITERIA	Requirement for at least a 6-month seizure free period has been added as inclusion criteria	As per FDA request
Protocol synopsis and Section 4.2.3. EXCLUSION CRITERIA	Patients with an identified genetic cause of ASD in their medical record will be excluded from the study.	As per FDA request
Section 11 APPENDIX 1 SCHEDULE OF ASSESSMENT	The suicidality assessments (C-SSRS, C-CASA) has been added to the follow-up visits.	As per FDA request
Section 4.6.1.8 STUDY-SPECIFIC ASSESSMENTS Section 11 APPENDIX 1 SCHEDULE OF ASSESSMENT	Drug accountability has been added to Day 7 and Day 14 visit	
Typos and clarifications.	New references have been added.	Minor corrections and clarifications.

Protocol Amendment 2 Summary of Changes (09 December 2020)

Section # and Name	Description of Change	Brief Rationale
Title Page, Protocol Approval Page, Footers, Protocol Amendment Summary of Changes.	Updated the protocol version and date.	To reflect the new version and date of the protocol.

Protocol synopsis and Section 4.2.2 Inclusion criteria.	BMI is being removed as an inclusion criterion.	BMI inclusion criterion was a European reference. Since the only enrolling site is in the US, where participants are more likely to have an increased BMI, this criterion is not truly applicable for this study. Additionally, there are no safety reasons for BMI limits given the profile of the drugs in STP1.
Typos and clarifications:	• Triplicate ECG on Day 1 (baseline) visit now clarified to be recorded 1 minute apart. • PK sampling points now include a 5-minutes window. • Appendix 1: Schedule of Assessments table now corrected to include orthostatic BP at Screening visit.	Minor corrections and clarifications.

Protocol Amendment 1 Summary of Changes (23 October 2020)

Section # and Name	Description of Change	Brief Rationale
Title Page, Protocol Approval Page, Footers, Protocol Amendment Summary of Changes.	Updated the protocol version and date.	To reflect the new version and date of the protocol.
Protocol synopsis and Section 4.2.2 Inclusion criteria	New inclusion criteria: Before enrolling in the study, subjects intending to rely on abstinence as a birth control method must still agree to use double-barrier birth control methods if they engage in intercourse.	As per FDA request.
Protocol synopsis and Sections 4.2.3 Exclusion Criteria, 4.6.1.5 Laboratory Assessments and 4.6.2 TIMING OF STUDY ASSESSMENT	HIV-1/2 antibody and HIV-2 antibody have been removed.	Very low incidence of such virus in US population.
Protocol synopsis and Sections 3.1.2 STOPPING RULES and 5.1.3 ADVERSE EVENTS OF SPECIAL INTEREST (IMMEDIATELY REPORTABLE	New definition of thrombocytopenia: Thrombocytopenia is defined as meeting one or both criteria:	As per FDA request.

TO THE SPONSOR)	• Platelet count below 150 × 10E9/L (or 150.000 platelets per microliter (mcL)) • Platelet count drop of ≥50% compared to baseline	
DATA SAFETY MONITORING BOARD (DSMB)	Now it says up to 4 members	To have enough quorum.
Typos and clarifications:	• Alcohol and substance abuse now in urine tests. • PK samples refer to blood samples (in Section 3.2.3 RATIONALE FOR PHARMACOKINETIC SAMPLING). • Efficacy population. • Urine samples for PK deleted on Day 7. • Renumbering sections from page 24 onwards. • Update Table of contents. • Three references mentioned in the body text now also added to the list of references.	Minor corrections and clarifications.

SYNOPSIS

Study Number	**STP1-C004**
Title	A Phase 1b, Double-Blind, Placebo-Controlled, First-in-Human Study to Evaluate Safety, Tolerability and Pharmacokinetics of a Two-Week Oral Treatment with STP1 in a Subgroup of Adult Patients with Autism Spectrum Disorder
Study Phase	1b
Objectives	**Primary Objective:** To determine the safety and tolerability of a 2-week twice a day (6 hours apart) treatment with STP1 (combined oral doses of [redacted]) in adult individuals with Autism Spectrum Disorder Phenotype 1 (ASD-Phen1). **Secondary Objective:** To determine the plasma pharmacokinetics of [redacted] when given orally in combination, as STP1, for 2 weeks. **Exploratory objectives:** - To evaluate the effect of a 2-week treatment with STP1 compared to placebo on cognition as assessed by subtests of [redacted] the Test of Attentional Performance for Children (KiTAP) test battery. - To evaluate the effect of a 2-week treatment with STP1 compared to placebo on maladaptive behavior as assessed by the Aberrant Behavior Checklist – Community (ABC-C). - To evaluate the effect of a 2-week treatment with STP1 compared to placebo change in the context of autism as measured by the change in the Ohio Autism Clinical Impression Scale (OACIS) change. - To evaluate the effect of a 2-week treatment with STP1 compared to placebo on the Children Sleep Habit Questionnaire (CSHQ) scores. - To evaluate the effect of a 2-week treatment with STP1 compared to placebo on the Clinical Global Impressions-Severity (CGI-S) scale, as reflected by the Clinical Global Impressions-Improvement (CGI-I) scale. - To evaluate the effect of a 2-week treatment with STP1 compared to placebo on brain electrophysiology parameters as measured by electroencephalogram (EEG). - To evaluate the effect of a 2-week treatment with STP1 compared to placebo on gaze aversion and social interest by assessing eye tracking tasks. - To evaluate the effect of a 2-week treatment with STP1 compared to placebo on Lactate/Pyruvate ratio (L:P).
Study Design	This is a randomized, double-blind, placebo-controlled, parallel-group, 2-dose ascending study (see Figure 2, Section 3.1.1). The first 8 eligible patients will be randomized and allocated to one of two groups with a 3:1 ratio:

	• One group will receive [redacted] mg twice a day (6 hours apart) oral [redacted] and [redacted] mg twice a day (6 hours apart) oral [redacted] (STP1 dose 1, N=6). • One group will receive matching oral placebo twice a day (6 hours apart) (N=2). Once first dose cohort (8 patients) finish 2 weeks of STP1 treatment and 2 weeks of follow up, a Data Safety Monitoring Board (DSMB) will evaluate the safety and tolerability data to decide whether the dose of ibudilast can be increased to 10 mg twice a day (6 hours apart) as planned, or the protocol should be modified or the study should be stopped. If the DSMB supports the start of the next dose level, up to 8 additional eligible patients will be assigned to the next dose level (patients that participated in the first cohort may be allowed to participate in the second part if no safety concern was identified): • [redacted] mg twice a day (6 hours apart) oral [redacted] (STP1 dose 2, N=up to 6). • One group will receive matching oral placebo twice a day (6 hours apart) (N=up to 2). The two drugs will be taken at the same time with food and water, and the two daily doses will be separated by approximately 6 hours. For all subjects, twice daily dosing will occur on Days 1 to 13, inclusive, and a final single dose administration will occur on the morning of Day 14 (morning dose only). On Days 1, 7 and 14, the morning dose will be taken on site. The screening period is considered to be between Day -14 and Day -1. The screening visit will include the following: written informed consent, updated medical history, physical examination, drugs of abuse, pregnancy test (females only), alcohol test, laboratory safety, coagulation, viral serology, urinalysis, and inclusion/exclusion criteria. Subjects will return to the study site at scheduled visits on Day 1, Day 7 Day 14/15 and, optionally, daily up to Day 18 for evaluation and pharmacokinetic sampling, and at approximately 14 days (Day 28) after the last dose for a post-treatment safety follow-up visit.
Study Duration	For each patient, the duration of the study will be approximately 6 weeks. This includes a 2-week screening period, 2 weeks of treatment and a safety follow-up visit 2 weeks after the last dose of investigational medicinal product (IMP). Doses will be administered twice daily (morning and afternoon) on Days 1 through 13 of the Treatment Period and only the morning dose will be administered on Day 14. The end of the study is defined as the date when the last patient, last observation (LPLO) occurs.

Number of patients	Approximately 20 subjects with ASD-Phen1 will be randomized, with the objective to obtain up to 8 patients completing each cohort of the study (2 cohorts). The number of patients may be increased if the targeted number of completers is not reached. As a minimum, 3 patients should be exposed to the highest dose of STP1
Subject Assignment	Randomization to each arm will be done on Day 1 through the SAS PROC PLAN software. Subjects will be randomized to the investigational treatment or placebo in a 3 to 1 ratio using a permuted-block design. The randomization plan will be generated by an independent statistician and none of the sponsor staff, investigators or study subjects will have access to the randomization schema prior to randomization and until the database is unblinded at the end of the study. A randomization list containing unblinded treatment codes will be provided to the unblinded personnel at the study site. Individual code break envelopes will be made available to the site in the event of the necessity of an emergency unblinding. The unblinded personnel at the site will store the randomization list in a location that is not accessible by blinded site staff. The unblinded personnel will be responsible for dispensing the assigned treatment.
Study Population	Male or female individuals, aged between 18 and 40 years, having been previously diagnosed with autism spectrum disorder (ASD) and meeting the 2 primary criteria for ASD-Phen1 (inclusion criteria #2 and #3).
Inclusion Criteria	Each patient must meet all of the following criteria to be enrolled in this trial: 1. Male or female individuals, aged between 18 and 40 years inclusive, previously diagnosed with ASD (based on Diagnostic and Statistical Manual of Mental Disorders, 5th Edition [DSM-5] criteria and ideally supported by either Autism Diagnostic Interview – Revised [ADI-R] or Autism Diagnostic Observation Schedule – Second Edition [ADOS-2] scores). 2. Patients must have [redacted] (according to the Centers for Disease Control and Prevention [CDC] growth charts [CDC, 2019]), or being diagnosed with macrocephaly. 3. Patients with systematic aggravation of ASD behavioral symptoms [redacted] as assessed by the ASD-Phen1 semi-structured interview form. 4. Patients must have a parent or reliable caregiver who agrees to provide information about the patient as required by the protocol. 5. The patient and/or caregivers should be fluent in English. 6. Patient willing and consenting or assenting to participate.

	7. If assent provided by the patient, parent or legal guardian willing to give written consent 8. Patients with ASD and comorbid seizure disorder should be seizure-free for at least 6 months prior to screening. 9. Before enrolling in the study, subjects must agree to use double-barrier birth control methods if they engage in intercourse. 10. Women of childbearing potential, defined as all women physiologically capable of becoming pregnant, can be enrolled if they are using highly effective methods of contraception during dosing and for 1 week after discontinuation of the investigational drug. 11. Sexually active males must use a condom during intercourse after the start of the IMP administration and for at least one week after stopping study medication and should not father a child in this period after completion of the study medication. A condom is required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid. In addition, male participants should not donate sperm for the time period specified above.
Exclusion Criteria	Individuals meeting any of the following criteria will not be included in the trial: 1. Patients with an identified genetic cause of ASD in their medical record will be excluded from the study. 2. History of traumatic head injury, cerebrovascular disorder, congestive heart failure, hepatic or renal disease. 3. Thrombocytopenia (platelet count < 100 000 mm^3) within 1 year prior to the screening visit. 4. Diabetes (type 1 diabetes mellitus or uncontrolled type 2 diabetes mellitus) or latent autoimmune diabetes of the adult (LADA). 5. Use of prohibited medications (Section 4.5.1) or herbal remedies within 2 weeks prior to randomization, or 5 half-lives (whichever is longer). 6. Alcohol and/or substance abuse/dependence within 12 months prior to screening. 7. A significant risk for suicidal behavior, in the opinion of the investigator and as assessed by the Columbia Severity Rating Scale (C-SSRS) or the simplified version adapted from the Columbia Classification Algorithm for Suicide Assessment (C-CASA). 8. Use of antioxidant supplements and/or vitamins within 2 weeks prior to randomization. 9. Initiation of, or a major change in psychological/behavioral intervention within 4 weeks prior to randomization. 10. Patient with any active infection, including but not limited to the following: o Bacterial infection; or o Fungal infection; or

	○ Positive result at screening for hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV)-1 and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). 11. Episode of fever (i.e., ≥100.5°F or 38.0°C) or clinically significant illness without fever (as judged by the investigator), within 10 days before Day 1. 12. Sustained resting systolic blood pressure (SBP) <80 mmHg or diastolic blood pressure (DBP) <40 mm Hg or a drop in SBP of ≥20 mm Hg, or in DBP of ≥10 mm Hg, or patient experiencing lightheadedness or dizziness during the orthostatic recordings. 13. Clinically relevant electrocardiogram (ECG) abnormalities; QTcF (Fredericia's correction) >450 ms (at screening or Day 1 pre-dose). 14. Confirmed clinically significant abnormal laboratory test results at screening (lactate and pyruvate not included) other than the conditions related to ASD-Phen1. 15. Patients with any active clinically significant disease (i.e. gastroenterological, endocrinological, renal, respiratory, cardiovascular, hepatic dysfunction, immunological or hematological disease) that following the judgment of the investigator may interfere with the conduct of the study, other than the conditions related to ASD-Phen1. 16. Patients with any history of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years, regardless of whether there is evidence of local recurrence or metastases. 17. Pregnant (confirmed by laboratory testing) or lactating female patient. 18. Participation in an investigational drug study within 30 days or 5 times its half-life, whichever longer, prior to randomization.
Test Product(s), Dose and Mode of Administration	[redacted] oral formulation will contain [redacted] mg of sustained release [redacted]. [redacted] oral formulation will contain [redacted] mg of the active substance. Matching placebos of [redacted] will be provided. On each dosing occasion, subjects will swallow the medications with water. The morning doses on Day 1, 7 and 14 will be administered by site staff, and the afternoon doses on Days 1 and 7 will be administered to the subject at home. Doses on all other days will be administered to the subject at home at approximately the same time each day. There are no restrictions with respect to food intake, other than subjects are not permitted to drink anything other than water while at the site on Days 1, 7, 14, 15 and 28. It is advised to take the medication with food. Subjects will continue to take their usual drug treatments (if any) from their own prescribed supply throughout the study unless prohibited (see section 4.5.1).

Endpoints	Primary Endpoints - Safety: The safety outcome measures for this study are as follows: • Incidence, nature and severity of adverse events (AEs). • Incidence, nature and severity of serious adverse events (SAEs). • Incidence, nature and severity of adverse events of special interest (AESIs). • Incidence, nature and severity of AEs leading to treatment discontinuation. • Changes from baseline in SBP, DBP, heart rate (HR) and respiratory rate. • Changes from baseline in orthostatic measurements of blood pressure (BP) and HR. • Changes from baseline in ECG parameters (HR, PQ, QRS, QT, RR and QTcF, along with information on T and U waves). • Incidence of clinically significant ECG abnormalities. • Hearing assessment. • Incidence of clinically relevant laboratory abnormalities, based on hematology, coagulation, blood chemistry and urinalysis test results: o Hematology: hemoglobin concentration, hematocrit level, erythrocytes and platelet counts, leucocytes differential (absolute) counts (neutrophils, eosinophils, lymphocytes, monocytes and basophils) o Coagulation: international normalized ratio (INR), activated partial thromboplastin time (aPTT) and prothrombin time (PT) o Blood chemistry: C-reactive protein (CRP test), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transferase (GGT), total and conjugated bilirubin, alkaline phosphatase (ALP), albumin, creatine phosphokinase (CPK), creatinine, urea, total protein, total cholesterol, triglycerides, glucose, sodium, chloride, calcium, magnesium, phosphate, potassium o Urinalysis: protein, blood, glucose, leucocytes, nitrites, pH and crystals. • Incidence of value outside of the normal ranges and of clinically relevant blood chemistry test results for electrolytes: o Sodium, chloride, calcium, magnesium, phosphate, potassium Secondary Endpoints - Pharmacokinetics: • [redacted] plasma concentration will be evaluated at Days 1, 7, and 14 to 18. • Plasma concentrations of [redacted] and associated PK parameters: Area under the plasma concentration-time curve ($AUC_{0\text{-}tau}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$), minimum plasma concentration prior to administration (C_{trough}), maximum plasma concentration (C_{max}, $C_{max,ss}$), time of

	maximum plasma concentration (T_{max}) and accumulation ratio, apparent plasma terminal elimination half-life (t1/2), apparent oral clearance and apparent volume of distribution (Vz/F). • Urine level of [redacted] will be assessed on Days 1 and 14/15. Exploratory Endpoints - Pharmacodynamics: • Change from baseline in subtasks from [redacted] and Cognition Battery and KiTAP test battery. • Global clinical change in the context of autism as measured by the change in the OACIS scale. • Change from baseline in ABC-C subscores. • Change from baseiline in the SRS2-subscores. • Change from baseline in the CSHQ scores. • Change from baseline in the CGI-S scale, reflected by the CGI-I scale. • Change from baseline in electrophysiology parameters as measured by EEG at resting state and during habituation and chirp tasks. • Change from baseline in eye gaze to eye regions during viewing of static faces and change in eye gaze to social scene viewing during viewing of dynamic video. • Change from baseline in Lactate/Pyruvate ratio (L:P)
Safety Assessments	Safety monitoring will begin when patient signs the informed consent form and continues through the end-of-study visit. All AEs and SAEs, whether reported by the patient/caregiver or noted by authorized study personnel, will be recorded in the patient's medical record and on the appropriate AE/SAE electronic case report form (eCRF). As for SAEs, AESIs have to be reported to the sponsor within 24 hours after learning of the event. An SAE is any AE that meets any of the following criteria: - Fatal (i.e., the AE actually causes or leads to death). - Life-threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death). This does not include any AE that, had it occurred in a more severe form or was allowed to continue, might have caused death. - Requires or prolongs in-patient hospitalization. - Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions). - Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug(s). - Significant medical event in the investigator's judgment (e.g., may jeopardize the patient's safety or may require medical/surgical intervention to prevent one of the outcomes listed above). AESI includes: - Electrolyte imbalance: hypokalemia (K^+ level <3.4 mEq/L), hyperkalemia (>5.0 mEq/L), hyponatremia (Na^+ level <135 mEq/L), hypochloremia (Cl^- level <97 mEq/L).

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- Thrombocytopenia is defined as meeting one or both criteria:
  - Platelet count below 150 × 10E9/L (or 150.000 platelets per microliter [mcL])
  - Platelet count drop of ≥50% compared to baseline
- Drug-induced liver injury (DILI).
- Dehydration and orthostasis (defined by a fall in systolic blood pressure overe 20 mm Hg or a fall in diastolic pressure over 10 mm Hg within 3 minutes of standing.

