SOARS-B SAP's and Protocols.

This supplement contains the following items:

Clarifications to Final SAP reflecting Primary Paper in the New England Journal of Medicine 05-15-2021:

1. Throughout the protocol and SAP, we refer to the ABC-SW, which is a modified subscale of the ABC-Lethargy, Social Withdrawal Subscale that eliminated questions 3, 32, and 53, which refer to lack of movement, in order to increase specificity for social reciprocity behaviors. In writing the primary paper, we wanted to eliminate any possible confusion with the original published ABC-Lethargy, Social Withdrawal subscale and referred to the modified scale as the ABC-modified Social Withdrawal subscale or ABC-mSW. We have changed our clinicaltrials.gov primary results to use the new nomenclature of ABC-mSW.
2. Reviewers pointed out that it was not entirely clear what our three secondary outcomes were. Therefore, we want to be clear that they are:
    a. the Sociability Factor (abbreviated SF) which combines the ABC-mSW and the PDD-BI screening version in order to assess both areas of social reciprocity challenges and social skills (there was an error in the original version of the final SAP in which the sociability factor was inadvertently omitted from section 4.1.2, which has been corrected)
    b.the Social Responsiveness Scale, 2nd Edition - Social Motivation Subscale (abbreviated SRS-2-SM);
    c. the Stanford Binet, 5th edition Abbreviated Intelligence Quotient (abbreviated ABIQ).
3. Reviewers requested that we clarify what our sensitivity analyses were. They follow
    a. Analysis using the original ABC-Lethargy, Social Withdrawal Subscale
    b. Analysis integrating baseline (or screen if no baseline was available) plasma oxytocin level, baseline oxytocin level x treatment, and baseline oxytocin x treatment x time into the MMRM model
    c. Analysis separately of the ABC-mSW outcome in the minimally verbal and fluently verbal subgroups.
    d. Analysis of the ABC-mSW outcome in the per proctocol population

4. In the primary paper we also clarify that the full analysis set is essentially a modified intent treat sample, and that age group was dropped from the final analysis model because it was not significant.

Initial Statistical Analysis Plan for SOARS-B

Version 1.0

March 21, 2013

Study of Oxytocin in ASD to Improve Reciprocal Social Behaviors (SOARS-B)

STATISTICAL ANALYSIS PLAN

A Phase II, Multicenter, Randomized, Double-blind, Flexible Dose, Placebo-controlled Study of the Efficacy, Tolerability, and Safety of Intranasal Oxytocin in Child Adolescent Participants with Autism Spectrum Disorders

Sponsor: National Institute of Child Health and Human Development

Version: Draft 1.0

Date: 03-21-2013

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Amendment The material information contained herein is subject to amendment at any time prior to the breaking of the blind.	

Table of Contents

LIST OF ABBREVIATIONS

Abbreviation or term	Explanation
ABC	Aberrant Behavior Checklist
ABC-SW	Aberrant Behavior Checklist – Social Withdrawal subscale
ADI-R	Autism Diagnostic Interview - Revised
ADOS-2	Autism Diagnostic Observation Schedule (version 2)
AE	Adverse Event
ALT	Alanine Aminotransferase
ASD	Autism Spectrum Disorder
AST	Aspartate Aminotransferase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CASI	Childhood Anxiety Symptom Inventory
CGI-I	Clinician Global Impression - Improvement
CGI-S	Clinician Global Impression - Severity
CI	Confidence Interval
cl	Chloride
CO2	Carbon Dioxide
CSQ	Caregiver Strain Questionnaire
CSS	Calibrated SeverityScore calculated from the ADOS-2
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
GUID	Global Unique Identifier
FAP	Full Analysis Population
IU	International Unit
K	Potassium
LS	Least Squares
OT	(intranasal) Oxytocin
M0	Month 0 Visit
M6	Month 6 Visit
M12	Month 12 Visit
M18	Month 18 Visit
MMRM	Mixed Model with Repeated Measures
mRNA	Messenger Ribonucleic Acid
Na	Sodium
NIH	National Institutes of Health
PDDBI-SV	Pervasive Developmental Disorders Behavior Inventory – Screening Version
PDDBI-Full	Pervasive Developmental Disorders Behavior Inventory – Full Version
PP	Per-protocol Population
OXTR	Human Oxytocin Receptor Gene
Q1	First Quartile
Q3	Third Quartile
QTcB	QT interval corrected for heart rate using Bazett’s formula

Abbreviation or term	Explanation
QTcF	QT interval corrected for heart rate using Fridericia's formula
RMET	Reading the Mind in the Eyes Test
SAP	Statistical Analysis Plan
SD	Standard Deviation
SF	Sociability Factor, a combined measure derived from the ABC-SW & PDDBI-SV
SLAES	Systematic Longitudinal Adverse Events Scale
SOARS-B	Study of Oxytocin in ASD to Improve Reciprocal Social Behaviors
SRS-2	Social Responsiveness Scale (version 2)
TEAE	Treatment-Emergent Adverse Event

INTRODUCTION

Purpose

The purpose of this SAP is to outline the planned analyses to support the completion of the primary manuscripts to be written for the SOARS-B Study. Exploratory analyses not identified or defined in this SAP may be performed to support the clinical trial. Any post-hoc or unplanned analyses performed and not identified in this SAP will be documented in the respective manuscript.

Study Objectives

Primary Objective

To determine the efficacy of intranasal oxytocin (OT) for the treatment of deficits of reciprocal social behaviors in child or adolescent participants with autism spectrum disorders (ASD) as measured by changes in the Aberrant Behavior Checklist- Social Withdrawal Scale (ABC-SW) and the sociability factor (SF), a combined measure formed from 13 items of the ABC-SW and the Pervasive Developmental Disorders Behavior Inventory - Screening Version (PDDBI-SV).

Secondary Objectives

To determine the safety and tolerability of OT in participants with ASD as measured by incidence of treatment emergent adverse events (TEAEs) and abnormal laboratory values, tolerability of OT dose, and time to early termination.

To determine the efficacy of OT as measured by changes in ABC-SW and SF scores in low-functioning and high-functioning strata separately.

To determine the efficacy of OT as measured by changes in Social Responsiveness Scale Version 2 (SRS-2) Social Motivation scores, Vineland 2 Adaptive Behavior Composite score and Nonverbal IQ or Mullen Early Learning Composite scores.

To determine the efficacy of OT (as measured by changes in ABC-SW scores, SF scores, SRS-2 Social Motivation scores, Vineland 2 Adaptive Behavior Composite, and Nonverbal IQ or Mullen Early Learning Composite scores) as a function of age.

To determine the efficacy of OT (as measured by changes in ABC-SW scores, SF, , SRS Social Motivation scores, Vineland 2 Adaptive Behavior Composite , and Nonverbal IQ or Mullen Early Learning Composite scores) as a function of baseline percent *OXTR* gene methylation.

Exploratory Objectives

To describe the influence of OT on the oxytocin system as measured by changes in *OXTR* gene methylation, mRNA expression of oxytocin relevant genes, levels of oxytocin in plasma and saliva, and whole blood serotonin levels over time.

To determine the efficacy of OT on other clinical assessments including the subscales of the ABC, SRS, Vineland, and PDDBI; the ADOS Calibrated Severity Score (CSS); the Clinical Global Impressions Severity and Improvement scores; self-reported social functioning and the Reading the Mind in the Eyes Task (RMET).

To examine the potential interaction between OT and various types of concurrent behavioral and/or

pharmacological treatments and social opportunities.

Study Design

This is a multicenter, randomized, double-blind, stratified, flexible dose, placebo-controlled study designed to evaluate OT in participants with ASD. OT will be given as an intranasal preparation with two different concentrations of 8 IU per insufflation or 24 IU per insufflation. Oxytocin will be administered daily, in single doses up to 40IU or divided doses in the morning and early afternoon as described in the protocol. Total daily dose is flexible, between 8 IU and 80 IU per day.

Participants who successfully complete screening and baseline assessments and are deemed eligible will be randomized to OT or placebo treatment in a 1:1 ratio, stratified by age group (3-6, 7-11, 12-17) and functioning (low, high), for a total of 6 strata. Randomization will not be stratified by center, as it was felt that additional stratification by center would lead to too many strata, a loss of degrees of freedom, and the potential for a grossly unequal randomization to treatments.

A schematic representation of the study design is shown in Figure 1. After screening and randomization at month 0 (M0), participants enter a double-blind treatment period of 6 months (M6), followed by open-label treatment with OT for 6 months, termination of OT at the end of month 12 (M12), and an 18 month (M18) follow-up visit 6 months after the month 12 visit.

Efficacy, safety, and tolerability are measured monthly throughout the treatment period. Participants who terminate early will be asked to provide safety data through the remainder of the study. The schedule of assessments is provided in Table 1.

Figure 1: Schematic Representation of the Study Design

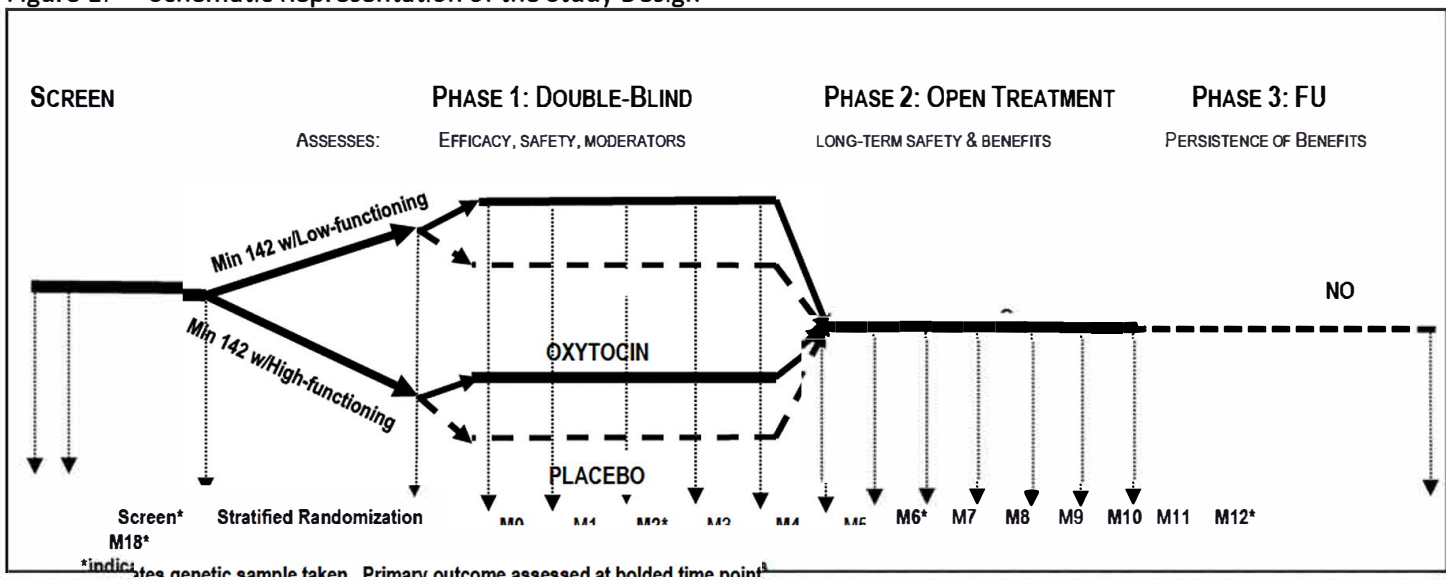

Table 1: Schedule of Visits and Assessments

SCHEDULE OF EVENTS	Screen	Double Blind Treatment							Open Treatment							Post Tx f/u
Procedure	1 or 2	M 0.5	M1	M2	M3	M4	M5	M6/ T	M 6.5	M7	M8	M9	M10	M11	M12/ T	M 18
ADOS-2	X							X							X	
ADI-R	X															
Stanford Binet/Mullen	X							X							X	X
DSM-V Checklist	X															
Inclusion/Exclusion	X															
Demographics																
Family Med History (NIH Form)	X															
Randomization Form																
GUID Acquisition Form																
GUID Record Form																
Con Meds	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Vital Signs	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Med/Psych History (SLAES)	X															
Adverse Effects (SLAES/Suicidality)			X	X	X	X	X	X		X	X	X	X	X	X	X
Physical Exam (NIH Form)	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Medical History (NIH Form)	X															
CGI S and I[0]			X	X	X	X	X	X		X	X	X	X	X	X	X
Social Skills Therapies			X	X	X	X	X	X		X	X	X	X	X	X	X
Psychosocial Therapies			X	X	X	X	X	X		X	X	X	X	X	X	X
Laboratory																
ECG	X							X							X	
Female Reproductive Status	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Urine/Serum Pregnancy[1]	X															
Safety labs[2]	X							X							X	
mRNA sample	X							X							X	
Methylation sample	X			X				X			X				X	X
Plasma/salivary oxytocin	X			X				X			X				X	X
Whole blood serotonin	X			X				X								
Genetic repository sample-subj[3]				X												
Genetic repository – parents[4]				X												
Parent Questionnaires																
Questionnaire Guidance Form			X	X	X	X	X	X		X	X	X	X	X	X	X

Social Opportunity Questionnaire			X	X	X	X	X	X		X	X	X	X	X	X	X
ABC				X		X		X			X		X		X	X
PDDBI-SV				X		X					X		X			
PDDBI-Full								X							X	X
CASI								X							X	X
Caregiver Strain								X							X	X
Vineland-II (Survey Form)					X			X				X			X	X
SRS-2					X			X				X			X	X
Subject Completed Questionnaires																
Reading Mind in Eyes Test[5]				X				X			X				X	X
Self-Rating of social function								X							X	X
Medication																
DVD training (med administration)*																
Med Administration Form*																
Dosing Guide[7]		X	X	X	X	X	X	X	X	X	X	X	X	X		
Risks Handout								X								
Medication Compliance[6]			X	X	X	X	X	X		X	X	X	X	X	X	
Oxytocin Dosing Log (staff)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Medication Diary Dispensed			X	X	X	X	X	X		X	X	X	X	X		

0-CGI-S only at baseline, CGI-S and I at all other visits 1-Required at screening but can be done at any time during the course of the study at physician discretion 2-Safety labs to include: glucose (random), CO2, Cl, K, Na, Creatinine, BUN, AST, ALT, urine specific gravity, urine pregnancy in pubertal girls. 3- Preferred time to collect, but can be done at any time. 4- One sample is requested from each biological parent, can be obtained at any point during the study 5- Assessment will be obtained only in participants who have fluent phrase speech & can define basic feelings and friendship. 6- . Completed each time the participant returns study medication
7- Dosing guide should be given at each visit (and also phone calls where the dosing has changed). Staff will circle the appropriate column for dosing that the patient should follow

*Must be given at baseline, but also can be given at any other time during the course of the study at study staff discretion

GENERAL STATISTICAL CONSIDERATIONS

Analysis Populations

In all populations, participants will be analysed in the treatment group into which they were originally randomized. Measurements from participants excluded from the pre-defined analysis populations or extra measurements (such as unscheduled or repeat assessments) will not be included in summary tables or figures unless specified otherwise.

