Document Type: Final Protocol

Document Date: Version 4 – 15 October 2021

Study Title: Randomized, Double-blind, Placebo-controlled, 28-Day Daily-dose Crossover Study of the Safety and Tolerability of SB-121 (Lactobacillus reuteri with Sephadex® and Maltose) in Subjects, ages 15 to 45 years, diagnosed with Autistic Disorder

Protocol Reference Number: SBI-SB121-20-01

NCT Number: NCT04944901

Scioto Biosciences

PROTOCOL

PRODUCT NAME/NUMBER:	SB-121
PROTOCOL NUMBER:	SBI-SB121-20-01
IND NUMBER:	[redacted]
DEVELOPMENT PHASE:	Phase 1
PROTOCOL TITLE:	Randomized, Double-blind, Placebo-controlled, 28-Day Daily-dose Crossover Study of the Safety and Tolerability of SB-121 (*Lactobacillus reuteri* with Sephadex® and Maltose) in Subjects, ages 15 to 45 years, diagnosed with Autistic Disorder.
PROTOCOL DATE:	Version 4 - 15 October 2021
COORDINATING/PRINCIPAL INVESTIGATORS:	Craig Erickson, MD Professor of Psychiatry Director, Fragile X Research and Treatment Center Medical Director, Psychiatry Neurobehavioral Care Continuum Director of Research, Division of Child & Adolescent Psychiatry and The Kelly O'Leary Center for Autism Spectrum Disorders Cincinnati Children's Hospital University of Cincinnati College of Medicine- Affiliated 3333 Burnet Avenue MLC 4002 Cincinnati, OH 45229 [redacted]
SPONSORED BY:	Scioto Biosciences Inc. [redacted]
CONTRACT RESEARCH ORGANIZATION:	[redacted]

This study will be performed in compliance with ICH Good Clinical Practices and applicable regulatory requirements, including the archiving of essential documents. Information contained in

this protocol is confidential in nature, and may not be used, divulged, published, or otherwise disclosed to others except to the extent necessary to obtain approval of the institutional review board or independent ethics committee, or as required by law. Persons to whom this information is disclosed should be informed that it is confidential and may not be further disclosed without the express permission of Scioto Biosciences Inc.

1. APPROVAL SIGNATURES

PROTOCOL NUMBER: SBI-SB121-20-01

PROTOCOL TITLE: Randomized, Double-blind, Placebo-controlled, 28-Day Daily-dose Crossover Study of the Safety and Tolerability of SB-121 (*Lactobacillus reuteri* with Sephadex® and Maltose) in Subjects, ages 15 to 45 years, diagnosed with Autistic Disorder.

I, the undersigned, have read this protocol and confirm that to the best of my knowledge it accurately describes the planned conduct of the study.

SIGNATURE **DATE:** 10/25/21

[redacted]

Joseph Trebley, PhD – Chief Executive Officer
Scioto Biosciences Inc.

2. PROTOCOL SUMMARY

2.1. Synopsis

PRODUCT NAME/NUMBER	SB-121
PROTOCOL NUMBER	SBI-SB121-20-01
DEVELOPMENT PHASE	Phase 1
PROTOCOL TITLE	Randomized, Double-blind, Placebo-controlled, 28-Day Daily-dose Crossover Study of the Safety and Tolerability of SB-121 (*Lactobacillus reuteri* with Sephadex® and Maltose) in Subjects, ages 15 to 45 years, diagnosed with Autistic Disorder.
INDICATION	Treatment of autistic disorder
OBJECTIVES	Primary Objective: • To evaluate the safety and tolerability of multiple doses of SB-121 in subjects with autistic disorder Secondary Objectives: • To monitor the elimination of Sephadex® from the GI tract in subjects with autistic disorder • To assess the impact of multiple doses of SB-121 on circulating oxytocin levels in subjects with autistic disorder • To assess the impact of multiple doses of SB-121 on tests of autistic disorder (AD) • To evaluate biomarkers of microbiota function, immune modulation, and inflammation following multiple doses of SB-121 in subjects with autistic disorder
STUDY DESIGN	This is a phase 1, single-center, randomized, double-blind, placebo-controlled cross-over study to evaluate the safety and tolerability of 28-days of once daily oral administration of a preparation of *L. reuteri* with Sephadex® and maltose (SB-121), to subjects with autistic disorder Up to 16 eligible subjects will be randomly allocated in a 1:1 ratio to receive treatment with either SB-121 or placebo. The investigational product will be provided to subjects in 2 bottles, the contents of which will be combined, allowed to sit for a period of 15-45 minutes and then added to a predefined drink. Subjects will undergo screening within ~14 days before administration of the investigational product (IP). All subjects will be required to provide written informed consent before any study-specific procedures are performed. Subjects will also be asked to provide consent to allow bio-banking of biological samples for future analysis. Subjects' eligibility for the study will be determined at Screening by assessment of inclusion and exclusion criteria. Eligible subjects will be provided with a stool sampling kit to collect a pretreatment stool sample within 48 hours before receiving the IP (timing of collection determined by the frequency of the subject's bowel movements). Subjects will return to the study site on Day 1. Following confirmation of eligibility criteria, they will be randomized to receive SB-121 or placebo.

	Following baseline tests, all subjects will be given their first dose of either SB-121 or placebo at the clinic then receive an additional 30 oral doses of SB-121 or placebo to be administered once daily at home, according to the treatment group they were randomized to. Refer to the Daily Medication Instructions for additional details. Subjects will undergo study-specific procedures on Day 1 in accordance with the Schedule of Events. Vital signs will be measured and ECG will be collected before administration of the IP. Vital signs will be measured post-dose. Subjects will be discharged after all Day 1 evaluations have been completed. Following the approximately 2-week washout period, all subjects will cross-over to the other treatment (SB-121 or placebo) and receive 30 oral doses to be administered once daily. Subjects will be instructed to collect a total of 5 stool samples at home according to the following schedule: • A pretreatment stool sample within approximately 48 hours before receiving the first dose of the IP. • Post-treatment samples: o Sample 1 should be taken no sooner than 21 days or later than 28 days into the first treatment period of the study (prior to the completion of the first treatment period) o Sample 2 should be taken approximately 7 days into the wash-out period after the first treatment period of the study and before the second treatment period o Sample 3 should be taken no sooner than 21 days or later than 28 days into the second treatment period of the study (prior to the completion of the second treatment period) o Sample 4 should be taken approximately 7 days after the completion of the second treatment period of the study Subjects will be provided with sampling kits to collect stool samples at home. Kits will include labels for sample containers, which should be completed with the time and date that the sample was obtained. Subjects will be instructed to return all stool samples to the responsible laboratory using pre-labeled shipping containers; alternative strategies for returning stool samples can also be discussed between the investigator and subjects. Subjects will be advised to contact the study site immediately in the event of symptoms consistent with bacteremia (e.g., fever, chills, diaphoresis) and to obtain appropriate medical care as indicated. In the event that subjects develop symptoms consistent with bacteremia, two blood samples will be collected, when feasible, for blood cultures and microbial identification. Follow-up visits will be conducted on Day 28, Day 1 of treatment period 2 and Day 28 of treatment period 2 (see 2.2 Schedule of Events). Details of AEs occurring throughout the study will be collected by study staff at each visit. Weekly telephone calls will be conducted by the investigator or designee between in-person visits during each treatment period. These calls will record and evaluate adverse events and will also determine IP compliance. In the case of adverse events identified during a phone call, the investigator or designee may determine that an unscheduled in-person study visit and/or lab tests may be required. Gastrointestinal events (nausea, vomiting, diarrhea, constipation,

	abdominal distention, abdominal pain/cramps, and flatulence) and systemic events (fever, chills, and diaphoresis) occurring at any time during the study and within 2 weeks after the last dose of IP will be considered AEs of special interest (AESIs).
PLANNED NUMBER OF SUBJECTS	The study will enroll up to 8 subjects in each group, for a total of up to 16 subjects.
STUDY ENTRY CRITERIA	*Inclusion criteria* A subject will be eligible for study participation if he or she meets all of the following criteria: 1. Subject and parent, caregiver or legally authorized representative has provided written informed consent for the study. 2. Subject is a male or female ≥15 and ≤45 years of age. 3. Diagnosis of Autistic Disorder as confirmed by gold standard clinical interview using DSM-5 criteria and administration of the Autism Diagnostic Observation Schedule-2 4. Subject, if female and of childbearing potential, is not lactating and not pregnant 5. Subject, if female, is either not of childbearing potential or is practicing an acceptable effective method of birth control. 6. Subject is willing to comply with all study requirements (including the requirements for stool sampling and biobanking) and to return to the study facility for the follow-up evaluations, as required. *Exclusion criteria* A subject will be excluded from the study if he or she meets any of the following criteria: 1. Subject has known allergy or significant adverse reaction to *L reuteri*, Sephadex®, maltose, or related compounds. 2. Subject has previously had GI surgery, intestinal obstruction, *Clostridium difficile* infection or diverticulitis. 3. Subject has travelled outside of the USA in the 30 days prior to screening. 4. Subject has had a diarrheal illness in 30 days prior to screening. 5. Subject currently has a fever or active/uncontrolled GI symptoms (e.g., nausea, vomiting, diarrhea, constipation, abdominal distention, abdominal pain/cramps, flatulence) or has had these within 14 days prior to screening. If the GI symptoms are stable, in the opinion of the investigator, the subject can be enrolled. 6. Subject has any immunological/autoimmune disorder including, but not limited to, systemic lupus erythematosis, rheumatoid arthritis, Sjögren's syndrome, inflammatory bowel disease, or immunoglobulin-deficiency disorder, that would increase the risk to the subject or interfere with the evaluation of SB-121. 7. Subject has a documented history of HIV, hepatitis B and/or hepatitis C 8. Subject has implanted prosthetic devices including prosthetic heart valves.