The progression from the first cohort to the second will be contingent upon DSMB review and agreement with the sponsor. The information to be reviewed will correspond to the safety and tolerability data from the first dose cohort (8 patients) who finish 2 weeks of treatment and 2 weeks of follow-up. The DSMB members will be independent from the study team.
</td></tr>
<tr><td>Pharmacokinetic Assessments</td><td>
Blood samples will be drawn on Days 1, 7, 14, 15 and, optionally, daily up to Day 18, and urine will be collected on Day 1 and Day 14 to determine the concentrations of [redacted].
The following sampling schedule will be applied:
<table>
<tr><td colspan="3">PK Schedule[c]</td></tr>
<tr><td>Time</td><td>Plasma[a]</td><td>Urine[b]</td></tr>
<tr><td>Day 1</td><td>0.25, 0.5, 1, 2, 4 and 6 h post-AM dose</td><td>0 to 6 h post-AM dose</td></tr>
<tr><td>Day 7</td><td>Pre-AM dose</td><td></td></tr>
<tr><td>Days 14 (only AM dose) and 15</td><td>Pre-AM dose and 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h post-AM dose</td><td>0-4, 4-8 and 8-12 h post-AM dose</td></tr>
<tr><td>Between Days 16 and 18[d]</td><td>48, 72, and 96 h post-AM dose of Day 14</td><td></td></tr>
<tr><td colspan="3">[a] both [redacted] will be analyzed from the same blood draw for each specified sampling time point
[b] Urine creatinine from the urine collection will be paired with a serum creatinine measurement to evaluate completeness of collection.
[c] All of these timepoints have a 5 minute window.
[d] Optional</td></tr>
</table>
Non-compartmental analysis (NCA) will be utilized to analyze data from the sampling schema.
Plasma PK parameters such as $AUC_{0\text{-}tau}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}inf}$, C_{max}, T_{max}, and $t_{1/2}$ at Day 1 and at Day 14 for both [redacted]. C_{trough} and $C_{max\ ss}$ will be calculated at Days 7 and 14, and accumulation ratio will be calculated at Day 14.
</td></tr>
<tr><td>Blinding</td><td>The study will be blinded for patients/caregivers, study personnel with direct interaction with the subjects and vendors, using placebo capsules and tablets that match the active drugs in appearance, labelling and packaging. The total number of capsules making up each dose will be identical for both STP1 dose levels and placebo.</td></tr>
</table>

	Study personnel responsible for receiving the randomization lists and dispensing the assigned treatment will be unblinded. An independent DSMB will be appointed. The DSMB will be blinded to treatment for safety/tolerability assessments (primary outcome) and will advise on the start of 2nd dose level.
Statistical Methods	For this Phase 1b study, descriptive statistics will be provided for all endpoints by treatment group. For continuous endpoints, summary statistics i.e., n (number of individuals included in the analysis), mean, SD, median, minimum, maximum, Quartile 1, and Quartile 3 will be provided. For categorical endpoints, the number and frequency in each category will be provided. All subjects who have received at least one dose of the combination, whether prematurely withdrawn from the study or not, will be included in the safety analysis. SAFETY and TOLERABILITY The number of patients who report the following events will be summarized for all treated patients: • AE and treatment-related AE. • AE and treatment-related AE that led to study treatment discontinuation. • SAE and treatment related SA. • SAE and treatment related SAE that led to study treatment discontinuation. • AESIs. Safety and tolerability are also measured by incidence and severity of AEs, physical examinations, ECG, vital signs, specific laboratory abnormalities and change from baseline in the CSHQ scores in all treated patients. As this study will provide descriptive statistics, there will not be any correction for multiple primary endpoints. PHARMACOKINETICS The PK analysis set will include subjects with at least one sample collected and analyzed for plasma drug concentration. Concentration of each drug in plasma and urine will be listed and summarized by visit and nominal time. The NCA using the sampling mode of PK data and standard descriptive statistics for PK parameters will be calculated using Phoenix WinNonlin software (Certara).
Sample size	The target number of completers for this Phase 1b study is up to 16 ASD-Phen1 individuals. Assuming a dropout rate of approximately 20%, the proposed sample size is up to 20 patients to be randomized. This sample size should provide the first evidence of safety, tolerability and pharmacokinetics of STP1 in individuals with ASD-Phen1 to inform potential future studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF ABBREVIATIONS

ABC-C	Aberrant behavior checklist-community
ADI-R	Autism diagnostic interview - revised
ADME	Absorption, distribution, metabolism, and excretion
ADOS-2	Autism diagnosis observation schedule ^{M}, second edition
AE	Adverse event(s)
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AOI	Areas of interest
aPTT	Activated partial thromboplastin time
ASD	Autism Spectrum Disorder
ASD-Phen1	ASD Phenotype 1
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
$AUC_{0\text{-}in}$	AUC extrapolated to infinity
$AUC_{0\text{-}last}$	AUC to the last measurable concentration
$AUC_{0\text{-}tau}$	AUC to the end of the dosing period
AZT	Azidothymidine
BCRP	Breast cancer resistance protein

GLOSSARY OF ABBREVIATIONS

BID	Twice daily
BMI	Body mass index
BP	Blood pressure
BPM	beats per minute
cAMP	Cyclic adenosine monophosphate
CARS	Childhood Autism Rating Scale
C-CASA	Columbia Classification Algorithm for Suicide Assessment
CDC	Centers for Disease Control and Prevention
CGI-I	Clinical Global Impressions-Improvement
CGI-S	Clinical Global Impressions-Severity
cGMP	Cyclic guanosine monophosphate
C_{max}	Maximum plasma concentration
$C_{max\ ss}$	Maximum plasma concentration at steady state
C_{trough}	Minimum plasma concentration prior to administration
CNS	Central nervous system
CPK	Creatine phosphokinase
CPT	Continuous Performance Tasks
CRO	Contract research organization
CRP	C-reactive protein
CSHQ	Children sleep habit questionnaire
C-SSRS	Columbia Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
CYP450	Cytochromes P450
DBP	Diastolic Blood Pressure
DCCS	dimensional change card sort
DDI	Drug-drug interaction
DILI	Drug-induced liver injury
DSM-5	Diagnostic and statistical manual of mental disorder, 5th edition
DSMB	Data safety monitoring board
DSUR	Development Safety Update Report
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic case report form
EEG	Electroencephalography
EDC	Electronic data capture
EF	Executive Function
EOS	End-of-study
ERP	Event Related Potentials
ESF	Eligibility screening form
FDA	Food and Drug Administration
FSIQ	Full scale intellectual quotient
GABA	Gamma aminobutyric acid
GCP	Good clinical practice
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
GLP	Good laboratory practice
HBV	Hepatitis B Virus
HBsAg	Hepatitis B surface antigen
HBcAb	Hepatitis B core antibody
HC	Head cicumferance

GLOSSARY OF ABBREVIATIONS

HCV	Hepatitis C Virus
hERG	Human ether-a-go-go-related gene
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HR	Heart rate
IBI	Intensive Behavior Intervention
IC_{50}	Concentration that gives half the maximum inhibition
ICH	International Concil on Harmonization
ICF	Informed consent form
IL-1β	Interleukin 1 beta
IL-10	Interleukin 10
IMP	Investigational medicinal product(s)
INN	International non-proprietary name
IND	Investigational new drug
INR	International normalized ratio
IRB	Institutional Review Board
IUD	intrauterine device
IUS	intrauterine system
IV	Intravenous
KiTAP	Test of Attentional Performance for Children
LADA	Latent Autoimmune Diabetes of the Adult
LCL	Lymphoblastoid cell line
LC-MS/MS	Liquid chromatography-mass spectrometry
L:P	Lactate-pyruvate ratio
LPLO	Last patient last observation
MedDRA	Medical Dictionary for Regulatory Activities Terminology
MEDNO	Medication number
MOS	Margin of safety
NCA	Non-compartmental analysis
NDDs	neurodevelopmental disorders
NIH	National Institute of Health
[redacted]	[redacted]
NKCC	Na-K-Cl cotransporter
NOAEL	No observed adverse effect level
[redacted]	[redacted]
OACIS	Ohio Clinical Impression Scale
OACIS-C	Ohio Clinical Impression Scale - Change
OACIS-S	Ohio Clinical Impression Scale - Severity
OAT	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
ORT	oral reading recognition
OTC	Over the counter
PCPS	pattern comparison processing speed
PD	Pharmacodynamic
PDE	Phosphodiesterase
PI	Principal investigator
PK	Pharmacokinetic
PKA	Protein kinase A
PT	Prothrombin time

GLOSSARY OF ABBREVIATIONS

PR	Pulse rate
PV	picture vocabulary
qPCR	Quantitative Polymerase Chain Reaction
SAE	Serious adverse event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SB5 ABIQ	Stanford Binet Intelligence Scales, Fifth Edition – Abbreviated Battery IQ
SBP	Systolic blood pressure
SD	Standard deviation
SI	Système international
SMT	Study management team
SOC	System Organ Classes
SRS-2	Social responsiveness scale – Version 2
SSPR	Social scene preference ratio
TAP	Tests of Attentional Performance
TK	toxicokinetic
TD	Typically developing
T_{max}	time of maximum plasma concentration
$t_{½}$	half-life
ULN	Upper limit of normal
U.S.	United States
Vz/F	Apparent volume of distribution
WCBP	Women patients of childbearing potential
WHO	World Health Organization

1. INTRODUCTION

Further information on the scientific rationale is provided in the Investigator's Brochure (STP1-IB).

1.1 BACKGROUND ON THE DISEASE

Autism spectrum disorder (ASD) comprises a group of lifelong neurodevelopmental conditions that, according to recent estimates, affect 1.5% of the population in developed countries [Baxter et al, 2015]. The prevalence of ASD has been estimated as 1 in 59 school-aged children in the United States US [Christensen et al, 2016]. ASD is characterized by both core symptoms and co-occurring conditions [Farmer et al, 2018]. Core symptoms are the fundamental aspects of the disorder that are required for diagnosis. According to the current diagnostic criteria (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5), [Baird, 2013]), an individual with ASD must show deficits in social interactions and communication, and deficits in at least 2 of the 4 subdomains of restricted or repetitive behaviors. Its manifestations must cause clinically significant impairment affecting the ability of patients to interact with others, especially people of their own age when referring to pediatrics. In addition to core symptoms, ASD is associated with a wide range of co-occurring conditions. In recent years, much progress has been made in understanding the prevalence and underlying biology of conditions co-occurring with ASD, such as seizures [Keller et al, 2017], sleep disorders [Deliens et al, 2015], gastrointestinal (GI) disorders [Hsiao, 2014; Vuong and Hsiao, 2017], psychiatric disorders (estimated to be around 70% of patients with ASD [Buck et al, 2014]), mitochondrial dysfunction [Legido et al, 2013], and immune system dysregulations [Gładysz et al, 2018]. Currently, there is no medicine approved for the core symptoms of ASD. The only approved drugs for ASD are aripiprazole (Abilify®) and risperidone (Risperdal®) to treat irritability associated with ASD.

ASD symptoms and their severity vary widely across the core and co-occurring symptoms. Thus, each individual with ASD has his or her own unique combination of symptoms and levels of functioning. This may result in relatively mild challenges for someone on the high functioning end of the spectrum, while symptoms may be more severe for others interfering with everyday life or even requiring full time lifelong care and support. Even within syndromic (genetically well-defined) ASD, there is a considerable variability in the range and severity of symptoms.

1.2 STRATIFICATION AND THERAPEUTIC STRATEGIES

The current version of the DSM abandoned classifications of subtypes of ASD to group them under one umbrella, mostly due to the lack of objective criteria to define these subtypes. However, ASD remains characterized by a high heterogeneity in its behavioral manifestations with very complex genetic underpinnings, suggesting the existence of subtypes of ASD. Therefore, efforts to categorize ASD are still of critical importance and must rely on defining a relationship between clinical symptoms and biological mechanisms to improve the outcome of clinical trials.

Patient stratification and precision medicine for ASD started with the increasing knowledge of genetic alterations related to risk for ASD. Medications targeting the neurohormonal oxytocin or vasopressin systems have failed. Encouraging results have also come from recent studies suggesting that only a subset of patients will respond to any given treatment [Beversdorf, 2016]. In clinical studies with D-cycloserine and memantine, positive effects on core symptoms were seen only in some adolescents and adults with ASD [Aman et al, 2017; Schade and Paulus, 2016]. In a study of a low dose of sertraline in young boys with fragile X syndrome, an increase in social interactions was observed in only boys who had ASD and specific brain-derived neurotrophic factor polymorphisms [AlOlaby et al, 2017]. This last result, in addition to other previous clinical failures such as the Phase 2 trial investigating arbaclofen in fragile X, indicates that stratification strategies using only genotype are likely to be insufficient to ensure successful treatment developments in ASD. Thus, a critical challenge is to determine sufficient number of biological markers to identify subgroups of potential best responders to a specific medication.

1.2.1 CLINICAL SIGNS AND SYMPTOMS OF ASD-PHEN1

STALICLA's approach develops on endophenotyping to define more specific ASD patient subgroups using a proprietary neurodevelopmental disorder specific integrative systems biology tool called DEPI. DEPI integrates large-scale systems biology data that include genetic, molecular, anatomical and clinical datasets from anonymous patient records and segregates patient populations based on the presentation of clinical signs and symptoms unique to each group of patients with ASD. DEPI also generates a specific molecular fingerprint (or 'disease node') for each patient cluster corresponding to common dysregulated cellular pathways, as a starting point to identify candidate clinical enrichment biomarkers. In a previous observational clinical trial sponsored by STALICLA and conducted at the Greenwood Genetic Center [STA-P1-C-001], the application of DEPI criteria allowed identification of a subpopulation within a general group of patients with idiopathic ASD. This population, identified as ASD-Phenotype 1 (ASD-Phen1), is characterized by the presence of two mandatory clinical criteria:

- [redacted]

and

- [redacted].

It is noteworthy that [redacted] is the most frequently reported quantitative trait seen in children who are subsequently diagnosed with ASD [Sacco et al, 2015]. It appears that [redacted] in this subcategory of patients with ASD patients undergoes a period of rapid and excessive acceleration overgrowth during the first year of life [Campbell et al, 2014; Courchesne, 2003; Lainhart et al, 1997]. In line with increased [redacted]

described above, structural magnetic resonance imaging studies of children with ASD also demonstrated an early phase of brain overgrowth [Hazlett et al, 2011; Shen et al, 2013; Aylward et al, 2002], followed by a decrease or even arrest in growth.

Likewise, immune dysfunction has been increasingly implicated as an important contributor to the severity and the pathogenesis of ASD phenotypes [Gładysz et al, 2018] and several clinical studies have demonstrated immune dysregulation in subsets of the ASD population. On pair with other neuropsychiatric diseases, dynamic interaction between the immune system and ASD symptoms have also been reported. While a subset of patients are reported to see their ASD core symptoms worsen under sickness [Shoffner et al, 2010], other subgroups have been described to see their core symptoms improve under sickness [Grzadzinski et al, 2018]. These data indicate that at least three subpopulations of ASD patients can be identified with regard to their behavior [redacted] 1) ASD patients [redacted] or 2) [redacted] and 3) ASD patients not affected [redacted], supporting the hypothesis of dysregulated interactions between immune and neuronal systems in subgroups of ASD, as recently proposed by Thom et al [Thom et al, 2019].

Very little is known about the underlying mechanisms leading to worsening or improvement of symptoms during episodes [redacted]. A small clinical trial involving 28 patients set to identify the potential relationship between ASD symptoms and mitochondrial dysfunctions reported that 70% of these patients show worsening of symptoms associated [redacted] [Shoffner et al, 2010]. Mitochondrial dysfunctions have long been associated with ASD [Rose et al, 2018] but also with an altered immune profile in patients with ASD. Analysis of the immune profile of ASD peripheral blood monocytes from ASD patients showed that changes in mitochondrial function observed in these patients are correlated with an affected interleukin 1 beta to interleukin 10 (IL-1β/IL-10) ratio, further strengthening the link between immune response, energy metabolism and ASD symptoms [Jyonouchi et al, 2019]. Importantly, these patients were also shown to worsen under immune insult [Jyonouchi et al, 2014].

In addition to providing information regarding stratification these results indicate that ASD-Phen1 immune clinical signs could also correlate with dysregulations in mitochondrial function and dysregulation of energy metabolism.

1.2.2 RATIONALE FOR STP1

STALICLA's first product is the combination of [redacted], called STP1. [redacted] is a brain penetrant phosphodiesterase 3 (PDE3), 4 (PDE4), 10 (PDE10) and 11 (PDE11) inhibitor marketed in [redacted] and is currently in development for chemotherapy-induced damage; glioblastoma (Phase 1/2); alcoholism; amyotrophic lateral sclerosis; multiple sclerosis; opioid abuse; substance-related disorders (Phase 2) and spinal cord disorders (Phase 3 in Europe). The use of [redacted] in neuroimmune and neuropsychiatric disorders is supported by the

drug's potential to control neuroinflammatory reactions through modulation of glial cell activation. This effect has also been proposed to support its use in several conditions including cerebral ischemia [Takuma et al, 2001; Wakita et al, 2003], pain [Hutchinson et al, 2009; Lilius et al, 2009], human immunodeficiency virus (HIV) infection related neuroinflammation [El-Hage et al, 2014], multiple sclerosis [Feng et al, 2004] and chemical substance abuse [Ray et al, 2017].

At the cellular level, [redacted] induces an increase in cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), leading to activation of intracellular signaling cascades (e.g., protein kinase A [PKA]) resulting in increased expression of the Na–K–Cl cotransporter (NKCC) 1. At the physiological level, these increased expression levels and activation of NKCC1 may/could lead to accumulation of chloride in cells and antagonize the gamma-aminobutyric acid (GABA) current in neurons, thereby decreasing inhibition and favoring excitation. This increased neuronal excitation can then lead to aberrant neuronal network activity and affect patient behavior.

To compensate this indirect effect of [redacted] the sponsor proposes to combine [redacted] for the treatment of ASD-Phen1 patients.

[redacted] has been first characterized as an inhibitor of NKCC2 co-transporters located on the loop of Henle [Russell, 2000]. [redacted] is prescribed for the treatment of edema associated with congestive heart failure or with hepatic or renal disease including the nephrotic syndrome. It is also currently in development for ASD in pediatric population (Phase 3), for pervasive child development disorders (Phase 3) and for Parkinson's disease (Phase 2) [Adis Insight Drug Profile, 2018].

Additional details on the expected mechanism of action are provided in the Investigator's Brochure [STP1-IB].

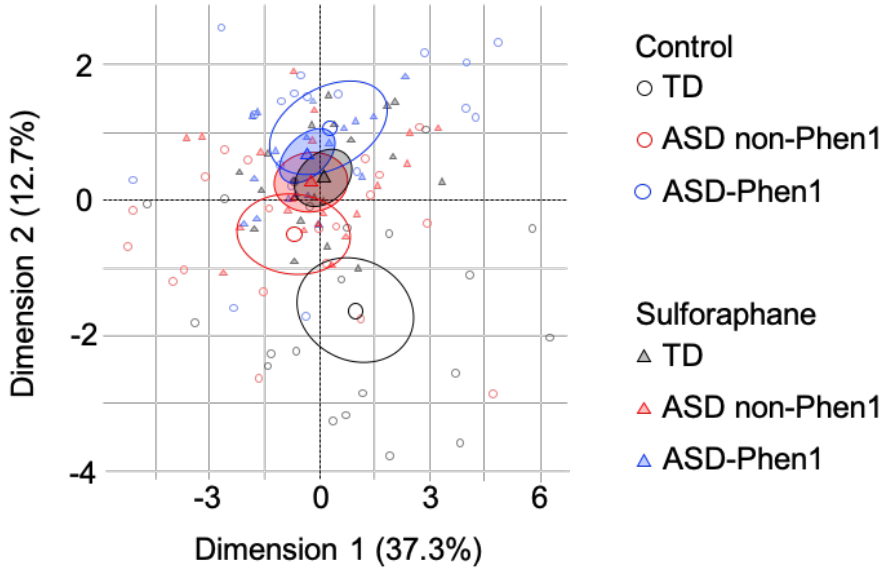
1.3 NON-CLINICAL EXPERIENCE

1.3.1 NON-CLINICAL PHARMACOLOGY

The systems biology approach has also generated a probabilistic model linking these two mandatory criteria, [redacted], to potential dysregulation in [redacted] related pathways. To investigate a potential stratification of ASD patients based on the known function of [redacted], the sponsor has designed an in vitro assay to monitor the bioenergetic profile of cells derived from patients' blood. Analysis of the bioenergetic profiles revealed differences between blood-derived cells from ASD-Phen1, ASD-non-Phen1 and typically developing individuals (TD). We then exposed the different cells to sulforaphane, an activator of [redacted], to investigate the relationship between [redacted] and the bioenergetic profile of the cells' phenotypes. Our results showed that cells derived from indistinct ASD patients have distinct metabolic profiles compared to cells derived from ASD-Phen1 patients or derived from TD individuals. The results also confirmed the existence of a relationship between [redacted] and ASD-Phen1 endophenotype, thus validating STALICLA's stratification models (Figure 1).

Comparing the bioenergetic profile of blood-derived cells from ASD-Phen1 patients with that of blood-derived cells from TD led the identification of key pathophysiological features associated with ASD-Phen1 including 1) a defect in the balance between glycolysis and pentose phosphate pathway activity and 2) a lack of [redacted] activation of the pentose phosphate pathway. Treating ASD-Phen1 cells with [redacted] led to an increase in glucose uptake, thus alleviating the defects in glycolysis. When related to ASD-Phen1 pathophysiology, the sponsor predicts that its beneficial effect will be to provide support for high-energy demand in different cellular subtypes, including active immune cells and neurons.