Safety Population

The safety population will include all participants who receive at least 1 dose study drug. The safety population will be used for all tabulations of demographics, baseline characteristics, and safety variables, unless otherwise specified in the SAP text or the table shell.

Full Analysis Set

The full analysis set (FAS) will consist of all evaluable participants, defined as all randomized participants who receive at least 1 dose of study drug and have at least 1 primary efficacy assessment on the two co-primary primary efficacy variables after administration of study drug. The FAS will be the primary efficacy population and used in efficacy analyses.

Per-Protocol Population

The per-protocol (PP) population is defined as a subset of FAS with no major protocol deviations. Protocol violators will be determined before database lock and the unblinding of treatment groups. The per-protocol population will be used only for supportive analyses of the primary efficacy endpoint.

General Summarization of Study Data

Unless otherwise specified, continuous variables will be summarized with descriptive statistics including n, mean, standard deviation (SD), median, first quartile (Q1) and third quartile (Q3) and minimum and maximum values. Categorical variables will be reported as number and percentage of participants in each category. Least squares (LS) means, standard errors, estimates of contrasts with 95% confidence intervals (CI), and p-values will be reported as appropriate.

All other statistical tests and confidence intervals will be 2-sided, unadjusted for multiplicity, and tested at $\alpha = 0.05$, except those explicitly stated to be otherwise. In cases where we have co-primary outcomes, the tests will be adjusted for the number of outcomes using a Bonferroni correction.

All analyses and summaries will be produced using SAS® version 9.3 (or higher).

Sequence of Planned Analyses

All analyses described in this SAP will be performed after data collection has concluded, the database locked, and the treatment blind has been broken. There will be no interim analyses of study data except for the summaries provided to the Data Safety Monitoring Board (DSMB).

An independent DSMB will meet three times yearly to review safety information for the study. The DSMB statistician will prepare summaries of data as described in the Safety Analyses section, separating them into treatment group A and treatment group B and will distribute them only to the members of the DSMB. The database manager will provide the DSMB with the treatments referred to by A and B if requested, but will not see the final reports. No study team member other than the DSMB statistician will see the summaries provided to the DSMB.

PARTICIPANT DISPOSITION ANALYSES

General Summaries

Disposition will be summarized for all enrolled participants. Summaries will include counts of:

Participants who were enrolled
Participants who were enrolled but not randomized
Participants who were randomized
Participants who were randomized but did not take study drug
Participants who were in the safety population
Participants who were in the FAS
Participants who were in the PP population
Participants who completed the study
Participants who terminated the study early
Participants with the each potential primary reason (i.e. adverse effect, inconvenience, administrative, lack of efficacy, noncompliance, lost to contact) for early termination

All summaries will be presented by treatment group and over all participants. A listing of disposition will be provided for all participants.

Protocol Deviations

Protocol deviations will be recorded by each site as they occur and/or captured during monitoring visits. Before database lock and treatment unblinding of the study, study investigators will review all protocol deviations and determine whether or not they are assessed to be major protocol deviations.

Major protocol deviations are defined as those that potentially impact participant safety and/or data integrity, and may include but are not limited to the following:

Dosing/randomization errors
Procedural errors
Participant did not meet all inclusion and exclusion criteria
Participant treatment assignment was unblinded during the study

All major protocol violations will be summarized and listed for the study.

Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized by treatment group and over all participants

for the safety population. Demographic characteristics will include sex, age, race, and ethnicity. Baseline efficacy variables (see Efficacy Analyses section for list), vital signs, and female reproductive status will also be summarized.

Age (months) will be calculated as the number of months between date of birth and date of informed consent.

Medical and psychiatric history will be summarized by treatment group and over all participants using the number of observations and percentage of participants reporting each category for the safety population.

Dose and Compliance

All dosing, exposure, and compliance information will be summarized by treatment group and over all participants for the safety population.

Summaries of dose will include the percent of participants not able to tolerate the target dose, mean six month and 12 month doses, mean maximal dose, and the percent of participants whose dose was a) not increased up to the target dose; b) was reduced to below the target dose; or c) was reduced but still at or above the target dose. A summary will also be provided of the percent participants whose six month and 12 month doses were given once a day versus twice a day.

Duration of exposure for study drug (days) will be determined by calculating the difference between the date of last dose known to be taken and date of the first day medication was taken inclusive (last treatment date – first treatment date) + 1.

Treatment compliance will be calculated using the following formula and categorized as <80%, 80% - 120%, or >120%:

$$\%\ \text{Compliance for study medication} = \left(\frac{\text{Number of days medication taken}}{\text{Number of days of exposure}} \right) \times 100$$

Concomitant Medications

Concomitant medications will be categorized into the following groups: **Neurobehavioral medications:** alpha adrenergic agonists, anti-anxiety medications, anti-cholinergics, anticonvulsants/mood stabilizers, antipsychotics, stimulants, and sleep aids and **Other medications:** acid reducers, anti-infection medications, cough/allergy/asthma medications, heart medications, inhalation steroids, nutritional supplements, systemic steroids, and other.

Medications stopped prior to the first date of study drug dosing will be considered prior medications. We do not plan to summarize these medications. Medications taken after the first date of study drug dosing will be considered concomitant medications.

Concomitant medications for each study treatment group will be summarized according to their membership in the groups noted above by . Upon request, a summary of each specific medication name will be provided.

In summaries, if a participant has taken a concomitant medication more than once, the participant will be counted only once in the total.

EFFICACY ANALYSES

The FAS will be used as the analysis population for all efficacy analyses unless otherwise specified.

Efficacy Variables

Detailed description of all efficacy variables is given in the protocol. All efficacy variables will be analyzed as change from baseline, except where noted.

Primary Efficacy Variables

The following variables will be analyzed as co-primary endpoints:

ABC-SW Total
Sociability Factor (SF)
The SF will be calculated by discarding the three lethargy items (3, 32, 53) from the ABC-SW, summing the 13 remaining items of the ABC-SW subscale, and adding the sum to the PDDBI-SV raw social deficits score.

Key Secondary Efficacy Variables

The following variables with be analyzed as jointly key secondary endpoints:

SRS-2 social motivation subscale score
Vineland 2 adaptive behavior composite score
Nonverbal IQ or Mullen Early Learning Composite scores

Exploratory Efficacy Variables

- Clinical Global Impression - Severity (CGI-S)
- Clinical Global Impression - Improvement (CGI-I)
- CGI-I will be analyzed as an endpoint mean instead of change from baseline.
- ABC subscales for irritability, stereotypy, hyperactivity, and inappropriate speech
- Calibrated Severity Score derived from the ADOS 2 (CSS)
- Scores from the ADOS will be used to derive a raw score for 14 specific questions according to the child's age, verbal abilities and the module used. A score of 8 will be converted to a score of 0. A score of 3 will be converted to a score of 2. These questions are listed in Table 2 below.
- The raw scores will then be used to derive a CSS which takes account of the child's age, module used and, for module 1, whether there are no words or single words using Table 3 below.
- The CSS for children 15-17 who are assessed with a module 1 will be derived using the 6-14 criteria.
- The CSS for 17 year olds assessed with module 2 or 3 will be derived using the 9-16 or 10-16 criteria respectively.
- PDDBI- Full Version subscales
- Caregiver strain questionnaire (CSQ) and its subscales
- Vineland 2 domains for communication, daily living skills, socialization, and maladaptive behaviors;

motor skills will not be analyzed since it is only valid in young children

- Participant rating of social function (only in verbally fluent individuals)
- Child and Adolescent Symptom Inventory disease categories
- Social Opportunity Questionnaire
- Reading the Mind in the Eyes test (only in individuals with fluent speech and understanding of basic emotions)
- OXTR gene methylation and mRNA expression
- Serum and salivary oxytocin levels
- Whole blood serotonin

Table 2: Questions for determining ADOS 2 Raw Severity Scores

Domain	Question #	Module 1, no words	Module 1, some words	Module 2, younger	Module 2, older	Module 3
Social	1	Unusual Eye contact	Unusual Eye contact	Unusual Eye contact	Unusual Eye contact	Unusual Eye contact
	2	Gaze & other behavior	Gaze & other behavior	Amt of social communication	Amt of social communication	Amt of social communicatio n
	3	Facial Expression	Facial Expression	Facial Expression	Facial Expression	Facial Expression
	4	Freq of Vocalizations	Freq of Vocalizations	Quality of Rapport	Quality of Rapport	Quality of Rapport
	5	Shared Enjoyment	Shared Enjoyment	Shared Enjoyment	Shared Enjoyment	Shared Enjoyment
	6	Qual of social overtures	Qual of social overtures	Qual of social overtures	Qual of social overtures	Qual of social overtures
	7	Response to joint atten	Pointing	Pointing	Pointing	Conversation
	8	Gestures	Gestures	Gestures	Gestures	Gestures
	9	Showing	Showing	Showing	Showing	Qual of social response
	10	Initiation of joint atten	Initiation of joint atten	Initiation of joint atten	Initiation of joint atten	Reporting of events
Restricted, Repetitive Behaviors	11	Intonation	Stereotyped language	Stereotyped language	Stereotyped language	Stereotyped language
	12	Unusual sensory interest	Unusual sensory interest	Unusual sensory interest	Unusual sensory interest	Unusual sensory interest
	13	Repetitive interests	Repetitive interests	Repetitive interests	Repetitive interests	Highly specific topics
	14	Hand Mannerisms	Hand Mannerisms	Hand Mannerisms	Hand Mannerisms	Hand Mannerisms

Table 3: Determining CSS from ADOS 2 Raw Severity Scores

ADOS class-ification	Calibrated severity score	Raw ADOS totals																	
		Module 1, No Words				Module 1, Single Words					Module 2, phrases						Module 3, fluent		
		2 years	3 years	4–5 years	6–14 years	2 years	3 years	4 years	5–6 years	7–14 years	2 years	3 years	4 years	5–6 years	7–8 years	9–16 years	2–5 years	6–9 years	10–16 years
NS	1	0–6	0–6	0–3	0–3	0–3	0–4	0–2	0–2	0–2	0–2	0–3	0–3	0–3	0–2	0–2	0–3	0–2	0–3
	2	7–8	7–8	4–6	4–6	4–5	5–6	3–4	3–4	3–5	3–5	4–5	4–5	4–5	3–5	3–5	4	3–4	4
	3	9–10	9–10	7–10	7–10	6–7	7	5–7	5–7	6–7	6	6	6	6–7	6–7	6–7	5–6	5–6	5–6
ASD	4	11–13	11–14	11–12	11–13	8–10	8–9	8–9	8–10	8–9	7–8	7–8	7	8	8	8	7	7	7
	5	14–15	15	13–15	14–15	11	10–11	10–11	11	10–11	9	9	8–9	–	–	–	8	8	8
AUT	6	16–19	16–20	16–19	16–19	12–13	12–14	12–15	12–16	12–18	10–11	10–12	10–13	9–14	9–14	9–14	9–11	9–10	9–10
	7	20–21	21–22	20–21	20–22	14–16	15–17	16–18	17–19	19–20	12	13–14	14–16	15–16	15–17	15–17	12	11–12	11–12
	8	22	23	22–23	23–24	17–19	18–19	19–20	20–21	21	13–14	15–16	17–18	17–20	18–21	18–20	13–15	13–14	13–14
	9	23–24	24	24–25	25	20–21	20–21	21–22	22–23	22–23	15–17	17–18	19–20	21–22	22–23	21–23	16–17	15–17	15–17
	10	25–28	25–28	26–28	26–28	22–28	22–28	23–28	24–28	24–28	18–28	19–28	21–28	23–28	24–28	24–28	18–28	18–28	18–28

General Modeling Considerations for Efficacy Analyses

Unless otherwise specified, efficacy analyses will fit a mixed longitudinal model with change from baseline to each post-baseline month for a response variable, low or high functioning group and age group as stratification variables, OT or placebo treatment group as the between-subjects factor, month as a within-subjects factor, treatment by month interaction, and baseline as a covariate. We will choose between two kinds of models: (1) a random coefficients model treating month continuously with random coefficients for the intercept, slope, and slope squared, and an unstructured covariance matrix among them, and (2) an mixed model with repeated measures (MMRM) treating month categorically and examining unstructured, Toeplitz, autoregressive, and compound symmetric covariance structures among months. We will choose among these candidate models using an information criterion (AIC) while remaining blind to the significance of the treatment effect to avoid bias in the choice of model. Distributional assumptions will be examined using residuals. These mixed models could fail to converge or encounter difficulties based on their use of asymptotics. If they do, we will attempt to simplify the models in order to eliminate the problems, and if necessary, move to analyses that assume compound symmetry, but use a Huynh-Feldt correction if compound symmetry fails.

Missing Data Considerations for Efficacy Analyses

The mixed models that will be used to evaluate efficacy variables are able to handle moderate amounts of missing data provided they are missing at random. We will examine the missing at random assumption by assessing baseline differences between dropouts and completers, as well as differences in response variables up to the point of premature withdrawal. If the missing at random assumption does not appear to be tenable, we will report the mixed models results but spend additional effort characterizing treatment effect at time of premature withdrawal.

Primary Efficacy Analysis

The co-primary efficacy response variables will be tested using a Bonferroni-corrected significance level of $\alpha = 0.025$. Longitudinal models as described in General Modeling Considerations will be fit with dependent variables of change from baseline to each post-baseline month up to month 6.

The following sample SAS code will be referenced for the random coefficients model.

```
PROC MIXED data = xxx ORDER=DATA;
CLASS treat functioning agegroup subject;
MODEL change= baseline agegroup treatment month treatment*month/ DDFM=KR;
RANDOM intercept month/ TYPE=UN SUBJECT=subject (treatment);
ESTIMATE “Treatment Diff at Month 6” treatment 1 -1 treatment*month 6 -6;
RUN;
```

The following sample SAS code will be referenced for MMRM model:

```
PROC MIXED data = xxx ORDER=DATA;
CLASS treat functioning agegroup month subject;
MODEL change= baseline agegroup treatment month treatment*month/ DDFM=KR;
REPEATED month/ TYPE=xx SUBJECT=subject (treatment);
LSMEANS treatment*month;
LSMEANS treatment*month / SLICE=month;
RUN;
```

The ESTIMATE statement in the random coefficients model and LSMEANS statement with the SLICE=month option in the MMRM will produce the test of treatment effect at month 6.

As a sensitivity analysis, the primary efficacy analysis will also be conducted in the per-protocol population.

Subgroup Analysis for Primary Efficacy Variables

As a secondary analysis for the primary efficacy variables, the primary efficacy variables will be analyzed within each of the low and high functioning stratum separately. This will be done using the same model as described in the primary efficacy analysis, but using contrasts to test the difference between the treatments on the month 6 change scores within each of the low and high-functioning strata.