	9. Subject has taken, or is taking, any of the following prohibited medications: a. Proton pump inhibitor within 2 weeks prior to screening b. Use of supplemental probiotics within 2 weeks prior to screening except for yogurt c. Current use of immunosuppressive medications, including corticosteroids d. Treatment with monoclonal antibodies within 4 weeks prior to screening e. Systemic antibiotics within 2 weeks prior to screening 10. Subject has diabetes mellitus or is pre diabetic. 11. Subject has received any IP (or investigational device) within 30 days prior to screening 12. Subject has any of the following laboratory test results at Screening: f. absolute neutrophil count of $<1.5 \times 10^9$/L g. alanine aminotransferase or aspartate aminotransferase $>1.5 \times$ upper limit normal (ULN), total bilirubin $>1.5 \times$ ULN (subjects with known Gilbert's Syndrome can be included) h. serum creatinine $>1.5 \times$ ULN i. any other abnormal laboratory test that is clinically significant in the judgment of the investigator. 13. Subject has an unstable medical condition or is otherwise considered unreliable or incapable, in the opinion of the investigator, of complying with the requirements of the protocol. 14. Subject tests positive for drugs of abuse in a urine drug screen at screening. 15. Subject has a history of alcohol abuse.
INVESTIGATIONAL PRODUCT	Name: SB-121 (*L. reuteri*) [redacted]
REFERENCE PRODUCT	[redacted]
TREATMENT REGIMENS	Eligible subjects will receive one dose of SB-121 or placebo daily for 28 days according to the treatment group to which they are allocated. 27 doses will be provided to the subject, prepared at home and combined with approximately 1 cup of a predefined drink. The first dose of IP will be administered at the clinic. Subjects will be required to refrain from using additional probiotics (except for yogurt) for the duration of their study involvement.
PLANNED STUDY SITES	The study will be conducted at a single study site: Cincinnati Children's Hospital (CCH), 3333 Burnet Ave, Cincinnati, OH 45229

CRITERIA FOR EVALUATION	Primary endpoints: • Incidence and severity of treatment-emergent AEs (TEAEs), serious AEs (SAEs), AESIs, and AEs leading to discontinuation from the study • Incidence of presence of Sephadex® microspheres in the stool • Incidence of symptomatic bacteremia with positive *L. reuteri* identification Secondary endpoints: Change from baseline in: • Clinical Global Impressions Severity and Improvement Subscales • Vineland Adaptive Behavior Scales (3rd edition) • Aberrant Behavior Checklist • Woodcock Johnson 3rd Edition • Repeatable Battery for Assessment of Neuropsychological Status • Test of Attentional Performance for Children (KiTap) • Neurophysiology Measures including EEG resting state, auditory habituation, and chirp modulated sweep auditory evoked response • Eye Tracking • Biomarkers in stool and blood samples: Plasma oxytocin, plasma vasopressin, serum hs-CRP, tumor necrosis factor-α, and stool biomarkers.
STATISTICAL METHODS	Summary statistics will be provided for the various parameters assessed during the trial. This will include summaries of AEs by severity and relationship to IP, changes from baseline in the AD measures, as well as changes from baseline in biomarkers.
SAMPLE SIZE DETERMINATION	The sample size was not determined based on statistical assumptions. Evaluation of up to 8 subjects per treatment group (16 subjects total) was considered sufficient to allow evaluation of the study's objectives.
STUDY AND TREATMENT DURATION	The overall study duration is expected to be approximately 14-weeks. The planned sequence and duration of the study periods will be as follows: 1. Screening: Up to 14 days 2. Study Period 1: Approximately 28 days 3. Approximately 14-day washout period 4. Study Period 2: Approximately 28 days 5. Post-treatment: Up to 14 days The maximum study duration for each subject is approximately 98 days.
PROTOCOL DATE	09 August 2021

2.2. Schedule of Events

	Study Period 1						WASH OUT (14-21 Days)	Study Period 2						Post-trial Washout
	Day -14 to 0	Day 1	Day 1	Day 7 (+/-2)	Day 14 (+/-2)	Day 21 (+/- 2)	Day 28 (+/-3)	Day 35 (+/-2)	Day 1[b]	Day 1	Day 7 (+/-2)	Day 14 (+/-2)	Day 21 (+/- 2)	Day 28 (+/- 3)	Day 42 (+4)
Measure	Screen	Visit 1: Pre-dose	Visit 1: Post-dose	Follow-Up 1 Phone	Follow - Up 2 Phone	Follow-Up 3 Phone	Visit 2: Final Visit of Period 1	Wash out period call	Visit 3: Pre-dose	Visit 3: Post-dose	Follow Up 1 Phone	Follow Up 2 Phone	Follow Up 3 Phone	Visit 4: End-of-Study/Early Termination Visit	
Informed Consent	X														
Med/Psych Hx	X														
Physical Exam[a]	X						X		X					X	
Safety Labs[b]	X						X		X					X	
Pregnancy Test (females)	X	X					X		X					X	
Biomarker Blood Draw	X						X		X					X	
Vital Signs	X	X	X				X		X	X				X	
ADOS-2[c]	X														
DSM-5 Checklist	X														
WASI-II		X													
SCQ		X													
CGI-S		X					X		X					X	
CGI-I							X							X	
Vineland-3		X					X		X					X	

	Day -14 to 0	Day 1	Day 1	Day 7 (+/-2)	Day 14 (+/-2)	Day 21 (+/- 2)	Day 28 (+/-3)	Day 35 (+/-2)	Day 1[b]	Day 1	Day 7 (+/-2)	Day 14 (+/-2)	Day 21 (+/- 2)	Day 28 (+/-3)	Post-trial Washout
Measure	**Screen**	**Visit 1: Pre-dose**	**Visit 1: Post-dose**	**Follow-Up 1 Phone**	**Follow-Up 2 Phone**	**Follow-Up 3 Phone**	**Visit 2: Final Visit of Period 1**	**Wash out period call**	**Visit 3: Pre-dose**	**Visit 3: Post-dose**	**Follow Up 1 Phone**	**Follow Up 2 Phone**	**Follow Up 3 Phone**	**Visit 4: End-of-Study/Early Termination Visit**	**Day 42 (+4)**
ABC		X					X		X					X	
ECG[d]	X	X					X		X					X	
EEG/ERP/Chirp		X					X		X					X	
Eye tracking		X					X		X					X	
WJ3 Subtests		X					X		X					X	
RBANS		X					X		X					X	
KiTap		X					X		X					X	
IP Compliance				X	X	X	X				X	X	X	X	
AE Review			X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication Review	X	X		X	X	X	X	X	X		X	X	X	X	
Stool Sample		X[e]					X	X[f]						X	X[g]

a) A full physical exam will be done at screening. A limited focused physical exam may be done in case of adverse events at all other visits, as determined by the investigator

b) Safety labs will include hematology, chemistry and urinalysis

c) ADOS 2 results can be obtained from the medical record and used for the study if completed within the previous 36 months. If results for the ADOS are available, it will not be completed.

d) ECG will be done at either the screening or baseline visit for participants with AD.

e) This is a pretreatment stool sample, to be collected within approximately 48 hours before receiving the first dose of the IP.

f) Should be taken approximately 7 days into the wash-out period after the first treatment period of the study and before the second treatment period

g) This is post treatment stool sample, to be collected approximately 7 days after the completion of the second treatment period of the study

h) Study Period 2 Begins on Day 42 of the study or up to 7 days later

ADOS-2= Autism Diagnostic Observation Schedule Modules 2, 3 or 4; WASI-II= Wechsler Abbreviated Scale of Intelligence Scale-Second Edition, SCQ= social communication questionnaire; CGI-S= clinical global impressions severity scale, CGI-I=clinical global impressions improvement scale; Vineland-3= Vinland Adaptive Behaviors Scales 3rd edition; PK= pharmacokinetics; EEG= electroencephalogram protocol; WJ3= Woodcock Johnson Spatial Relations and Auditory Attention subtests; RBANS= repeatable battery of neuropsychological status; KiTap= computerized test of attentional performance in children; ABC= aberrant behavior checklist;

3. TABLE OF CONTENTS

3.1. Table of Tables

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4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ABBREVIATION	EXPLANATION
AE	adverse event
AD	autistic disorder
AESI	adverse event of special interest
ASD	Autism Spectrum Disorder
ATCC	American Type Culture Collection
BMI	body mass index
CFR	Code of Federal Regulations
CFU	colony forming units
CRA	clinical research associate
CSR	clinical study report
DBP	diastolic blood pressure
DMP	data management plan
DSM	Diagnostic and Statistical Manual of Mental Disorders
EDC	Electronic Data Capture
ET	Early Termination
FDA	Food and Drug Administration
eCRF	electronic case report form
GCP	Good Clinical Practice
GI	gastrointestinal
GRAS	generally-recognized-as-safe
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IDS	Investigational Drug Services
IL	interleukin
IND	investigational new drug
IP	investigational product
IRB	institutional review board
L. reuteri	*Lactobacillus reuteri*
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight

ABBREVIATION	EXPLANATION
MedDRA	Medical Dictionary for Regulatory Activities
MRS	De Man, Rogosa and Sharpe broth
NIH	National Institutes of Health
PT	preferred term
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
SD	standard deviation
SOC	system organ class
TEAE	treatment-emergent adverse events
TMF	trial master file
UAE	unexpected adverse event
ULN	upper limit of normal
USA	United States of America
WHO-DD	World Health Organization Drug Dictionary

5. INTRODUCTION

5.1. Background and Rationale

Autistic disorder (AD) manifests as difficulties in social interaction, speech and nonverbal communication, and restricted/repetitive behaviors. This is usually first diagnosed in childhood with many of the features presenting around 2-3 years of age. It is estimated that that one in 59 children have autism.

The management of autistic disorder is complex. There are medications that can help with some of the clinical manifestations of the disease, but there are currently no approved medications to treat the core impairments of autism.

Recent research suggests *L. reuteri* can be used to treat social deficits in genetic, environmental, and idiopathic mouse models of AD and that this rescue depends upon the vagus nerve as well as the oxytocinergic and dopaminergic signaling in the brain (Sgritta 2018).

Oxytocin is a hypothalamus derived, posterior pituitary stored nonapeptide which has gained recent interest as an important neuropsychiatric and metabolic hormone, beyond its classic role in lactation and parturition. Oxytocin is central to complex social cognition and behaviors, such as attachment, social exploration, and recognition. In healthy humans, oxytocin binds to receptors in social brain regions such as amygdala and anterior cingulate cortex (Mu 2018). It is a key component of the network regulating social brain functions such as modulation of social stress, emotion recognition and memory formation (Agostoni 2010). In AD, intranasal administration of oxytocin in several (Hay 2010, Neu 2008, Mshvildadze 2008) but not all (Salminen 2004) studies reported improvement in emotion recognition. Oxytocin treatment may be most efficacious in a subset of individuals with AD who have insufficient or low basal oxytocin levels (Neu 2008, Penders 2006).

Circulating oxytocin is a useful marker of central oxytocin activity because the peptide is synthesized by the same neurons with axons that terminate in the posterior pituitary and secrete the hormone into the blood and axons that project to social brain regions. Oxytocin is secreted in pulses (Wang 2014) and the peptide has a very short half-life. Further, oxytocin does not cross the blood-brain barrier when systemically administered. This could potentially be overcome by intranasal administration. However, intranasal oxytocin is unable to achieve sustained physiological levels for pulsatile oxytocin signaling. This may be accomplished by oral delivery of SB-121, that contains an activated *L. reuteri* and could stimulate the release of oxytocin via the gut-brain axis.