Figure 1 Principal Component Analysis of the Bioenergetic Profile of Untreated and Sulforaphane-treated Blood-derived Cells.



TD=typical development ; ASD =autism spectrum disorder

For further information, please refer to the STP1 Investigator's Brochure [STP1-IB].

1.3.2 NON-CLINICAL PHARMACOKINETICS AND METABOLISM

There are no available data on the Absorption, distribution, metabolism, and excretion (ADME) profile of STP1 at pharmacological doses. Available toxicokinetic (TK) data with STP1 are completed with relevant information from the pharmacokinetic (PK) profile, distribution and metabolism from in vivo studies with individual drugs that is available in the literature. This section also summarizes in vitro Drug Metabolism and Pharmacokinetics (DMPK) studies that were performed with [redacted].

STP1:

STP1 was orally administered during 28 days in a twice a day regimen in rat and a once daily regimen in dog and TK profile were analyzed in both species.

In the rat, oral administration of STP1 led to a higher exposure to [redacted] in females versus males, while there was no sex difference in [redacted] exposure. Both compounds reached a C_{max} at 0.5 hour post-administration. [redacted] was cleared from rat plasma with a $t_{1/2}$ in the range of 4.2 to 6.4 hour following single-day administration of STP1 and [redacted] had a $t_{1/2}$ that varied between 4.7 to 8.1 h following multiple STP1 administrations. Repeated administration of STP1 led to a decrease in [redacted] exposure, sign of a metabolism induction in the rat. In contrast, there was evidence of increased [redacted] exposure following repeat dosing.

In the dog, there was considerable variability in the TK data due to the prevalence of emesis and the resulting impact on exposure in dogs. As such, the TK results should be interpreted with caution and definitive conclusions on dose proportionality, sex differences or the potential for accumulation should be avoided in this species. There was no clear trend relating exposure to sex for both compounds. Exposure to [redacted] in terms of AUC_{tlast} and C_{max}, generally increased at rates greater than dose proportional, while same parameters for [redacted] were generally similar at all dose levels on Day 1 and nearly dose proportional on Day 28. Accumulation of [redacted] and [redacted] in terms of AUC_{tlast}, is difficult to assess by evaluating accumulation ratios due to high variability and a better indicator might be predose values on Day 28: for both compounds, although predose concentrations were often detected on Day 28, the levels were relatively low and unlikely to have a meaningful impact on exposure from the subsequent dose.

[redacted]

[redacted] is rapidly absorbed and has variable bioavailability in nonclinical species, often being subjected to extensive first pass metabolism following oral dosing. [redacted] plasma concentrations decline in a biphasic manner with elimination half-life ranging from 1 to 12 hours across the species. It is highly bound to plasma protein and distributes well into tissues, including the CNS. [redacted] undergoes metabolism by cytochromes P450 (CYP450) enzymes followed by uridine diphosphate glucuronidation. CYP3A4 likely responds to >50% of the hepatic metabolism of [redacted] in humans, but various other isoforms are also capable of metabolizing the drug. The major metabolite observed in plasma in various species is the 6,7-dihydrodiol-[redacted] There are no human disproportionate metabolites based on reaction phenotyping studies in vitro that compare metabolism from rat, dog and human hepatocytes. [redacted] is predominantly excreted in the feces in rats and in the urine in dogs, monkey and human. CYP450 induction has been reported following repeated administration in rats, and there is potential for CYP2B6 induction at the proposed clinical doses based on in vitro studies. In contrast, CYP450 inhibition is unlikely. [redacted] did not display substrate activity for common drug transporters but inhibits Organic Anion Transporter (OAT3), Organic Cation Transporter (OCT1), P-glycoprotein (P-gp) and Breast cancer resistance protein (BCRP) in vitro. OAT3 and OCT1 inhibition by [redacted] in vivo

is unlikely, but the potential for inhibition of P-gp and BCRP in vivo warrants further investigation.

[redacted] is rapidly absorbed and rapidly cleared from plasma in rats and dogs. In rats, the absorption seems to be saturable. [redacted] is highly bound to proteins in plasma and shows limited distribution into tissues, with the exception of kidneys and liver. Cross-species metabolism and reaction phenotyping studies confirmed extensive metabolism of [redacted] by rat hepatocytes and relative higher metabolic stability in dogs and humans. The enzyme responsible for [redacted] metabolism in humans is not identified. There are no human disproportionate metabolites based on reaction phenotyping studies in vitro that compare metabolism from rat, dog and human hepatocytes. In vivo metabolism and excretion data from literature suggests the presence of various metabolites in rats, with the 3'-acid being the most abundant in pooled urine and feces samples. Many of the same metabolites observed in rats are also described in dog excreta, albeit at much lower levels. [redacted] is mostly excreted via the feces in rats, and nearly equally excreted via the urine and feces in dogs. CYP450 inhibition or induction are not expected. [redacted] is a substrate for OAT1, OAT3, Organic Anion Transporting Polypeptide (OATPB1) and OATPB3 and inhibits OAT3, OCT2 and BCRP in vitro, of which, BCRP could potentially be inhibited in vivo from extrapolated exposure data from literature.

For further information, please refer to the STP1 Investigator's Brochure [STP1-IB].

1.3.3 TOXICOLOGY, SAFETY PHARMACOLOGY AND GENOTOXICITY

Toxicology studies:

In the pivotal 4-week Good Laboratory Practice (GLP) rat toxicity study [Study 8400256], the following treatment groups were compared to the control group: STP1 at 100:10 or 50:5 mg/kg twice a day [redacted]. The main findings of this study were decreased body weight gain and food consumption, minor clinical pathology changes and high liver weight associated with centro-lobular hepatocyte hypertrophy. This later finding was likely an adaptive mechanism in the rats. Most of these findings were reversible, were clinically silent and not considered adverse. Thus, STP1 at 100/10 mg [redacted] was considered the no observed adverse effect level (NOAEL) in the rats. This dose corresponds to a 100-fold safety margin for a human dose of 10 mg twice a day of [redacted] and of 1 mg twice a day of [redacted] based on allometric scaling.

In the pivotal 4-week GLP dog toxicity study [Study 01405001], the following once daily treatments were compared to the control group: STP1 at 8:0.8, 6:0.6 and 3/0.3 mg/kg/day [redacted]. The primary findings were emesis, decreased food consumption and body weight gain, suspected moderate dehydration and minor clinical pathology changes. Most of these findings were reversible. The primary target organ at necropsy was the kidney where minimal to

moderate degeneration/regeneration was noted at all STP1 dose levels. These histology changes were not observed at the end of the recovery period but resulted in minimal to mild interstitial fibrosis. As moderate tubular degeneration/regeneration was considered adverse, which was found in females in the low dose group, a NOAEL could not be determined for females. Therefore, the NOAEL was considered to be 3/0.3 mg/kg/day STP1 ( ) in male dogs only. This dose corresponds to a 10-fold safety margin for a human dose of 10 mg twice a day of and to a 5-fold safety margin for a human dose of 1 mg twice a day of based on allometric scaling.

Safety pharmacology studies:

A battery of safety pharmacology studies that included an in vitro human ether-a-go-go-related gene (hERG) assay with ibudilast, CNS and respiratory assessments in rats and cardiovascular assessments in telemetered dogs with STP1. There were no findings that were considered adverse, as the findings were either transient, of modest magnitude, within normal ranges or of unlikely clinical significance at the doses evaluated. In addition, all the findings were readily monitorable in the clinical setting. The key findings are summarized below:

- did not inhibit the hERG channel current at clinically relevant concentrations.
- In a CNS study in rats, a single oral dose of STP1 resulted in a transient and dose dependent reduction in locomotor activity (>10:1 mg/kg ibudilast/bumetanide) and body temperature (>50/5 mg/kg ibudilast/bumetanide).
- In a respiratory study in rats, a single oral dose of STP1 resulted in higher respiratory rates, with minimal changes to tidal volume, resulting in higher minute volume. These changes occurred primarily at achieved STP1 dose levels of 50/5 and 200/20 mg/kg .

In a cardiovascular study in dogs, an increase in heart rate was observed following STP1 dose levels >3/0.3 mg/kg . Changes in diastolic pressure, mean arterial pressure, pulse pressure, PR interval and QT interval were considered to secondary to heart rate, the act of vomiting/retching or a combination of the two and not a direct effect of STP1 administration. There was no effect on heart rate-corrected QT interval, Van de Water correction formula (QTcV).

Genotoxicity studies:

Based on the long clinical experience with and the full genotoxicity package that has been performed to evaluate the genotoxic potential of [Studies 8400253, 8400254 and 8400255], it is concluded that STP1 is not expected to have any genotoxic potential. STP1 has not yet been evaluated for carcinogenic or for reproductive and development toxicity potential.

For further information, please refer to the STP1 Investigator's Brochure [STP1-IB].

1.4 PREVIOUS CLINICAL EXPERIENCE

At the time of writing, STP1 has not been investigated clinically and Study STP1-C004 is the first in-human study. However, both compounds have been developed for different indications and safety and pharmacokinetic data have been published for both compounds.

[redacted] has been marketed in 10-mg capsules by [redacted] for the treatment of [redacted]

[redacted] is a loop diuretic that has been marketed in the US [redacted]. It is prescribed for the treatment of e[redacted].

1.4.1 CLINICAL PHARMACOKINETICS

[redacted] is an orally bioavailable drug with high plasma protein binding (>95%) and good distribution to peripheral tissues [Rolan et al, 2008]. It is metabolized by several cytochrome P450 enzymes with CYP3A4 counting for more than 50% and has an oxidative di-hydroxylated metabolite, 6,7-dihydrodiol [Rolan et al, 2009]. Food has no significant effect on [redacted] exposure. The primary elimination pathway for [redacted] is renal clearance. According to the drug label, following a single oral 10-mg dose in healthy adult volunteers, approximately 60% of the dose is recovered in urine as metabolite by 72 hours. There is currently no information available in the public domain concerning clinical drug-drug interaction (DDI) studies in humans for potential inhibition or induction of CYP450 enzymes and/or interaction of [redacted] with drug transporters. Results from our in vitro experiments indicate that ibudilast is not expected to cause clinically relevant inhibition of cytochrome P450 in humans. Consistent induction of CYP2B6 has been observed with [redacted] on human hepatocytes, however for an equivalent dose beyond the 10 mg twice a day dose which will be the highest dose tested in this study (see Section 1.3.2). After a single oral dose of 30 mg (generic delayed-release [redacted] product, [redacted]) in healthy volunteers, the median time to maximal concentration (t_{max}) was approximately 4 to 5 hours, the mean C_{max} was 32 ng/mL and the apparent elimination half-life ($t_{1/2}$) approximately 19 hours. The T_{max} and $t_{1/2}$ were similar after multiple dosing for 14 days [Rolan et al, 2008]. Negligible amount of drug was found in the urine and the 6,7-dihydrodiol metabolite was readily detected in plasma. The steady state geometric mean $AUC\tau_{ss}$ during twice a day dosing (516.6 ng.h/mL for day 6 and 506.5 ng.h/mL for Day 16) was about 10% smaller than the $AUC_{0-\infty}$ after a single dose, which corresponded to an approximately 0.9 ratio. This ratio was <1.0 showing that there was no accumulation of [redacted] from the single dose to the multiple doses, and the multiple dose plasma concentrations

were as predicted under linear PK. Based on these data and assuming a similar bioavailability between [redacted] capsule, the expected mean $C_{max,ss}$ with the planned dose of [redacted] mg twice a day should be close to 10 ng/mL and the expected mean $AUC\tau_{ss}$ should not exceed 170 ng.h/mL.

[redacted]

In humans, the bioavailability of [redacted] is reported to range from 59 to 89% with a median value of about 80% [Brater, 1991]. Plasma protein binding of [redacted] is high (from 93 to 97%) [Turmen et al, 1982]. The observed [Tissue]/[Plasma] ratios for [redacted] are low including low brain levels after systemic administration [Löscher et al, 2013]. To date, the main enzymes responsible for [redacted] metabolism in humans have not been identified and described in the literature. The drug is rapidly eliminated by urinary excretion ($t_{1/2}$ between 1 and 1.5 hours), with approximately 80% of an oral dose of ^{14}C-labeled [redacted] in human volunteers recovered in urine and with 45% eliminated as unchanged drug. Approximately 2% of the administered dose is eliminated by biliary excretion, suggesting that liver biliary excretion plays a minor role in drug elimination [Burinex Product Monograph, 2018]. Results from in vitro experiments indicate that [redacted] is not expected to cause clinically relevant inhibition of cytochrome P450 in humans [Study 8400152]. After single oral dose of [redacted] in healthy volunteers, the T_{max} occurred between 0.5 and 2 hours post-dose, with mean C_{max} values between 31 and 48 ng/mL following 1 mg of oral [redacted] [Holazo et al, 1984; Yagi et al, 1999]. The $t_{1/2}$ ranged between 0.7 and 1.5 hours in the majority of studies following both oral or intravenous administration and the $AUC_{0-\infty}$ observed for oral doses of 1 and 2 mg were around 100 and 140 ng.h/mL.

Because [redacted] is a diuretic drug, it may have an effect on the renal clearance of [redacted] and could cause a reduction in its plasma level. Thus, urinary drug levels of [redacted] will be measured in this study.

1.4.2 SAFETY AND TOLERABILITY

There is no safety data from the administration of STP1 in humans, but it is available for each of its components.

[redacted]

From the clinical Japanese studies, the most common adverse events (AEs) recorded in approximatively 15,000 adult patients with either bronchial asthma or complications following cerebral infarction were anorexia (0.58%), nausea (0.56%) and increased liver enzyme levels (0.36%); rare cases of thrombocytopenia were also reported [redacted]. Ledeboer et al. have summarized the safety data from studies in asthma and post-stroke dizziness from Japanese articles [Ledeboer et al, 2007]. In a double-blind, placebo-controlled study of 238 patients with post-stroke dizziness, [redacted] given at 30 mg once daily for 8 weeks did not show AE differences compared with placebo. The most common AEs were GI effects (11.2%), including anorexia (4.3%) and hepatic dysfunction (4.3%). In a 2-

year, open-label study of the recurrence of stroke where [redacted] (N=937) was given at 30 mg/day, 2.3% showed GI effects; [redacted] was discontinued in 1.8% of the patients. More recently, [redacted] was tested at dosages up to 100 mg/day in patients with multiple sclerosis [Fox et al, 2018] or opioid abusers [Cooper et al, 2017]. [redacted] was well tolerated, with an increased incidence of either headache or GI effects (i.e., nausea, diarrhea) compared with placebo. Table 1 summarizes the published clinical studies with the most frequently reported AEs for [redacted]

[redacted] doses from 0.5 to 2 mg twice a day for up to 3 months were studied in two randomized, double-blind, placebo-controlled trials in children and adolescents (2 to 18 years old) with ASD [Lemonnier et al, 2012; Lemonnier et al, 2017]. The frequency of AEs was correlated with the [redacted] dose. The most frequent AEs were hypokalemia, increased diuresis, loss of appetite, dehydration and asthenia. Hypokalemia occurred mainly at the beginning of treatment with the 1.0 and 2.0 mg twice a day doses and improved gradually with oral potassium supplementation. The minimally effective and safe [redacted] dose in the treatment of ASD was [redacted] mg twice a day; however, a [redacted] dose of [redacted] mg twice a day for 6 months is currently being evaluated in ongoing confirmatory trials in pediatric subjects.

In this trial, a [redacted] mg dose twice a day will be given over 2 weeks and laboratory safety monitoring will be implemented. If needed, oral potassium supplementation will be provided.

AE=adverse event; BID=twice daily; CO=crossover; DB=double-blind; GI=gastrointestinal; HV = healthy volunteers; MAD=multiple ascending dose; MS=multiple sclerosis; N/A=not available; NCT=National Clinical Trial number OLE=open-labeled extension; PC=placebo-controlled; PI=principal. investigator PK=pharmacokinetics; PD=pharmacodynamic; R=randomized; TID=three times daily; y=age.

1.5 STUDY RATIONALE AND BENEFIT-RISK ASSESSMENT

1.5.1 STUDY RATIONALE

ASD is characterized by a high heterogeneity in its behavioral manifestations matched, by very complex genetic underpinnings, suggesting the existence of subtypes of ASD. Therefore, efforts to categorize autism are still of critical importance and must rely on defining a relationship between clinical symptoms and biological mechanisms to improve on the outcome of clinical trials. STALICLA has identified a first subpopulation with idiopathic ASD (called ASD-Phen1) defined by clear clinical signs and symptoms that can be linked to dysregulation in key intracellular pathways and transcription factors as well as immune response dysregulations. The combination of ibudilast and bumetanide, called STP1, has been identified as the most promising drug candidate to modulate the identified dysregulations in ASD-Phen1 patients. [redacted] is expected to be the main driver of the potential effect of STP1. Its anti-inflammatory effect as a PDE4 and PDE10 inhibitor should exert beneficial immunomodulatory, and cognitive and behavioral effects by regulating energy metabolism. [redacted] although not an ASD-Phen1-selective compound, has been identified as a relevant candidate to maintain the effect of [redacted] over time in this specific patient population. Promising results have confirmed the existence of such an ASD subpopulation at the cellular level (see Section 1.3.1). *In vitro*, ibudilast has demonstrated its potential to modulate the identified dysregulated pathways in ASD-Phen1 cell lines (for further information, please refer to the STP1 Investigator's Brochure [STP1-IB]). In addition, STP1 has shown a good safety profile in nonclinical toxicology studies. Taken individually, both compounds have also demonstrated good clinical safety and tolerability profiles over a broad range of doses and indications. The present 2-week treatment, 2-dose level study will assess for the first-time tolerability, safety and PK profiles of STP1 in adult individuals with ASD-Phen1. The study was designed as a randomized, double-blind, placebo-control, 2-dose, ascending study.

The use of a placebo-control arm allows for a fair comparison to assess safety, tolerability and potential pharmacodynamic (PD) effect of STP1.

Despite the amount of supporting clinical safety data available for both compounds (see Section 1.4.2), this is the first time that STP1 will be tested in humans. Therefore, a stepwise ascending dose approach was chosen, starting with a "low" dose of [redacted] mg twice a day (i.e., below the dose typically prescribed for other indications and at the lower range of the Concentration that gives half the maximum inhibition [IC_{50}] for PDE4). Once the first dose cohort (8 patients) finishes 2 weeks of treatment and 2 weeks of follow up, the Data Safety Monitoring Board (DSMB) will advise STALICLA whether the study can pursue with the second dose level of [redacted] mg twice a day [6 hours apart]). In order to obtain sufficient safety and tolerability data to support a Phase 2 trial with a similar or lower dose of [redacted] the dose of [redacted] will be kept at 1 mg twice a day in both cohorts.

1.5.2 BENEFIT-RISK ASSESSMENT

[redacted] is a brain penetrant PDE3, PDE4, PDE10 and PDE11 inhibitor. [redacted] has been marketed [redacted]. Recommended dosing is [redacted] mg orally twice a day for asthma. In published studies of [redacted] with doses up to 100 mg/day, the most common AEs were headaches and GI effects (e.g., nausea,

diarrhea, and elevated liver enzymes). Rare cases of thrombocytopenia have also been reported. [redacted] has anti-inflammatory effects and is predicted to have therapeutic effects for patients with ASD-Phen1 because these patients are thought to have an inflammatory-specific phenotype. Beneficial activity has been reported in rodent models of inflammatory-based brain disorders.