Addition of Covariates to Primary Efficacy Analysis Model

As additional secondary analyses for the primary efficacy variables, the following groups of covariates will be added separately to the model in the primary efficacy analysis and tested for their significance:

Baseline age, treatment-by-age interaction, treatment-by-age-by-month interaction.
Baseline percent OXTR gene methylation, treatment-by-baseline-methylation-interaction, treatment-by-baseline-methylation-by-month interaction

In both cases, the primary test will be the significance of treatment-by-variable-by-month interaction, and if not significant, the treatment-by-variable interaction.

The predictor for age group listed in the description of the model fit in primary efficacy analysis will be

removed from the model adding baseline age and interactions as covariates.

Analyses of Key Secondary Efficacy Variables

The three key secondary efficacy variables will be tested using a Bonferroni-corrected significance level of $\alpha = 0.0167$ using the same model as in the primary efficacy analysis. Supplemental analyses will include separate analyses in low and high-functioning strata and inclusion of baseline age and baseline percent OXTR methylation covariates, as described in the primary efficacy analysis.

Analyses of Exploratory Efficacy Variables

Exploratory efficacy variables will be analyzed the same way as the primary and secondary efficacy variables except with no Bonferroni correction for type I error.

Analyses of Month 12 and Month 18 Follow-up Data

Following the 6-month double blind phase, all participants will receive 6 months of OT during an open label phase. Therefore, participants randomized to OT during the 6 month double-blind phase will receive 12 months of OT treatment, and participants randomized to placebo will received 6 months of OT treatment.

The longitudinal models used in previous analyses will be fit for the following time periods and participant groups. All analyses will be considered exploratory and will not include any correction for type I error:

Change from 6 months to 12 months in participants receiving OT during the double-blind phase.
Change from baseline to 12 months in participants receiving OT during the double-blind phase.
Change from 6 months to 12 months in participants receiving placebo during the double-blind phase.
Change from baseline to 12 months in participants receiving placebo during the double-blind phase.
Change from 6 months to 12 months compared to change from baseline to 6 months in participants receiving placebo during the double-blind phase.
Change from baseline to 6 months of OT treatment in either the double-blind or open treatment periods. This will combine baseline to 6 months data for participants receiving OT during the double-blind phase with 6 months to 12 months data (relabeled as baseline to 6 months) in participants receiving placebo in the double-blind phase.
Change from 12 months to 18 months in participants receiving OT during the double-blind phase.
Change from baseline to 18 months in participants receiving OT during the double-blind phase.
Change from 12 months to 18 months in participants receiving placebo during the double-blind phase.
Change from baseline to 18 months in participants receiving placebo during the double-blind phase.

SAFETY ANALYSES

Safety and tolerability will be evaluated based on the incidence of TEAEs, incidence of elicited reports of suicidal ideation or statements, self-injurious behaviors, and suicidality, incidence of AEs leading to termination, vital signs measurements, abnormal laboratory test results, and ECG findings. All safety data will be presented using the safety population.

Treatment Emergent Adverse Events (TEAEs) and Potential Suicidality

Adverse events will be monitored continuously from the time a participant signs the informed consent

document until the completion of the final follow-up visit. Similarly reports of suicidal ideation or statements, self-injurious behaviors, and suicidality elicited from participants who are able to speak and understand the concepts and all caregivers will be elicited at every contact and followed continuously

Medical and behavioral conditions that are present at screening and/or baseline will only be considered treatment emergent adverse events if their severity increases significantly after the participant has taken at least one dose of study treatment. Intermittent conditions such as seasonal allergies will only be considered TEAEs if the severity or frequency is significantly greater than in the previous two years. Only TEAEs will be considered in the adverse event safety analyses.

TEAEs will be coded using the Systematic Longitudinal Adverse Event Scale (SLAES). Additional details about this scale are given in the protocol.

Severity of adverse events will be categorized as mild, moderate, severe, life-threatening, or resulting in death. Relationship to treatment will be categorized as unknown or not applicable relationship, unrelated, possibly related, probably related, or certainly related.

Overall summary of TEAEs, such as all TEAEs, severe TEAEs, TEAEs by severity, TEAEs by relationship to treatment, TEAEs leading to study termination, serious TEAEs, life-threatening TEAEs and TEAEs resulting in death, will be summarized by category and preferred term for each treatment group and over all participants. All TEAEs with moderate or greater severity will be listed for each participant. Severe TEAEs, TEAEs leading to study termination, serious TEAEs, and TEAEs resulting in death will be listed separately.

All reports of suicidal ideation or statements, self-injurious behaviors, and suicidality will be summarized at each time point. In addition the proportion of these that represent a significant change in frequency or severity from the child's baseline level of functioning as determined by the caregiver will be summarized. In addition, the proportion of these events that the steering committee judges represent stereotypic behaviors will be summarized. For each participant with a report of potential suicidality, the sequence of their reports for each study visit will be provided.
No formal hypothesis-testing analysis of TEAEs or suicidality incidence rates will be performed.

Vital Signs

Vital signs (systolic and diastolic blood pressure, pulse, and body temperature) will be measured monthly at every study visit.

Blood pressure and pulse will be measured with the participant as calm as possible and sitting.

Vital sign measurements will be summarized at each time point for the absolute value and for changes from baseline by treatment group and over all participants. Alarm values will be determined prior to unblinding and the percent of participants with values in the alarm range will be summarized at each time point by treatment group.

All vital signs data will be available upon request.

Weight and Body Mass Index

Weight and Height are measured monthly at every study visit. Weight and BMI will be summarized at each

time point for the absolute value and for changes from baseline by treatment group and over all participants. All weight and BMI data will be available upon request.

Clinical Laboratory Evaluations

Blood and urine samples for safety laboratory assessments will be collected at screening, month 6, and month 12. To the extent possible, blood samples will be obtained at approximately the same time of day and under non-fasting conditions. All safety labs except prolactin will be processed by LabCorp.

Laboratory test results, including prolactin results, will be summarized by treatment and over all participants at each time point for the absolute value itself and for changes from baseline. We will also summarize the percent of participants in each treatment group with values within alarm ranges, which will be defined prior to breaking the blind. The mean value and SD of the labs within alarm ranges will be summarized at each time point. All measurements of the out of range value for each participant with an out of range value will be listed.

12-lead Electrocardiogram (ECG)

A 12-lead ECG will be performed at screening, month 6, and month 12. To the extent possible, ECGs will be performed after the participant has rested quietly for at least 5 minutes in the supine position. QT intervals will be automatically corrected by the ECG machine using Bazett's formula (QTcB) and Fridericia's formula (QTcF). It will be noted if the participant was agitated or in a nonsupine position.

Interval data will be summarized at each time point for the absolute value and for changes from baseline by treatment group and over all participants. Intervals that are outside of alarm ranges will be summarized. Overall assessment of ECG as normal or abnormal will also be summarized by treatment group using counts and percentage.

Safety Data Reported to the DSMB

- Safety data will be submitted to the independent DSMB three times per year. The following summaries will be provided for review at each meeting:
- Summary table of participant disposition including number screened, number randomized, number completed, percent early terminated, and reasons for early termination.
- Listing of all participants terminating the study early with duration of participation in the study and duration of treatment and reason for study discontinuation.
- Listing of all serious TEAEs.
- Summary table of the number and percent of participants with TEAEs by severity.
- Summary table of the number and percent of participants with TEAEs by relationship to study drug.
- Summary table of the number and percent of participants with TEAEs leading to study early termination.
- Listing of all TEAEs leading to study early termination.
- Summary table of the number and percent of participants with TEAEs by preferred term.
- Summary table of the number and percent of participants with TEAEs by category.
- Listing of all severe TEAEs.
- Summary table of the number and percent of participants with of suicidal ideation or vocalizations, self-injurious behaviors and suicide attempts by treatment group.

- Listing of all suicidal ideation or vocalizations, self-injurious behaviors and suicide attempts by treatment group.
- Summary of the proportion of suicidal ideation or vocalizations, self-injurious behaviors and suicide attempts by treatment group that are judged to be more severe than the participant's baseline level of such behaviors.
- Additional summaries of other study data, including efficacy data, may be provided if requested by the DSMB.

SAMPLE SIZE AND STATISTICAL POWER CONSIDERATION

We will use a mixed longitudinal model for our primary analyses (see Efficacy Analyses section). Since some aspects of the model such as covariance structure are unknown, we performed power and sample size calculations using a more conservative, simplified model corresponding to a two-group t-test on change scores. We plan to analyze two co-primary outcomes measured as change from baseline to month 6: the ABC-SW, which will provide consistency with pivotal trials of other medications hypothesized to improve ASD social symptoms, and the combined social score, which integrates ABC-SW symptoms with the new PDDBI-SV in order to fully capture the range of impairments in reciprocal social behaviors observed in ASD. We will use an alpha of 0.025 to correct for two co-primary outcomes. SOARS-B is powered to allow independent evaluation of oxytocin efficacy in low and high-functioning youth.

Standard deviations of change scores for the ABC-SW range from ~5 to 9 in several large ASD intervention trials (Shea 2004; McCracken 2002; Owen 2009; Marcus 2009, King 2009; Aman 2009). We consider a between groups difference of 5-7 points in ABC-SW change scores to be clinically meaningful. In our power calculations, we use conservative estimates of 9 points for the SD of ABC-SW change and 5 points for between group differences in ABC-SW changes (differential improvement on ~1/3 of items). To achieve 80% power with an alpha of 0.025 on the ABC-SW, we will require 71 participants in each treatment group within the two strata. Thus, our total required sample to evaluate the ABC-SW separately in low and high-functioning strata is 284. We examined change in the combined social score in 30 3-17 year olds with ASD, 20 treated with aripiprazole and 10 medication-free controls. The mean baseline value was 33.5 (17.2 SD) and the change overall was -10.7 (SD 13.0), with a SD of change in each group of 11.9. Within each treatment group, changes in the combined social score paralleled those in the ABC-SW and PDDBI-SV, but had less variability (Sikich, personal data). We consider a between groups difference in the combined social measure changes of 10-12 to be clinically meaningful. Conservative estimates using a SD of 15 and a between groups difference of 10 in the combined social score changes results in a larger effect size for the combined social measure than for the ABC-SW. Consequently, we should have at least 80% power for tests of the combined social score. A sample size of 300 will allow for 5% attrition between randomization and the first post-randomization visit. The primary objective considers all participants, resulting in much greater power than for analyses in separate strata. The power of moderator analyses depends on the distribution of participant characteristics and variability in methylation and mRNA expression observed, which can't be estimated reliably at this time.

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FINAL STATISTICAL ANALYSIS PLAN FOR PRIMARY ANALYSIS OF SOARS-B TRIAL

Version 2.0

Dated: February 19, 2018

Study of Oxytocin in ASD to improve Reciprocal Social Behaviors (SOARS-B)

STATISTICAL ANALYSIS PLAN

A Phase II, Multicenter, Randomized, Double-blind, Flexible Dose, Placebo-controlled Study of the Efficacy, Tolerability, and Safety of Intranasal Oxytocin in Child Adolescent Participants with Autism Spectrum Disorders

Sponsor: National Institute of Child Health and Human Development

Version: Draft 2.0

Date: 02-19-2018

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Amendment

The material information contained herein is subject to amendment at any time prior to the breaking of the blind.

Table of Contents

Abbreviation or term	Explanation
ABC	Aberrant Behavior Checklist
ABC-SW	Aberrant Behavior Checklist – Social Withdrawal subscale
ADI-R	Autism Diagnostic Interview - Revised
ADOS-2	Autism Diagnostic Observation Schedule (version 2)
AE	Adverse Event
ALT	Alanine Aminotransferase
ASD	Autism Spectrum Disorder
AST	Aspartate Aminotransferase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CASI	Childhood Anxiety Symptom Inventory
CGI-I	Clinician Global Impression - Improvement
CGI-S	Clinician Global Impression - Severity
CI	Confidence Interval
cl	Chloride
CO2	Carbon Dioxide
CSQ	Caregiver Strain Questionnaire
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
GUID	Global Unique Identifier
FAS	Full Analysis Set Population
IU	International Unit
K	Potassium
LS	Least Squares
OT	(intranasal) Oxytocin
MMRM	Mixed Model with Repeated Measures
Na	Sodium
NIH	National Institutes of Health
PDDBI-SV	Pervasive Developmental Disorders Behavior Inventory – Screening Version
PP	Per-protocol Population
OXTR	Human Oxytocin Receptor Gene
Q1	First Quartile
Q3	Third Quartile
RMET	Reading the Mind in the Eyes Test
SAP	Statistical Analysis Plan
SD	Standard Deviation
SF	Sociability Factor, a combined measure derived from the ABC-SW & PDDBI-SV
SLAES	Systematic Longitudinal Adverse Events Scale
SOARS-B	Study of Oxytocin in ASD to Improve Reciprocal Social Behaviors
SRS-2	Social Responsiveness Scale (version 2)
TEAE	Treatment-Emergent Adverse Event
VABS	Vineland Adaptive Behavior Scales 2nd edition

1. INTRODUCTION

1.1 PURPOSE

The purpose of this SAP is to outline the planned analyses to support the completion of the primary manuscript to be written for the SOARS-B Study. Other analyses not identified or defined in this SAP may be performed to support the clinical trial, and will be described in separate SAPs. Any post-hoc or unplanned analyses performed and not identified in this SAP will be documented in the respective manuscript.

1.2 STUDY OBJECTIVES

1.2.1 PRIMARY OBJECTIVE

- To determine the efficacy of intranasal oxytocin (OT) for the treatment of deficits of reciprocal social behaviors in child or adolescent participants with autism spectrum disorders (ASD) as measured by changes in the Aberrant Behavior Checklist- Social Withdrawal Scale (ABC-SW) .

1.2.2 SECONDARY OBJECTIVES

- To determine the efficacy of OT as measured by changes in ABC-SW scores as a function of baseline plasma oxytocin levels, age and functional level and the interaction of each with treatment, and with treatment and week.
- To determine the safety and tolerability of OT in participants with ASD as measured by incidence of treatment emergent adverse events (TEAEs) and abnormal laboratory values, tolerability of OT dose, and time to early termination.
- To determine the efficacy of OT as measured by changes in the sociability factor (SF), a combined measure formed from 13 items of the ABC-SW and the Pervasive Development Disorders Behavior Inventory –Screening Version (PDDBI-SV).
- To determine the efficacy of OT as measured by changes in ABC-SW scores in low-functioning and high-functioning strata separately.
- To determine the efficacy of OT as measured by changes in Social Responsiveness Scale Version 2 (SRS-2) Social Motivation scores and Stanford Binet-5 Abbreviated IQ standard scores.