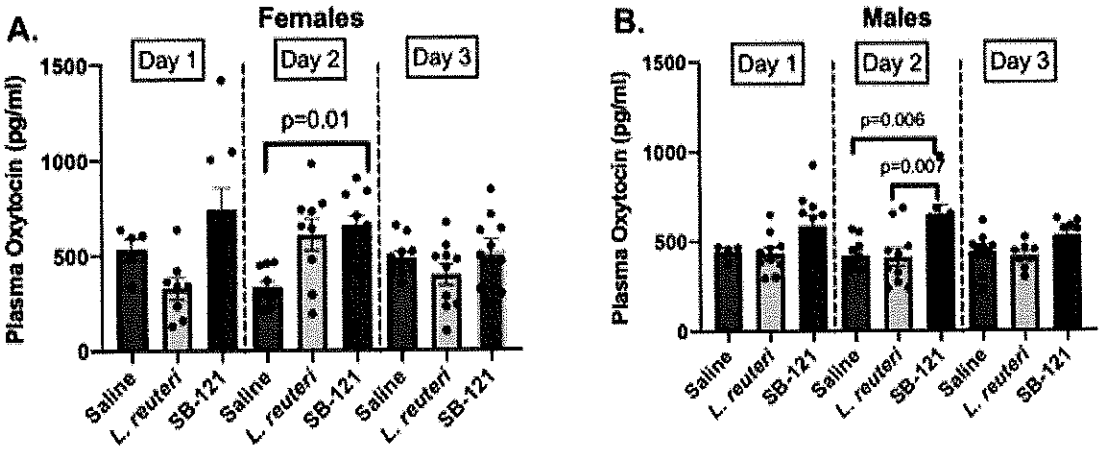
L. reuteri is a bacterium that naturally colonizes the outer mucous layer of the intestines. It stimulates production of mucin by goblet cells, and protects intestinal cells from opportunistic pathogens. *L reuteri* also stimulates oxytocin release into the circulation and onto neurons in the ventral tegmental area of the brain (Vlasova 2016, Van Tassell 2011, Mack 2003, He 2001). Studies of AD animal models demonstrate that impairments in social interactions can be overcome through oral administration of *L. reuteri.* Mechanistic studies revealed that this was due to an increase in oxytocin facilitated through vagus nerve signaling (Van Tassell 2011). Administration of *L. reuteri* has also been shown to enhance wound healing in both animal models and human subjects by increasing circulating oxytocin levels via afferent vagus nerve signaling (Vlasova 2016, Mack 2003).

It is hypothesized that SB 121, which contains *L.reuteri,* can have a role in the management of AD, and that this is potentially mediated through oxytocin signaling.

5.2. Nonclinical Experience

Both male and female immature rat pups have low baseline circulating oxytocin levels. Pups were treated once via oral gavage (300µl) at day 15 of age with either saline, planktonic *L. reuteri*, or SB-121 (2 x 10^9 CFU *L. reuteri*, 20 mg of Sephadex® and 28.8 mg maltose in 1 mL saline). Blood was collected on days 1, 2 and 3 post-treatment and plasma oxytocin was measured. The results suggest SB-121 is capable of increasing circulating oxytocin levels with no differences by sex (Figure 1).

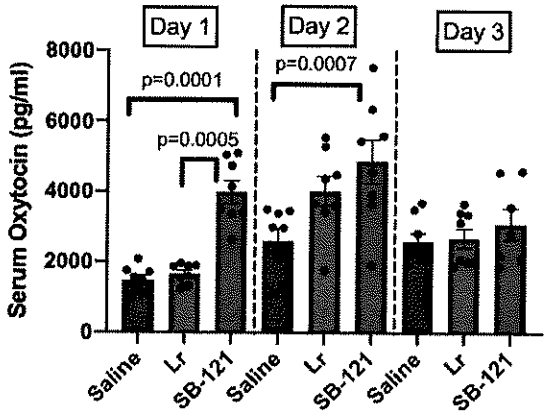
Figure 1: Plasma Oxytocin Levels in Pups Following Treatment with SB-121, Planktonic *L. reuteri*, or Saline



Oxytocin levels were measured in both females (A) and males (B) and values are shown as an average ± SEM for the first three days post-treatment. ANOVA followed by Tukey's multiple comparison test was used to compare groups.

Assessments were also performed in adult female rats after a single dose of SB-121, planktonic *L. reuteri* or saline. At day 1, 2 and 3 post-dosing, and plasma oxytocin levels were determined by enzyme immunoassay (EIA; Assay Designs). On day 1 post-dose, plasma oxytocin levels in SB-121 treated animals were significantly higher than that of animals treated with planktonic *L. reuteri* or saline (Figure 2). Oxytocin levels between the groups were similar by day 3. This study showed that a single dose of SB-121 is effective at stimulating circulating oxytocin on day 1, which was maintained on day 2. Additionally, planktonic *L. reuteri* showed a trend in its ability to stimulate oxytocin levels on day 2, but levels were not significantly different than the saline-treated group.

Figure 2: Plasma Oxytocin Levels in Female Rats Following Treatment with SB-121, Planktonic *L. reuteri* (Lr), or Saline

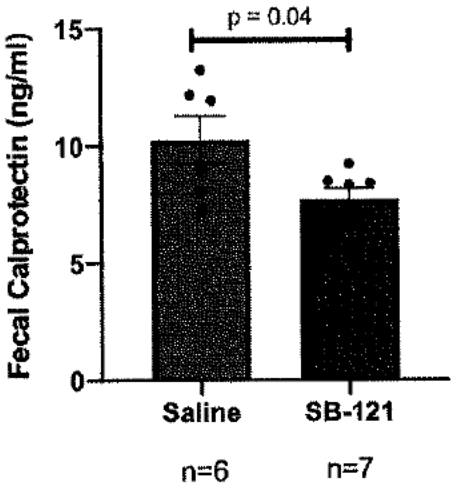


Oxytocin levels are shown as an average ± SEM for the first three days post-treatment. ANOVA followed by Tukey's multiple comparison was used to compare groups.

Impact of *L. reuteri* / Sephadex® Biofilm on GI inflammation

GI symptoms, including constipation, abdominal pain, flatulence, and diarrhea, are often associated with AD with a prevalence range from 23% to 70% (Milani 2017, Nuriel-Ohayon 2016). In healthy adult C57B6/J male mice, administration of SB-121 results in a decrease of fecal calprotectin levels (Figure 3), a biomarker of GI inflammation, suggesting that SB-121 may reduce clinical or subclinical gut inflammation and may improve GI symptoms commonly associated with AD.

Figure 3: Fecal Calprotectin Levels in Male Mice After 4 days of SB-121 or Saline Administration



Feces were collected on the next day from the distal colon at study termination. Bars are the mean ± SEM. Unpaired t-test was used to compare the 2 groups.

5.3. Clinical Experience

This is the first study of SB-121 in humans.

5.4. Summary of Potential Risks and Benefits

SB-121 is a preparation of *L. reuteri* with Sephadex® and maltose.

[redacted]

5.4.1 *L. reuteri* Risk Assessment

L. reuteri is a human commensal. In over 25 clinical trials of *L. reuteri* in a diverse set of patient populations, targeting a wide variety of indications and including full-term and premature infants, adverse events (AE) associated with the use of *L. reuteri* have been limited to abdominal cramps.[15] These studies included patient populations ranging from very low birthweight infants to adults 65 years and older, as well as individuals with cystic fibrosis, catheter-dependent females with spina bifida, and individuals with human immunodeficiency virus (HIV). A vast majority of these studies followed a daily dosing regimen ranging from 2-weeks to 3 months.

Long-term follow-up data on patients administered *L. reuteri* are limited, and longer term safety is not known. However, a detailed health risk assessment of *L. reuteri* was performed by the Norwegian Scientific Committee for Food Safety (Narvhus 2016). They noted the following:

- **Undesirable short-term side-effects:** *L. reuteri* has been granted generally-recognized-as-safe (GRAS)-status by FDA (GRAS Notice Numbers 254, 409, 410, 440) and qualified-presumption-of-safety (QPS)-status by the European Food Safety Authority (European Food Safety Authority 2018) and has never caused systemic infections in humans. It has been extensively used as a probiotic delivered orally at daily doses ranging from 1 x 10^8 to

1 x 10^{10} CFU. Generally, patients are dosed daily for 4 weeks or longer. No AEs attributed to *L. reuteri* have, to our knowledge, been reported.

- **Undesirable long-term side-effects:** In 2013 Abrahamsson *et al* followed up their 2007 study where 1 x 10^{8} CFU of *L. reuteri* ATCC 55730 was administered to the mother 4 weeks before term until birth, and to 1 year for their babies (Abrahamsson 2013). No severe AEs, neither short-term nor long-term, were reported, and there was no significant difference in the prevalence of GI symptoms between the treatment groups. We are not aware of any other long-term follow-up data.

In this study SB-121 will be given once a day for 28 days.

5.4.2 Antibiotic Susceptibility Profile

[redacted] All testing followed CLSI M45 guidelines.

Minimum inhibitory concentration (MIC) values were determined by broth microdilution for these antibiotics using panels prepared at IHMA, Inc. following CLSI guidelines. The panels were incubated at 35°C in 5% CO2 for 24 hours before reading the MIC endpoints. Quality control (QC) testing was performed each day of testing as specified by the CLSI using *Streptococcus pneumoniae* ATCC 49619.

[redacted]

5.4.3 Sephadex® Risk Assessment

Sephadex® Safety

Sephadex® G25 has been proved to be safe when applied directly to open wounds in humans and is marketed as Debrisan® Absorbent pad for acute and chronic wounds.

The Sponsor is not aware of any clinical trials treating either infants or adults with an oral therapeutic containing Sephadex®. Unlike the nonclinical safety studies and clinical trials with Debrisan® that applied Sephadex® chronically to wounds, the dextranomer spheres of Sephadex® are unlikely to be in contact with gut tissues for prolonged periods after oral administration. Sephadex® is not absorbed systemically. Preclinical studies with SB-121 demonstrate that Sephadex® is cleared in feces from 24 to 48 hours as intact microspheres (Scioto Biosciences Inc. SB-121 Investigator's Brochure. Edition 1).

6. OBJECTIVES

6.1. Primary Objective

- To evaluate the safety and tolerability of multiple doses of SB-121 in subjects with AD

6.2. Secondary Objectives

- To monitor the elimination of Sephadex® from the GI tract in subjects with AD
- To assess the impact of multiple doses of SB-121 on circulating oxytocin levels in subjects with AD
- To assess the impact of multiple doses of SB-121 on tests of AD
- To evaluate biomarkers of microbiota function, immune modulation, and inflammation following multiple doses of SB-121 in subjects with AD

6.2.1.1 Endpoints

Endpoints in this study are as follows:

Primary endpoints:

- Incidence and severity of treatment-emergent AEs (TEAEs), serious AEs (SAEs), AESIs, and AEs leading to discontinuation from the study
- Incidence of presence of Sephadex® microspheres in the stool
- Incidence of symptomatic bacteremia with positive *L. reuteri* identification

Secondary endpoints:

Change from baseline in:

- Clinical Global Impressions Severity and Improvement Subscales
- Vineland Adaptive Behavior Scales (3rd edition)
- Aberrant Behavior Checklist
- Woodcock Johnson Test (3rd Edition)
- Repeatable Battery for Assessment of Neuropsychological Status
- Test of Attentional Performance for Children (KiTap)
- Neurophysiology Measures including EEG resting state, auditory habituation, and chirp modulated sweep auditory evoked response
- Eye Tracking
- Biomarkers in stool and blood samples: Plasma oxytocin, plasma vasopressin, serum hs-CRP, tumor necrosis factor-α, and stool biomarkers.