[redacted] marketed in the United States [redacted] is a potent NKCC1 inhibitor that acts as a loop diuretic. It has been administered in doses of 0.5, 1, or 2 mg twice a day for up to three months in a randomized, double-blind, placebo-controlled trial of 88 children and adolescents (ages 2 to 18 years) with ASD [Lemonnier et al, 2017]. Statistically significant improvements on an autism rating scale (Childhood Autism Rating Scale [CARS]) and an autism severity scale (Social Responsiveness Scale, 2nd Edition [SRS-2]) were reported with [redacted] therapy over placebo among study completers. AEs were dose-related, the most common being hypokalemia, increased diuresis, loss of appetite, dehydration and asthenia. Six of 21 patients in the 2 mg twice a day group dropped out because of an AE, most frequently hypokalemia. Hypokalemia occurred generally at the beginning of treatment and improved gradually with oral potassium supplementation and potassium-rich foods. The [redacted] twice a day dose appeared to provide the best balance between safety and efficacy. To minimize the risk of hypokalemia, concomitant use of other loop diuretics (e.g., furosemide) will not be permitted during the study and electrolyte levels will be closely monitored. In the event of confirmed hypokalemia, potassium supplementation will be prescribed.

The 4-week rat and dog toxicology packages support the current clinical trial starting with a combination of [redacted] twice a day and [redacted] twice a day in cohort 1, followed by [redacted] twice a day and [redacted] twice a day in cohort 2. The pivotal toxicity studies performed in rats and dogs have shown that dog in the most sensitive species to STP1 treatment: the NOAEL was 3 mg/kg/day in male dogs which represents a 5-fold safety margin based on allometric scaling.

[redacted] has immunomodulatory and anti-inflammatory properties and, as such, should not be taken while an acute infection is ongoing. Therefore, patients with active infection, including (but not limited to) bacterial, fungal or viral infection, will not be included in the trial. Rare cases of thrombocytopenia have been reported with the use of [redacted] even though not expected at the dose levels used in this study and the short treatment duration, platelet count will be monitored throughout the study and patients with history of thrombocytopenia within the past year will not be included in the study. The use of other medication that could worsen possible thrombocytopenia will be used with precaution. [redacted] is predominantly cleared by CYP3A4 metabolism with to a lesser extent other CYP450 pathways (see Section 1.3.2). In order to avoid interference with the PK of STP1, moderate or strong CYP3A4 inhibitors (e.g., ketoconazole) are excluded. As a potent loop diuretic, [redacted] may trigger hypokalemia and hypotension. Changes in electrolyte levels and blood pressure (including orthostatic blood pressure [BP]) will be monitored at each post-dose study visit and other loop diuretics will be prohibited. PK drug-drug interactions between [redacted] are not expected since both compounds are metabolized by different pathways. Both drugs are inhibitor of BCRP transporter at subclinically relevant levels, but the effect of drug combination on BCRP is unknown and drugs that are substrate of BCRP should be used with caution during this clinical trial. It is

possible that because [redacted] is a diuretic, an effect on the renal clearance of [redacted] may be observed, leading to a reduction in [redacted] plasma level. Urinary drug levels of [redacted] will be assessed in this study to evaluate potential effects of [redacted] clearance.

Overall, the risk to patients with ASD-Phen1 treated with STP1 is considered small. There is limited or no direct benefit expected for the patients enrolled in this trial, designed to assess safety, tolerability and PK of 2 doses of STP1 given twice a day for 2 weeks. However, the unmet medical need for effective treatment in Autism is enormous. Given the potential of STP1 to significantly improve the identified dysregulated biological pathways in ASD-Phen1, the potential benefit for future patients (including pediatrics) would be significant.

2. OBJECTIVES OF THE STUDY

2.1 PRIMARY OBJECTIVE

The primary objective of the study is:

- To determine the safety and tolerability of 2-week twice a day (6 hours apart) treatment with STP1 (combined oral doses of [redacted]) in adult patients with ASD-Phen1.

2.2 SECONDARY OBJECTIVE

The secondary objective of the study is:

- To determine the plasma PK of multiple dosing and to determine the concentration in the urine of [redacted] when given orally in combination, as STP1, for 2 weeks.

2.3 EXPLORATORY OBJECTIVES

The exploratory objectives of this study are as follows:

- To evaluate the effect of a 2-week treatment with STP1 compared to placebo on cognition as assessed by subtests of the [redacted] and the Test of Attentional Performance for Children (KiTAP) test battery.
- To evaluate the effect of a 2-week treatment with STP1 compared to placebo on maladaptive behavior as assessed by the Aberrant Behavior Checklist–Community (ABC-C).
- To evaluate the effect of a 2-week treatment with STP1 compared to placebo change in the context of autism as measured by the change in the Ohio Autism Clinical Impression Scale (OACIS) change.
- To evaluate the effect of a 2-week treatment with STP1 compared to placebo on the Children Sleep Habit Questionnaire (CSHQ) scores.
- To evaluate the effect of a 2-week treatment with STP1 compared to placebo on the Clinical Global Impressions-Severity (CGI-S) scale, also reflected by the Clinical Global Impressions-Improvement (CGI-I) scale.
- To evaluate the effect of a 2-week treatment with STP1 compared to placebo on brain electrophysiology parameters as measured by electroencephalogram (EEG).
- To evaluate the effect of a 2-week treatment with STP1 compared to placebo on gaze aversion and social interest by assessing eye tracking tasks.
- To evaluate the effect of a 2-week treatment with STP1 compared to placebo on Lactate/Pyruvate ratio (L:P).

3. INVESTIGATIONAL PLAN

3.1 STUDY DESIGN

This is a randomized, double-blind, placebo-controlled, parallel-group, 2-dose ascending study cohort (first and second), to assess the safety, tolerability and PK of 2-week treatment with STP1 in adult patients with ASD-Phen1.

3.1.1 OVERVIEW OF STUDY DESIGN

Figure 2 gives an overview of the study design. If eligible, the first dose cohort (8 patients) will be randomized and allocated to one of two groups with a 3:1 ratio:

- One group will receive [redacted] mg oral twice a day (6 hours apart) oral [redacted] and [redacted] mg oral twice a day (6 hours apart) oral [redacted] (STP1 dose 1, N=6).
- One group will receive matching oral placebo twice a day (6 hours apart) (N=2).

Once the first dose cohort (8 patients) finishes the 2 weeks of STP1 treatment and the 2 weeks of follow up, a DSMB will evaluate the safety and tolerability data to decide whether the dose of [redacted] can be increased as planned to [redacted] mg, or the study should be modified or be stopped.

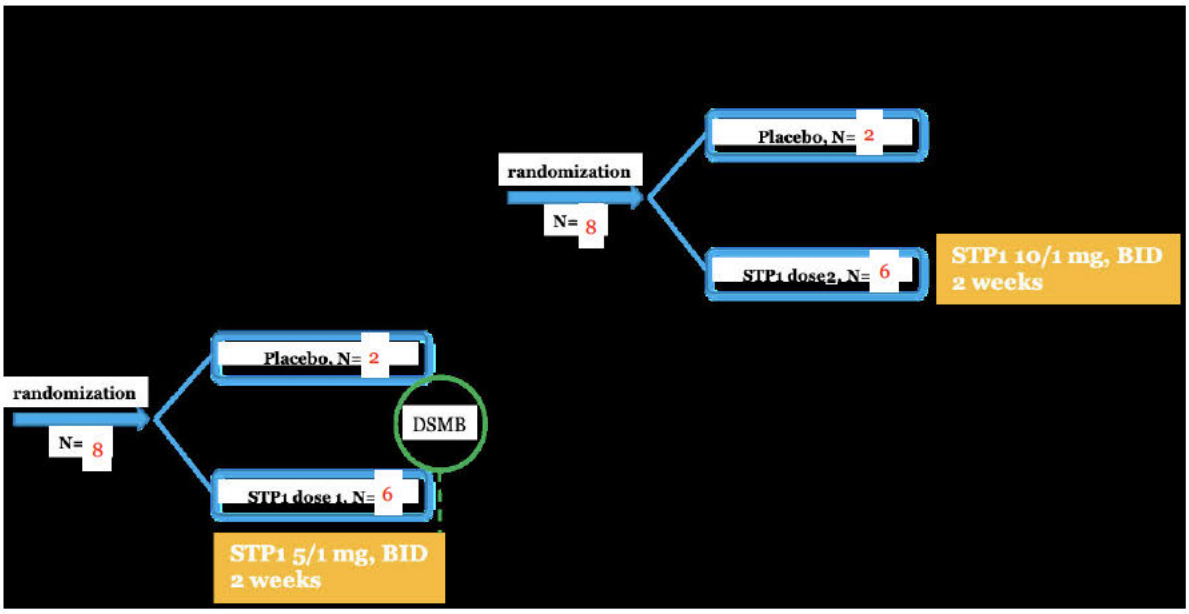
If the analysis supports the start of the next dose level, up to 8 additional eligible patients will be assigned to the next dose level (patients that participated in the first cohort maybe allowed to participate in the second if no safety concern):

- One group will receive [redacted] mg oral twice a day (6 hours apart) oral [redacted] and [redacted] mg oral twice a day (6 hours apart) oral [redacted] (STP1 dose 2, N= up to 6).
- One group will receive matching oral placebo twice a day (6 hours apart) (N= up to 2).

For each dose cohort, the total duration of the study for each patient will be up to 6 weeks divided as follows:

- A screening phase of 2 weeks (from Day -14 to Day -1).
- A double-blind treatment phase of 2 weeks (from Day 1 to Day 14).
- A follow-up phase of 2 weeks after treatment discontinuation (from Day 15 to Day 28).

Figure 2 Overview of Study Design



For STP1 dose 2: N= up to 2 placebo and up to 6 STP1 dose 2

3.1.2 STOPPING RULES

Study Stopping rules

During and after the administration of STP1, the patient will be closely monitored for clinically significant signs and symptoms potentially related to the study drugs (ibudilast and bumetanide), in particular for signs and symptoms GI and dehydration and for clinically significant changes in the electrocardiogram (ECG). Any AE should be followed up until resolution. If not resolved, STALICLA shall be contacted and the patient may be considered to withdrawn from the trial depending on the severity of the event/s.

The DSMB will perform reviews of safety data throughout the study.

Enrolment in the study will be placed on hold and no further dosing will occur pending a full safety review if:

- One fatal or life-threatening SAE occurs, that is considered by the investigator as potentially or possibly related to SPT1 and later confirmed the patient received investigational medicinal product (IMP).
- STALICLA, investigators and/or the DSMB considers that the number and/or severity of AEs, abnormal safety monitoring tests or abnormal laboratory findings justify putting the study on hold. Examples are:
    - One severe systemic-related reaction occurs and does not resolve within 24 hours.
    - Three or more similar severe AEs occur as defined by the Common Terminology Criteria for Adverse Events (CTCAE v5.0), which are judged related to STP1.

- If 2 patients at any dose level discontinue treatment due to changes in vital signs, ECG intervals or findings on continuous ECG monitoring no further patients will be enrolled at this dose level pending review by DSMB.

The DSMB can recommend (i) for the study to continue without amendment, (ii) to continue the study with modifications to the protocol or (iii) to stop the study.

The study may continue after the safety review, if the DSMB and STALICLA agree it is safe to proceed.

Individual stopping rules

If a patient experiences an AE following administration of study drug (i.e., post-treatment) that is judged to be related to the study drug, and is graded as severe or SAE, no further doses of study drug will be administered.

If a patient experiences an AE that is judged to be related to the study drug, and is graded as mild or moderate, the patient may receive a further dose of study drug following discussion with the investigator and sponsor, dependent on the nature of the AEs reported. Expected transient GI pain requiring 48 hours stop and not resuming with reinitiation of treatment is commonly observed. The number of subjects reporting similar AEs and reports of the same or similar AEs in a patient, will form part of the assessment to determine if patients should be re-challenged.

Changes in vital signs and ECG intervals

Patients will be discontinued if after repeated measurements, have pulse/heart rate, blood pressure or ECG interval measurements that fulfil one or more of the criteria listed below.

Vital signs

- Pulse/heart rate <40 or >120 beats per minute (BPM)
- Systolic BP <90 or >180 mm Hg
- Diastolic BP <50 or >100 mm Hg

Laboratory tests

- Severe hypokalemia (defined as less than 2.5 mEq/L) or persistent hypokalemia despite potassium supplementation
- Thrombocytopenia is defined as meeting one or both criteria:
    - Platelet count below 150 × 10E9/L (or 150.000 platelets per microliter (mcL))
    - Platelet count drop of ≥50% compared to baseline
- Severe drug-induced liver injury (DILI).
- Dehydration and orthostasis (defined by a fall in systolic blood pressure over 20 mm Hg or a fall in diastolic pressure over 10 mm Hg within 3 minutes of standing.

3.1.3 DATA SAFETY MONITORING BOARD (DSMB)

Blinded safety data will be reviewed by the sponsor on an ongoing basis throughout the study period. In addition, an independent group of experts (DSMB) will have a

safety oversight responsibility and will advise STALICLA. The DSMB shares with STALICLA the responsibility for regularly monitoring the overall safety of the patients in the trial by carefully reviewing overall rates of AEs including SAEs and other specified safety events. The DSMB will help STALICLA to minimize patient exposure to unnecessary risk.

The primary responsibilities of the DSMB are to 1) review and evaluate the accumulated study data for patient safety, study conduct and progress, and 2) make recommendations to STALICLA concerning the continuation, modification, or termination of the trial. The DSMB will consider study-specific data as well as relevant background knowledge about the disease, STP1, and patient population under study.

The DSMB recommendation should focus on safety data. There is extensive experience and available data with the use of [redacted] ([redacted]) and of [redacted] (in the U.S.) alone for other indications and the AE profile of both individual drugs is known. The DSMB should take into consideration this clinical experience to evaluate overall rates of AEs reported for this study. Should the DSMB consider that the combination of [redacted] ([redacted] mg twice a day) and [redacted] ([redacted] mg twice a day) is safe for the study population, they should recommend continuing the study according to the protocol.

The DSMB consist of a minimum of 3 and up to 4 STALICLA-independent members. They will be independent from the study. The board will consist of:

- 2 MDs specialized in ASD/neuro-immunology/neurodevelopmental disorders (NDDs).
- One MD or PhD with extensive experience in drug development in ASD or neurodevelopmental disorders, and
- Optionally 1 Clinical Pharmacologist.

Once the first dose cohort (i.e., 8 patients) has finished the 2 weeks of treatment and the 2 weeks of follow-up, the DSMB will initially review and evaluate the blinded study data but has the right to request individual unblinded data when justified. The blinded study data will include:

- Interim/cumulative data for evidence of study drug-related AEs:
- Interim/cumulative safety laboratory data with a more specific evaluation of potential electrolyte imbalance as described in the study protocol and coagulation parameters.
- ECG and vital signs data (including orthostatic blood pressure changes).
- Data quality, completeness and timeliness.
- Adherence to the protocol.

The DSMB will specifically assess the changes from baseline all safety laboratory data with a more specific evaluation of potential electrolyte imbalance and coagulation parameters.

The DSMB will provide recommendations to STALICLA as to whether the study should continue according to the protocol (i.e., proceeding with the recruitment for the second dose level of [redacted] mg twice a day (6 hours apart) of [redacted] mg twice a day (6 hours apart) of [redacted]) or be terminated.

3.1.4 END OF STUDY

The end of the study is defined as the date when the last patient, last observation (LPLO) occurs. LPLO is expected to occur approximately 4 weeks after enrolment of the last patient.

3.2 RATIONALE FOR STUDY DESIGN

There is currently no data on the safety and PK of [redacted] given in combination. Therefore, the present study evaluates safety and tolerability as the primary outcome, using doses which are used clinically, with the goal of confirming the good safety and PK profiles of the combination in this study population. This is a parallel-group, multiple dose study whereby the dose of [redacted] will only be increased if the safety/tolerability results support the start of the second dose level as evaluated by an independent DSMB. The use of a placebo-controlled arm allows for a fair comparison to assess safety and potential PD effect of STP1. Potential clinical improvement of the patients will be explored and may help identify the endpoints most sensitive to change. The outcomes will be used to design a larger dedicated Phase 2 trial in adults and adolescents.

3.2.1 RATIONALE FOR DOSAGE SELECTION AND TREATMENT DURATION

As [redacted] is expected to be the main driver of the STP1 effect, only the dose of [redacted] will be increased between the two cohorts while the dose of [redacted] will be fixed to [redacted] twice a day (6 hours apart).

The starting dose of [redacted] mg of [redacted] twice a day (6 hours apart) was chosen based on the PK and safety data available from the literature. The margins of safety (MOS) associated with the starting dose is [redacted] and was derived from the preclinical toxicokinetic data and the estimated NOAEL in the toxicology studies performed in the dog, the most sensitive species to STP1. The second dose level of [redacted] mg twice a day (6 hours apart) is expected to allow [redacted] to reach a C_{trough} of approximately 13 ng/mL, corresponding to 56 nmol which is within the range of PDE4 IC_{50} [Huang et al, 2006]. Hence, the 5 and 10 mg twice a day (6 hours apart) doses are expected to modulate PDE4 and might already show positive PD effects (e.g., on EEG endpoints or on processing speed, as assessed by a computerized cognitive battery). The safety / tolerability profile should remain acceptable as per extensive data collected in [redacted] and from clinical trials and summarized in Table 1, with nausea as the most frequent AE reported with doses of 30 mg/day. In this study, the maximum dose planned is 20 mg/day for 2 weeks.

[redacted] alone given to children and adolescents with ASD, aged 2 to 18 years, has been shown to improve core symptoms in a subset of patients with an acceptable risk/benefit ratio at a dose of 1 mg twice a day (6 hours apart) for 3 months [Lemonnier et al, 2017].

The [redacted] steady state concentration should be achieved after 4 days of treatment. Hence, a 2-week treatment duration with STP1 was considered appropriate to provide sufficient safety, tolerability and PK information for both [redacted], after multiple dosing of STP1. Beneficial impacts of PDE4 inhibitors on cognition, and more specifically processing speed, have been recently described in different studies involving healthy volunteers [Chiu et al, 2014; Heckman et al, 2018; Van Duinen et al, 2018]. Two weeks of treatment are thought to be sufficient to observe potential PD

effects on EEG recordings, or cognition and processing speed (as assessed by the [redacted] or KiTAP test battery).

3.2.2 RATIONALE FOR STUDY POPULATION

Adult male or female individuals with idiopathic ASD and meeting the 2 primary criteria for ASD-Phen1 will be enrolled in this study.

Since this is the first time that STP1 will be tested in humans, only adult patients with ASD-Phen1 will be included in the study. In addition, no prospect of direct clinical benefit of STP1 has been demonstrated yet and a treatment duration of 2 weeks will not allow for a potential impact on functional outcomes.

STP1 is specifically designed to alleviate the cellular and molecular perturbations identified in patients with ASD-Phen1; therefore, only individuals with idiopathic ASD matching the 2 ASD-Phen1 primary criteria will be enrolled in this trial. This subpopulation is defined by 1) [redacted] nd 2) [redacted]. Identifying the right subpopulation is critical not only to increase the chance to respond to STP1 but also to avoid enrolling patients with ASD for whom STP1 may worsen their symptoms. In fact, another subpopulation of patients with ASD showed improvement in their symptoms when presenting with fever [Grzadzinski et al, 2018].

3.2.3 RATIONALE FOR PHARMACOKINETIC SAMPLING

Blood PK samples will be collected from all patients for measurement of plasma and urine concentration of [redacted]. These STP1 PK data will be used to evaluate potential correlation between blood exposure and AE incidence, will be compared with PK data [redacted] described in the literature, and will be used to explore the potential impact of [redacted] (potent diuretic) on the PK and excretion of [redacted]. Combined with the safety, tolerability and PD data collected, these data will help identify the dose levels to be used in a subsequent Phase 2 trial.

3.2.4 RATIONALE FOR BIOMARKER ASSESSMENTS

- Electroencephalographic (EEG) recordings

Gamma band activity has established neural mechanisms, which include the local circuit glutamate/GABA interactions [Cardin et al, 2009; Lally et al, 2014]. During sensory processing, for instance, gamma is associated with bottom-up sensory processing of basic stimulus properties [Brosch et al, 2002]. In addition, enhancing effects of the PDE4 inhibitor roflumilast on sensory gating in healthy volunteers were recorded using an Event Related Potential (ERP) paradigm [Heckman et al, 2018].