1.2.3 EXPLORATORY OBJECTIVES

- To determine the efficacy of OT on other clinical assessments including the subscales of the ABC, SRS, Vineland Adaptive Behavior Scale 2nd edition (VABS), and Caregiver Strain Questionnaire, the PDDBI-SV; the Clinical Global Impressions Severity and Improvement scores and, in the high functioning participants only, the Reading the Mind in the Eyes Test (RMET).

1.3 STUDY DESIGN

This is a multicenter, randomized, double-blind, stratified, flexible dose, placebo-controlled study designed to evaluate OT in participants with ASD. OT will be given as an intranasal preparation with two different

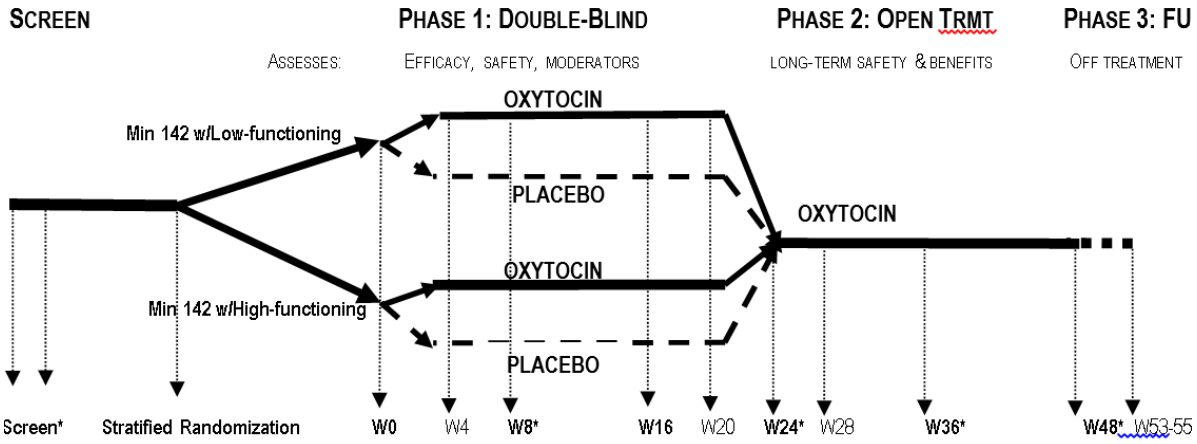
concentrations of 8 IU per insufflation or 24 IU per insufflation. Oxytocin will be administered daily, in single doses up to 40 IU or divided doses in the morning and early afternoon as described in the protocol. Total daily dose is flexible, between 8 IU and 80 IU per day.

Participants who successfully complete screening and baseline assessments and are deemed eligible will be randomized to OT or placebo treatment in a 1:1 ratio, stratified by age group (3-6, 7-11, 12-17) and functioning (low, high), for a total of 6 strata. Randomization will not be stratified by center, as it was felt that additional stratification by center would lead to too many strata, a loss of degrees of freedom, and the potential for a grossly unequal randomization to treatments.

A schematic representation of the study design is shown in Figure 1. After screening and randomization at week 0 (W0), participants enter a double-blind treatment period of 24 weeks (W24), followed by open-label treatment with OT for 24 weeks, termination of OT at the end of week 48 (W48), and a week 53-55 (W53-55) or week 72 OFF treatment follow-up . Although the study design requires patient follow-up from week 0 to week 53-55, this SAP focuses on data analyses for the double-blind treatment period (week 0 to week 24).

Efficacy, safety, and tolerability are measured every four weeks throughout the treatment period. Participants who terminate early are asked to provide safety data through the remainder of the study. The schedule of assessments is provided in Table 1.

Figure 2: Schematic Representation of the Study Design



*indicates safety labs and genetic sample taken. Primary outcome assessed at bolded time points.

Table 1: Schedule of Assessments

6.0 Schedule of Events	Scrn	Double Blind Treatment								Open-label Treatment			FU
Procedure		W0	W2	W4	W8	W12	W16	W20	W24/T	W28	W36	W48	W53-55
ADOS-2	X								X[7]				
ADHR	X[7]												
Stanford Binet/Mullen	X								X				
DSM-V Checklist	X												
Inclusion/Exclusion	X	X											
Demographics		X											
Family Med Hx (NIH Form)	X												
Randomization Form		X											
GUID Acquisition Form/GUID Record		X											
Con Meds	X	X		X	X		X	X	X	X	X	X	
Vital Signs	X	X		X	X		X	X	X	X	X	X	
Med/Psych History (SLAES)	X	X											
Adverse Effects (SLAES/Suicidality)	X	X		X	X		X	X	X	X	X	X	X
Full Physical Exam (NIH Form)	X												
Focused Clinical Physical Exam		X		X	X		X	X	X	X	X	X	
Medical History (NIH Form)	X												
CGI S and I[6]		X		X	X		X	X	X	X	X	X	
VAS Change (Clinician and Coord)		X		X	X		X	X	X	X	X	X	
Social Skills Therapies		X		X	X		X	X	X	X	X	X	
Psychosocial Therapies		X		X	X		X	X	X	X	X	X	
Laboratory													
ECG	X								X				
Female Reproductive Status	X	X		X	X		X	X	X	X	X	X	
Urine/Serum Pregnancy[1]	X												
Safety labs[2]	X								X		X	X[7]	
mRNA sample	X	X**			X				X		X		
Methylation sample	X	X**			X				X		X		
Plasma/Salivary oxytocin	X	X**			X				X		X		
Whole blood serotonin	X				X				X				
Parent Questionnaires													
Questionnaire Guidance Form		X		X	X	X	X	X	X	X	X	X	
Social Opportunity Questionnaire		X		X	X	X	X	X	X	X	X	X	
ABC		X		X	X	X	X	X	X	X	X	X	
PDDBI-SV		X		X	X	X	X	X	X	X	X	X	
CASI-5		X							X			X	
Caregiver Strain		X							X			X	
Vineland-II (Survey Form)		X							X			X	
SRS-2		X				X			X		X	X	
Subject Completed Questionnaires													
Reading Mind in Eyes Test[3]		X			X				X			X	
Self-Rating of social function		X							X			X	
Medication													
Oxytocin Administration Instructions*		X											
SOARS-B Parent Dose Sheet[5]		X	X	X	X		X	X	X	X	X	X	
Risks Handout		X							X				
Medication Compliance[4]				X	X		X	X	X	X	X	X	X
Oxytocin Dosing Log (staff)		X	X	X	X		X	X	X	X	X	X	X
Medication Diary Dispensed		X		X	X		X	X	X	X	X	X	

0-CGI-S only at baseline, CGI-I and CGI-S at most other time points (office visits)
1-Required at screening but can be done at any time during the course of the study at physician discretion. Must have a negative pregnancy test within two weeks of baseline.2-Safety labs to include: glucose (random), CO2, Cl, K, Na, Creatinine, BUN, AST, ALT, full urinalysis, urine pregnancy in pubertal girls. Screening safety labs must be repeated if more than 12 months between screening and baseline.
3- Assessment will be obtained only in participants who have fluent phrase speech & can define basic feelings and friendship.
4- Completed each time the participant returns study medication
5- The appropriate SOARS-B Parent Dose Sheet should be given at each time dose changes to parents
6-Stanford Binet or Mullen may be completed at physician discretion
7- At physician discretion
*Must be given at baseline, but also can be given at any other time during the course of the study at study staff discretion
**Optional redraw of mRNA, plasma/salivary oxytocin, and methylation at baseline for those who may have had a lengthy time between screening and baseline (not safety labs). Procedures for visits are allowed to be completed on separate days, however, subjects may not be dispensed open label medication until all week 24 procedures are completed

2.GENERAL STATISTICAL CONSIDERATIONS

2.1 Analysis Populations

In all populations, participants will be analysed in the treatment group into which they were originally randomized. Measurements from participants excluded from the pre-defined analysis populations or extra measurements (such as unscheduled or repeat assessments) will not be included in summary tables or figures unless specified otherwise.

2.1.1. Safety Population

The safety population will include all participants who receive at least 1 day of study drug. The safety population will be used for all tabulations of safety variables, unless otherwise specified in the SAP text or the table shell.

2.1.2. Full Analysis Set

The full analysis set (FAS) will consist of all evaluable participants, defined as all randomized participants who receive at least 1 day of study drug and have a baseline assessment on the ABC-SW and at least 1 primary efficacy assessment on the ABC-SW after administration of study drug. The FAS will be used for all tabulation of demographics and baseline characteristics, and the primary efficacy population and used in efficacy analyses.

2.1.3. Per-Protocol Population

The per-protocol (PP) population is defined as a subset of FAS who complete the full 24 weeks of double blind treatment with study drug (including those who stayed on the study drug for at least 22 weeks with 80% treatment compliance) and all assessments of the ABC-SW at baseline, Weeks 4, 8, 12, 16, 20 and 24. The per-protocol population will be used only for supportive analyses of the primary efficacy endpoint.

2.2 General Summarization of Study Data

Unless otherwise specified, continuous variables will be summarized with descriptive statistics including n, mean, standard deviation (SD), median, first quartile (Q1) and third quartile (Q3) and minimum and maximum values. Categorical variables will be reported as number and percentage of participants in each category. Least squares (LS) means, standard errors, estimates of contrasts with 95% confidence intervals (CI), and p-values will be reported as appropriate.

All other statistical tests and confidence intervals will be 2-sided, unadjusted for multiplicity, and tested at $\alpha = 0.05$, except those explicitly stated to be otherwise.

All analyses and summaries will be produced using SAS® version 9.3 (or higher).

2.3 Sequence of Planned Analyses

All analyses described in this SAP will be performed after data collection has concluded, the database locked, and the treatment blind has been broken. There will be no interim analyses of study data except for the summaries provided to the Data Safety Monitoring Board (DSMB).

An independent DSMB will meet twice yearly to review safety information for the study. The study's DSMB statistician will prepare summaries of data as described in the Safety Analyses section, separating them into treatment group A and treatment group B and will distribute them only to the members of the DSMB. No study team member other than the DSMB statistician will see the summaries provided to the DSMB.

Analyses for the primary manuscript as described in this SAP will focus on analyzing data during the 24-week double-blinded treatment period. Analyses on open-label treatment period (24-48 weeks) and the off-treatment follow-up period (>48 weeks) will be specified in separate analysis plans.

When performing analyses for the primary efficacy variable (ABC-SW), additional models with baseline serum oxytocin, age, and functionality as covariate will be examined and tested prior to analyzing data on the secondary efficacy variables.

2.4 Summary of Data Sources

Data collected at each center by investigators are entered into the ReDCAP database, and parents/guardians' responses on various measurements are entered into the Qualtrics database. The RMET data are entered into the Qualtrex participant database. Both RedCAP data and Qualtrics data are used for analysis.

3. PARTICIPANT DISPOSITION ANALYSES

3.1 General Summaries

Disposition will be summarized for all enrolled participants. Summaries will include counts of:

- Participants who were enrolled
- Participants who were enrolled but not randomized
- Participants who were randomized
- Participants who were randomized but did not take study drug
- Participants who were in the safety population
- Participants who were in the FAS
- Participants who were in the PP population
- Participants who completed the double blind portion of the study
- Participants who terminated the study early
- Participants with the each potential primary reason for early termination (i.e. adverse effect, inconvenience, administrative, lack of efficacy, noncompliance, lost to contact)

All summaries will be presented by treatment group and over all participants. A listing of disposition will be provided for all participants who did not complete the double blind portion of the study.

3.2 Protocol Deviations

Protocol deviations will be recorded by each site as they occur and/or captured during monitoring visits. All protocol deviation data will be available upon request.

3.3 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized by treatment group and over all participants for the FAS population. Demographic characteristics will include sex, age, race, and ethnicity. Clinical characteristics will include Autism Diagnostic Observation Scale (ADOS) severity scores. Baseline efficacy variables (see Efficacy Analyses section for list), vital signs, and female reproductive status will also be summarized.

Age (months) will be calculated as the number of months between date of birth and date of baseline visit. .

3.4 Dose and Compliance

All dosing, exposure, and compliance information will be summarized by treatment group and over all participants for the FAS population.

Summaries of dose will include the percent of participants not able to tolerate the target dose, defined as those who were not at 48 IU TDD or higher at week 24, mean 24 week dose, and mean maximal dose.

Medication compliance will be evaluated each time the participant returns study medication at week 4, 8, 16, 20 and 24 using the formula below. The average of non-missing percent compliance per subject will be used to report mean treatment compliance for each treatment group.

$$\left[\left(\frac{\text{TOTAL Number of Sprays Prescribed}}{}\right) \text{ minus } \left(\frac{\text{TOTAL Number of Missed Sprays}}{}\right)\right] \div \frac{\text{TOTAL Number of Sprays Prescribed}}{} \times 100 = \frac{}{\text{Percent Compliance}}$$

Definitions:

TOTAL Number of Sprays Prescribed = Total number sprays prescribed per day x number of days since the last visit. NOTE: be sure to only count ½ days if subject has already taken a morning dose.

TOTAL Number of Missed Sprays = Total number of missed sprays according to the parent completed dosing diary. If parent does not return dosing diary, the parent can be asked how many sprays were missed.

3.5 Concomitant Medications

Concomitant medications will be categorized into the following groups and subgroups: **Neurobehavioral medications:** alpha adrenergic agonists, anti-anxiety medications, anti-cholinergics, anticonvulsants/mood stabilizers, antidepressants, antipsychotics, stimulants, other attention deficit hyperactivity disorder medications and sleep aids and **Other medications:** medications for gastrointestinal disorders, anti-infection medications, cough/allergy/asthma medications, heart medications, inhalation steroids, nutritional supplements, systemic steroids, and other.

Medications stopped prior to the first date of study drug dosing will be considered prior medications. We do not plan to summarize these medications. Medications taken after the first date of study drug dosing will be considered concomitant medications.

Concomitant medications for each study treatment group will be summarized according to their membership in the groups noted above. Upon request, a summary of each specific medication name will be provided.

In summaries, if a participant has taken a concomitant medication more than once, the participant will be counted only once in the total.

4. EFFICACY ANALYSES

The FAS will be used as the analysis population for all efficacy analyses unless otherwise specified.

4.1 Efficacy Variables

Detailed description of all efficacy variables is given in the protocol. All efficacy variables will be analyzed as change from baseline, except where noted.

4.1.1 Primary Efficacy Variable

The ABC-SW score will be analyzed as the primary endpoint.