7. STUDY DESIGN

7.1. Overall Study Design and Plan

This is a phase 1, single-center, randomized, double-blind, placebo-controlled cross-over study to evaluate the safety and tolerability of 28-days of once daily oral administration of SB-121 (a preparation of *L. reuteri* with Sephadex® and maltose), to subjects with AD.

Up to 16 eligible subjects will be randomized 1:1 to receive treatment with either SB-121 or placebo for 28 days. After a washout period of ~14 days, the subjects will be crossed over to the alternate treatment for 28 days.

Subjects will undergo screening within ~14 days before the first administration of the investigational product (IP). All subjects (or parent/authorized designee) will be required to provide written informed consent before any study-specific procedures are performed. Subjects will also be asked to provide consent to allow storage of biological samples for future analysis that will not involve genetic tests. Subjects' eligibility for the study will be determined at Screening by assessment of inclusion and exclusion criteria. Eligible subjects will be provided with a stool sampling kit to collect a pretreatment stool sample within 48 hours before receiving the IP.

Subjects will return to the study site (clinic) on Day 1. Following confirmation of eligibility criteria, they will be randomized to receive SB-121 or placebo. Following baseline tests, all subjects will be given their first dose of either SB-121 or placebo at the clinic.

Subjects will undergo study-specific procedures on Day 1 in accordance with the schedule of events and will be discharged after all Day 1 evaluations have been completed. They will then take the IP at home once a day for the remaining 27-days. They will then return to the clinic on Day 28.

Following the ~14 day washout period, all subjects will cross-over to the other treatment (SB-121 or placebo) for 28-days and the study procedures will be repeated.

Subjects will be instructed to collect a total of 5 stool samples at home according to the following schedule:

- A pretreatment stool sample within approximately 48 hours before receiving the first dose of the IP.
- Post-treatment samples:
    - Sample 1 should be taken no sooner than 21 days or later than 28 days into the first treatment period of the study (prior to the completion of the first treatment period)
    - Sample 2 should be taken approximately 7 days into the wash-out period after the first treatment period of the study and before the second treatment period
    - Sample 3 should be taken no sooner than 21 days or later than 28 days into the second treatment period of the study (prior to the completion of the second treatment period)
    - Sample 4 should be taken approximately 7 days after the completion of the second treatment period of the study

Subjects will be advised to contact the study site immediately in the event of symptoms consistent with bacteremia (e.g., fever, chills, diaphoresis) to obtain appropriate medical care as indicated. In the event that subjects develop symptoms consistent with bacteremia, 2 blood samples will be collected, when feasible for blood cultures, and microbial identification.

Follow-up visits will be conducted on Day 28 of treatment period 1, Day 1 of treatment period 2 and Day 28 of treatment period 2 as per the Schedule of Events.

Details of AEs occurring throughout the study will be captured at each visit, additionally AEs of special interest will be captured in the Patient Dairy. Weekly telephone calls will be conducted by the study team between in person visits during each treatment period. These calls will record and evaluate adverse events and will also determine IP compliance. In the case of adverse events identified during a phone call, the study team may determine that an unscheduled in-person study visit and/or lab tests may be required. Gastrointestinal events (nausea, vomiting, diarrhea, constipation, abdominal distention, abdominal pain/cramps, and flatulence) and systemic events (fever, chills, diaphoresis) occurring at any time during the study and within 2 weeks after the last dose of IP will be considered AEs of special interest (AESIs), and will be solicited.

The investigational product will be provided to subjects in 2 bottles, the contents of which will be combined, allowed to sit for a period of 15-45 minutes at room temperature, then combined with a predefined drink for consumption. See the Daily Medication Instructions for additional details.

7.2. Rationale and Discussion of Study Design

SB-121 is being developed for use in the treatment of AD in patients age 36 months and above.

This study is a multiple-dose, randomized, double-blind, placebo-controlled, cross-over single-site Phase I study. The primary objective is to evaluate the safety and tolerability of multiple doses of SB-121 in subjects ages 15 to 45 years with AD.

Additionally, multiple behavioral and physiological measures of AD, as well as mechanistic biomarkers, will be assessed in order to inform later stage trials.

A placebo control is used in the present study to enable assessment of safety of SB-121.

The study is being conducted in a double-blind fashion to reduce the risk of bias in the reporting of study outcomes by subjects and investigators. A cross-over design will enable each patient to be a control for her/himself, thus increasing the power to detect target engagement in a first in disease study while also increasing available safety data by exposing all subjects to the IP.

7.3. Selection of Doses in the Study

[redacted] *L. reuteri* strains are safe with minimal reported adverse events in clinical trials using doses as high as 10^{10} CFU.(Di Nardo 2014, Mobini 2017) Maltose is an accepted food additive and naturally occurs in gram levels in foods such as cooked sweet potatoes, edamame, broccoli, barley and others. The safety of Sephadex® delivered orally has yet to be tested clinically. [redacted] A formulation consisting of $2x10^9$ CFU *L. reuteri* + 800 mg Sephadex® (largest possible level without formation of a gel) + 1152 mg maltose in 1 ml saline was tested in rats that were dosed with 500 µl by oral gavage daily for 2

weeks. There were no treatment-related abnormalities in any gut, liver, lung or kidney tissue as determined by an independent pathologist. In addition, clearance of the Sephadex® microparticles into feces was at a rate to that did not indicate any accumulation in the gut with daily dosing.

Preclinical studies of SB-121 demonstrate efficacy in rodents using doses between 100 µl and 500 µl. [redacted]

7.4. Study Site(s)

The study will take place at a single site in the United States.

7.5. End of Study Definition

This clinical trial will be considered completed 14 days after the end of study period 2.

8. SUBJECT POPULATION

8.1. Selection of Study Population

The present study will be conducted in subjects with AD ages 15-45 years.

A sufficient number of subjects will be enrolled to ensure up to 8 subjects are randomized per treatment group for a total of up to 16 subjects.

8.2. Study Entry Criteria

8.2.1 Inclusion Criteria

A subject will be eligible for study participation if he or she meets all of the following criteria:

1. Subject/parent (or authorized designee) has provided written informed consent for the study.
2. Subject is ≥15 and ≤45 years of age at the time of enrollment.
3. Diagnosis of AD as confirmed by the gold standard clinical interview using DSM-5 criteria and administration of the Autism Diagnostic Observation Schedule-2.
4. Subject, if female and of childbearing potential, is not lactating or pregnant.
5. Subject, if female, is either not of childbearing potential or is practicing an acceptable effective method of birth control.
6. Subject is willing to comply with all study requirements (including the requirements for stool sampling and biobanking) and to return to the study facility for the follow-up evaluations, as required.

8.2.2 Exclusion Criteria

A subject will be excluded from the study if he or she meets any of the following criteria:

1. Subject has known allergy or significant adverse reaction to *L reuteri*, Sephadex®, maltose, or related compounds.
2. Subject has previously had GI surgery, intestinal obstruction, *Clostridium difficile* infection or diverticulitis.
3. Subject has travelled outside of the USA in the 30 days prior to screening.
4. Subject has had a diarrheal illness in 30 days prior to screening.
5. Subject currently has a fever or active/uncontrolled GI symptoms (e.g., nausea, vomiting, diarrhea, constipation, abdominal distention, abdominal pain/cramps, flatulence) or has had these within 14 days prior to screening. If the GI symptoms are stable, in the opinion of the investigator, the subject can be enrolled.
6. Subject has any immunological/autoimmune disorder including, but not limited to, systemic lupus erythematosis, rheumatoid arthritis, Sjögren's syndrome, inflammatory bowel disease, or immunoglobulin-deficiency disorder, that would increase the risk to the subject or interfere with the evaluation of SB-121.
7. Subject has a documented history of HIV, hepatitis B and/or hepatitis C
8. Subject has implanted prosthetic devices including prosthetic heart valves.
9. Subject has taken, or is taking, any of the following prohibited medications:
    a. A proton pump inhibitor within 2 weeks prior to screening
    b. Use of supplemental probiotics within 2 weeks prior to screening except for yogurt

c. Current use of immunosuppressive medications, including corticosteroids
d. Treatment with monoclonal antibodies within 4 weeks prior to screening
e. Systemic antibiotics within 2 weeks prior to screening

10. Subject has diabetes mellitus or is prediabetic.
11. Subject has received any IP (or investigational device) within 30 days prior to screening.
12. Subject has any of the following laboratory test results at Screening:
    a. An absolute neutrophil count of $<1.5 \times 10^9$/L
    b. alanine aminotransferase or aspartate aminotransferase >1.5 × upper limit normal (ULN), total bilirubin >1.5 × ULN (subjects with known Gilbert's Syndrome can be included)
    c. serum creatinine >1.5 × ULN
    d. any other abnormal laboratory test that is clinically significant in the judgment of the investigator.
13. Subject has an unstable medical condition or is otherwise considered unreliable or incapable, in the opinion of the investigator, of complying with the requirements of the protocol.
14. Subject tests positive for drugs of abuse in a urine drug screen at screening.
15. Subject has a history of alcohol abuse.

8.3. Subject Discontinuation/Withdrawal from the Study

Subjects are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a subject from the study for any reason, including the following:

- Pregnancy
- Significant study noncompliance
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject
- If the subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the eCRF.

Whenever possible and reasonable, the evaluations that were to be conducted at the completion of the study should be performed at the time of early discontinuation.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit the following business day, as well as counsel the subject on the importance of maintaining the assigned visit schedule
- Before a subject is deemed lost to follow-up, a member of the study team will make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary,

a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file.

- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

Subjects who are unable to attend the End of Study Visit will be contacted by telephone for reporting of AEs.

8.4. Subject Replacement Criteria

Subjects who withdraw consent, are withdrawn, or discontinue from the study may be replaced.

Randomized subjects withdrawn from the study may not re-enter. The subject number for a withdrawn subject will not be reassigned to another subject.

9. TREATMENTS

9.1. Identification of Investigational Product(s)

SB-121 is a preparation of *L. reuteri* with Sephadex® and maltose.

L. reuteri is a Gram-positive nonsporulating facultative anaerobic bacteria and the strain utilized in SB-121 is *L. reuteri* ATCC 23272, grown in De Man, Rogosa and Sharpe (MRS) broth and lyophilized in the presence of cryoprotectants (sorbitol and maltodextrin).

[redacted]

The Daily Medication Instructions will detail the steps for storage, preparation and consumption of the IP.

[redacted]

9.1.1 Rescue Medication

Not applicable.

9.2. Selection of Timing of Dose for Each Subject

This is a daily-dose study. The first dose of SB-121 or placebo will be administered at the study site, according to the treatment group to which the subjects are allocated, on the morning of the Day 1 and after confirmation that the subject remains eligible.