Altogether, altered inhibitory/excitatory networks in ASD-Phen1 and potential impact of STP1 could be evaluated using electrophysiology as a target engagement biomarker, but also the findings may be predictive of clinical/behavioral responses relevant to STP1 drug development.

- Lactate to pyruvate ratio (L:P)

Mitochondrial dysfunctions could explain, at least in part, the energy metabolism dysfunction observed in the ASD-Phen1 subpopulation (as described in Section 1.2.1 and 1.3.1). Whole blood lactate and pyruvate levels combined with the L:P is used to assess mitochondrial function. An elevated L:P ratio (>20) may indicate inherited

disorders of the respiratory chain complex, tricarboxylic acid cycle disorders and pyruvate carboxylase deficiency. An L:P ratio below 10 (disproportionately elevated pyruvic acid) may indicate an inherited disorder of pyruvate metabolism, such as defects of the pyruvate dehydrogenase complex. Exploratory analysis of these parameters should help understanding whether the energy metabolism impairment observed is linked to mitochondrial activity defect.

3.3 OUTCOME MEASURES

3.3.1 SAFETY OUTCOME MEASURES

The safety outcome measures for this study are as follows:

- Incidence, nature and severity of AEs.
- Incidence, nature and severity of SAEs.
- Incidence, nature and severity of AESIs.
- Incidence, nature and severity of AEs leading to treatment discontinuations.
- Changes from baseline in systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and respiratory rate.
- Changes from baseline in orthostatic measurements of BP and HR.
- Changes from baseline in ECG parameters (HR, PQ, QRS, QT, RR and QTcF, along with information on T and U waves).
- Incidence of clinically significant ECG abnormalities.
- Hearing assessment
- Incidence of clinically relevant laboratory abnormalities, based on hematology, blood chemistry and urinalysis test results:
    - Hematology: hemoglobin, hematocrit, erythrocytes, platelet count, leucocytes differential (absolute) counts (neutrophils, eosinophils, lymphocytes, monocytes, basophils).
    - Blood chemistry: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total and conjugated bilirubin, alkaline phosphatase (ALP), albumin, creatine phosphokinase (CPK), creatinine, urea, total protein, total cholesterol, triglycerides, sodium, chloride, calcium, magnesium, phosphate, potassium, glucose.
- Incidence of value outside of the normal ranges of blood chemistry test results for electrolytes:
    - Sodium, chloride, calcium, magnesium, phosphate, potassium
- Urinalysis: protein, blood, glucose, leucocytes, nitrites, pH, and presence of crystals.

3.3.2 PHARMACOKINETIC OUTCOME MEASURES

Plasma concentrations of [redacted] will be used and a non-compartmental analysis (NCA) will be applied to analyze data from the sampling

schema. Urine concentration of [redacted] will be determined over a given period.

Plasma PK parameters such as $AUC_{0\ tau}$, AUC_{ast}, C_{max}, T_{max}, C_{trough}, apparent oral clearance, apparent volume of distribution and accumulation ratio will be calculated at steady state for both parent drugs. If data permitted, the elimination half-life at steady state will be estimated.

3.3.3 EXPLORATORY OUTCOME MEASURES

- Cognition, as assessed by subtasks [redacted] and the KiTAP battery test.
- Global clinical change in the context of autism as measured by the change in the OACIS scale.
- Maladaptive behavior, as assessed by the ABC-C.
- Change from baseline in the CGI-S scale (reflected by the CGI-I scale).
- Quality of sleep as assessed by the CSHQ.
- Electrophysiology parameters as measured by EEG at rest and during habituation and chirp tasks.
- Eye tracking.
- Change from baseline in blood L:P.

4. MATERIALS AND METHODS

4.1 CENTER

This is a single-center study to be conducted in the U.S. An additional site(s) may be included for back-up purposes and may be activated if needed.

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

4.2 STUDY POPULATION

This study will include male and female individuals between 18 to 40 years of age (inclusive) with ASD-Phen1.

4.2.1 RECRUITMENT PROCEDURES

An observational study [Study STP1-C003, NCT04273087] is currently ongoing at Cincinnati Children's Hospital Medical Center. This study allows early identification of adult patients with ASD-Phen1 who could be potential patients in this clinical trial. However, participation in this observational study [Study STP1-C003] does not guarantee or mandate participation in this treatment protocol [Study STP1-C004].

A total of up to approximately 20 patients will be enrolled with the objective to obtain a maximum of 16 patients completing the 2-week treatment duration. The number of patients may be increased if the actual dropout rate is higher than expected, to obtain up to 16 evaluable patients. Patients must meet all the inclusion criteria and none of the exclusion criteria in order to qualify for the study, as described in Section 4.2.2 and Section 4.2.3. Unless otherwise stated, inclusion and exclusion criteria refer to screening. Under no circumstances are individuals permitted to be re-randomized to receive a second course of treatment within the same cohort.

Subjects will be identified for potential recruitment per site-specific recruitment plans prior to consenting to take place on the study. Any recruitment materials for subjects and their families, will receive institutional review board (IRB) approval prior to use.

4.2.2 INCLUSION CRITERIA

Study patients must meet the following criteria for study entry:

1. Male or female (from 18 to 40 years old) individuals previously diagnosed with ASD (based on DSM-5 criteria as confirmed by the principal investigator [PI] or supported by either Autism Diagnostic Interview – Revised [ADI-R)] or Autism Diagnostic Observation Schedule – Second Edition [ADOS-2] scores).
2. Patients must have a documented [redacted] (according to the Centers for Disease Control and Prevention [CDC] growth charts, [CDC, 2019]) or being diagnosed [redacted].
3. Patients with systematic aggravation of ASD behavioral symptoms occurring [redacted] as determined by the ASD-Phen1 semi-structured interview form.
4. Patients must have a parent or reliable caregiver who agrees to provide information about the patient as required by the protocol.
5. The patients and caregivers should be fluent in English.

6. Patient willing and consenting or assenting to participate.
7. If assent provided by the patient, parent or legal guardian willing to give written consent.
8. Patients with ASD and comorbid seizure disorder should be seizure-free for at least 6 months prior to screening.
9. Before enrolling in the study, subjects must agree to use double-barrier birth control methods if they engage in intercourse.
10. Women of childbearing potential, defined as all women physiologically capable of becoming pregnant, can be enrolled if they are using highly effective methods of contraception during dosing and for 1 week after discontinuation of the investigational drug. Highly effective contraception methods include:
    - Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy or tubal ligation at least 6 weeks before taking the investigational drug. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment.
    - Male sterilization (at least 6 months prior to screening). For female patients on the study the vasectomized male partner should be the sole partner for that patient.
    - Use of oral (estrogen and progesterone), injected or implanted hormonal methods of contraception or placement of an intrauterine device (IUD) or intrauterine system (IUS) or other forms of hormonal contraception that have comparable efficacy (failure <1%), for example hormone vaginal ring or transdermal hormone contraception. Progesterone containing hormonal tablets must be associated with inhibition of ovulation in order to qualify as highly effective.
    - In case of use of oral contraception women should have been stable on the same pill for a minimum of 3 months before taking the investigational drug. If local regulations deviate from the contraception methods listed above and require more extensive measures to prevent pregnancy, local regulations apply and will be described in the informed consent form (ICF).

    Women are considered post-menopausal and not of childbearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g., age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least 6 weeks before taking the investigational drug. In case of oophorectomy alone the reproductive status of the woman must be confirmed by follow up hormone level assessment.

    Sexually active males must use a condom during intercourse after the start of the IMP administration and for at least one week after stopping study medication and should not father a child in this period after completion of the study medication. A condom is required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid. In addition, male

participants should not donate sperm for the time period specified above.

4.2.3 EXCLUSION CRITERIA

Study patients who meet any of the following criteria will not be included in the study:

1. Patients with an identified genetic cause of ASD in their medical record will be excluded from the study.
2. History of traumatic head injury, cerebrovascular disorder, congestive heart failure, hepatic or renal disease.
3. Thrombocytopenia (platelet count <100,000 mm^3) within 1 year prior to the screening visit.
4. Diabetes (type 1 diabetes mellitus or uncontrolled type 2 diabetes mellitus) or latent autoimmune diabetes of the adult (LADA).
5. Use of prohibited medications (Section 4.5.1) or herbal remedies within 2 weeks prior to randomization, or 5 half-lives (whichever is longer).
6. Alcohol and/or substance abuse/dependence within 12 months prior to screening.
7. A significant risk for suicidal behavior, in the opinion of the investigator and as assessed by the Columbia Severity Rating Scale (C-SSRS) or the simplified version adapted from the Columbia Classification Algorithm for Suicide Assessment (C-CASA).
8. Use of antioxidant supplements and/or vitamins within 2 weeks prior to randomization.
9. Initiation of, or a major change in psychological/behavioral intervention within 4 weeks prior to randomization.
10. Patient with any active infection, including but not limited to the following:
    - Bacterial infection; or
    - Fungal infection; or
    - Positive result at screening for hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV)-1 and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
11. Episode of fever (i.e., ≥100.5°F or 38.0°C) or clinically significant illness without fever (as judged by the Investigator), within 10 days before Day 1.
12. A SBP <80 mm Hg or a (DBP) <40 mm Hg or a drop in SBP of ≥20 mm Hg, or in DBP of ≥10 mm Hg, or patient experiencing lightheadedness or dizziness during the orthostatic recordings.
13. Clinically relevant ECG abnormalities; QTcF (Fredericia's correction) >450 ms (at screening or Day 1 pre-dose).
14. Confirmed clinically significant abnormal laboratory test results at screening (lactate and pyruvate not included) other than the conditions related to ASD-Phen1.
15. Patients with any active clinically significant disease (i.e. gastroenterological, endocrinological, renal, respiratory, cardiovascular, hepatic dysfunction,

immunological or hematological disease) that following the judgment of the investigator may interfere with the conduct of the study, other than the conditions related to ASD-Phen1.

16. Patients with any history of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years, regardless of whether there is evidence of local recurrence or metastases.
17. Pregnant (confirmed by laboratory testing) or lactating female patient.
18. Participation in an investigational drug study within 30 days or 5 times its half-life, whichever longer, prior to randomization.

4.3 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Each dose level will include up to 8 patients (up to 6 subjects assigned to STP1 and up to 2 assigned to placebo). The list of randomized treatment assignments will be generated by the sponsor or its designee, distributed to the unblinded site personnel, and incorporated into double-blind labeling. The qualified individual responsible for dispensing the study drug supplies will prepare the correct medication supply required according to the randomization schedule. This individual will write the date dispensed and Patient Number on the study drug labels and on the Drug Accountability Records. This individual will also record the study drug batches or lot numbers received by each subject during the study.

The randomized treatment assignment will be allocated from the list sequentially to subjects in the order in which they are enrolled. The unblinded personnel will be responsible for dispensing the assigned treatment.

With the exceptions described below, the randomization list will not be available to the project team at STALICLA or to any blinded personnel at the study centers.

The randomization list will be made available to the individual responsible for PK sample bioanalysis. The list will also be provided to the contract research organization (CRO) unblinded independent statisticians and programmers as needed to create unblinded data displays for the DSMB review(s).

Emergency Unblinding:

As per Health Authority reporting requirements, the sponsor will break the treatment code for all unexpected SAEs (see Section 5.1) that are considered by the investigator to be related to study drug(s).

The PIs will receive a set of sealed treatment codes. If the identity of the test medication needs to be known in order to manage the patient's condition, the treatment code for that patient may be broken.

In the event of an emergency, the treatment code for an individual patient will be readily available to the investigator and sponsor through on-site code break envelopes, as well as through communication with the unblinded, independent statistician responsible for generating the randomization list.

All such occurrences should be documented in the study file. Treatment codes should not be broken except in emergency situations and, if possible, the Chief Medical Officer/Clinical Trial Manager (STALICLA) should be contacted before the code is opened. At the final monitoring visit, the unused treatment codes will be counted and

checked and a statement to the effect that all are intact (or not as the case may be) will be made by the monitor; this statement will be included or referred to in the final study report. All treatment codes will be returned to the sponsor or designee.

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

4.4 INVESTIGATIONAL MEDICINAL PRODUCTS

4.4.1 FORMULATION, PACKAGING AND HANDLING

In this study, STP1 will consist of co-administration of [redacted]. [redacted] will be administered as a sustained-release granule encapsulated formulation containing either [redacted] mg or [redacted]. The placebo will be provided in a matching formulation, to be administered according to the same schedule as the study drug.

[redacted] re-packaged with a new label. Tablets with a dose strength of [redacted] mg will be used in this study. The placebo will be provided in a matching formulation, to be administered according to the same schedule as the study drug.

Study drugs packaging will bear a label with the identification required by local law, the protocol number, drug identification and dosage. The packaging and labeling of the study medication will be in accordance with local regulations.

The study drugs must be stored according to the details on the product labels. Study drugs should be stored below 25°C and protected from intense light in the containers provided. Upon arrival of investigational products at the site, site personnel should check them for damage and verify proper identity, quantity, integrity of seals and temperature conditions, and report any deviations or product complaints to the monitor upon discovery.

For further details, see the STP1 Investigator's Brochure [STP1-IB].

4.4.2 DOSAGE, ADMINISTRATION AND COMPLIANCE

[redacted] are to be taken orally at the same time, twice a day (6 hours apart) over 2 weeks. The drugs should be taken during food intake.

The first doses of medication will be administered at the study center on study Day 1, once all pre-dose assessments have been conducted and eligibility has been confirmed. For the subsequent study visits (Day 7 and Day 14), the morning dose will be taken at the study center. On Day 14, [redacted] will be given once (morning dose only).

Patients will be provided with a sufficient amount of medication to cover study treatment until the next site visit (including some overage). The qualified individual responsible for dispensing the study drug supplies will prepare the correct medication supply required according to the randomization schedule. This individual will write the date dispensed and Patient Number on the study drug labels and on the Drug Accountability Records. This individual will also record the study drug batches or lot numbers received by each subject during the study.

4.4.3 INVESTIGATIONAL MEDICINAL PRODUCTS ACCOUNTABILITY

[redacted] and the corresponding placebos will be provided by the sponsor. The investigational site will acknowledge receipt of investigational medicinal products

(IMP), 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 IMP must be maintained. The Drug Dispensing Logs must be kept current and should contain the following information:

- The identification of the Patient to whom the study drugs were dispensed (for example, patient initials and date of birth).
- The date(s), quantity of the study drugs dispensed to the Caregiver.
- The date(s) and quantity of the study drugs returned by the Caregiver.
- All records and drug supplies must be available for inspection by the Study Monitor [at every monitoring visit].

IMP will either be disposed 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 investigational medicinal product 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, 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 (e.g., Medication number [MEDNO]) of investigational product(s) destroyed.
- Quantity of investigational product(s) destroyed.
- Date of destruction.
- Method of destruction.

4.4.4 POST-TRIAL ACCESS TO STP1

The sponsor does not intend to provide STP1 or other study interventions to patients after conclusion of the study or any earlier patient withdrawal.

4.5 CONCOMITANT THERAPIES AND FOOD

Concomitant therapy includes any medication, e.g., prescription drugs, over-the-counter (OTC) 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 patient from screening until the follow-up visit. All concomitant therapies taken within 30 days of the screening visit should be reported to the investigator and recorded on the Concomitant Medications electronic Case Report Form (eCRF).

All medications administered to manage AEs should also be recorded on the AE eCRF.

Allowed concomitant therapies, including medications used for the treatment of stable medical conditions other than ASD that are allowed, must be on a stable dosing regimen for 6 weeks prior to screening and remain stable throughout the study (from screening to last follow-up visit).

High antioxidant containing food – including broccoli sprouts- considered a major source of sulforaphane and high glutamate containing food -e.g., parmesan, should be avoided.

This section is not meant to be exhaustive and any questions or concerns around individual concomitant therapy should be referred to the Study Management Team.

4.5.1 PROHIBITED THERAPIES

[redacted] have been used in clinic for many years and prohibited medication for both individual drugs is known. In vitro DMPK studies (see IND Section 4.3 of the Investigator's Brochure [STP1-IB]) showed that the risk for DDI between [redacted] is negligible to low.

Prohibited therapies should not be taken during the time period from at least 2 weeks (or 5 half-lives, whichever is longer) prior to initiation of study treatment until the end of the follow-up period.

4.5.1.1 POTENTIAL INTERACTIONS WITH IBUDILAST

- Selective PDE3, PDE4 inhibitors: theophylline, amrinone, rolipram, should be avoided to prevent pharmacodynamic drug interaction.

4.5.1.2 POTENTIAL INTERACTIONS WITH BUMETANIDE

- Especially in the presence of impaired renal function, the use of parenterally administered [redacted] in patients to whom aminoglycoside antibiotics are also being given should be avoided, except in life-threatening conditions.
- Drugs with nephrotoxic potential: there has been no experience with the concurrent use of [redacted] with drugs known to have a nephrotoxic potential. Therefore, the simultaneous administration of these drugs should be avoided.
- Lithium: lithium should generally not be given with diuretics (such as [redacted] because they reduce its renal clearance and significantly increase the risk of lithium toxicity.
- Probenecid: pretreatment with probenecid reduces both the natriuresis and hyperreninemia produced by [redacted] This antagonistic effect of probenecid on [redacted] natriuresis is not due to a direct action on sodium excretion but is probably secondary to its inhibitory effect on renal tubular secretion of [redacted] Thus, probenecid should not be administered concurrently with [redacted]
- Indomethacin: indomethacin blunts the increases in urine volume and sodium excretion seen during [redacted] treatment and inhibits the [redacted] increase in plasma renin activity. Concurrent therapy with [redacted] is thus not recommended.
- Antihypertensives: [redacted] may potentiate the effect of various antihypertensive drugs, necessitating a reduction in the dosage of these drugs

4.5.1.3 MEDICATIONS TO BE AVOIDED WITH STP1

- BCRP substrates: both [redacted] are inhibitors of the BRCP transporter. Although BCRP inhibition by each individual drug might not be of clinical relevance at the dose level used, it is difficult to predict the effect of both drugs administered together on the BRCP transporter activity. Therefore, drugs that are substrates of BCRP will be prohibited during this clinical trial. The list includes [Mao and Unadkat, 2015]:
    - Anthracenes: mitoxantrone, bisantrene and aza-anthrapyrazole.
    - Camptothecin derivates: topotecan, SN-38, irinotecan and diflomotecan.
    - Polyglutamates: methotrexate, methotrexate-Glu2 and methotrexate-Glu3.
    - Nucleoside analogs: azidothymidine (AZT), AZT 5′-monophosphate and lamivudine (3TC).
    - Other drugs: prazosin, indolocarbazole, flavopiridol, canertinib, imatinib mesylate, gefitinib, nilotinib, glyburide, cimetidine, sulfasalazine, nitrofurantoin, rosuvastatin and pantoprazole.
    - The concomitant treatment with CYP3A4 moderate or strong inhibitors is excluded. Medicines that are potent CYP3A4 inhibitors include (but are not limited to) clarithromycin, diltiazem, erythromycin, itraconazole, ketoconazole, ritonavir, telithromycin, and verapamil. Grapefruit juice is also a strong CYP3A4 inhibitor.

4.5.1.4 MEDICATIONS TO BE AVOIDED IN THE STUDY POPULATION

- Potent [redacted] activators: potent [redacted] activators such as sulforaphane should be avoided in ASD-Phen1 subpopulation as it is predicted that it would increase the patient ASD symptoms.

4.5.2 PRECAUTIONS

Consumption of food and beverages containing caffeine or methylxanthines (e.g., coffee, tea, cola, chocolate) is permitted during the study but meals served at the clinic should not contain caffeine or methylxanthines.

Vitamin/mineral supplements and occasional use of paracetamol (up to 1,000 mg/day) are allowed up until 24 hours before dosing.