4.1.2 Secondary Efficacy Variables

The following variables will be analyzed as secondary endpoints:

- Sociability Factor
- SRS-2 social motivation subscale score
- Stanford Binet-5 Abbreviated IQ (ABIQ)

4.1.3 Exploratory Efficacy Variables

- Clinical Global Impression - Severity (CGI-S)
- Clinical Global Impression - Improvement (CGI-I)
- CGI-I will be analyzed as an endpoint mean instead of change from baseline.
- ABC subscales for irritability, stereotypy, hyperactivity, and inappropriate speech
- PDDBI-SV total score
- Caregiver strain questionnaire (CSQ) and its subscales
- Vineland 2 adaptive behavior composite score, and domain scores for daily living skills, communication and socialization. Motor skills will not be analyzed since it is only valid in young children
- Reading the Mind in the Eyes test (only in individuals with fluent speech and understanding of basic emotions)

4.2 General Modelling Considerations for Efficacy Analyses

Unless otherwise specified, the primary model is mixed model with repeated measures (MMRM), with change from baseline to each post-baseline timepoint for a response variable, functioning (low versus high) as the stratification variable, center as a blocking factor, treatment (oxytocin versus placebo), week as a continuous variable, treatment and week interaction, and baseline value as a covariate, with random coefficients for the intercept and week, with an unstructured structure among the random coefficients. We will investigate the interaction of treatment, week, and functioning and adopt the most parsimonious model as the final model. Distributional assumptions will be examined using residuals. These mixed models could fail to converge or encounter difficulties based on their use of asymptotics. If they do, we will attempt to

simplify the models in order to eliminate the problems, and if necessary, move to analyses that assume compound symmetry, but use a Huynh-Feldt correction if compound symmetry fails.

4.3 Missing Data Considerations for Efficacy Analyses

If there is <5% of subjects with covariate data points missing, then no additional sensitivity analyses will be performed. If there are >=5% of subjects with missing covariate data, we will perform multiple imputation (MI). We will use the full conditional specification method (FCS) to impute missing data. If data have a monotone missing pattern, we will use linear regression to impute continuous variables, logistic regression to impute binary/ordinal variables, and the discriminant function to impute nominal variables. We will check normality of continuous variables. If very asymmetric, variables will be transformed. We will create 20 imputed datasets including all variables intended to be used in subsequent statistical analysis as a minimum. We will analyze the complete datasets using standard statistical analyses. Finally, the results from each model will be combined to produce inferential results.

If there is <5% of subjects with efficacy outcome data points missing, this will be considered a negligible amount and no additional sensitivity analyses will be performed. If there are >=5% of subjects with missing outcome data, the Mixed-Effect Model Repeated Measure (MMRM) approach is able to handle moderate amounts of missing data provided they are missing at random. However, it is difficult to rule out the possibility that at least some of the missing data are missing not at random (MNAR). We will apply a pattern-mixture approach to investigate the MNAR pattern. Specifically, we will define the missed visit indicator (1 if missing and 0 if observed). We fit a pattern-mixture MMRM model with this defined missed visit indicator. The coefficient of the missed visit indicator (e.g., gamma) is estimated and tested for statistical significance. If this coefficient is not statistically different from zero, missing at random assumption is valid. Otherwise, the missing is not at random. If the latter, we conduct an extensive sensitivity analysis by pre-specifying the value of gamma, which plays the role of sensitivity parameter to the MAR assumption. We define a grid in a plausible range of sensitivity parameter. For each pre-specified value, we investigate the treatment effect. When the pre-specified value is zero, the analysis assumes MAR.

4.4 Primary Efficacy Analysis

The primary efficacy response variable will be tested at the level of $\alpha = 0.05$. Longitudinal models as described in General Modeling Considerations will be fit with dependent variable of change from baseline to each post-baseline timepoint (weeks) up to week 24. The primary hypothesis test of interest will be a test of treatment effect at week 24.

As a sensitivity analysis, the primary efficacy analysis will also be conducted in the per-protocol population.

4.5 Subgroup Analysis for Primary Efficacy Variable

As a secondary analysis for the primary efficacy variable, the interaction of treatment-by-functionality-by-week will be examined. In addition, the primary efficacy variable will be analyzed within the low and high functioning stratum separately.

4.6 Addition of Covariates to Primary Efficacy Analysis Model

As additional secondary analyses for the primary efficacy variable, the following groups of covariates will be added separately to the model in the primary efficacy analysis and tested for their significance:

Baseline age, treatment-by-age interaction, treatment-by-age-by-week interaction.
Baseline serum oxytocin, treatment-by-oxytocin interaction, treatment-by-oxytocin-by-week interaction.

In both cases, the primary test will be the significance of treatment-by-variable-by-week interaction, and if not significant, the treatment-by-variable interaction.

4.7 Analyses of Key Secondary Efficacy Variables

The key secondary efficacy variables will be tested using the same model as in the primary efficacy analysis. For variables that were only measured at baseline and Week 24 (or the latest visit during the randomized treatment period), the change scores will be reported, and treatment group differences will be tested using two sample t-test.

4.8 Analyses of Exploratory Efficacy Variables

Exploratory efficacy variables will be analyzed the same way as the primary and secondary efficacy variables.

5. SAFETY ANALYSES

Safety and tolerability will be evaluated based on the incidence of TEAEs, incidence of elicited reports of suicidal ideation or statements, self-injurious behaviors, and suicidality, incidence of AEs leading to termination and changes in vital signs, modified BMI z-score , ECG findings and laboratory measurements, and incidence of pre-specified abnormal laboratory test results. All safety data will be presented using the safety population.

5.1 Treatment Emergent Adverse Events (TEAEs) and Potential Suicidality

Adverse events will be monitored continuously from the time a participant signs the informed consent document until the end of double-blind randomized treatment period (week 24). Similarly, reports of suicidal ideation or statements, self-injurious behaviors, and suicidality elicited from participants who are able to speak and understand the concepts and from all caregivers will be elicited at every contact and followed continuously during the double-blind treatment.

Medical and behavioral conditions that are present at screening and/or baseline will only be considered treatment emergent adverse events if their intensity increases significantly (i.e. numeric rating of intensity is increased) after screening and/or baseline.

TEAEs will be coded using the Systematic Longitudinal Adverse Event Scale (SLAES) mapping to MedDRA terms and MedDRA terms for TEAEs not mapped in the SLAES. Additional details about this scale are given in the protocol.

Intensity of adverse events will be categorized as mild, moderate, severe, life-threatening, or resulting in death. Relationship to treatment will be categorized as unknown or not applicable relationship, unrelated, possibly related, likely related, or certainly related. For FDA reporting, all possibly related, likely related and certainly related TEAEs will be considered related.

Overall summary of TEAEs, such as all TEAEs, TEAEs by intensity and TEAEs by relationship to treatment, will be summarized by category and preferred term for each treatment group and over all participants. TEAEs leading to participant withdrawal from the study, and TEAEs resulting in death will be listed separately.

Serious adverse events (SAE), defined using generally accepted criteria as stated in the protocol, will be listed and summarized by treatment group and participant experiencing at least one SAE. Relationship to treatment and outcome will be included in each listing.

All reports of suicidal ideation or statements, self-injurious behaviors, and suicidality will be listed by participant and treatment group.
No formal hypothesis-testing analysis of TEAEs or suicidality incidence rates will be performed.

5.2 Vital Signs

Vital sign measurements will be summarized at each time point for the absolute value and for changes from baseline by treatment group. Alarm values will be determined prior to unblinding and the percent of participants with values in the alarm range will be summarized at each time point by treatment group.

All vital signs data will be available upon request.

5.3 Weight and Body Mass Index

Weight and Height are measured at every study visit. These values will be used to determine the BMI and modified BMI z score at each time point. Changes in modified BMI z-score from baseline to Week 24 by treatment group will be summarized. All BMI data will be available upon request.

5.4 Clinical Laboratory Evaluations

Blood and urine samples for safety laboratory assessments will be collected at screening, and week 24. To the extent possible, blood samples will be obtained at approximately the same time of day and under non-fasting conditions. All safety labs will be processed by LabCorp.

Laboratory test results, including prolactin results, will be summarized by treatment at baseline and for changes from baseline. We will also summarize the percent of participants in each treatment group with values within alarm ranges, which will be defined prior to breaking the blind. The mean value and SD of the labs within alarm ranges will be summarized at each time point. All measurements within alarm ranges for each affected participant will be listed.

5.5 12-lead Electrocardiogram (ECG)

A 12-lead ECG will be performed at screening, and week 24.

Interval data will be summarized at baseline and week 24 for the absolute value and for changes from baseline by treatment group. Intervals that are within alarm ranges will be summarized and listed by participant. Overall assessment of ECG as normal or abnormal will also be summarized by treatment group using counts and percentages.

5.6 Safety Data Reported to the DSMB

Safety data will be submitted to the independent DSMB twice per year. The following summaries will be provided for review at each meeting, separated by treatment group:

- Summary table of participant disposition including number screened, number randomized, number completed, percent early terminated, and reasons for early termination.
- Listing of all participants terminating the study early with duration of treatment and reason for study discontinuation.
- Listing of all SAEs.
- Summary table of the number and percent of participants with TEAEs by severity.
- Summary table of the number and percent of participants with TEAEs by relationship to study drug.
- Summary table of the number and percent of participants with specific TEAEs leading to participant early withdrawal. .
- Summary table of the number and percent of participants with TEAEs by system organ class and preferred term by treatment group.
- Listing of all severe TEAEs.
- Summary table of the number and percent of participants with suicidal ideation or vocalizations, self-injurious behaviors and suicide attempts by treatment group.
- Listing of all suicidal ideation or vocalizations, self-injurious behaviors and suicide attempts by treatment group.
- Summary of the proportion of suicidal ideation or vocalizations, self-injurious behaviors and suicide attempts by treatment group that are judged to be more severe than the participant's baseline level of such behaviors.

Additional summaries of other study data, including efficacy data, may be provided if requested by the DSMB.

6. SAMPLE SIZE AND STATISTICAL POWER CONSIDERATION

We will use a mixed longitudinal model for our primary analyses (see Efficacy Analyses section). Since some aspects of the model such as covariance structure are unknown, we performed power and sample size calculations using a more conservative, simplified model corresponding to a two-group t-test on change scores. SOARS-B is powered to allow independent evaluation of oxytocin efficacy in low and high-functioning youth.

Standard deviations of change scores for the ABC-SW range from ~5 to 9 in several large ASD intervention trials (Shea 2004; McCracken 2002; Owen 2009; Marcus 2009, King 2009; Aman 2009). We consider a between groups difference of 5-7 points in ABC-SW change scores to be clinically meaningful. In our power calculations, we use conservative estimates of 9 points for the SD of ABC-SW change and 5 points for between group differences in ABC-SW changes (differential improvement on ~1/3 of items). To achieve 90% power with an alpha of 0.05 on the ABC-SW, we will require 71 participants in each treatment group within the two strata. Thus, our total required sample to evaluate the ABC-SW separately in low and high-functioning strata is 284. A sample size of 300 will allow for 5% attrition between randomization and the first post-randomization visit. On 3/28/2016, the study team reduced the attrition rate to 1% based on attrition observed in the study, which gives a final sample size of 290. The primary objective considers all participants, resulting in much greater power than for analyses in separate strata.

7. REFERENCES

Aman, M. G., C. J. McDougle, et al. (2009). "Medication and Parent Training in Children With Pervasive Developmental Disorders and Serious Behavior Problems: Results From a Randomized Clinical Trial." Journal of the American Academy of Child & Adolescent Psychiatry 48(12): 1143-1154.

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McCracken, J. T., J. McGough, et al. (2002). "Risperidone in Children with Autism and Serious Behavioral Problems." New England Journal of Medicine 347(5): 314-321.

Owen, R., L. Sikich, et al. (2009). "Aripiprazole in the Treatment of Irritability in Children and Adolescents With Autistic Disorder." Pediatrics 124(6): 1533-1540.

Shea, S., A. Turgay, et al. (2004). "Risperidone in the Treatment of Disruptive Behavioral Symptoms in Children With Autistic and Other Pervasive Developmental Disorders." Pediatrics 114(5): e634-e641.

Agreement to Planned Analyses

Trial: SOARS_B

Analysis Plan Date: 2/19/2018

Purpose of analysis plan(s):

- ☐ DMC/Interim analysis or safety report
- ☐ Substudy analysis
- ☒ Final (main trial) analysis
- ☐ Confirmatory analysis
- ☐ Operational reporting
- ☐ Central Statistical Surveillance
- ☐ Outcomes registry analysis
- ☐ Addendum to analysis plan version Click here to enter text.
- ☐ Other: Click here to enter text.

In signing this document, I am confirming that I have reviewed and approve the analysis plan(s) referenced above.

Faculty/Reviewing Biostatistician (signature): S. Luo	Faculty/Reviewing Biostatistician's Title: Associate Professor of Biostatistics & Bioinformatics, Duke Clinical Research Institute
Printed name: Sheng Luo	Date: 2/21/2018

Principal Investigator (signature): L. Sikich MD	Principal Investigator's Title and Affiliation: Associate Professor, Department of Psychiatry and Behavioral Sciences; Associate Director, Duke Center for Autism and Brain Development
Printed name: Linmarie Sikich	Date: 21 Feb 2018

Sponsor Representative (signature):	Sponsor Representative's Title and Institution:
Printed name:	Date:

Sponsor approval required whenever sponsor requests to review the analysis plan.

Summary of Changes to SAP for SOARS-B Primary Analysis

High Level Summary of Changes in the SAP for the SOARS-B Study Primary Analysis

Changes from version dated 3-21-2013 to final version 2.0 dated 2-19-2018

1. Added new statistical analysis team due to Dr. Hamer's death and Dr. Johnson's departure from academia. The new team consists of Drs. Sheng Luo and Lilin She, who modified the final version of the consent in collaboration with lead PI Dr. Sikich.
2. Changes in Treatment Site Principal Investigators
3. **Limited SAP to analysis to primary manuscript**, due to DCRI SOP's and financial limitations. Noted additional SAPs would be written for subsequent analyses. This included r**emoval of all analyses involving genetic measures, salivary oxytocin levels, serotonin levels, changes in plasma oxytocin after baseline, concurrent behavioral and pharmacological interventions, the Child and Adolescent Symptom Index (CASI) and novel assessments including self-reported social functioning, social opportunities** Section 4.9 of original SAP was deleted.
4. Removed the sociability factor (SF) as a primary outcome. The SF is also referred to as combined measure of ABC-SW and PDD-BI Screening version. The SF was removed due to emerging evidence that it was not more sensitive to changes than either measure alone.
5. Added specification of covariates in the model including baseline plasma oxytocin levels. Note that there is an error in final SAP in that predefined age group was used in these analyses rather than an actual age in months.
6. Removed inclusion of cognitive functioning assessed by the Mullen instead focusing only on cognitive functioning assessed by the Stanford Binet 5th edition due to the small number of participants completing Mullen and issues converting to a common measurement unit.
7. Deleted secondary analysis of Vineland measures.
8. Updated study design figure and schedule of procedures to reflect changes to visit schedule (note there is an error in the Figure such that Week 12 visit is missing).
9. Eliminated examination of baseline percent *OXTR* gene methylation as a covariate in analyses due to challenges in quantification of percent methylation given multiple possible methylation sites and ongoing challenges with assessing methylation of the *OXTR* gene.
10. Added that the full analysis set will only include participants who have both a baseline and post-baseline ABC-SW, rather than just a post-baseline assessment of the primary outcome. (This reflects deletion of SF as a primary outcome measure.)
11. Specified that the demographics and baseline symptom measures will reflect only the full analysis set.
12. Clarified that age will reflect age at time of baseline visit rather than screening since there was often a lag of several months between screening and baseline for the early participants in the trial due to issues with study drug.
13. Deleted reporting of psychiatric history.
14. Will no longer adjust for multiplicity
15. Eliminated the following exploratory efficacy measures: Calibrated ADOS severity score, PDD-BI subscales replacing with PPD-BI screening version.
16. Eliminated Vineland 2 two domain composite score and maladaptive behaviors subscales, as well as measures listed in item 3.(above) from exploratory efficacy variables.
17. In Section 4.2, eliminated age group as a stratification variable, eliminated evaluation of two kinds of models instead using a mixed model with repeated measures (MMRM)
18. Specified thresholds for conducting sensitivity analyses for missing data.
19. Provided more detail related to SAS programming for primary analyses.
20. In Section 4.6 of SAP deleted examination of *OXTR* gene methylation and instead added examination of baseline serum oxytocin level.
21. Section 4.7 of SAP, removed bonferroni correction using simple two sample t-test instead
22. Error in section 4.8 of final SAP. Exploratory efficacy variables were analyzed in the same way as the key secondary efficacy analyses.
23. Section 5.1 changed severity of TEAEs to intensity paralleling changes in the SLAES.
24. Section 6, eliminated use of two primary outcomes.