Starting on Day 2, the daily dose of study medication is to be taken by mouth (consumed orally), in a single sitting at home. The study medication should only be taken once a day by the study subject. If at all possible, the study medication should be taken at the same time every day (for example, every day around breakfast).

9.3. Dose Adjustment Criteria

Dose adjustment is not allowed in this study.

9.3.1 Stopping Rules

The clinical severity of an AE will be graded according to the NCI-CTCAE (version 5.0, 27-Nov-2017). If an NCI-CTCAE scale is not available for a given AE. Refer to Section 11.2.2.1.The trial will be stopped in the event of ≥1 subject reporting a Grade 4 laboratory and/or clinical AE considered related to SB-121 or ≥2 subjects reporting Grade 3 laboratory and/or clinical AE considered related to SB-121.

9.4. Treatment Compliance

All subjects will receive the first dose of IP at the study site under the supervision of appropriate study personnel. Administration details and IP batch or lot number(s) will be recorded in the subject's eCRF. Information regarding subsequent doses is to be recorded in the Patient Diary and returned to the study site.

9.5. Study Blind

This is a double-blind, placebo-controlled cross-over study. The study team and subjects will be blinded to the randomized study treatment assignments. The study blind will be maintained throughout the duration of the clinical trial. Only the dispensing pharmacist will be aware of study drug assignment.

9.6. Procedure for Breaking the Study Blind

In a medical emergency where knowledge of the subject's treatment assignment may influence the subject's clinical care, the investigator may access the subject's treatment assignment. Under these circumstances, the investigator will contact the un-blinded pharmacist to get the subject's treatment assignment. Emergency unblinding can thus be made for any subject without affecting the double-blind nature of the study. The Medical Monitor will be notified of any emergency unblinding requests. The investigator should make every effort to discuss the rationale for emergency unblinding with the Medical Monitor prior to unblinding the individual subject. Once a subject is unblinded, he/she must be withdrawn from the study. The subject's treatment assignment should not be shared with the sponsor/designee and other study team members. The investigator must record the reason for the emergency unblinding in the source documents and record it in the electronic database.

9.7. Permitted and Prohibited Therapies

All concomitant medications used (including over-the-counter medications and herbal supplements) will be recorded in the source document and on the appropriate eCRF. Information on prior and concomitant medications will be collected at Screening; any changes to concomitant medications will be reported at each subsequent study visit.

Medications that started prior to treatment will be considered prior medications whether or not they were stopped prior to treatment. Any medications continuing or starting post-treatment will be considered to be concomitant. If a medication starts prior to treatment and continues after treatment with IP is started, it will be considered both prior and concomitant.

9.7.1 Prohibited Therapies

The following therapies are prohibited both before and during the study:

- Proton pump inhibitor within 2 weeks prior to screening
- Use of systemic antibiotics within 2-weeks prior to Screening
- Use of supplemental probiotics within 2 weeks prior to screening except for yogurt
- Current use of immunosuppressive medications, including corticosteroids
- Monoclonal antibodies (e.g., adalimumab) within 4-weeks of screening

- Any IP (or investigational device) within 30 days before enrollment in this study.

For subjects receiving excluded therapies after randomization, eligibility for study continuation, will be determined on a case-by-case basis at the discretion of the medical monitor.

9.7.2 Permitted Therapies

Other concomitant medications (i.e., those not listed as prohibited in Section 9.7.1) are allowed, but should be limited to those medications considered necessary in the investigator's judgment. Subjects can continue on stable doses of their medications for AD and associated symptoms, as determined by the investigator.

9.8. Dispensing and Storage

[redacted]

The investigator must confirm the receipt of the IP with his or her signature. The investigator must keep a copy of this receipt and another copy will be stored at Scioto Biosciences Inc.

[redacted]

9.9. Drug Accountability

The investigator must maintain adequate records showing the receipt, dispensing, return, or other disposition of the IP, including the date, quantity, batch or code number, and identification of subjects (subject number) who received the IP. The IP may not be relabeled or reassigned for use by other subjects. If any of the IP are not dispensed, are lost, stolen, spilled, unusable, or are received in a damaged container, this information must be documented and reported to the sponsor.

9.10. Labeling and Packaging

[redacted]

9.10.1 Labeling

The IP will be provided as a kit, with a label affixed that meets the applicable regulatory requirements and may include the following:

- Protocol number
- Unique kit identifier
- Subject number (record at the time of dispensing)
- Manufacture and expiry date
- Package contents
- Storage instructions
- Caution: "New Drug – Limited by United States Law to Investigational Use" and "Keep out of reach of children"
- Sponsor name and address

Other information may be included as required.

All empty packaging or packaging containing unused SB-121 components should be saved for final disposition by the sponsor or designee.

9.10.2 Packaging

10. STUDY PROCEDURES

Written informed consent must be obtained before any study-related procedures are initiated, including the cessation of prohibited concomitant therapy.

For the timing of assessments and procedures throughout the study, refer to the Schedule of Events (Section 2.2). Throughout the study, every reasonable effort should be made by study personnel to follow the timing of assessments and procedures in the Schedule of Events for each subject. If a subject misses a study visit for any reason, the visit should be rescheduled as soon as possible.

10.1. Study Duration

The overall study duration is expected to be approximately 14-weeks.

The planned sequence and duration of the study periods will be as follows:

1. Screening: Up to 14 days
2. Study Period 1: 28 days
3. 14-day washout period
4. Study Period 2: 28 days
5. Post-treatment: Up to 14 days

The maximum study duration for each subject is approximately 98 days.

10.2. Study Periods and Visits

10.2.1 Screening

The subject will be screened within 14 days before randomization in the study. After informed consent, the following procedures will be performed at screening:

Medical and Psychiatric History: A medical and psychiatric history will be completed by a member of the study team to assess that the subject meets study eligibility criteria. The following will be part of this:

1. Autism Diagnostic Observation Schedule - 2 (ADOS-2) Modules 2, 3 or 4 (Lord 2000, Lord 1989). The ADOS-2 is an investigator-based AD diagnostic assessment that places the participant in naturalistic situations demanding specific social and communication responses. It covers social communication, social relatedness, play, and repetitive behaviors. Participants who are able to complete Modules 2, 3 or 4 will be enrolled in the study. Modules 2, 3 and 4 of the ADOS were chosen for this project to accommodate the age range in this study. Additionally, they accommodate verbal skills such as sentence speech and phrase speech. Furthermore, these modules assess individuals' ability to report on an event through several opportunities (e.g., description of a picture). This information will be vital in assessment of participants' ability to report adverse events.

2. DSM-5 Checklist: A DSM-5 checklist will be completed by a member of the study team conducting the diagnostic assessment. The checklist includes a listing of the current symptom criteria for AD in the DSM-5 (American Psychiatric Association 2013).

Concomitant medications will be recorded.

Physical Examination: A study physician or other qualified study team member will complete a general physical examination to assess that the subject meets study eligibility criteria. This includes checking the vital signs (pulse, BP and oral temperature) and height and weight.

Screening Labs: Blood samples for hematology and chemistry will be collected. A urine sample will be collected for urine analysis from all subjects, and a urine sample will be collected from females of child bearing potential for a pregnancy test.

Eligible subjects will be provided with a stool sampling kit to collect a pretreatment stool sample within approximately 48 hours before receiving the first dose of IP.

Rescreening: Rescreening of a subject may be allowed once under circumstances where the subject passed the screening but could not be randomized within the 14-day screening window due to logistical, personal or other unforeseeable reasons. For rescreening in these circumstances, all screening measures will be repeated with the exception of the DSM and ADOS-2.

10.2.2 Post-screening

A pretreatment stool sample within approximately 48 hours before receiving the first dose of the IP.

For all eligible subjects the following tests will be performed by a study team member as indicated in the Schedule of Events (Section 2.2):

10.2.2.1 Autism Pathophysiology Measures

Wechsler Abbreviated Scale of Intelligence Scale-Second Edition (WASI-II): The WASI-II (Wechsler 2017) is a tool used to evaluate an individual's cognitive functioning and generate IQ scores. There are 4 subtests: Block Design, Vocabulary Matrix, Reasoning, and Similarities. This measure will only be done at baseline, prior to the first dose.

Social Communication Questionnaire (SCQ): The SCQ, originally called the Autism Screening Questionnaire, is a continuous measure of AD behaviors and symptoms based on the Autism Diagnostic Interview- Revised (ADI-R) (Johnson 2011, Chandler 2007). This measure will only be done at baseline, prior to the first dose.

Clinical Global Impressions Severity (CGI-S) and Improvement (CGI-I) Subscales: The clinician-rated CGI-S is rated on a 7 point Likert scale from minimally to the most severely affected, anchored to core symptoms of AD. Clinical response to drug exposure will be assessed with the clinician-rated CGI-I (Guy 1976). The CGI-I is a 7-point scale designed to measure symptomatic change that compares the patient's clinical condition to the baseline . The CGI-I assessment will focus on the core symptoms of AD. CGI-I is a gold standard measure of potential change with treatment in placebo-controlled pharmacotherapy trials in AD.

Vineland Adaptive Behavior Scales (3rd edition): The Vineland™-3 measures communication, daily living skills and socialization skills. The Vineland™-3 will be administered to a subject's reliable caregiver in this study, during which the rater from the study team will ask the caregiver open ended questions relating to the subject's activities and behavior.

Aberrant Behavior Checklist (ABC): The ABC is an informant-based questionnaire consisting of 58 items subdivided amongst 5 scales: irritability, lethargy and social withdrawal, stereotypic behavior, hyperactivity/non-compliance, and inappropriate speech (Aman 1985). A score for each item ranges from 0 indicating "no problem" to 3 indicating "severe problem". Scale scores are calculated by summing the items within that scale. Higher scores indicate greater impairment.

Woodcock Johnson 3rd Edition (WJ-III): The Spatial Relations and Auditory Attention subtests of the WJ-III will be administered. The Spatial Relations subtest will assess the visual spatial thinking domain with a task that requires the identification of parts needed to form a complete shape. Responses can be oral or motoric (pointing). The Auditory Attention subtest will assess auditory processing (speech/sound discrimination) requiring the identification of orally presented words amid increasingly intense background noise. Examinees point to a picture of the word presented.

Repeatable Battery for Assessment of Neuropsychological Status (RBANS): The RBANS is a neuropsychological battery for persons with neurological disorders. The RBANS covers five domains including Immediate Memory, Language, Attention, Visuospatial/Constructional, and Delayed Memory (Randolph 1998).

Test of Attentional Performance for Children (KiTap): The KiTap is an automated computer based assessment of attentional performance developed and normed for the pediatric population. Despite its development in pediatrics, the KiTap is well suited for use in developmental disabilities across all age ranges and has been normed specifically in FXS in adults and youth (Knox 2012). The task presents an enchanted castle animation and investigates performance in a number of areas including Alertness, Vigilance, Visual Scanning, Distractibility, Attention, Flexibility, and Sustained Attention.