Because of a theoretical DDI with [redacted] i.e., risk or severity of bleeding can be increased secondary to thrombocytopenia, Non-steroidal anti-inflammatory drugs, acetylsalicylic acid and other drugs that might have an impact on coagulation parameters should be used with caution during this clinical trial.

4.5.3 BEHAVIORAL AND COGNITIVE INTERVENTIONS

Behavioral or cognition-oriented interventions should not be changed or initiated during the study. Similarly, initiation of new medication or other treatments intended to improve cognition is not permitted.

4.5.4 SPECIAL CONSIDERATIONS AND FASTING

Subjects will be required to refrain from intense physical exercise within 72 hours before the screening visit and throughout the study and should inform the investigator of any event which could interfere with the conduct or interpretation of the study.

Laboratory safety assessments should be conducted after subjects have been fasted for a minimum of 4 hours.

4.6 STUDY PROCEDURES

4.6.1 DESCRIPTION OF STUDY ASSESSMENTS

At each visit, assessments and examinations will be conducted as described in the Schedule of Assessments (Appendix 1).

The total volume of blood loss for all the laboratory assessments throughout the duration of the study, including blood collection for PK, for biomarkers and biobanking, will be up to approximately 90 mL, per participant enrolled in a cohort of the study, but may be more if unscheduled laboratory tests need to be performed.

4.6.1.1 MEDICAL/FAMILY HISTORY AND DEMOGRAPHIC DATA

Medical history includes clinically significant diseases, prior medical interventions (e.g., vaccines, surgeries) or smoking/substance use history. All medication (e.g., prescription drugs, OTC drugs, herbal/homeopathic remedies, nutritional supplements) used by the patient within 4 weeks leading up to the screening visit will be collected.

Demographic data collected will include age, sex, race/ethnicity (as reported by the caregiver and where applicable), and [redacted] value within the first 2 years of age used for inclusion or a diagnosis of [redacted]

4.6.1.2 PHYSICAL EXAMINATION

A general physical examination will be performed as outlined in the Schedule of Assessments (see Appendix 1).

Any abnormality identified should be recorded on the Medical History eCRF page. Any new or worsened clinically significant abnormality should be reported as an AE on the AE eCRF page. Height and weight will be recorded, and body mass index (BMI) will be calculated.

4.6.1.3 VITAL SIGNS

Vital signs will include measurements of temperature, HR, respiratory rate and SBP and DBP, and will be recorded at the time-points specified in the Schedule of Assessments (Appendix 1). BP and HR measurements should be obtained in a quiet room at a comfortable temperature, with the patient's arm unconstrained by clothing or other material. All measurements will be obtained from the same arm (when possible) and, with the same cuff size, using a well-calibrated automatic instrument with a digital readout, throughout the study. Vital signs should be measured prior to blood draw or at least 10 minutes after the last blood draw.

After the patient has been resting for approximately 5 minutes in a supine position, BP and HR will be recorded. The subject will then be asked to stand, and BP and HR measurements will be repeated after standing 1 and 3 minutes.

A drop in systolic BP of ≥20 mm Hg, or in diastolic BP of ≥10 mm Hg will be recorded as abnormal and any dizziness, weakness, or visual changes associated with position change will be recorded as an AE.

4.6.1.4 ELECTROCARDIOGRAMS

ECG recordings will be performed as specified in the Schedule of Assessments. Whenever possible, the same brand/model of a standard high-quality, high-fidelity

ECG machine equipped with computer-based interval measurements should be used for each patient. The conditions of the post-dose recordings should be as close as possible to pre-dose time points; this includes but is not limited to food intake, activity level, stressors and room temperature. To minimize variability, the ECG will be recorded in triplicate at baseline (recorded 1 minute apart) and it is important that patients be in a resting position for at least 10 minutes prior to each ECG evaluation. Body position should be consistently maintained for each ECG evaluation to prevent changes in HR. 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 meals and 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 patient's permanent study file at the site.

ECG characteristics, including heart rate, QRS duration and HR, and QT intervals will be recorded on the eCRF. QTcF (Fredericia's correction) and RR will be recorded on the eCRF. Changes in T-wave and U-wave morphology and overall ECG interpretation will be documented on the eCRF. T-wave information will be captured as normal or abnormal, U-wave information will be captured in two categories: absent/normal or abnormal.

4.6.1.5 LABORATORY ASSESSMENTS

Normal ranges for the study laboratory parameters must be supplied to the sponsor before the study starts. Laboratory safety tests shall be collected at time-points specified in the Schedule of Assessments (Appendix 1).

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 patient's safety. Where the clinical significance of abnormal laboratory results is considered uncertain, screening laboratory tests may be repeated before randomization to confirm eligibility. If there is an alternative explanation for a positive urine or blood test for drugs of abuse, e.g., previous occasional intake of 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. 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.

When a fasted state is required, subjects have to be fasted for at least 4 hours.

Blood samples for the following laboratory tests will be sent to the local laboratory(ies) for analysis.

- Hematology (5 mL per sample): hemoglobin, hematocrit, erythrocytes, platelet count, leucocytes differential (absolute) counts (neutrophils, eosinophils, lymphocytes, monocytes, basophils).

- Blood chemistry (5 mL per sample): C-reactive protein (CRP test), AST, ALT, GGT, total and conjugated bilirubin, alkaline phosphatase (ALP), lactate, pyruvate, albumin, creatine phosphokinase (CPK), creatinine, urea, total protein, total cholesterol, triglycerides, sodium, chloride, calcium, magnesium, phosphate, potassium, glucose.
- Coagulation (2 mL per sample): international normalized ratio (INR), activated partial thromboplastin time (aPTT), prothrombin test (PT).
- Viral serology (2 mL per sample):
    - HIV (specific tests HIV-1 antibody).
    - Hepatitis B surface antigen (HBsAg).
    - Total hepatitis B core antibody (HBcAb).
    - Hepatitis C virus (HCV) antibody.

To test for ongoing infection to the SARS-CoV-2, the Food and Drug Adminiastration (FDA)'s approved biosamples (nasal swab, saliva) will be collected and quantitative Polymerase Chain Reaction (qPCR) test will be performed on this sample.

Urine samples will be used for analyses of protein, blood, glucose, leucocytes, nitrites, pH and presence of crystals. A urine pregnancy test will also be done for female patients.

Urine tests for barbiturates, benzodiazepines, amphetamines (including ecstasy), opiates (including methadone), cocaine, and cannabinoids will also be done. It will be performed at the site's local laboratory.

An alcohol test in urine will be performed at the screening visit and at the discretion of the investigator for the subsequent visits.

4.6.1.6 PHARMACOKINETICS ASSESSMENTS

Blood samples (2 samples of 2 mL) for determination of plasma concentration of [redacted] (and metabolite if appropriate) and [redacted] will be collected as specified in Appendix 1 and Table 2.

Table 2 PK sampling times

PK Schedu e[c]		
T me	P asma[a]	Ur ne[b]
Day 1	0.25, 0.5, 1, 2, 4 and, 6h post-AM dose	0-6 h post-AM dose
Day 7	Pre-AM dose	
Days 14 (on y AM dose) and 15	Pre-AM dose and 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h post-AM dose	0-4, 4-8 and 8-12 h post-AM dose
Between Days 16 and 18[d]	48, 72 and 96 h post-AM dose of Day 14	

[a] both [redacted] be ana yzed from the same b ood draw for each spec f ed samp ng t me po nt
[b] ur ne creat n ne from the ur ne co ect on w be pa red w th a serum creat n ne measurement to eva uate comp eteness of co ect on
[c] a these t mepo nts have a 5 m nutes w ndow
[d] Opt ona

Plasma and urine concentration of [redacted] will be measured by a specific and validated liquid chromatography-mass spectrometry (LC-MS/MS) method.

The actual dosing time of the preceding dose before each study visit (ie, time of PM dosing for Day 6 and Day 13) will be captured in the subject's medication diary (see Section 4.6.1.8) and in the relevant page of the eCRF. The actual time of the PK blood sampling needs to be precisely entered into the corresponding eCRF section.

4.6.1.7 BIOMARKER SAMPLING

- **Lactate to pyruvate ratio (L:P)**

Blood levels of lactate and pyruvate will be measured as an exploratory tool to further characterize the specificity of the ASD-Phen1 subpopulation, and as a potential biomarker of STP1 activity (lactate and pyruvate are part of the laboratory assessments list in Section 4.6.1.5). Whole blood lactate to pyruvate ratio (L:P) will be used to assess the mitochondrial function as detailed in Section 3.2.4.

4.6.1.8 STUDY-SPECIFIC ASSESSMENTS

- **Stanford Binet Intelligence Scales, Fifth Edition – Abbreviated Battery IQ (SB5 ABIQ)**

The Stanford–Binet Intelligence Scales (or more commonly the Stanford–Binet) is an individually administered cognitive ability and intelligence test that is used to diagnose developmental or intellectual deficiencies in young children. The ABIQ consists of two routing subtests from the full battery (Full Scale IQ – FSIQ). This abbreviated battery is based on Nonverbal Fluid Reasoning (Object Series/Matrices) and Verbal Knowledge (Vocabulary) [Janzen et al, 2004]. The test measures five weighted factors: knowledge, quantitative reasoning, visual-spatial processing, working memory and fluid reasoning [Nicolas et al, 2013]. It takes approximately 15 to 30 minutes to administer and will be administered at baseline only (screening).

- **Aberrant Behavior Checklist – Second Edition – Community (ABC-C)**

The Aberrant Behavior Checklist-Community, ABC-C, [Aman et al., 1985; Kaat et al, 2014] is a 58-item, parent or caregiver-rated questionnaire rating scale that measures

the severity of a range of problem behaviors commonly observed in individuals with intellectual and developmental disabilities, across 5 domains: irritability, agitation and crying; lethargy/social withdrawal; stereotypic behavior; hyperactivity/noncompliance; and inappropriate speech. Behaviors are rated on a 4-point scale from 0 to 3, with higher scores indicating more severity of the behavior specified. It will provide information about the range and severity of maladaptive behaviors in ASD-Phen1 patients. The ABC-C should take approximately 15 minutes to be completed and will be administered at baseline (screening) and at D14 and D28.

- **Social Responsiveness Scale, 2nd Edition (SRS-2)**

The Social Responsiveness Scale™, Second Edition, or SRS-2, identifies social impairment associated with ASD and quantifies its severity. It is a 65-item parent/caregiver rating scale used to assess the severity of social impairment within patients with ASD. Social awareness, social motivation and restricted interests and repetitive behavior are the domains explored by the scale [Constantino et al, 2013]. Standardized scores are derived (total score and 5 subscores). It is sensitive enough to detect even subtle symptoms but specific enough to differentiate clinical groups, the SRS-2 can be used to monitor symptoms throughout the life span. It allows to assess social impairment in natural settings—teachers, parents and others are asked to rate symptoms that they have noticed over time at home, in the classroom or elsewhere. Raters evaluate symptoms using a quantitative scale representing a range of severity. In addition to a total score reflecting severity of social deficits in the autism spectrum, five treatment subscale scores are provided: social awareness, social cognition, social communication, social motivation and restricted interests and repetitive behavior (for details see https://www.parinc.com/Products/Pkey/426). The questionnaire should take approximately 20 minutes to be completed and will be administered at baseline (screening) and Day 14 visits.

- **Ohio Autism Clinical Impression Scale – Severity (OACIS severity) and Improvement (OACIS change)**

The Ohio Autism Clinical Impressions Scale (OACIS) is an autism-specific clinical global impression scale, used as an outcome measures in psychopharmacology clinical trials. The OACIS is composed of two scales: the OACIS-Severity scale (OACIS-S), which measures global severity of illness at a given point in time as well as scores for 9 anchors: social interactions; aberrant/abnormal behaviors; repetitive/ritualistic behaviors; verbal communication; non-verbal communication; hyperactivity/inattention; anxiety/fears; sensory sensitivities; restricted and narrow interests. The OACIS improvement scale (OACIS-C), which measures change from the baseline state at following visits. The OACIS-C rating scale permits a global evaluation by the clinician of the subject's improvement over time. The OACIS-S is a 7-point scale ranging from 1 (no symptoms) to 7 (very severe). The OACIS-C is a seven-point scale, ranging from 1 (very much improved) to 7 (very much worse). The OACIS-S will be administered at baseline (screening) and Day 14 and the OACIS-C will be administered at the end of the study treatment only (D14).

A single clinician will rate the OACIS for each subject. In addition, the clinician must be blinded for side effects since potential diuretic effect of [redacted] may unblind the clinician. It is highly recommended that the clinician who will complete the OACIS is not involved in any other cognitive assessments or should complete the OACIS before any other interviews or cognitive testing.

- [redacted]

It includes over 100 stand-alone measures, also available in 30-minute batteries to assess:

- [redacted]

- **KiTAP Test Battery**

The Test of Attentional Performance for Children (Testbatterie zur Aufmerksamkeitsprüfung für Kinder; KiTAP) is a computer-based Continuous Performance Tasks (CPT) and Executive Function (EF) battery test, adapted for use in children from the Tests of Attentional Performance (TAP), a test used since the late 1990s to measure attention and EF performance in adults with various medical, neurological, and psychiatric conditions. The KiTAP is composed of eight tests that vary in length and difficulty level, thus it may be possible to use this test to measure attentional function over the broad range of intellectual ability present in a cohort with a disorder such as fragile X syndrome [Akshoomoff et al, 2014; Hessl et al, 2016].

- **Clinical Global Impressions-Improvement (CGI-I) Scale**

The CGI-Improvement (CGI-I) is simple in its format. Each time the patient is seen after medication has been initiated, the clinician compares the patient's overall clinical condition to the one-week period just prior to the initiation of medication use (the so-called baseline visit). The CGI-S score obtained at the baseline (initiation) visit serves as a good basis for making this assessment. Again, only the following one query is rated on a seven-point scale: “Compared to the patient's condition at admission to the

project [prior to medication initiation], this patient's condition is: 1=very much improved since the initiation of treatment; 2=much improved; 3=minimally improved; 4=no change from baseline (the initiation of treatment); 5=minimally worse; 6= much worse; 7=very much worse since the initiation of treatment."

- **Clinical Global Impressions-Severity (CGI-S) Scale**

The CGI-S score is often obtained, by researchers conducting psychopharmacology trials, at the baseline (initiation) visit and serves as a good basis for making this assessment. An illness severity rating is made on a scale of 1 to 7, with 1 being "normal not at all mentally ill" and 7 being "among the most extremely ill patients". Subsequently, the patient's condition on the study drug (or placebo) is compared to the patient's condition before the initiation of the study drug (or placebo) (baseline) via additional CGI-S ratings or the CGI-I item.

- **EEG**

Acquisition: Human recordings are acquired through 128-channel Phillips/EGI HydroCel nets with a Phillips/EGI Netamp 400 Amplifier (EGI, Eugene, OR). Data are recorded using Phillips/EGI Netstation acquisition software at a sampling rate of 1,000 Hz with saline based net electrodes (impedance under 10 KΩ). Systematic behavioral interventions have been developed by the site including social stories, visual schedules and demo nets/parent training to optimize successful data collection from our target population. In our experience, >95% of adults with ASD tolerate the procedure and provide usable EEG data following the implementation of these preparation/training procedures. EEG studies are conducted in sound attenuated and electrically shielded private rooms dedicated for EEG/ERP studies. Acoustic and tactile stimuli are generated using MATLAB (MathWorks, Natick, MA) and presented using Presentation 17 software (Neurobehavioral Systems, Berkeley, CA). Sound delivery is synchronized with the EEG recording using a Presentation 17 generated TTL pulse to mark the onset of each stimulus. During experimental neurophysiological procedures, when appropriate, subjects sit while watching a silent video (i.e., cartoon movie). The silent video will be standardized across all patients and used to facilitate cooperation in ASD.

1. Resting State: Continuous data will be recorded for 4 minutes eyes open and 4 minutes eyes closed (dependent on patient cooperation).

2. Auditory Chirp: Auditory "chirp" stimuli will be delivered at 65 dB SPL through standardized headphones with a 1,000-Hz carrier tone amplitude modulated (AM) by a chirp sinusoid linearly increasing in frequency from 0 to 100 Hz over 2,000 ms. Two hundred stimuli will be separated by a 1.5 to 2 second inter-trial interval, for 12.5 minutes of testing.

EEG will be recorded before and after morning dosing on Day 1 and after dosing on Day 14 and Day 28.

- **Eye Tracking**

Eye tracking data will be collected using a Tobii (Stockholm, Sweden) T120 infrared binocular eye tracker sampling at a rate of 120 Hz to record X and Y coordinates of eye position and pupil diameter along with gaze duration. The paradigms will be run on an integrated 17-inch flat-panel monitor (1,280 × 1,024 pixels resolution) running Tobii Studio (Version 3.0, Tobii Technology, Sweden).

Paradigm 1. Social scene preference ratio

Rectangular areas of interest (AOIs) will be created around both the social scene and geometric pattern videos. Social scene preference ratio (SSPR) will be calculated by dividing the time spent viewing the social scene videos by the total time spent viewing the social scene or geometric pattern videos (Eq. 1). Only the silent side-by-side video paradigm will be used in this analysis as audio from the social scene video was found to have a significant impact on selective preference towards the coinciding video.

$$\text{Eq. 1: SSPR} = \frac{\text{Viewing Time}_{\text{Social Scenes}}}{\text{Viewing Time}_{\text{Total}}}$$

Paradigm 2. Emotional faces

Stimuli will consist of 12 colored photographs of adult human faces (equal numbers of males and females) from the NimStim Face Stimulus Set [Tottenham et al, 2009], each showing a calm, happy, or fearful facial expression. Each emotional face will be presented on the screen for 5 s. Prior to presenting the emotional faces, a scrambled version of the face image will be presented for 1 s. Similar to Farzin et al, [2009, 2011], each face and corresponding scrambled image will be matched on mean luminance, and equivalence will be confirmed using a photometer (Minolta, LS-100, Osaka, Japan). Face images will subtend a 12.12° by 17.19° region (the size of an actual human face) when viewed from a distance of 60 cm and will be presented on a standard 50% gray background (RGB: 128, 128, 128).

- **Children Sleep Habit Questionnaire (CHSQ)**

The Children Sleep Habits Questionnaire (CSHQ) is a retrospective, 45-item parent questionnaire that has been used in a number of studies to examine sleep behavior in young children. The CSHQ includes items relating to a number of key sleep domains that encompass the major presenting clinical sleep complaints in this age group: bedtime behavior and sleep onset; sleep duration; anxiety around sleep; behavior occurring during sleep and night wakings; sleep-disordered breathing; parasomnias; and morning waking/daytime sleepiness. Parents are asked to recall sleep behaviors occurring over a “typical” recent week. Items are rated on a three-point scale: “usually” if the sleep behavior occurred five to seven times/week; “sometimes” for two to four times/week; and “rarely” for zero to one time/week. Some items were reversed in order to consistently make a higher score indicative of more disturbed sleep [Owens et al, 2000]. The abbreviated version of this questionnaire includes 22 items assessing sleep habits and possible difficulties with sleep [Owens et al, 2000]. Unless noted, “always” refers to something occurring every night, “usually” if it occurs 5 or 6 times a week, “sometimes” if it occurs 2 to 4 times a week, “rarely” if it occurs once a week and “never” if it occurs less than once a week. Since the questionnaire is designed for children, the questionnaire will be answered by the parent/caregiver whenever possible. It should take approximately 5 to 10 minutes to be completed.

- **Clinical Assessment of Suicidality**

The assessment of suicidality will be collected for all patients at all visits using the Columbia Suicide Severity Rating Scale (C-SSRS) or the Columbia Classification Algorithm for Suicide Assessment (C-CASA).