Original SOARS-B Protocol, Version 1.0

February 7, 2013

Study of Oxytocin in Autism to improve Reciprocal Social Behaviors
(SOARS-B)

Protocol Version: 1.0

Sponsored by the Eunice Kennedy Shriver Institute for Child Health and Development
Under
Cooperative Agreement 1U01HD073984-01

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Katerina Tsilou, MD, Scientific Collaborator, NICHD
Alice Kau, PhD, Program Officer, NICHD

Cheryl Alderman, BS, CCRP, Project Manager

STATEMENT OF COMPLIANCE

This study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following *(use applicable regulations depending on study location and sponsor requirements; samples follow):*

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (May 9, 1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

*Site Investigator: _____ (name)

Signed: _____ Date: _____

** The protocol should be signed by the clinical site investigator who is responsible for the day to day study implementation at his/her specific clinical site; i.e., if Investigational New Drug (IND) study, the individual who signs the Form FDA 1572.*

TABLE OF CONTENTS

PROTOCOL SUMMARY

Title:	Study of Oxytocin in Autism to improve Reciprocal Social Behaviors
Synopsis:	We propose to conduct this Phase 2 study to evaluate oxytocin as a supplemental treatment for improving social difficulties in individuals with autism.
Objectives:	We are proposing to randomly assign 300 individuals between the ages of 3 -17 years old with an autism spectrum disorder to 6 months of treatment with oxytocin or a matched placebo across five sites in the United States. Subsequently all participants will receive open label oxytocin for 6 additional months. Post- treatment assessments will be done 6 months after treatment stops. We will also determine *OXTR* methylation status at baseline, 2 months, and end of treatment to explore potential relationships with baseline severity of social problems and/or treatment response.
Population:	300 subjects, male or female, ages 3-17 with an autism spectrum disorder
Phase:	II
Number of Sites:	5 treatment sites
Description of Agent or Intervention:	Oxytocin, intranasal (8IU to 80IU daily)

Key Roles

Individuals:

Overall Principal Investigator:
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Associate Professor
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1 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

There is a tremendous unmet need for accessible treatments that address core symptoms of ASD and are safe for sustained use, especially for the population of nonverbal individuals who require the most intensive lifelong support. We have created the ACE SOARS Network to provide the infrastructure necessary to meet this need on an ongoing basis. Our initial clinical trial, described in this application, is SOARS-B (the Study of Oxytocin in ASD to improve Reciprocal Social Behaviors). SOARS-B will test a very promising potential treatment−intranasal oxytocin−for ASD's fundamental social communication deficits in a large, highly generalizable group of verbal and nonverbal children. SOARS-B will also provide information about the regulation of DNA methylation and transcription of the oxytocin receptor gene (*OXTR*), as well as other genes relevant to oxytocin's CNS activity, as a function of time and in response to oxytocin treatment. These data will fill a key gap in our understanding of oxytocin's role in ASD and its ability to alter epigenetic modifications of *OXTR*.

1.2 Rationale

THE SOCIAL MOTIVATION MODEL OF ASD PATHOPHYSIOLOGY

Difficulties in social orienting are evident across auditory and visual modalities and across the lifespan in ASD (Dawson 1998, 2004; Whitehouse 2008; Sasson 2007). Electrophysiologic studies have found that individuals with ASD can attend to social stimuli if instructed to do so, but that they do not do so spontaneously (Ceponiene PNAS 2003). In contrast, typically developing individuals give preferential attention to social stimuli, processing them faster with greater brain activation in prefrontal cortex than nonsocial stimuli (Goren 1975; Greene 2011). Youth with ASD show slower processing of social versus nonsocial stimuli and the rate inversely correlates with the magnitude of social impairments (Dawson 2004). Individuals with ASD also appear to experience reduced relative rewards from interpersonal interactions. In contrast to their typically developing peers, children with ASD prefer looking at pictures of inanimate objects to looking at pictures of people (Sasson 2008, 2011). They also fail to activate the ventral striatum, which is the center of the brain's reward circuit, in response to social rewards whereas high levels of activation are evoked by social rewards in typically developing children (Scott-Van Zeeland 2010). Further, when presented with social stimuli, children with ASD show reduced rather than increased activity in the prefrontal cortex, which assesses the relative value of a reward (Greene 2011). The extent of reduction correlates with the severity of social and communication impairments in children with ASD (Ohnishi 2000; Dawson 1998). These differences, as well as ASD's clinical presentation, have led to the hypothesis that ASD is related to a fundamental impairment in social motivation (Waterhouse 1996; Dawson 2002, 2005; Grelotti 2002).

THE ROLE OF OXYTOCIN IN SOCIAL BEHAVIOR

Oxytocin is the brain's most abundant neuropeptide. It can act as a classical neurotransmitter, a neuromodulator and a hormone with actions throughout the body (Gimple 2001, Veening 2010, Baskerville 2010). Oxytocin's half-life in the plasma is 1-2 minutes compared to ~30 minutes in the CSF. Central release of oxytocin is dependent upon CD38 and dramatically stimulates further release of oxytocin (~1000 fold) and increases the number of oxytocin-containing cells in the periventricular nucleus. Together these factors lead to long lasting oxytocin elevations throughout the brain following acute increases in CSF oxytocin. One of oxytocin's major central actions is to activate the brain's reward circuit by increasing dopamine release from the ventral tegmental area to the ventral striatum, amygdala and hippocampus. Its neuromodulatory actions appear to result primarily from somatodendritic release with binding to oxytocin receptors that are widely distributed throughout the limbic region and prefrontal cortex. Differential species-specific patterns of social behavior appear related to the distribution and density of oxytocin and vasopressin receptors in the brain (Insel 2010). Oxytocin can bind vasopressin receptors, although it is unknown to what it extent it does so normally. Oxytocin also can increase expression of oxytocin and vasopressin receptors in the brain although these effects are often sexually dimorphic and highly regulated by hormones and interleukins (Moos 1989, Morris 2004, Miyan 2003, Tribollet 1989, Bales Hormone Behavior 2007, Bales Neuroscience 2007, Carter 2009, Carter 2007). Social isolation and social stress somewhat later, during the post-weaning period, also have sexually dimorphic effects on the number of oxytocin and vasopressin neurons (Bales Devel Psychobiology 2007, Tanaka 2010).

In animal models including primates, oxytocin has been demonstrated to increase eye contact, social approach, social recognition, social memory, and generosity and to reduce stress responses (Takayangi 2005; Liu 2008, Insel 2010). Oxytocin also influences social behavior in people (MacDonald 2010). Exogenous oxytocin increases gaze to eye regions, social cognition, social memory, positive communication, empathy, perceptions of trustworthiness, and cooperation within one's own group (Guastella 2008, 2010; Domes 2007; Di Simplicio 2008; Fischer-Shofty 2010; Keri 2009; Unkelbach 2008; Savaskan 2008; Rimmele 2009; Dizten 2008; Zak 2005, 2007; Theodoridou 2009; Petrovic 2008; Kosfeld 2005, Baumgartner 2008; De Dreu, 2010). Intranasal oxytocin also reduces cortisol; perceived stress, amygdala activation to threatening social images, and tolerance of ethnic differences (Heinrichs 2003; Kirsch 2005; Petrovic 2008; De Dreu 2011). However, some of these effects, including enhancement of social memories and secure attachment, may be limited to individuals with less robust prosocial behaviors initially (Bartz 2010a, 2010b, Buchheim 2009). Allelic variations in *OXTR* have also been correlated with infant attachment, social auditory processing, empathy and prosocial decision making (Lerer 2010; Tops 2011, Chen 2011).

OXYTOCIN'S POTENTIAL ROLE IN ASD

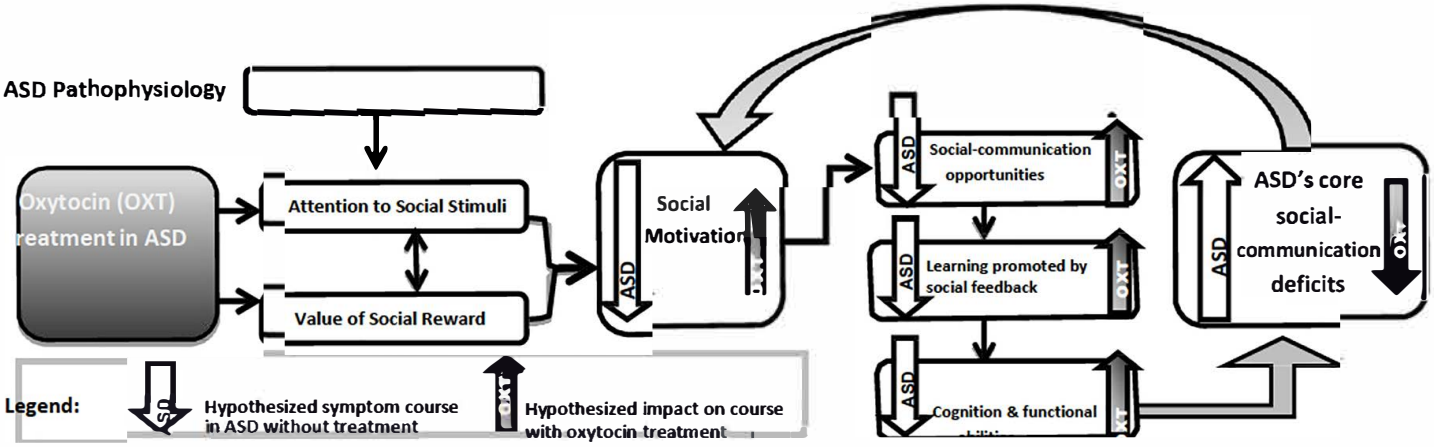
Multiple variations in the oxytocin signaling system have been associated with ASD relative to controls. Allelic variations in the CD38 gene, which is required for central release of oxytocin, and in *OXTR* have been identified, some of which also have been correlated with cognition and functioning in individuals with ASD (Green 2001; Andari 2010; Munesue 2010; Wu 2005; Jacob 2007; Lerer 2010; Yrigollen 2008; Tansey 2010; Wermter 2010; Liu 2010; Campbell 2011; Riebold 2011). Modestly greater (~ 20-40%) *OXTR* methylation relative to controls has been found in two small, independent ASD samples (Gregory 2009). Reduced plasma oxytocin and CD38 mRNA expression have also been reported in ASD (Modahl 1998, Jin 2007).

Oxytocin has shown promise for modifying social behavior in mouse models for ASD and in people with ASD and related disorders. In the *Otxr* null mouse, a single dose of oxytocin improved social deficits, reduced aggression and reduced vulnerability to drug-induced seizures, apparently acting through the vasopressin 1A receptor. Further, in these mice, repeated doses of oxytocin improved reversal learning, indicating enhanced cognitive flexibility (Sala 2011). A single dose of oxytocin also improved social deficits in *Cd38* null mice (Higashida 2010). In Fragile X, which is frequently comorbid with ASD, oxytocin improved gaze avoidance and reduced cortisol elevations elicited by social interaction (Hall 2011). In high functioning individuals with ASD, single doses of oxytocin enhanced attention to faces and eyes, visual and auditory affect recognition, the ability to distinguish whether others were being cooperative, and preference for interacting with receptive individuals (Andari 2010; Guastella 2010; Hollander 2007). Oxytocin challenge also reduced self-reported compulsive and repetitive behaviors (Hollander et al., 2003). Sustained oxytocin treatment has been reported to improve ASD symptoms in one child (Munesue 2010). In the only pilot study of sustained oxytocin treatment currently completed, affect recognition accuracy improved three times as much with 6 weeks of oxytocin treatment as had been reported in Andari's single dose study (Anagoustou in preparation, network preliminary data). Within the next 6 months, an 8-week blinded study of oxytocin in 24 children with ASD will be completed at UNC.

Our Working Model

The above evidence regarding impairments in social orienting and social reward in ASD, oxytocin's role in social orienting and social motivation, and variability in various aspects of oxytocin signaling among people with ASD, together with extensive preliminary evidence that supplemental oxytocin can impact social behaviors in ASD, have led to the formulation of our working model (Figure 1). We postulate that ASD pathophysiology acts to fundamentally alter social orienting and decrease the relative value ascribed to social rewards resulting in limited social motivation. Reduced social motivation leads to a vicious cycle of reduced social opportunities, reduced learning from social feedback, reduced skills and functional abilities and worsening of ASD's core social communication symptoms, which further reduces social motivation. We hypothesize that sustained intranasal oxytocin treatment will positively modify the earliest parts of this cycle – social orienting and the value of social rewards – and disrupt the cycle leading to improvements in the core social and communication impairments of ASD.

Figure 1: Model of ASD Social Symptom Pathophysiology & Proposed Mechanism of Oxytocin Treatment



1.3 Potential Risks

There is a dearth of information about the safety of sustained use of oxytocin, although more information is emerging on a daily basis. Indeed, the objective of specific Aim 2 is to provide such information, gathered prospectively in a systematically elicited fashion.

Oxytocin has been reported to exert a large number of effects in the body and the brain (Gimpl & Fahrenholz 2001). The classic physiological effects of oxytocin in humans as well as all placental mammals are uterine contractions and milk ejection. These are conclusively known to occur only under the hormonal conditions at the end of pregnancy and postpartum when oxytocin receptors proliferate in the uterus and mammary tissue and lactogenesis occurs. Theoretically, oxytocin administration could cause uterine contractions during phases of the menstrual cycle when estrogen levels are relatively high or in women who are receiving estrogen. This, however, has never been demonstrated or reported. IV administration of Oxytocin is FDA approved for induction or facilitation of labor and postpartum contraction of the uterus. Intranasal oxytocin spray is also available in other countries to facilitate nursing in new mothers. Intranasal oxytocin also was FDA approved as a lactation aid in the United States until 1995 when it was withdrawn from the US market by the company that manufactured it, Novartis. This was a business decision and the FDA has confirmed no safety concerns were involved in this decision.