EEG Measures: Subjects will complete EEG evaluations prior to the first dose of IP (baseline) and following four weeks of chronic dosing in each treatment period. The procedure will be performed on all subjects but inability to complete the procedure will not be considered a deviation.

The EEG tests include:

1. EEG resting state task: Participants will complete a 5-minute resting state EEG protocol.
2. Auditory habituation Evoked Response Potential (ERP): ERPs will be recorded during passive listening.
3. Chirp modulated sweep: Subjects will passively listen to auditory stimuli consisting of a amplitude modulated chirps.

Eye Tracking/Pupillometry Measures: Infrared eye tracking will be used as a measure of eye gaze and pupillary reactivity in response to visual processing of human faces and of social scenes at prior to the first dose of IP (baseline) and following four weeks of chronic dosing in each treatment period.

10.2.2.2 Stool Sampling

Following screening, all subjects who meet eligibility criteria, will be provided with sample kits comprising a commode specimen collection system with closed container storage, biohazard bags, and with labels for the sample containers, which subjects should complete with the time and date that the sample was obtained. Styrofoam coolers with ice-packs will be provided to subjects to keep samples cool during transport. Instructions for stool collection and shipping will be provided.

Stool samples will be collected for determination of the presence of Sephadex® microspheres. The stool samples will also be used for the evaluations of potential biomarkers of inflammation, based on changes from baseline (pre-treatment).

Subjects will be instructed to collect a total of 5 stool samples at home according to the following schedule:

- A pretreatment stool sample within approximately 48 hours before receiving the first dose of the IP.
- Post-treatment samples:
    - Sample 1 should be taken no sooner than 21 days or later than 28 days into the first treatment period of the study (prior to the completion of the first treatment period)
    - Sample 2 should be taken approximately 7 days into the wash-out period after the first treatment period of the study and before the second treatment period
    - Sample 3 should be taken no sooner than 21 days or later than 28 days into the second treatment period of the study (prior to the completion of the second treatment period)
    - Sample 4 should be taken approximately 7 days after the completion of the second treatment period of the study

10.2.2.3 Sampling

Samples for changes from baseline will be collected for the evaluation of the following systemic biomarkers: Plasma oxytocin, plasma vasopressin, serum hs-CRP, tumor necrosis factor-α, and stool biomarkers.

10.2.3 Treatment Visit – Day 1 (See Schedule of Events Section 2.2)

Following confirmation of eligibility criteria, subjects will be randomized to receive SB-121 or placebo. Subjects will undergo study-specific procedures on Day 1 in accordance with the Schedule of Events.

Following baseline tests, all subjects will be given their first dose of either SB-121 or placebo at the clinic. The IP will be prepared according to the provided Daily Medication Instructions.

All subjects receive an additional 27 doses of IP to be self-administered once daily at home at about the same time every day (e.g. around breakfast). In addition to the 27 oral doses, additional material will be provided for use in the event of spillage. Refer to the Daily Medication Instructions

for details on IP storage, reconstitution and consumption. All subjects will be instructed to capture the date and time of each dose of IP on the Patient Diary.

Subjects will be discharged after all Day 1 evaluations have been completed.

10.2.4 Follow-up Visits (See Schedule of Events – Section 2.2)

Follow-up clinic visits will be conducted on Day 28, Day 1 of treatment period 2 and Day 28 of treatment period 2. Subjects will undergo study-specific procedures in accordance with the Schedule of Events.

Telephone Calls: Weekly telephone calls will be conducted by a member of the study team between in person visits during each treatment period in accordance to the Schedule of Events. These calls will be to assess IP compliance and to capture any adverse events. If a concerning adverse event is identified during a phone call, the study staff will consult with the investigator and an unscheduled in-person study visit and/or lab work may be added.

10.2.5 End of Study / Early Termination

An End-of-Study Visit will be conducted on Day 28 of Study Period 2. Subjects will undergo study-specific procedures in accordance with the Schedule of Events.

Subjects who discontinue the study early for any reason will be asked to attend the study site for an Early Termination (ET) Visit; procedures performed at the ET visit will be the same as those conducted for subjects attending the End-of-Study Visit.

Subjects who are unable to attend the End of Study Visit/Early Termination Visit will be contacted by telephone for reporting of AEs. However, investigators should make every effort to ensure that subjects do attend.

The Post-Treatment Period will last up to 14 days, wherein subjects will not receive any study drug. During this time, subjects will be contacted by telephone for reporting of AEs. A post treatment stool sample is to be collected approximately 7 days after the completion of the second treatment period of the study

10.2.6 Safety Variables

Safety assessments will include the evaluation of AEs, clinical laboratory assessments and vital signs.

10.2.6.1 Adverse Events

The definitions and management of AEs, and any special considerations for AEs, are provided in Section 11.

Details of AEs occurring throughout the study will be collected by a member of the study team at each visit. Weekly telephone calls will be conducted by a member of the study team between in person visits during each treatment period. These calls will record and evaluate AEs and will also determine IP compliance. In the case of adverse events identified during a phone call, the investigator or designee may determine that an unscheduled in-person study visit and/or lab tests may be required. Gastrointestinal events (nausea, vomiting, diarrhea, constipation, abdominal distention, abdominal pain/cramps, and flatulence) and systemic events (fever, chills, and

diaphoresis) occurring at any time during the study and within 2 weeks after the last dose of IP will be considered AEs of special interest (AESIs). If a subject develops fever, chills or abnormal sweating they will be instructed to contact the study team as soon as possible.

During clinic and telephone visits, according to the Schedule of Events (Section 2.2), a study team member complete a check list, which will be used for the solicited collection of specific AEs (adverse events of special interest) occurring since their previous study visit. These AEs include the following:

- Gastrointestinal events: nausea, vomiting, diarrhea, constipation, abdominal distention, abdominal pain/cramps, and flatulence
- Systemic events: fever, chills and diaphoresis

Additionally, all subjects will be instructed to write down any adverse events of special interest that they may be having on the Patient Diary.

10.2.6.2 Clinical Laboratory Safety Assessments

10.2.6.2.1 Clinical Laboratory Tests to be Performed

Samples for the following laboratory tests will be collected at the timepoints specified in the Schedule of Events (Section 2.2):

Hematology:	hemoglobin, hematocrit, red blood cell (RBC) count, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, white blood cell count including differential (to also include absolute neutrophil, lymphocyte and eosinophil counts)
Serum Chemistry:	albumin, total and direct bilirubin, total protein, calcium, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, glucose, sodium, potassium, chloride, bicarbonate, lactate dehydrogenase, uric acid, highly sensitive C-reactive protein
Urinalysis (dipstick):	pH, specific gravity, blood, glucose, protein, ketones (microscopic evaluation will be conducted in case of abnormal findings)
Urine Pregnancy Test:	for women of childbearing potential only
Urine Drug Screen (Screening only):	amphetamines, barbiturates, benzodiazepines, cocaine, opiates, and phencyclidine

Subjects are not required to be fasted at the time of sampling for clinical laboratory tests.

Blood samples will be up to 20 mL in volume. The total amount of blood to be drawn will be a maximum of 80 mL per subject.

The normal ranges of values for the clinical laboratory assessments in this study will be provided by the responsible laboratory. They will be regarded as the reference ranges on which decisions will be made.

All laboratory values out of the reference range, are not necessarily clinically relevant. The investigator must evaluate the out-of-range values and record this assessment of the clinical relevance in the appropriate eCRF. All clinical laboratory values that, in the investigator's opinion, show clinically relevant changes must be reported as AEs up to 14 days after the second study period.

10.2.6.2.2 Biological Markers or Biopsies

Details of the analysis of biomarkers in plasma and stool samples are provided in (Section 10.2.2.1).

10.2.6.2.3 Microbe Identification in for Suspected Bacteremia

In the event that a subject develops symptoms consistent with bacteremia (e.g., fever, chills, diaphoresis), attempts will be made to collect appropriate blood samples for microbial identification using culture bottles; subjects should attend clinic at their earliest convenience to provide a blood sample. Details for the correct handling of the samples will be provided in the laboratory manual.

[redacted]

10.2.6.2.4 Storage of Biological Samples

Subjects will be required to consent to storage of unused blood/plasma and/or stool samples.

Unused biological samples will be stored at Scioto Biosciences Inc. for potential analysis of AD and its response to SB-121 as well as other tests related to the mechanism of action of SB-121. Genetic tests on these samples will not be permitted. Samples will be stored for up to 5 years.

Sample labeling will include the following minimum information:

- Study sponsor and study identification number
- Subject identification
- Date and time of sample collection

10.2.6.3 Clinical Examinations

10.2.6.3.1 Vital Signs

Vital signs, including pulse rate, SBP, DBP, and oral temperature will be measured after the subject has been in a supine position for 5 minutes.

10.2.6.3.2 Height, Weight and Body Mass Index

Subjects' height and body weight will be measured at Screening, and BMI calculated.

10.2.6.3.3 Physical Examination

A complete physical examination (excluding breast, pelvic, rectal and genitourinary examination, unless clinically indicated) will be performed at Screening). During all other study visits a targeted physical exam, including vital signs, may be performed as needed to evaluate adverse events.

10.2.7 Clinical Pharmacology

Not applicable for this study.

11. ADVERSE EVENTS

11.1. Definitions

11.1.1 Adverse Events

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Pre-existing diseases or conditions will not be considered AEs unless there is an increase in the frequency or severity, or a change in the quality, of the disease or condition.

Any AE occurring after obtaining the informed consent will be recorded.

11.1.2 Unexpected Adverse Event

An expected AE is one for which the nature or severity is consistent with the known AE profile of the IP.

An unexpected adverse event (UAE) is one for which the nature or severity is not consistent with the applicable product information (Investigator's Brochure in the case of SB-121).

There are no expected adverse events for SB-121 at this time.

11.1.3 Serious Adverse Events

A serious adverse event (SAE) is any AE that:

- results in death
- is life-threatening
  NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing hospitalization
  An elective hospital admission to treat a condition present before exposure to the IP does not qualify the condition or event as an SAE.
- results in persistent or significant disability/incapacity
- is a congenital anomaly
  NOTE: A congenital anomaly in an infant born to a mother who was exposed to the IP during pregnancy is an SAE
- is an important medical event
  NOTE: Medical and scientific judgment should be exercised in deciding whether it is appropriate to consider other situations as serious, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events include intensive treatment in an

emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency, or drug abuse.

Each SAE considered to be possibly, probably, or definitely related to the IP must be assessed for expectedness to determine whether it is reportable as a SUSAR (Suspected Unexpected Serious Adverse Reaction). The Reference Safety Information for this study, against which expectedness assessments are made, can be found in the Investigator's Brochure Edition 1). Determination of the expectedness of an event is the responsibility of the Sponsor.

11.1.4 Treatment-Emergent Adverse Events

An AE is defined as a treatment emergent adverse event (TEAE) if the first onset or worsening is after the first administration of IP.