- Columbia Severity Rating Scale (C-SSRS): The Columbia Suicide Severity Rating Scale, or C-SSRS, is a suicidal ideation and behavior rating scale created by researchers at Columbia University, University of Pennsylvania, University of Pittsburgh and New York University to evaluate suicide risk. It rates an individual's degree of suicidal ideation on a scale, ranging from "wish to be dead" to "active suicidal ideation with specific plan and intent and behaviors". Questions are phrased for use in an interview format, but the C-SSRS may be completed as a self-report measure if necessary. The scale identifies specific behaviors which may be indicative of an individual's intent to complete suicide. An individual exhibiting even a single behavior identified by the scale was 8 to 10 times more likely to complete suicide (for details see https://cssrs.columbia.edu/).
- Simplified version adapted from the Columbia Classification Algorithm for Suicide Assessment (C-CASA). The C-CASA is a classification system that utilizes definitions of suicidality derived from empirical findings on the phenomenology of suicidality and identified predictive and risk factors. The criteria for a suicide attempt include both self-injurious behavior and suicidal intent (at least some intention to commit suicide). Intent to die portends a risk for future suicide and repeated attempts and can be reliably obtained. Inclusion of intent in the definition of suicide allows a distinction between those who self-injure in an attempt to die and those who self-injure for purely other non-suicidal reasons (e.g., to manage affect). The C-CASA has eight categories that distinguish suicidal events from non-suicidal events and indeterminate or potentially suicidal events [Posner et al, 2007].
- **Suicidal Ideation:** Has the patient wished he/she were dead or wished they could go to sleep and not wake up?
- **Suicidal Behavior:** Has the patient made a suicide attempt? Has the patient done anything to harm himself or herself?
- **Self-injurious Behavior:** Has the patient engaged in non-suicidal self-injurious behavior?
- If the investigator determines that any of these questions can be asked directly to the subject based on the subject's ability to understand and to react to the questions, then those questions should be directed to the subject. If the answer is "yes" to any of these 3 questions, then the investigator will further evaluate the suicidal risk of the patient.

- **Subject Medication Diary**

All enrolled (i.e., randomized patients) will be asked to maintain a "Subject Medication Diary". This template diary will be provided during the Baseline/Randomization visit to keep track of the daily doses and of the timing of study medications. Patients will be asked to bring this diary along with the study medication bottles to the Day 7 and Day 14 study visits to assess for compliance and drug accountability. If this is not done at the Day 14 visit for any reason, this can also be completed at Day 28 visit.

4.6.2 TIMING OF STUDY ASSESSMENTS (SEE APPENDIX 1)

The patients willing to participate in the study will only be included when all screening examination procedures have demonstrated that all inclusion criteria and none of the

exclusion criteria apply. The patients will be assigned a patient number within the study.

Screening (Visit 1, Day -14 to Day -1)

During the screening visit the following assessments will be performed:

- Informed consent to be obtained from the patient, parent or legal guardian .
- Eligibility criteria including all inclusion and exclusion criteria.
- Demographics .
- Medical history: including all clinically significant diseases, prior medical interventions (e.g., vaccines, surgeries) or smoking/substance use history. All medication (e.g., prescription drugs, OTC drugs, herbal/homeopathic remedies, nutritional supplements) used by the patient within 4 weeks leading up to the screening visit will be collected.
- Physical examination: a general physical examination will be performed.
- Vital signs: including temperature, HR, respiratory rate, orthostatic SBP and DBP.
- ECG-12 lead.
- Serology: HIV (specific tests HIV-1 antibody), Hepatitis B (HBsAg, HBsAb) and hepatitis C virus (HCV) antibody.
- Pregnancy test (urine) in females.
- SARS-Cov-2 test (nasal swab, saliva).
- Substance abuse history, and test urine
- Blood sampling for hematology, chemistry, and coagulation.
- Urine sampling for urinalysis.
- Alcohol (urine test).
- Suicidality assessment.
- ABC-C test.
- SRS-2 test.
- SB5 ABIQ test.
- OACIS-severity assessment.
- NIH toolbox assessment.
- KiTAP assessment.
- CSHQ assessment.
- CGI-S assessment.
- AEs
- Previous and concomitant treatments.

Treatment period

The treatment period consists of 3 visits (Days 1, 7 and 14). After screening assessments, the enrolment and treatment period will be started. Patients meeting all inclusion and none of the exclusion criteria will be enrolled.

Patients will then receive the study medication, orally, twice daily (6 hours apart) for 14 days. The patient will be closely observed during and after the administration of the study medication. The following procedures and assessments will be performed during and after the treatment: Please refer to the Schedule of Assessment (Appendix 1).

Visit 2 (Day 1)

During the Day 1 visit, defined as the first day of the study drug administration, the following assessments will be performed:

- Randomization.
- Physical examination: a general physical examination will be performed.
- Vital signs: including temperature, HR, respiratory rate, SBP and DBP.
- Orthostatic BP assessment.
- ECG-12 lead.
- Substance abuse history, and test (urine)
- Hearing assessment.
- Administration of the study medication.
- Study medication supply.
- Blood and urine sampling for PK.
- Suicidality assessment.
- [redacted]
- KiTAP assessment.
- Eye tracking assessment (pre- and post-dose).
- EEG (pre- and post-dose).
- CSHQ assessment.
- AEs.
- Previous and concomitant treatments.

Visit 3 (Day 7)

- Physical examination: a general physical examination will be performed.
- Vital signs: including temperature, HR, respiratory rate, SBP and DBP.
- Orthostatic BP assessment.
- ECG-12 lead.
- Blood sampling for hematology, chemistry and coagulation .
- Urine sampling for urinalysis.
- Administration of the study medication.
- Blood sampling for PK at pre-AM dose.
- Suicidality assessment.
- Hearing assessment.
- AEs.
- Previous and concomitant treatments.

Visit 4 (Day 14)

- Physical examination: a general physical examination will be performed.
- Vital signs: including temperature, HR, respiratory rate, SBP and DBP.
- Orthostatic BP assessment.
- ECG-12 lead.
- Blood sampling for hematology, chemistry, and coagulation.
- Urine sampling for urinalysis.
- Administration of the study medication.
- Blood and urine sampling for PK
- Suicidality assessment.
- ABC-C test.

- SRS-2 test.
- OACIS-severity assessment.
- OACIS-change assessment.
- [redacted]
- KiTAP assessment.
- Eye tracking assessment.
- EEG.
- CSHQ assessment.
- CGI-I assessment.
- CGI-S assessment.
- Hearing assessment.
- AEs.
- Previous and concomitant treatments.

Visit 4 (Day 15) and optional visit 5/6/7 (Day 16/17/18) (adults)

Patients will be given the option to have their follow-up PK sampling visit between Day 16 and Day 18.

- Blood sampling for PK.
- Urine sampling for PK (visit 4 [Day 15] only).
- Suicidality assessment.
- AEs.
- Previous and concomitant treatments.

End-of-study (EOS) follow-up visit (visit 8, Day 28)

The following assessments will be performed for each patient on Day 28, at the end of the follow-up:

- Physical examination: a general physical examination will be performed.
- Vital signs: including temperature, HR, respiratory rate, SBP and DBP.
- Orthostatic BP assessment.
- ECG-12 lead.
- Pregnancy test (urine) in females.
- Blood sampling for hematology, chemistry, and coagulation.
- Urine sampling for urinalysis.
- Blood sampling for PK at any time.
- Suicidality assessment.
- ABC-C test.
- SRS-2 test
- [redacted]
- KiTAP assessment.
- Eye tracking assessment.
- EEG.
- CSHQ assessment.
- CGI-I assessment.
- CGI-S assessment.
- Hearing assessment.
- AEs.
- Previous and concomitant treatments.

At visit days when multiple assessments involving the patient coincide, the following sequence should be followed:

- Vital signs and ECG recordings.
- Fasting laboratory samplings (and PK sampling where applicable).
- Break.
- EEG and eye tracking recordings (on Day 1 only).
- Break, snack and dose administration.
- Cognitive assessment.
- EEG and eye tracking recordings.

The cognitive testing should be performed at a similar time of day and in the same sequence across all study visits. Breaks can be allowed between the cognitive tasks if deemed necessary by the clinician/rater.

4.6.2.1 SCREENING AND PRE-TREATMENT ASSESSMENTS

Written informed consent for participation in the study must be obtained before performing any study-specific screening tests or evaluations. Informed Consent Forms (ICFs) for enrolled patient and for patients 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 patients meet all eligibility criteria. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure.

An Eligibility Screening Form (ESF) documenting the investigator's assessment of each screened patient with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator and kept at the investigational site. Where the clinical significance of an abnormal screening test result (e.g., laboratory test) is considered uncertain, the test should be repeated to confirm the result.

Subjects who participated in the observational study [STP1-C003, NCT04273087] will not have to redo the ASD-Phen1 semi-structured interview form. Data from the STP1-C003 study will be transferred or entered into this study database.

An abbreviated rescreening may be allowed under circumstances where the patient is screen-passed but could not be randomized within the 2-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 rescreening visit exceed 2 months, otherwise a full screening visit will be required. The abbreviated rescreening will include the following: a written informed consent, updated medical history, physical examination, drugs of abuse, pregnancy test (females only), alcohol urine test, laboratory safety, coagulation, viral serology, urinalysis, and inclusion/exclusion criteria.

4.6.2.2 ASSESSMENTS DURING TREATMENT

All assessments must be performed according to the Schedule of Assessments (Appendix 1). Under no circumstances will patients who enroll in this study and have completed treatment as specified, be permitted to be allocated a new randomization number and re-enroll in the study within the same cohort.

4.6.2.3 FOLLOW-UP ASSESSMENTS

Patients who complete the Day 15 study visit or discontinue from the study early, will be asked to return to the clinic on Day 28 (±2 days) for the follow-up visit. After the study completion, AEs should be followed as outlined in Section 5.6.

4.7 PATIENT, STUDY, AND SITE DISCONTINUATION

4.7.1 PATIENT DISCONTINUATION

The investigator has the right to discontinue a patient from STP1 or withdraw a patient from the study at any time. In addition, patients have the right to voluntarily withdraw from the study at any time for any reason. Reasons for discontinuation of STP1 or withdrawal from the study may include, but are not limited to, the following:

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

Patients who fulfill the criteria described in the Stopping Rules section (as defined in Section 3.1.2) should be discontinued from the study.

4.7.1.1 DISCONTINUATION FROM STP1

Patient must discontinue STP1 if they experience any of the following:

- Pregnancy.
- Patient unable to continue to comply with study requirements.
- Unblinding of a patient.

Patients who discontinue STP1 prematurely will be asked to return to the clinic for a follow-up visit (see Section 4.6.2.4). The primary reason for premature STP1 discontinuation should be documented on the appropriate eCRF page. Patients who discontinue STP1 prematurely may be replaced to achieve a total of 16 patients.

4.7.1.2 WITHDRAWAL FROM STUDY

Every effort should be made to obtain information on patients who withdraw from the study. The sponsor or a designee should follow up by telephone or electronic mail. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. The Study Monitor should include reviewing of subject withdrawals as part of the monitoring visit scope and Monitoring Plan.

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

Patients who withdraw consent may be replaced to achieve a total of 16 patients.

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 AE in this or other studies indicates a potential health hazard to patients.
- Patient enrollment is unsatisfactory.
- Important new information becomes available that may be relevant to the subject's consent.

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 replace a site at any time. Reasons for replacing 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 (GCP).

5. ASSESSMENT OF SAFETY

5.1 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording the incidence, nature and severity of AEs, including SAEs and AEIs; measurement of protocol-specified safety laboratory assessments; and measurement of protocol-specified vital signs, ECGs and other protocol-specified tests described in Section 5.1.4.

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 GCP, an AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition).
- 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) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug(s).
- AEs 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)

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

- Fatal (i.e., the AE actually causes or leads to death).
- Life-threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death). This does not include any AE that, had it occurred in a more severe form or was allowed to continue, might have caused death.
- Requires or prolongs in-patient hospitalization (see Section 5.3.5.10).
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions).
- Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug(s).
- Significant medical event in the investigator's judgment (e.g., may jeopardize the patient's safety or may require medical/surgical intervention to prevent one of the outcomes listed above).

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (rated as mild, moderate or severe, or according to a pre-defined grading criterion; see Section 5.3.3); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

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

SAEs are required to be reported by the investigator 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 ADVERSE EVENTS OF SPECIAL INTEREST (IMMEDIATELY REPORTABLE TO THE SPONSOR)

AESIs 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). AESIs for this study include the following:

- Electrolyte imbalance: hypokalemia (K+ level <3.4 mEq/L), hyperkalemia (>5.0 mEq/L), hyponatremia (Na+ level <135 mEq/L), hypochloremia (Cl- level <97 mEq/L)
- Thrombocytopenia is defined as meeting one or both criteria:
    - Platelet count below 150 × 10E9/L (or 150.000 platelets per microliter (mcL))
    - Platelet count drop of ≥50% compared to baseline
- Drug-induced liver injury (DILI)
- Dehydration and orthostasis (defined by a fall in systolic blood pressure over 20 mm Hg or a fall in diastolic pressure over 10 mm Hg within 3 minutes of standing.

5.1.4 OTHER SAFETY ASSESSMENTS

Additional safety data will be obtained on the following:

- Changes from baseline in SBP, DBP, HR and respiratory rate.
- Changes from baseline in orthostatic measurements of BP and HR
- Changes from baseline in ECG parameters (HR, PQ, QRS, QT, RR and QTcF, along with information on T and U waves)
- Incidence of clinically significant ECG abnormalities
- Hearing assessment
- Incidence of laboratory abnormalities, based on hematology, blood chemistry, coagulation and urinalysis test results, as described in Section 4.6.1.5.

5.2 SAFETY PLAN

The Safety Plan considers observations made in non-clinical investigations including GLP toxicology studies in rats and dogs and the observations made in clinical trials. Hypothetical considerations are included in the interpretation of non-clinical and clinical data.

The safety monitoring and the frequency of the scheduled visits are described in Appendix 1. All safety data will be reviewed throughout the study by the sponsor in a treatment-blinded manner. However, the DSMB will review the blinded safety and tolerability data obtained from the first dose cohort (8 patients) who finish the 2 weeks of treatment and the 2 weeks of follow-up. The following sections specify some of the monitoring measures that will be taken to maximize the patient's safety in this study.

5.2.1 MANAGEMENT OF SPECIFIC ADVERSE EVENTS

5.2.1.1 ABNORMAL LABORATORY FINDINGS

Laboratory abnormalities reported with [redacted] included hyperuricemia (18.4%) and electrolytes imbalance (e.g., hypochloremia for 14.9% or hypokalemia for 14.7%). Hypokalemia was also observed in a [redacted] dose-dependent manner in the BUMEA trial [NCT01078714; Lemonnier et al, 2017]. In the current study, laboratory assessment will be done every week during the treatment period and at the end of the follow-up period. If hypokalemia is observed, potassium supplements should be administered, and patients should be advised to eat potassium-rich foods.

5.2.1.2 CHANGES IN BLOOD PRESSURE

As a potent loop diuretic compound, [redacted] is as an anti-hypertensive drug. Hypotension and dizziness have been reported in approximately 1% of the patients taking [redacted]. In this study, assessment of BP, including orthostatic BP, will be performed at each study visit. If considered needed, symptomatic treatment should follow standard of care. Additional assessments of vital signs might be considered by the investigator.

5.2.1.3 SUICIDALITY

Clinical data does not point to any suicidality liability of [redacted]. However, monitoring for suicidality is mandatory in clinical trials of CNS-active molecules and will be implemented as outlined in Section 4.6.1.8 and Appendix 1. The Investigator is asked to assess individually appropriate next steps in case a suicidality alert arises.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The investigator is responsible for ensuring that all AEs (see Section 5.1.1 for definition) are recorded on the AE eCRF page and reported to the sponsor in accordance with instructions provided in this section and in Sections 5.4 through Section 5.6.

For each AE recorded on the AE eCRF page, 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 AEs at each patient contact. All AEs, whether reported by the patient or the caregiver or noted by study personnel, will be recorded in the patient's medical record. AEs will then be reported on the AE eCRF page as follows:

After informed consent has been obtained but prior to initiation of study drug(s), only SAEs caused by a protocol-mandated intervention should be reported (e.g., SAE related to invasive procedures such as biopsies).

After initiation of study drug(s), all AEs, regardless of relationship to study drug(s), will be reported until the follow-up visit (i.e., 2 weeks after the last dose of study drug[s]).

At the end of the 2-week follow-up period, investigators should report any deaths, SAEs, or other AEs of concern that are believed to be related to prior treatment with study drug(s) (see Section 5.6).

5.3.2 ELICITING ADVERSE EVENT INFORMATION

A consistent methodology of non-directive questioning should be adopted for eliciting AE information at all patient 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 3 provides guidance for assessing AE severity.

Table 3 Adverse Event Severity Grading Scale

Severity	Description
Mild	A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. Discomfort noticed, but no disruption of normal daily activity.
Moderate	A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject. Discomfort sufficient to reduce or affect normal daily activity.
Severe	A type of AE that interrupts usual activities of daily living, significantly affects clinical status, may require intensive therapeutic intervention, or is incapacitating with inability to work or to perform normal daily activity. The term “severe” does not necessarily equate to “serious”.

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 patient, the circumstances surrounding the event and an evaluation of any potential alternative causes to determine whether or not an AE is considered to be related to the study drug(s), indicating "yes" or "no" accordingly as per below definitions:

- **Related:** The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

- **Not related:** There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset or an alternate etiology has been established.

The following guidance should be taken into consideration:

- Temporal relationship of event onset to the initiation of study drug(s).
- Course of the event, considering especially the effects of dose reduction, discontinuation of study drug(s), or reintroduction of study drug(s) (where applicable).
- Known association of the event with the study drug(s) or with similar treatments.
- Known association of the event with the disease under study.
- Presence of risk factors in the patient 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 patient receiving combination therapy, causality will be assessed individually for each protocol-mandated therapy.

5.3.5 PROCEDURE FOR RECORDING ADVERSE EVENTS

Investigator(s) should use correct medical terminology/concepts when recording AEs on the AE eCRF page. Avoid colloquialisms and abbreviations.

Only one AE term should be recorded in the event field on the AE eCRF page.

5.3.5.1 DIAGNOSIS VERSUS SIGNS AND SYMPTOMS

A diagnosis (if known) should be recorded on the AE eCRF page 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 AE eCRF page. If a diagnosis is subsequently established, all previously reported AEs based on signs and symptoms should be nullified and replaced by one AE report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

5.3.5.2 ADVERSE EVENTS OCCURRING SECONDARY TO OTHER EVENTS

In general, AEs 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 AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the AE eCRF page. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe GI hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and subsequent fracture, all three events should be reported separately on the eCRF.

All AEs should be recorded separately on the AE eCRF page if it is unclear as to whether the events are associated.

5.3.5.3 PERSISTENT OR RECURRENT ADVERSE EVENTS

A persistent AE is one that extends continuously, without resolution, between patient evaluation time points. Such events should only be recorded once on the AE eCRF page. 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 AE eCRF page should be updated to reflect this.

A recurrent AE is one that resolves between patient evaluation time-points and subsequently recurs. Each recurrence of an AE should be recorded separately on the AE eCRF page.

5.3.5.4 ABNORMAL LABORATORY VALUES

Not every laboratory abnormality qualifies as an AE. A laboratory test result should be reported as an AE 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 AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., ALP and bilirubin 5 times the upper limit of normal (ULN) associated with cholecystitis), only the diagnosis (i.e., cholecystitis) should be recorded on the AE eCRF page.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the AE eCRF page, 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 AE. For example, a 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 AE eCRF page, 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 AE. A vital sign result should be reported as an AE 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 AE.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high BP), only the diagnosis (i.e., hypertension) should be recorded on the AE eCRF page.

Observations of the same clinically significant vital sign abnormality from visit to visit should not be repeatedly recorded on the AE eCRF page, 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 elevated liver tests, like:

- ALT and/or AST >3xULN or
- ALP >1.5xULN or
- TBL to >2xULN or
- AT >3xULN accompanied by elevated TBL >2xULN and not primarily cholestatic: ATx / ALPx <2 (Hy's Law case definition) or
- Elevation of AT in temporal association with nausea, vomiting, anorexia, abdominal pain, or fatigue, or
- Possibly liver-related deaths and liver-related treatment discontinuations.

is considered to be an indicator of severe liver injury.