A number of adverse effects have been reported in the context of these two clinical applications, primarily with intravenous administration of oxytocin, which include anaphylactic reaction, hypertension, hypotension, cardiac arrhythmias, nausea and vomiting, afibrinogenemia and associated bleeding and, in the context of prolonged intravenous administration of oxytocin, hyponatremia. There has also been at least one report of life-threatening anaphylaxis during surgery when multiple drugs were being intravenously infused simultaneously (D. Pant, et al, 2009). Symptoms that may result from anaphylaxis include generalized hives, itchiness, or flushing; crampy abdominal pain, diarrhea, and vomiting; a feeling of anxiety and impending doom; swelling of the lips, tongue, throat resulting in shortness of breath, wheezes or stridor, and low oxygen; a drop in blood pressure that may result in a feeling of lightheadedness and loss of consciousness; reduced muscle tone with possible loss of bladder control; and, most seriously, coronary artery spasm may occur with subsequent myocardial infarction or cardiac dysrhythmia and possible death. There is also a case report of psychosis occurring during oxytocin treatment of a man with obsessive compulsive disorder (Ansseau et al, 1987), although there are two completed pilot studies in schizophrenia that have reported reduction of psychotic symptoms (Feifel et al, 2010 – using a TDD of 40 IU during week 1, followed by a TDD of 80 IU for 2 weeks in 16 people; Pedersen et al., 2011 using TDD 48 IU for 2 weeks in 7 people) with no significant adverse events. There are a limited number of published, sustained oxytocin treatment studies in adults with other diseases (Den Boer 1992, Epperson 1996a, Epperson 1996b, Ohlsson 2005).

Evidence for safety of sustained intranasal oxytocin treatment in ASD

Placebo controlled safety data of sustained intranasal oxytocin treatment in people with ASD is available from three currently unpublished trials:

- the Anagnostou adult study (10 on TDD 48 IU oxytocin for 6 weeks, 9 on placebo),
- a trial conducted by Adam Guastella, personal communication 2012 (26 teens on total daily dose [TDD] 24 IU oxytocin for 8 weeks, 24 on placebo), and
- the UNC Autism Speaks pilot trial in 3-17 year olds (12 on oxytocin with TDD ranging between 4 IU and 64 IU for 8 weeks and 13 on placebo) and 24 on open label oxytocin TDD up to 48 IU for 8 weeks. Adverse events observed during the double blind and open-label phases of the UNC oxytocin pilot study are shown in Table 1.

Table 1. Treatment Emergent Adverse Effects in UNC Pilot study of children with ASD

Treatment Emergent, Moderate or Severe Adverse Events	8 Wks Oxytocin N =12, N (%)	8 Wks Placebo N = 13,N (%)	16 Wks Oxytocin N = 11, N (%) (only inW8-16)	8 Wks Placebo 8 Wks Oxytocin N = 13, N (%) (only in W8-16)
Aggression or Hostility	1 (8.3%)	4 (30.8%)	1 (9.1%)	1 (7.7%)
Infection	1 (8.3%)	1 (7.7%)	0	0
Agitation	2 (16.7%)	1 (7.7%)	1 (9.1%)	1 (7.7%)
Anger/Irritability	1 (8.3%)	3 (23.1%)	1 (9.1%)	3 (23.1%)
Insomnia Mid cycle or other	3 (25%)	2 (15.4%)	0	0
Disinhibited Behavior	1 (8.3%)	1 (7.7%)	0	0
Mood Lability	1 (8.3%)	1 (7.7%)	1 (9.1%)	3 (23.1%)
Oppositional	2 (16.7%)	3 (23.1%)	1 (9.1%)	2 (15.4%)
Hyperactivity	0	1 (7.7%)	0	0
Crying Increased	1 (8.3%)	1 (7.7%)	0	1 (7.7%)
Insomnia, Initial	3 (25.0%)	2 (15.4%)	0	0
Insomnia, Terminal	0	0	1 (9.1%)	0
Low frustration tolerance	1 (8.3%)	2 (15.4%)	0	1 (7.7%)
Diarrhea	2 (16.7%)	0	0	0
Constipation	0	1 (7.7%)	0	0
Appetite Increased	1 (8.3%)	1 (7.7%)	0	0
Weight Increased	1 (8.3%)	0	0	0
↓Attention or Concentration	1 (8.3%)	2 (15.4%)	0	0
Rituals or Repetitive Behaviors	0	0	0	1 (7.7%)

Silliness	1 (8.3%)	1 (7.7%)	0	0
Anxiety	1 (8.3%)	0	0	0
Apathy	0	0	0	1 (7.7%)
Accidental Injury	1 (8.3%)	2 (15.4%)	0	0
Allergies	1 (8.3%)	0	1 (9.1%)	1 (7.7%)
Restlessness/Akathisia	1 (8.3%)	1 (7.7%)	0	0

We have been able to access the raw adverse event data from these three trials and have aggregated it to provide adverse event data on 48 individuals with ASD exposed to oxytocin for at least 6 weeks and 46 individuals with ASD treated with placebo. This aggregation, shown in Table 2, found low rates of adverse events in both oxytocin and placebo groups and no signal for greater adverse events with oxytocin treatment. None of these studies identified any significant or systematic changes in electrolytes or vital signs. The apparently higher rates in the UNC Pilot study probably reflect differences in the methods of assessing adverse events. The UNC pilot study used systematic elicitation of adverse events (same instrument as proposed for SOARS-B trial) and the other studies used spontaneous report or much more limited questionnaires.

Table 2. Aggregated adverse events with sustained intranasal oxytocin treatment in ASD

Adverse Event	**6-8 wks Oxytocin (n=48) %**	**Placebo (n=46) %**
Increased Anxiety/Panic Attack	0	2%
Increased Social Withdrawal	0	2%
Increased Tics	0	2%
Decreased Attention	2%	2%
Increased Restlessness	2%	2%
Disinhibition	2%	2%
Low Frustration Tolerance	2%	2%
Agitation	4%	2%
Oppositionality	4%	7%
Irritability	6%	7%
Mood Lability	4%	7%
Increased Crying	2%	2%
Aggression	2%	9%
Insomnia	6%	4%
?Absence Seizure	2%	0
Fatigue	2%	9%
Increased appetite	0	2%
Decreased appetite	0	2%
Increased thirst	8%	17%
Increased urination	8%	11%
Lightheaded	4%	7%
Vomiting	4%	2%
Nausea	6%	2%
Diarrhea	0	4%

Headache	6%	7%
Shortness of Breath	4%	2%
Increased allergies	6%	2%
Cough	0	2%
Rash	0	2%

There is also preliminary safety data regarding even more extended oxytocin treatment for up to 6 months in children with ASD available by personal communication from from an ongoing trial funded by DOD being conducted by Evdokia Anagostou in 11 youth 10-17 years treated for up to 6 months treated with oxytocin yielding a total of 22 youth. There have been no significant lab abnormalities in these participants. Three (14%) have shown increases in irritability or mood lability and one has had increased allergy symptoms.

Blood Draw Risks:

During laboratory blood draws the risks involved are pain, bruising, and rarely, infection at the location where the blood was taken. This risk will be minimized by having a trained professional take each participant's blood. We will attempt to avoid these risks by using aseptic techniques, and applying pressure after the phlebotomy. A local anesthetic may be used to reduce associated pain if the participant wishes to use it. If a subject becomes nervous or agitated about the phlebotomy procedures, we will utilize an anti-anxiety medication to calm subjects who may be nervous.

Psychological/Psychiatric Risk:

This patient population can be at risk for worsening of psychiatric/psychological symptoms that may be attributed to their mental illness alone, medication non-adherence, or a number of other causes that may not be related to this study. The Principal Investigator will use clinical judgment to assess each treatment emergent adverse event and determine intensity and relatedness to the study. In cases of worsening of symptoms patients may require inpatient hospitalization. If the study doctor feels that the subject is at serious risk for hurting themselves or others, he/she can ask a judge to allow the participant to be hospitalized against his/her will. Subjects may become frustrated during study procedures. Research staff will work to ensure that all directions and questions are easy to understand for subjects who have intellectual difficulties. Subjects will be allowed breaks, if needed.

Since this is a large trial of oxytocin that will require patient involvement for up to 52 weeks (1 year), a subject's psychiatric care will be assumed by the study physician.

Confidentiality Risk

The potential indirect risks are related to loss of confidentiality and could include someone such as an insurance agency or employer learning that the participant has a serious mental illness with resulting potential stigmatization. It is also possible that public knowledge of this diagnosis or its documentation in the medical record could lead to inability to obtain insurance coverage at reasonable rates in the future.

Additional risk for OPTIONAL biomarker/genetics portion:
There are no additional needle sticks for this portion of the study. However, the additive in the DNA collection tube can be irritating if it contacts the skin so special care will be taken so that, during the blood draw, the additive in the tube doesn't flow out into the collection needle.

2 OBJECTIVES

The ACE SOARS Network's immediate goals are to translate these exciting findings regarding oxytocin's neurobehavioral effects into an evidence-based, widely accessible intervention for ASD's fundamental impairments in reciprocal social behaviors and to identify factors that differentially influence response to oxytocin treatment in ASD. Our central hypothesis is that intranasal oxytocin will partially reverse the early pathophysiologic alterations in social orienting and the salience of social rewards present in ASD, which lead to decreased social motivation and ultimately to ASD's core social communication impairments, thereby enhancing reciprocal social behaviors (see Figure 1, in *Impact section*). Sustained improvements in social motivation and social reciprocity are expected to facilitate communication and learning and ultimately improve functioning. We will accomplish our immediate goals by conducting SOARS-B, a large (n=300) randomized, double-blind trial of sustained (six month) treatment with intranasal oxytocin in children 3-17 years old with ASD and examining clinical and biological factors that may predict or enhance response. Regardless of SOARS-B's outcome, its results will significantly impact the care of people with ASD by definitively testing a very promising translational treatment strategy in a highly generalizable sample. The moderator analysis is likely to support treatment personalization. The exploratory epigenetic studies will enhance understanding of the regulation of key biological pathways in ASD and facilitate development of future treatments.

2.1 Specific Aims

Specific Aim 1: **Determine the efficacy of intranasal oxytocin treatment in children with ASD.**
Analysis 1a: Compare oxytocin treatment to placebo for improving reciprocal social behaviors.
Hypothesis: oxytocin will reduce maladaptive social behaviors and increase prosocial behaviors.
Analysis 1b: Compare oxytocin treatment to placebo for improving reciprocal social behaviors separately in subgroups of children who have significant language impairment and intellectual disability and those whose language and intellectual abilities are in the normal range at baseline.
Hypothesis: oxytocin's actions on the social reward system are unrelated to verbal or cognitive abilities.
Analysis 1c: Compare the treatments' enhancement of social motivation, cognitive skills and functioning. *Hypothesis: oxytocin will increase social motivation, improving acquisition of cognitive and functional skills.*

Specific Aim 2: Provide information about the safety and tolerability of intranasal oxytocin.
Analysis 2a: Compare incidence and severity of treatment emergent adverse effects, clinically significant laboratory and electrocardiogram changes, serious adverse events and treatment discontinuation due to tolerability issues over six months in the oxytocin and placebo groups.
Analysis 2b: Tabulate these safety indicators during six months of open oxytocin treatment

<u>Specific Aim 3</u>: Identify clinical or biological traits that preferentially influence response to oxytocin.
Analysis 3a: Determine if baseline age influences behavioral changes more in the oxytocin group. *Hypothesis: the magnitude of changes observed depends on both intervention efficacy and brain plasticity.* **Analysis 3b:** Determine if baseline *OXTR* methylation levels predict level of behavioral improvement.
Working hypothesis: greater OXTR methylation decreases oxytocin receptor density and increases the amount of oxytocin required to bind available receptors and elicit optimal reciprocal social behaviors.
Analysis 3c: Determine if other baseline traits or intervening experiences interact preferentially with oxytocin

<u>Exploratory Aim 4:</u> Describe the changes in *OXTR* methylation and mRNA expression of genes related to oxytocin signaling occurring over six months with oxytocin and placebo treatment.
Rationale: understanding the regulation of oxytocin signaling will facilitate development of novel treatments.

2.2 Study Outcome Measures

2.2.1 Primary Outcome Measures

Our primary outcome is reciprocal social behaviors, which we will assess using two co-primary measures. The first measure is the ABC-SW subscale, which is being used in other clinical trials focusing on the core social and communication symptoms of autism. The other measure is a combined social score from the ABC-SW, which primarily captures aloof, and avoidant behaviors, and the Pervasive Developmental Disorders Behavior Inventory-Screening Version (PDDBI-SV, Cohen 2011), a recently developed measures which assesses both maladaptive social problems and social skills. The PDDBI-SV is comprised of 18 items included in the older, more comprehensive Pervasive Developmental Disorders Behavior Inventory (PDD-BI, Cohen 2003 (x2)). The PDDBI-SV assesses both social impairments typically associated with the active but odd subtype of ASD and development of pro-social skills that are integral to improved reciprocal social behavior. The PDDBI-SV results in a raw SOCDEF score, which is the sum of the reverse-scored social skills and the regularly-scored problems, and a SOCDEF T score. In marked contrast to the PDD-BI's age-based standard scores (for children 1.5 and 12.5 years old), the PDDBI-SV SOCDEF score does *not* change with age in individuals with ASD. The PDDBI-SV has been validated in original PDD-BI development sample of 311 children 1-17 years old and a recently-acquired sample that includes 145 youth between 13 and 15 years old, 90 between 16 and 19 years, and 59 between 20 and 40 years (Cohen 2011, personal communication). The combined social score will be calculated by discarding the three lethargy items from the ABC-SW (questions 3, 32, and 53, which all assess reduced physical movement), summing the 13 remaining items of the ABC-SW subscale, and adding it to the PDDBI-SV raw SOCDEF score. The combined social score is derived from two well-validated instruments and has face validity for capturing the full range of impairments in reciprocal social behaviors observed in ASD. We chose not to use the Social Responsiveness Scale (SRS), which was developed to provide a quantitative measure of social impairments typically observed in ASD in children 3-18 years old, because it has been demonstrated to be

quite stable over time (Constantino 2000, 2009) and no intervention studies have clearly demonstrated its sensitivity to change.