11.2. Event Assessment and Follow-up of Adverse Events

All AEs will be captured on the appropriate eCRF. Information to be collected includes event description, time of onset, investigator's assessment of severity, relationship to IP, and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or stabilization, as determined by the investigator.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's pre-existing medical condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of onset and duration of each episode.

The study team will record all AEs with start dates occurring any time after informed consent is obtained until 14 days after study treatment. At each study visit, the study team will ask about the occurrence of AEs since the last visit whether in the clinic or on the phone. Any SAEs considered to be related to study treatment that occur beyond 14 days after study treatment will also be reported.

Subjects who discontinue IP early will continue to be monitored for 14 days from last treatment for AEs, unless they withdraw consent or are lost to follow-up.

11.2.1 Assessment

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE described previously.

Adverse events of special interest will be solicited and captured in the same manner as any other AE (see Section 11.3.1).

11.2.2 Evaluation

11.2.2.1 Severity of Adverse Events

The clinical severity of an AE will be graded according to the NCI-CTCAE (version 5.0, 27-Nov-2017). If an NCI-CTCAE scale is not available for a given AE, the following definitions will be used:

Grade 1 (mild)	asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 (moderate)	minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living
Grade 3 (severe)	medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
Grade 4 (life-threatening)	Life-threatening consequences; urgent intervention indicated
Grade 5	Death related to AE

11.2.2.2 Seriousness

The investigator is to evaluate whether the AE meets the criteria for a SAE, as described in Section 11.1.3.

11.2.2.3 Outcome at the Time of Last Observation

The outcome at the time of last observation will be classified as:

- Recovered/resolved
- Recovered/resolved with sequelae
- Recovering/resolving
- Not recovered/not resolved
- Fatal
- Unknown

11.2.2.4 Adverse Event Relationship to Investigational Product

The investigator must make an assessment of each TEAE's relationship to the IP. The categories for classifying the investigator's opinion of the relationship are as follows:

Not related	An AE with sufficient evidence to accept that there is no causal relationship to IP administration (e.g., no temporal relationship to drug administration, because the drug was administered after onset of event; investigation shows that the drug was not administered; another cause was proven).
Unlikely related	An AE with a temporal relationship to IP administration that makes a causal relationship improbable, and in which other drugs, events, or underlying disease provide plausible explanations.
Possibly related	An AE with a reasonable time sequence to administration of the IP, but that could also be explained by concurrent disease or other drugs or events. Information on drug withdrawal may be lacking or unclear.
Probably related	An AE with evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event occurs within a reasonable time after administration of the IP, is unlikely to be attributed to concurrent disease or other drugs.
Definitely related	An AE with a biologically plausible relationship occurring in a plausible time relationship to IP administration and that cannot be explained by a concurrent disease or other drugs or events.

11.2.3 Documentation

All AEs that occur within the period of observation for the study must be documented in the eCRF with the following information, where appropriate.

- AE name or term
- When the AE first occurred (start date and time)
- When the AE stopped (stop date and time) or an indication of "ongoing" if not resolved
- Severity of the AE

- Seriousness of the AE
- Actions taken including concomitant medications
- Outcome
- Investigator opinion regarding the AE relationship to the IP(s)

11.2.4 Treatment of Adverse Events

Adverse events that occur during the study will be treated, if necessary and as determined by the investigator, by established standards of care. If such treatment constitutes a deviation from the protocol, the subject may continue in the study after consultation between the investigator and the medical monitor.

11.2.5 Follow-up

All AEs will be followed to adequate resolution or stabilization, as determined by the investigator. All findings relevant to the final outcome of an AE must be reported in the subject's medical record and recorded on the eCRF page.

11.2.6 Reporting

11.2.6.1 Serious Adverse Events

The investigator or designee must report all SAEs within 24 hours of first becoming aware of the event by completing, signing, and dating the Serious Adverse Event Report Form, verifying the accuracy of the information recorded in the form with the source documents, and sending the SAE form to [redacted]

At the time of first notification, the investigator or designee should provide the following information, as available:

- Protocol number
- Investigator's name
- Reporter name
- Subject number
- Subject's year of birth
- Subject's gender
- Date of first dose of IP(s)
- Date of last dose of IP(s), if applicable
- Adverse event term
- Date of occurrence of the event
- A brief description of the event, outcome to date, and any actions taken
- The seriousness criteria(on) that were met

- Concomitant medication at onset of the event and any medications to treat the event
- Relevant medical history information
- Relevant laboratory test findings
- Investigator's opinion of the relationship to IP(s) (not related, unlikely related, possibly related, probably related, or definitely related)
- Whether and when the investigator was unblinded as to the subject's treatment assignment

Any missing or additional relevant follow-up information concerning the SAE should be sent via the same contact details as above as soon as possible on a follow-up SAE Report Form.

Further information may be requested from the site by using a follow-up request form or via email communication.

The investigator is required to comply with applicable regulations (including local laws and guidance) regarding the notification of the SAE to health authorities, and institutional review board (IRB).

The sponsor is responsible for sending Investigational New Drug (IND) safety reports to the FDA in accordance with applicable regulations.

11.3. Special Considerations

11.3.1 Adverse Events of Special Interest

Gastrointestinal events (nausea, vomiting, diarrhea, constipation, abdominal distension, abdominal pain/cramps, and flatulence) and systemic events (fever, chills, diaphoresis) will be considered as AESIs.

11.3.2 Pregnancy

All women of childbearing potential who participate in the study will be counseled on the need to use acceptable, effective contraceptive methods for the duration of the study (from screening until 14 days after the end of study period 2) and on the importance of avoiding pregnancy during study participation. Women should be instructed to contact the study team immediately if pregnancy occurs or is suspected.

A urine pregnancy test will be conducted at Screening and prior to administration of the IP on every woman of childbearing potential. A woman who is found to be pregnant at the Screening Visit will be excluded from the study and considered to be a screening failure.

A woman who becomes pregnant during the study will be immediately discontinued from further study participation. The investigator must report the pregnancy within 24 hours of learning of the pregnancy, to [redacted] using the Pregnancy Data Collection Form via the same contact information as for SAE reporting.

Early Termination Visit assessments are required as soon as possible after learning of the pregnancy. The investigator should request additional information following delivery, including status of the newborn. These findings must be reported on the Pregnancy Data Collection Form and forwarded. A pregnancy meets the SAE criterion only if it results in a spontaneous abortion

or a congenital anomaly. An induced therapeutic abortion to terminate a pregnancy because of complications or medical reasons must be reported as an SAE. The underlying medical diagnosis for this procedure should be reported as the SAE term.

11.3.3 Overdose

The maximal dose of SB-121 should not be exceeded during the study. Overdose that occurs during the study will be treated and documented as an AE/SAE if it fulfills the definitions/criteria for AE/SAE. If the overdose does not result in an AE, it should be reported in written form to the Sponsor. The information contained therein should include study site identification, reporter identification, subject number, dose of IP any action taken (e.g., supportive measures or therapy), and any comments.

12. DATA SAFETY MONITORING BOARD

A data safety monitoring board will not be used in this study. Sponsor will continuously monitor outcomes to ensure ongoing patient safety.

13. STATISTICS

13.1. Statistical Analysis

This section presents a summary of the planned statistical analyses. A statistical analysis plan (SAP) that describes the details of the analyses to be conducted will be written and finalized prior to database lock.

Summary statistics will be provided for the variables described below. For continuous variables, these statistics will typically include the number of subjects, mean, standard deviation (SD), median, minimum, and maximum. For categorical variables, these statistics will typically include the number and percentage of subjects in each category.

For subjects who withdraw from the study or are replaced for any reason, all available data will be included in applicable analyses.

13.1.1 Analysis Populations

The following population is planned for this study:

- The Safety Population will consist of all subjects who receive at least one dose of IP. This population will be used for all safety analyses.

13.1.2 Study Subjects and Demographics

13.1.2.1 Disposition and Withdrawals

The numbers of subjects randomized and completing or withdrawing, along with reasons for withdrawal, will be tabulated overall and by treatment group.

13.1.2.2 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. Corrective actions for deviations are to be developed by the site and implemented promptly.

It is the responsibility of the site investigator to use continuous vigilance to identify protocol deviations. Major protocol deviations are those that impact subject safety and/or can affect the integrity of the trial. These could include, but are not be limited to:

- Conduct of human subjects research without appropriate IRB approval
- Failure to obtain consent and/or authorization from subjects, including obtaining consent from someone who cannot legally consent for the subject
- Enrolling a subject who does not meet eligibility criteria
- Dosing errors
- Repeated occurrence for a subject of study visits outside of the protocol-specified timeframes or missed study visits

Protocol deviations that are to be promptly (within time-frames mandated by the IRB) reported to the IRB are:

- Conduct of human subjects research without appropriate IRB approval
- Dosing errors that result in overdose and are associated with an AE
- Enrolling a subject who does not meet eligibility criteria
- Failure to obtain consent and/or authorization from subjects, including obtaining consent from someone who cannot legally consent for the subject
- Incidents that may compromise information security, subject privacy, and/or confidentiality (e.g., subject data breach). These incidents should also be reported to the applicable Information Policy Office and/or HIPAA Privacy Office.

Protocol deviations will be provided in a listing. Major protocol deviations will be tabulated by treatment group.

13.1.2.3 Demographics and Other Baseline Characteristics

Demographic and baseline characteristics (including age, sex, race, ethnicity, weight, height, and body mass index) will be summarized using descriptive statistics.

Prior and concomitant medications will be summarized by treatment group, by the number and percentage of subjects taking each medication, classified using World Health Organization Drug Dictionary (WHO-DD) Anatomical Therapeutic Chemical (ATC) classes and preferred terms.

13.1.3 Exposure and Compliance

Medication compliance will be monitored via Patient Diary as well as during follow-up calls and at the end of each study treatment phase, as noted in Schedule of Events (Section 2.2). Subjects will be asked to return all their medication vial(s), including all empty vials, at the end of each treatment period. Study drug compliance will be assessed by the study staff by recording bottle counts of study treatments.

13.1.4 Safety and Tolerability Analyses

Safety analyses will be conducted using data from the safety population. Safety variables include TEAEs; discontinuations because of AEs, clinical laboratory values that are abnormal and considered clinically significant, vital signs, and physical examination results. No formal inferential analyses will be conducted for safety variables.

13.1.4.1 Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 18.0 or higher.

Treatment-emergent AEs are defined as:

- AEs with onset at the time of or following the start of treatment with IP, or

- AEs starting prior to the start of treatment but increasing in severity or relationship at the time of, or following, the start of treatment with IP.

The number and percentage of subjects with AEs will be displayed for each treatment group by system organ class (SOC) and preferred term (PT). Summaries of AEs by severity and relationship to IP will also be provided. Serious adverse events and AEs resulting in discontinuation of IP will be summarized separately in a similar manner. Adverse events of special interest will also be summarized by SOC and PT.

Subject listings of AEs, SAEs, and AEs causing discontinuation of IP will be produced.