5.3.5.7 DEATHS

All deaths that occur during the protocol-specified AE reporting period (see Section 5.3.1), regardless of relationship to study drug, must be recorded on the AE eCRF page and immediately reported to the sponsor (see Section 5.4).

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 AE eCRF page. Generally, only one such event should be reported. The term "sudden death" should only be used for the occurrence of an abrupt and unexpected death due to presumed cardiac causes in a patient with or without preexisting heart disease, within one hour of the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the patient was last seen alive and stable. If the cause of death is unknown and cannot be ascertained at the time of reporting, "unexplained death" should be recorded on the AE eCRF page. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death.

5.3.5.8 PREEXISTING MEDICAL CONDITION

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

A preexisting medical condition should be recorded as an AE only if the frequency, severity or character of the condition worsens during the study. When recording such

events on the AE eCRF page, 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 WORSENING OF SYMPTOMS ASSOCIATED WITH ASD

Worsening of symptoms associated with ASD should be recorded as an AE if judged by the investigator to have unexpectedly worsened in severity or frequency or changed in nature at any time during the study. When recording an unanticipated worsening of symptoms associated with ASD on the AE eCRF page, it is important to convey the concept that the condition has changed by including applicable specific descriptors (e.g., exacerbation of repetitive behaviors or worsening of verbal communication).

A worsening of symptoms of ASD does not generally constitute a reason for withdrawal of the subject from the study. This must be discussed with and agreed to by the medical monitor/sponsor and should be recorded in the treatment section of the AE eCRF form.

5.3.5.10 HOSPITALIZATION OR PROLONGED HOSPITALIZATION

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE (per the definition of SAE in Section 5.1.2), except as outlined below.

The following hospitalization scenarios are not considered to be SAEs:

- Planned hospitalization required by the protocol.
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
    - The patient has not suffered an AE.
    - Prolonged hospitalization due to psychosocial issues such as lack of home care facilities, caregiver issues, transport issues, etc.

The following hospitalization scenarios are not considered to be SAEs, but should be reported as AEs instead:

- Hospitalization for an AE that would ordinarily have been treated in an out-patient setting had an out-patient clinic been available.

A hospitalization of the patient does not necessarily constitute a reason for withdrawal of the patient from the study (for instance, a short hospitalization for psychosocial reasons may be acceptable). Each case should be discussed with the medical monitor/sponsor. It should be determined if the patient can continue the study medication while hospitalized. In case study medication cannot be continued while the patient is hospitalized, it should be determined whether the hospitalization is of such duration that a continuation in the study after discharge can be justified. The decision should be recorded in the eCRF.

5.3.5.11 OVERDOSES

Study drug(s) overdose is the accidental or intentional use of the drug in an amount higher than the dose being studied. An overdose or incorrect administration of study drug(s) is not an AE unless it results in untoward medical effects.

Any study drug(s) overdose or incorrect administration of study drug(s) should be noted on the Study Drug Administration eCRF page.

All AEs associated with an overdose or incorrect administration of study drug(s) should be recorded on the AE eCRF page. If the associated AE fulfills serious 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).

Appropriate supportive treatment should be initiated according to the individual's clinical signs and symptoms and in accordance with best medical practices. In case of [redacted] overdosage, for instance, treatment may consist of electrolyte supplementation and/or replacement of fluid and electrolyte losses by careful monitoring of the urine and electrolyte output and serum electrolyte levels.

5.3.5.12 CAREGIVER/PARENT-REPORTED OUTCOME DATA

In this trial, caregiver/parent questionnaires are administered. AE reports will not be derived from these questionnaires. However, if any responses suggestive of a possible AE are identified during site review of questionnaires, site staff will alert the investigator, who will determine if the criteria for an AE have been met and will document the outcome of this assessment in the patient's medical record per site practice. If the event meets the criteria for an AE, it will be reported on the AE eCRF page.

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 or designee within 24 hours after learning of the event, regardless of relationship to study drug(s):

- SAEs
- AESIs
- Pregnancies

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 SAE to the local Health Authority and IRB.

5.4.1 EMERGENCY MEDICAL CONTACTS

To ensure the safety of study patients, access to the medical monitors is available 24 hours a day 7 days a week. Medical monitors contact details are listed in the "Protocol Administrative and Contact Information & List of investigators".

5.4.2 REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS AND ADVERSE EVENTS OF SPECIAL INTEREST

For reports of SAEs and AESIs (see Sections 5.1.2 and 5.1.3), investigators should record all case details that can be gathered on the SAE Reporting Form and forward this form to the sponsor and independent medical monitor and follow the safety reporting process as instructed in the Safety Management Plan, within 24 hours to:

	Phone	email
SAE Reporting Line	+1 513-309-9633 or +1 513-858-2989 (ext. 304 or 320)	STP1Safety@statkingclinical.com

5.4.3 REPORTING REQUIREMENTS FOR PREGNANCIES

5.4.3.1 PREGNANCIES IN FEMALE PATIENTS

Women patients of childbearing potential (WCBP) will be instructed to immediately inform the investigator if they become pregnant during the study or within 2 weeks after the last dose of study drug(s). 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 AE eCRF page. The investigator should discontinue study drug(s) and counsel the patient/caregiver, 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 STUDY SUBJECTS

Male patients will be instructed through the ICF to immediately inform the investigator if their partner becomes pregnant during the study or within 2 weeks after the last dose of study drug(s). 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. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male study subject exposed to study drug(s). The pregnant partner will need to sign an Authorization For Use and Disclosure of Pregnancy Health Information to allow for follow-up on her pregnancy. Once the authorization has been signed, the investigator will update the Clinical Trial Pregnancy Reporting Form with additional information on the course and outcome of the pregnancy. An investigator who is contacted by a male study subject or his pregnant partner may provide information on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the treating physician and/or obstetrician.

5.5 FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

5.5.1 INVESTIGATOR FOLLOW-UP

The investigator should follow each AE 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-u, or the patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to study drug(s) or trial-related procedures until a final outcome can be reported.

During the study period, resolution of AEs (with dates) should be documented on the AE eCRF page 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 AE eCRF page.

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 SAEs, AESIs, and pregnancies, the sponsor or a designee may follow-up by telephone, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries or consultant 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 patients for AEs after the end of the AE reporting period (defined as 2 weeks after the last dose of the study drug).

If the investigator becomes aware of any other SAEs occurring after the end of the AE reporting period, if the event is believed to be related to prior study drug treatment, the event should be reported directly to the sponsor or its designee, either by scanning and emailing the SAE Reporting Form using the email address provided to investigators.

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

The sponsor will promptly evaluate all SAEs and AESIs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, IRB and applicable Health Authorities based on applicable legislation.

To determine reporting requirements for single AE cases, the sponsor will assess the expectedness of these events using the STP1 Investigator's Brochure [STP1-IB].

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.

6. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

6.1 DETERMINATION OF SAMPLE SIZE

The primary objective of this study is to evaluate the safety and tolerability profile of 2 dose levels of STP1 in patients with ASD-Phen1. A maximum of approximately 20 patients will be enrolled in this study, to ensure that up to 16 patients (up to 8 per dose level) will have complete dataset after 2 weeks of treatment and 2 weeks of follow-up. They will be randomized with a 3:1 ratio to one of the 2 groups (active/placebo) for each dose level.

Up to six patients on active and up to 2 on placebo per dose level (maximum 4 on placebo in total) was considered sufficient for the primary objective.

6.2 SUMMARIES OF CONDUCT OF STUDY

To determine whether the integrity of the study was maintained, listing/summary of data referring to the general conduct of the study (such as enrollment, protocol violations, use of prohibited co-medication, treatment code break) will be generated.

6.3 ANALYSIS POPULATIONS

6.3.1 SAFETY ANALYSIS POPULATION

All patients who have received at least one administration of the study treatments, whether prematurely withdrawn from the study or not, will be included in the safety analysis. For the safety analysis population, data will be analyzed according to the treatment actually taken.

6.3.2 EFFICACY ANALYSIS POPULATION

This study has not been designed for efficacy analysis, but exploratory endpoints will be analyzed to look at early efficacy and/or to allow selection of relevant endpoints for a Phase 2 study. The efficacy population will consist of all randomized patients who meet the inclusion/exclusion criteria, received full course of the study drug as per randomization, have completed the main relevant visits and without any major protocol violations which would render the data unreliable.

6.3.3 PHARMACOKINETIC ANALYSIS POPULATION

All patients who have received at least one administration of the study treatments and with at least one sample collected and analyzed for plasma drug concentration, whether prematurely withdrawn from the study or not, will be included in the PK analysis.

6.4 SUMMARIES OF TREATMENT GROUP COMPARABILITY

Demographics, baseline characteristics and all baseline laboratory values will be summarized descriptively by treatment using frequency tables and summary statistics as appropriate. Placebo data will be pooled from the 2 dose groups.

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

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

- Incidence of AEs (overall, by intensity and by relationship to study medications).
- Incidence of SAEs.
- Incidence of AESIs.
- Incidence of discontinuations due to AEs.

- Incidence of laboratory abnormalities (including hematology, clinical chemistry, coagulation and urinalysis parameters).
- Incidence of BP abnormalities.
- Incidence of ECG abnormalities as well ECG changes as compared to baseline measurements.

Further details on the safety parameters are given in Section 5.1. Safety data will be summarized using descriptive statistics using the safety analysis population, which will include all patients treated. Additional safety analyses may be performed as stated in the Statistical Analysis Plan (SAP) for the study.

6.4.1 ADVERSE EVENTS

The original terms recorded on the eCRF by the investigator for AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized according to the MedDRA System Organ Classes (SOC) and preferred terms within SOC.

AEs will be summarized by mapped term and appropriate thesaurus level.

6.4.2 CLINICAL LABORATORY TEST RESULTS

All clinical laboratory data will be stored on the database in the units in which they were reported. Patient 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.

6.4.3 VITAL SIGNS

Vital signs data will be presented by individual listings with flagging of values outside the normal ranges. In addition, tabular summaries will be used as appropriate.

6.4.4 ECG DATA

ECG data will be presented by individual listings with flagging of values outside the normal ranges. In addition, tabular summaries will be used as appropriate.

6.4.5 CONCOMITANT MEDICATIONS

The original terms recorded on the patients' eCRF by the investigator for concomitant medications will coded using World Health Organization (WHO) Drug dictionary.

Concomitant medications will be presented in summary tables and listings.

6.5 EFFICACY ANALYSES

This study has not been designed for efficacy analysis, but exploratory endpoints will be analyzed to look at early efficacy and/or allow selection of relevant endpoint for a phase II study.

6.6 PHARMACOKINETIC ANALYSES

Individual plasma and urine concentrations of [redacted] will be listed. NCA using Phoenix 64, Build 8.0.0.3176 (or later), WinNonlin will be used to analyze the dose-concentration-time data of [redacted]. As far as possible, individual pharmacokinetic parameters will be estimated and the influence of various covariates (such as body weight) on these parameters will be investigated in an

exploratory way. When applicable, the following secondary PK parameters will be derived from the model for each individual included in the PK analysis and will be presented descriptively: $AUC_{0\ tau}$, $AUC_{0\ ast}$, C_{max}, T_{max}, C_{trough}, $t_{1/2}$, apparent oral clearance and apparent volume of distribution (Vz/F) and accumulation ratio.

6.7 EXPLORATORY ANALYSES

6.7.1 LACTATE AND PYRUVATE BLOOD LEVELS

Individual and mean blood levels (including change from baseline) of lactate, pyruvate, and L:P ratio will be presented by listings and descriptive summary statistics, by treatment group.

6.7.2 OTHER EXPLORATORY ENDPOINTS

Individual and mean data (including change from baseline) will be presented as listings and descriptive summary statistics, by treatment group. Graphical displays may be added where applicable.

- Change from baseline in subtask scores of [redacted] and KiTAP test battery.
- Global clinical change in the context of autism as measured by the change in the OACIS scale.
- Change from baseline in the ABC-C sub-scores.
- Change from baseline in the SRS-2 subscores.
- Change from baseline in the CSHQ.
- Change from baseline in eye tracking.
- Change from baseline in selected EEG parameters at resting state and during habituation and chirp tasks.
- Change from baseline in the CGI-S (reflected by the CGI-I) scale.

7. DATA COLLECTION, MANAGEMENT AND QUALITY ASSURANCE

7.1 SOURCE DATA DOCUMENTATION

Where applicable, the data will be transcribed by the study center from the paper source documents into the eCRF.

A CRO identified by 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 Electronic Data Capture (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 study coordinator. Accurate and reliable data collection will be assured by verification and cross-checking of the eCRF against the investigator's records by the study monitor (source document verification), and the maintenance of a study drug dispensing log by the investigator.

The CRO will produce a Data Handling Manual and a Data Management Plan that describes the quality checking to be performed on the data. Data collected electronically will be sent to the CRO, using the CRO's standard procedures to handle and process the electronic transfer of these data. Before study initiation, data to be entered directly into the eCRF (i.e., no prior written or electronic record of the data) and considered source data must be defined in the Study Monitoring Plan. 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.

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 7.2. To facilitate source data verification, the investigators and institutions must provide the CRO direct access to applicable source documents and reports for trial-related monitoring, CRO audits, and IRB review. The investigational site must also allow inspection by applicable Health Authorities. Throughout the study, the study management team (SMT) will review data according to the Data Handling Manual.

For classification purposes, preferred terms will be assigned by the CRO to the original terms entered on the eCRF, using the most up-to-date version of the Medical Dictionary for Regulatory Activities (MedDRA) terminology for AEs and diseases and the International Non-proprietary Name (INN) Drug Terms and Procedures Dictionary for treatments and surgical and medical procedures.

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

7.2 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRF, electronic Patient Reported Outcomes data (if applicable), ICF,

laboratory test results and medication inventory records, must be retained by the PI 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.

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 US or under a US Investigational New Drug (IND) application will comply with US Food and Drug Administration (FDA) regulations and applicable local, state, and federal laws.

8.2 INFORMED CONSENT

The sponsor’s sample ICF (and ancillary sample ICF such as Caregiver's ICF, if applicable) will be provided to each site. The sponsor or its designee must review and approve any proposed deviations from the sponsor's sample ICF or any alternate consent forms proposed by the site (collectively, the “Consent Forms”) before IRB submission. The final IRB-approved Consent Forms must be provided to the sponsor or its designee for Health Authority submission purposes according to local requirements.

In this study, caregiver specific information and consent may be collected, if separate from the legal guardian.

The Consent Forms must be signed and dated by the patient or the patient’s legally authorized representative before his or her participation in the study. The case history or clinical records for each patient 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 patient to participate. The final revised IRB-approved Consent Forms must be provided to the sponsor for Health Authority submission purposes.

Patients 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 policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient 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 patient or the patient’s legally authorized representative. All signed and dated Consent Forms must remain in each patient’s study file or in the site file and must be available for verification by study monitors at any time.

Each Consent Form may also include patient authorization to allow use and disclosure of personal health information in compliance with the US Health Insurance Portability and Accountability Act (HIPAA) of 1996. If the site utilizes a separate Authorization Form for patient 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 ICFs, any information to be given to the patient and relevant supporting information must be submitted to the IRB by the PI and reviewed and approved by the IRB before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB.

The PI is responsible for providing written summaries of the status of the study to the IRB/ethics committee (EC) annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB. Investigators are also responsible for promptly informing the IRB of any protocol amendments (see Section 9.5).

In addition to the requirements for reporting all SAEs to the sponsor, investigators must comply with requirements for reporting SAE to the local Health Authority and IRB. 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 and archived in the site's study file.

8.4 CONFIDENTIALITY

The sponsor (or its designee) maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in data sets that are transmitted to any sponsor location.

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

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

Data generated by this study must be available for inspection upon request by representatives of the US FDA and other national and local Health Authorities, sponsor monitors, representatives, and collaborators, and the IRB 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., LPLO).

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, ICF and documentation of IRB and governmental approval. In addition, at the end of the study, the investigator will receive the patient data, which includes an audit trail containing a complete record of all changes to data.

The sponsor shall also submit a Development Safety Update Report (DSUR) once a year to the IRB/EC and Health Authorities according to local regulatory requirements and timelines of each country participating in the study.

It is the understanding of the sponsor that this protocol (and any modifications) as well as appropriate consent procedures and advertisements, will be reviewed and approved by an IRB. This board must operate in accordance with the current Federal Regulations. The sponsor will be sent a letter or certificate of approval prior to initiation of the study, and also whenever subsequent amendments/modifications are made to the protocol. The sponsor shall also submit an IND Annual Report to the FDA according to local regulatory requirements and timelines.

9.2 SITE INSPECTIONS

Site visits will be conducted by the sponsor or an authorized representative for inspection of study data, patients' 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.

9.3 ADMINISTRATIVE STRUCTURE

The sponsor of the trial is STALICLA SA. This study will be conducted using the services of a CRO managed by STALICLA. If needed, the CRO will manage other vendors required to provide services such as cardiac monitoring, event planning, central laboratories, scale management and cognitive testing and STALICLA will maintain oversight.

9.4 PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings by the sponsor or designated investigators. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor prior to submission. All publications have to be approved by the sponsor. 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.

The sponsor will comply with the requirements for publication of study results. 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 may be designated by mutual agreement.

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.

Authorship will be determined by mutual agreement and in-line with International Committee of Medical Journal Editors authorship requirements.

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

Requests from investigators to modify the protocol to ongoing studies will be considered only by consultation between an appropriate representative of the sponsor and the investigator [investigator representative(s) in the case of a multicenter trial]. Any substantial protocol amendments will be prepared by the sponsor and reviewed and approved by the Chief of Translational Development and study Biostatistician.

All protocol modifications must be submitted to the appropriate IRB for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be obtained before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial patients, or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in monitor[s], change of telephone number[s]).

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11. APPENDIX 1 SCHEDULE OF ASSESSMENTS

PERIOD	SCREENING	TREATMENT PERIOD			FOLLOW-UP VISITS	
Day	D-14 to D-1	Day 1	Day 7	Day 14	Day 15-18[e]	Day 28
Visit Window			+/-1	+/-1	+/-1	+/-2
Assessments						
Informed Consent	x					
Eligibility	x					
Randomization		x				
Demography	x					
Medical History	x					
Physical Examination	x	x	x	x		x
Vital Signs	x	x[a]	x	x[a]		x
Orthostatic BP	x	x[a]	x	x[a]		x
ECG-12 Lead	x	x[a]	x	x[a]		x
Hearing Assessment		x	x	x		x
Serology	x					
Pregnancy Test (urine)[b]	x					x
SARS-Cov-2 Test (nasal swab, saliva)	x					
Substance Abuse (urine test)	x	x				
Blood Chemistry	x		x	x		x
Hematology	x		x	x		x
Coagulation	x		x	x		x
Urinalysis	x		x	x		x
Alcohol (urine test)	x					
Administration of Study Medication[c]		x	x	x		
Study medication supply		x				
Drug Accountability			x	x		x[f]
Blood PK Sample[d]		x	x	x	x	
Urine PK Sample[d]		x		x		
Suicidality (C-SSRS, C-CASA)	x	x	x	x	x	x
ABC-C	x			x		x
SRS-2	x			x		
SB5 ABIQ	x					
OACIS-Severity	x			x		
OACIS-Change				x		
[redacted]	x	x		x		x
KiTAP	x	x		x		x
Eye Tracking		x		x		x
EEG		x		x		x
CSHQ	x	x		x		x
CGI-I				x		x
CGI-S	x			x		x
Adverse Events	x	x	x	x	x	x
Previous and Concomitant Treatments	x	x	x	x	x	x

a To be performed pre-dose and between 4 and 5 hours post-dose as specified in the protocol.

b Females only.

c Patients will receive their AM and PM dose of study drug during the Day 1, Day 7 and Day 14 visits to allow for proper PK sampling.

d PK samples will be collected as described in the PK assessment section. A 5 minutes window for each timepoint is allowed

e Day 16 to Day 18 are optional

f If not done at Day 14 visit