13.1.4.2 Clinical Laboratory Evaluations

Descriptive summaries (mean, SD, median, minimum, and maximum) of actual (absolute) values and changes from baseline values will be presented for clinical laboratory values for each treatment group at each timepoint.

Laboratory values that are outside the normal range will also be flagged in the data listings and presented with the corresponding normal ranges. Any out of range values that are identified by the investigator as being clinically significant will also be shown in a data listing.

Incidence of presence of Sephadex® microspheres in the stool and incidence of symptomatic bacteremia with positive *L. reuteri* identification will be summarized and listed.

13.1.4.3 Vital Signs

Descriptive summaries (mean, SD, median, minimum, and maximum) of actual values and changes from baseline will be calculated for SBP, DBP, pulse rate, and oral temperature.

13.1.5 Stool Sample Analyses

Qualitative assessments of levels of Sephadex® microspheres in stool samples will be conducted. Results will be summarized using descriptive statistics.

13.1.6 Secondary Endpoints

Changes from baseline in the AD measures will be summarized using descriptive statistics. The measured value for each subject will be the change from baseline in the CGI-S, ABC, WJ3 Spatial Relations and Auditory Attention subtests, KiTap, RBANS, and eye tracking. The CGI-I in itself assesses change from a subject's baseline. Details of the analyses will be described in a separate statistical analyses plan focused on the Autism Pathophysiology Measures.

Changes from baseline in biomarkers of inflammation (Section 10.2.2.1) will be summarized using descriptive statistics.

13.2. Sample Size Determination

The sample size was not determined based on statistical assumptions. Evaluation of up to 8 subjects per treatment group (16 subjects total) was considered sufficient to allow evaluation of the study's objectives.

14. STUDY CONDUCT

14.1. Sponsor and Investigator Responsibilities

14.1.1 Sponsor Responsibilities

The sponsor is obligated to conduct the study in accordance with strict ethical principles. The sponsor reserves the right to withdraw a subject from the study, to terminate participation of a study site at any time and/or to discontinue the study.

The sponsor is responsible for sending IND safety reports to the FDA in accordance with 21 CFR 312.32.

14.1.2 Investigator Responsibilities

The trial will be conducted in accordance with ICH GCP and applicable USA Code of Federal Regulations (CFR).

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those participants who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol (Section 16.1).

Investigator(s) should ensure that all persons who are delegated study-related responsibilities are adequately qualified and informed about the protocol, the IP, and their specific duties within the context of the study. Investigator(s) are responsible for providing Scioto Biosciences Inc. with documentation of the qualifications, GCP training, and research experience for themselves and their staff.

To ensure compliance with the guidelines, the study may be audited by an auditor. The investigator agrees, by written consent to this protocol, to cooperate fully with any audits.

14.1.3 Management of Clinical Study Procedures During COVID-19 Pandemic

During this global public health crisis, pragmatic and harmonized actions are required to ensure the necessary flexibility and procedural simplifications that are needed to maintain the integrity of the clinical studies and to ensure the rights, safety, and wellbeing of study participants and the safety of clinical study staff. The safety of the trial participants is of primary importance, and risks of involvement in the trial, in particular with added challenges due to COVID-19, will be weighed against anticipated benefit for the trial participants and society.

The measures detailed in the Guidance for Clinical Research Studies During COVID-19 Pandemic and its iterations (provided by Cincinnati Children's Hospital) will be implemented on until the pandemic is considered resolved by governmental and public health organizations, as applicable.

14.1.4 Confidentiality and Privacy

Subject confidentiality and privacy is strictly held in trust by the participating investigator(s), their staff, and the sponsor and their representatives/designees. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to study subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The sponsor/designees, the study monitor(s), other authorized representatives of the sponsor, representatives of the IRB, regulatory authorities, may inspect all documents and records required to be maintained by the investigator, including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, institutional policies, or sponsor requirements.

Personal study subject data collected and processed for the purposes of this study will be managed by the Investigator and the investigational site staff with adequate precautions to ensure the confidentiality of these data, and in accordance with applicable national and/or local laws and regulations on personal data protection.

Study monitors, auditors, and other authorized agents of the Sponsor; the IRB(s)/EC(s) approving this research; and any applicable regulatory authorities will be granted direct access to the study subjects' original medical records for verification of clinical trial procedures and data without violating the subjects' confidentiality, to the extent permitted by the law and regulations. In any presentation of the results of this study at meetings or in publications, the subjects' identity will remain confidential.

14.2. Site Initiation

Study personnel may not screen or enroll subjects into the study until after receiving notification from the sponsor or its designee that the study can be initiated at the study site. The study site will not be authorized for study initiation until:

1. The study site has received the appropriate IRB approval for the protocol and the appropriate ICF.
2. All regulatory documents have been submitted to and approved by the sponsor or its designee.
3. The study site has a Clinical Trial Agreement in place.
4. Study site personnel, including the investigator, have participated in a study initiation meeting.

14.3. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demographics, and screen failure reasons (e.g. eligibility criteria).

Subjects who fail inclusion and/or exclusion criteria may be rescreened once for the study at the discretion of the investigator and with the approval of the medical monitor. This can occur 30 days or more after the original Screening Visit. If a subject is eligible to enter the study after having previously failed screening, the subject will be assigned a new subject identification number.

14.4. Study Documents

All documentation and material provided by the sponsor/designee for this study are to be retained in a secure location and treated as confidential material. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, institutional policies, or sponsor requirements.

14.4.1 Informed Consent

Consent forms describing in detail the study intervention, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to any study procedure.

Consent forms containing all required regulatory elements will be IRB-approved and the subject will be asked to read and review the document. The investigator/designee will explain the research study to the subject and answer any questions that may arise. An oral explanation will be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Subjects will have the opportunity to carefully review the written consent form and ask questions prior to signing. The subjects should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. Signed ICFs will remain a part of the investigator files; a signed copy of the ICF and HIPAA Authorization Form will be given to the subjects for their records. The informed consent process will be conducted and documented in the source document. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

For subjects under the age of 18 years, the subject's legally authorized representative (e.g. parent) will sign the consent form after the subject has provided assent.

Some of the AD assessment instruments require responses regarding the perceptions of the parents/caregivers. Hence, signed consents will be required from them.

14.4.2 Investigator's Regulatory Documents

The regulatory documents must be received from the investigator and reviewed and approved by the sponsor/ designee before the study site can initiate the study and before Scioto Biosciences Inc. will authorize shipment of IP to the study site. Copies of the investigator's regulatory documents must be retained at the study site in a secure location. Additional documents, including a copy of the protocol and applicable amendment(s), the SB-121 IB, eCRF completion guidelines, copies of regulatory references, copies of IRB correspondence, and IP accountability records should also be retained as part of the investigator's regulatory documents. It is the investigator's responsibility to ensure that copies of all required regulatory documents are organized, current, and available for inspection.

14.4.3 Premature Study Termination

The study may be temporarily suspended or terminated prematurely if there is sufficient reasonable cause at any time by the sponsor, the IRB, regulatory authorities, or the investigator.

Conditions may arise during the study that could prompt the sponsor to halt the study or to terminate study conduct at a study site. Conditions that may prompt such considerations include, but are not limited to, the following events:

- The discovery of unexpected, serious, or unacceptable risk to the subjects enrolled in the study
- A decision on the part of sponsor to suspend, discontinue, or shorten the study
- Failure of the Investigator to enroll eligible subjects into the study
- Failure of Investigator to comply with International Conference of Harmonisation Tripartite Guideline: Guideline For Good Clinical Practice E6 (R1) (ICH-GCP) guidelines or applicable regulations

14.4.4 Sample Retention

Samples obtained during the study (including safety laboratory samples and stool samples) may be used for purposes related to this research. The samples will be stored until the sponsor has determined that specimens are no longer needed, and the decision has been made that none of the samples needs to be reanalyzed, but no longer than 5 years. In addition, identifiable samples can be destroyed at any time at the request of the subject.

With the subject's consent and as approved by the IRB, samples will be stored at the Scioto Biosciences Inc for a maximum period of 5 years.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research.

When the study is completed, access to study data and/or samples will be provided through Scioto Biosciences Inc.

14.5. Use of Information and Publication

All information concerning SB-121, Scioto Biosciences Inc.'s operations, patent applications, formula, manufacturing processes, basic scientific data, and formulation information supplied by the sponsor/ designee to the investigator, and not previously published, is considered confidential and remains the sole property of Scioto Biosciences Inc.

The information developed in this study will be used by Scioto Biosciences Inc. in connection with the continued development of SB-121 and thus may be disclosed as required to other clinical investigators or government regulatory agencies.

The information generated by this study is the property of Scioto Biosciences Inc. Publication or other public presentation of SB-121 data resulting from this study requires prior review and written approval of Scioto Biosciences Inc. Abstracts, manuscripts, and presentation materials should be provided to Scioto Biosciences Inc. for review at least 30 days prior to the relevant submission deadline.

It is agreed that the results of the study will not be submitted for presentation, abstract, poster exhibition, or publication by the investigator without the permission of Scioto Biosciences Inc. If applicable, this study will be registered at www.ClinicalTrials.gov, and results information from this study will be submitted to www.ClinicalTrials.gov.

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APPENDICES

The following appendices are provided:

A. Regulations and Good Clinical Practice Guidelines

A. Regulations and Good Clinical Practice Guidelines

1. Regulations

Refer to the following United States Code of Federal Regulations (CFR):

- FDA Regulations 21 CFR, Parts 50.20 – 50.27
  Subpart B – Informed Consent of Human Subjects
- FDA Regulations 21 CFR, Parts 56.107 – 56.115
  Part 56 – Institutional Review Boards
  Subpart B – Organization and Personnel
  Subpart C – IRB Functions and Operations
  Subpart D – Records and Reports
- FDA Regulations 21 CFR, Parts 312.50 – 312.70
  Subpart D – Responsibilities of Sponsors and Investigators

2. Good Clinical Practice Guidelines

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use GCP guidelines can be found at the following URLs:

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf

16. ATTACHMENTS

16.1. Investigator's Agreement

PROTOCOL NUMBER: SBI-SB121-20-01

PROTOCOL TITLE: Randomized, Double-blind, Placebo-controlled, 28-Day Daily-dose Crossover Study of the Safety and Tolerability of SB-121 (*Lactobacillus reuteri* with Sephadex® and Maltose) in Subjects, ages 15 to 45 years, diagnosed with Autistic Disorder.

FINAL PROTOCOL: Version 2.0, 03 June 2021

I have read this protocol and agree to conduct this clinical study as outlined herein. I will ensure that all sub investigators and other study staff members have read and understand all aspects of this protocol. I agree to cooperate fully with Scioto Biosciences Inc. and their representatives during the study. I will adhere to all FDA, ICH, and other applicable regulations and guidelines regarding clinical studies on an IP during and after study completion.

Principal Investigator:

Printed Name: CRAIG GRICKSON MD

Signature: [redacted]

Date: Dec 2, 2021

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