2.2.2 Secondary Outcome Measures

Secondary Outcome Measures: We will use the SRS Social Motivation subscale to assess oxytocin's hypothesized mechanism of action. Cognitive skills will be assessed using the Stanford Binet-5th Edition (SB-5) (Roid). If a child cannot complete the routing tests on the SB-5, they will be assessed using the Mullen. Functional skills including communication will be assessed using the standard score of the Vineland 2, the Caregiver Strain Questionnaire (Brannan 1997) and, in verbally fluent youth, a 7 item questionnaire being piloted to assess participant satisfaction with their social relationships, which is included in the appendix. The specific components of these measures tested in Aim 1c are SRS Social Motivation subscale, the NV IQ or Mullen Early Learning Composite standard score, and the Vineland Adaptive Behavior Composite. Other subscales of these measures and the other functional measures will be analyzed in an exploratory way.

2.2.3 Additional Exploratory Outcome Measures

Additional Clinical Assessments: In order to provide a comprehensive assessment of the clinical features of children in the trial we will also perform several other assessments. The Clinical Global Impressions – Improvement score, which is routinely used in pharmacologic clinical trials, will capture the study physician's global impression of response. Other symptom domains in ASD will be assessed with the original PDD-BI using raw scores since normed scores are only provided for children younger than 13, the other subscales of the ABC, and the severity score derived from the ADOS (**Gotham** 2009). We will also obtain the complete SRS to specifically assess its sensitivity to change in comparison to other outcomes. All concomitant medications and behavioral or alternative medication therapies will be recorded at each visit. Assessment of treatment-emergent adverse effects will utilize the systematic longitudinal adverse effects scale developed by Sikich and described in the oxytocin treatment section.

Exploratory Behavioral Measures: We will include two exploratory behavioral measures that seek to address gaps in currently validated measures (eg. ASD appropriate, participant evaluation of social satisfaction) or to objectively assess a social behavior that we hypothesize will be improved by oxytocin treatment. This measure is most likely to be useful in adolescents as younger children, but has not been validated in anyone above 12. We will also incorporate the Reading the Mind in the Eyes Task (**Baron-Cohen**) into our analysis, which has been shown to be sensitive to both single and sustained doses of oxytocin. However, its use is restricted to high-functioning individuals who can verbally identify specific emotions.

Biologic Outcome Measures: We will obtain blood, urine and vital signs from participants at regular intervals in order to assess the safety of oxytocin. In addition, we obtain blood from participants at screening and months 2, 6, 8, 12 and 18 that will used to assess *OTXR* differential methylation status and to assess mRNA expression. We will also obtain a blood sample from participants (and their biological parents with appropriate consent) to donate to the NIMH genetic repository for establishment of lymphoblast cell lines

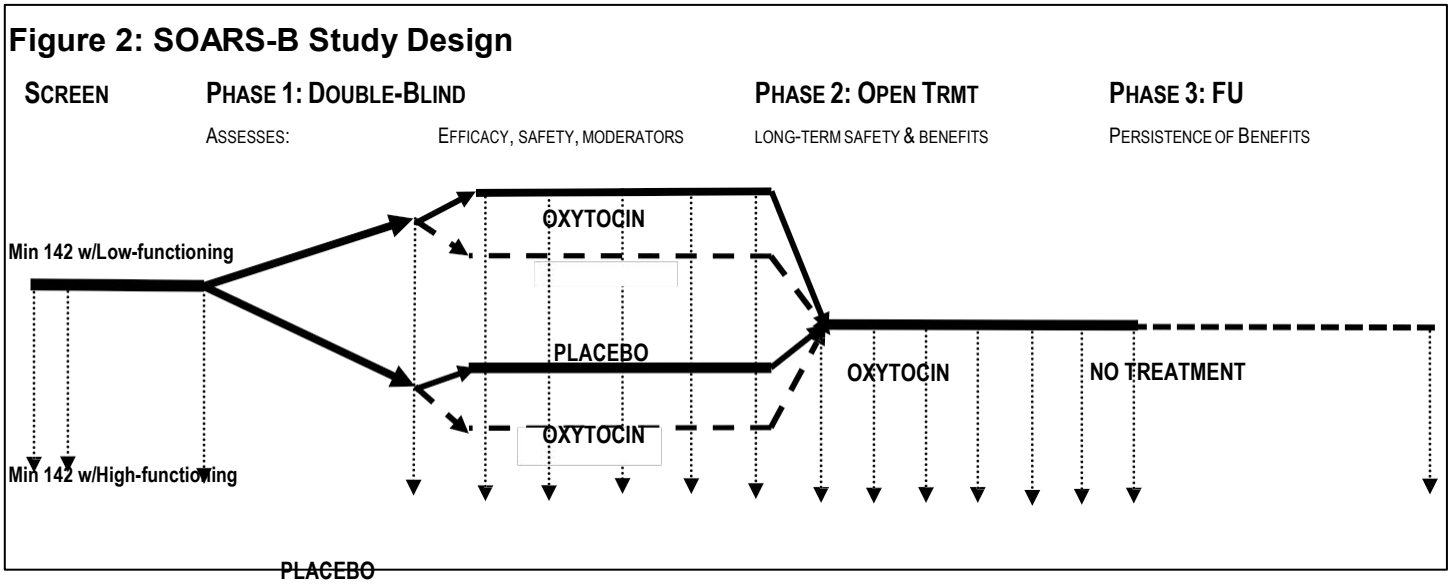
Potential interacting factors: We will assess potential environmental factors that may interact with oxytocin treatment through caregiver reports. Our main focus is on social skills treatments, interventions the child receives outside of the school setting and social opportunities outside of school settings for interactions with peers, adults who are not caregivers/treatment providers, and caregivers/treatment providers.

3 Study Design

SOARS-B is a large (n =300) randomized, placebo-controlled trial of sustained (6 month), flexible dose intranasal oxytocin treatment in children 3 to 17 years old with ASD. The primary aim is to determine the efficacy of oxytocin for improving reciprocal social behaviors in the entire sample and the low- and high-functioning subgroups. Social motivation, cognitive skills and functional behaviors will be secondary outcomes. We will examine safety during the double-blind phase and the six month open extension phase. The second major aim of the study is to identify whether participant baseline clinical and biological characteristics such as age and extent of OXTR methylation preferentially influence response to supplemental oxytocin. In post-hoc exploratory analyses, we will examine the influence of intervening processes, such as concomitant antipsychotic or social skills treatments that are likely to be randomly distributed between the oxytocin and placebo groups, on oxytocin response. Finally, participants will be assessed six months after stopping oxytocin to assess maintenance of treatment effects

See figure 2 for overall study design.

Figure 2: SOARS-B Study Design



Screen* **Stratified Randomization** **M0** M1 **M2*** M3 **M4** M5 **M6*** M7 **M8** M9 **M10** M11 **M12*** **M18***

***indicates genetic sample taken. Primary outcome assessed at bolded time points.**

4 STUDY ENROLLMENT AND WITHDRAWAL

4.1 Subject Inclusion Criteria

- Be between the ages of 3 years 0 months and 17 years 11 months at the time of randomization
- Be diagnosed by clinician experienced in assessment of ASD with autistic disorder, Asperger's syndrome, or PDD-NOS using DSM-V-TR criteria
- Must have clinical diagnosis of ASD confirmed using the Autism Diagnostic Observation Scale (ADOS, Lord et al., 2001)
- Must have clinical diagnosis of ASD confirmed using the Autism Diagnostic Interview-Revised (ADI-R, Rutter, 2003). ASD criteria proposed by Risi (2006). Specifically, subject must be within 1 point of autism criteria on both social and communication domains of the ADI **or** meet autism criteria in one of these ADI domains and come within 2 points of autism criteria in the other
- Have a guardian who is able to provide informed consent
- If cognitively able, subject must be able to provide informed assent/consent

4.2 Subject Exclusion Criteria

- Have a known diagnosis of Rett Syndrome or Childhood Disintegrative Disorder, or have marked sensory impairment such as deafness or blindness
- Have active cardiovascular disease or renal disease that is not controlled by medication
- Subjects who are pregnant, lactating, or who refuse to practice contraception if sexually active
- Subjects who have had changes in allied health therapies, behavioral or educational interventions within the two months prior to randomization other than those associated with school holidays
- Subjects who have had changes in medications or medication doses of risperidone, aripiprazole, other antipsychotic medications, clonidine, guanfacine, stimulants or anti-convulsants within four weeks of randomization
- Subjects who have had previous chronic treatment with oxytocin
- Subjects who are currently being treated with an agent that affects brain serotonin levels, including St. John's Wort
- Subjects who use benzodiazepines on a regular basis (i.e. 3 of 7 days in a week)
- Subjects who have caretakers who are unable to speak English, be consistently present at visits to report on symptoms, or are otherwise judged as unable to comply with the protocol by the data collection site team

4.3 Rationale for Inclusion and Exclusion Criteria

Frequently when large trials are done with a very specific subpopulation, no further studies are done to explore response in other subgroups because of the large cost of such trials and public frustration with studies perceived as duplicative and incremental. However, families and clinicians caring for individuals from untested subgroups often struggle to make treatment decisions without any specific evidence regarding efficacy or safety. Therefore, we have made

the entry criteria for participation in this trial as broad and generalizable as possible. We will conduct secondary post-hoc analyses of various clinical and biological factors that might typically be excluded (eg. concomitant antipsychotic treatment, Fragile X) instead. This approach will provide the greatest amount of clinically relevant information regarding sustained oxytocin treatment in shortest amount of time possible.
We also will allow psychotropic medications with strong evidence of efficacy for treating problem behaviors or psychiatric disorders frequently associated with ASD (specifically antipsychotics, anticonvulsants, stimulants, and other medications approved for ADHD) to increase generalizability. Agents that influence serotonergic neurotransmission or specifically impair reward pathways in the brain (eg. naltrexone) will be excluded because they lack evidence supporting their use in ASD and are likely to interfere with neural systems affected by oxytocin.
We chose not to require a minimal baseline score on the Aberrant Behavior Checklist –social withdrawal subscale (ABC-SW, **Aman** 1994) due to concerns this would exclude many people with ASD, even though such a threshold would increase comparability with an ongoing trial also targeting ASD's core social behaviors and might increase the probability of observing ABC-SW changes. Also, diagnosis of ASD requires significantly impaired reciprocal social behavior.

We are including very young children because we believe they are likely to demonstrate significantly greater functional improvements than older children are as a result of increased intervention services and greater brain plasticity. We believe that the careful titration schedule and close adverse effect monitoring (phone calls at Month 0.5 and 6.5, along with a dosing administration handout that staff will provide to participants from Baseline (Month 0) to Month 11 to monitor progress between office visits) will provide sufficient safety for this vulnerable population. Further, the prospective, systematically elicited information about safety in young children gained in this study will inform the design of future trials and off label use in young children.

We are including children with low cognitive functioning because there is no rationale for believing that they would be less likely to respond to oxytocin treatment. Such children are seldom included in clinical trials and desperately need effective treatments to reduce their suffering. We expect that we will still be able to measure social reciprocity which will be valuable in studying the effects of oxytocin on children with autism of varying cognitive functioning.

We will exclude subjects with non-English speaking caretakers because our staff is not qualified (i.e. they are not fluent in other languages) to explain the study to non-English speaking subjects. In addition, many of the pencil and paper assessments used to diagnose the included diagnoses have not been normed with non-English speaking populations. Thus, the measures may not be as appropriate, valid, and/or reliable for non-English speaking subjects.

4.4 Strategies for Recruitment and Retention

Before the study is initiated, it will be approved by the Institutional Review Board (IRB) at each treatment site and the Network DSMB. All of the subjects and their parent/guardian will provide consent/assent for participation in this study. In addition to the informed consent requirements,

per the Privacy Rule (HIPAA) regulations, research participants will provide a written "authorization" to use their Protected Health Information (PHI) in connection with research. In requesting an authorization from potential participants, investigators will specify how the information will be used and how the privacy of that information will be protected. The researcher obtaining Informed Consent will thoroughly review the consent form with the child and his or her parents, including study procedures and potential risks and benefits of study participation. The child and parents will be encouraged to ask questions throughout the process. It will be emphasized that research is voluntary and that the subject can opt out of this project at any time without jeopardizing his/her treatment. Participants who turn 18 during their participation in the study and who have the cognitive capacity to read and understand the consent form, and whose parents do not have legal guardianship of them, while participating in this study will be re-approached by one of the physician investigators. The investigator will continue the informed consent process acknowledging that the individual is now legally an adult and will ask the participant if they voluntarily wish to continue their participation in the study. If he/she does, the participant will be asked to sign the informed consent document and a new HIPAA authorization. If the individual does not wish to continue participation they will be withdraw from study treatment just as any other individual who chooses to withdraw consent from participation.

This study will be registered in ClinicalTrials.gov no later than 21 days after the first subject is enrolled. Summary results information (including adverse events) will be reported no later than 1 year after the completion date for the study. Grant and progress report forms shall include a certification that the investigators have made all required submissions to ClinicalTrials.gov.

4.5 Treatment Assignment Procedures

4.5.1 Randomization Procedures

We expect to screen ~ 400 children in order to randomize 300. Individuals who meet all inclusion criteria and none of the exclusion criteria, will be categorized by the data base according to the following criteria to determine if they belong in the high or low functioning stratum. Using a centralized, randomization scheme with permuted blocks of 4 or 6, stratified only by functional status, the database will randomly assign the treatment which will be communicated to the site investigational pharmacy. Enrollment within the two strata will be monitored to ensure that there are at least 142 participants in each and that at least 21% of the participants within each stratum fall into each of the specified age groups 3-6, 7-11, 12-17. The minimal representation of these age groups should ensure that the potential moderating effects of age can be fully assessed. We will not stratify by age due to the potential for very small cell sizes with large numbers of strata. If a functional or age subgroup appears to be under represented after 50%, 75% and 90% of participants are randomized, recruitment efforts will be focused on the under represented subgroups. Children will be considered low-functioning if they have significant language impairment and intellectual disability (NV IQ or Mullen Early Learning Composite Standard Score < 70). We define significant language impairment in participants 72 months or older, as having a lack of fluent phrase speech reflected by use of ADOS Module 1 or 2. In participants younger than 6 years, significant language impairment will be defined as lack of phrase speech

and Mullen receptive language subtest score more than 1 SD below the mean for age. Participants will be considered as high-functioning if they have nonverbal IQ ≥ 70 and significant language skills, which we define as fluent phrase speech reflected by use of ADOS Module 3 or 4, *or*, if younger than 6 years, Mullen receptive language subtest score within 1 SD of the mean for age or higher. Participants who fail to meet both the language and cognitive criteria for low- or high-functioning will be stratified according to their language abilities, which more strongly predict long-term outcome (Venter 1992; Szatmari 2003; Sallows 2005; Billstedt 2007).

4.5.2 Masking Procedures

The spray and a normal saline nasal solution (the placebo) will be packaged in indistinguishable nasal administration bottles. There will be four possible bottles: Low concentration oxytocin/placebo with yellow labels and High concentration oxytocin/placebo with blue labels

5 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

5.1 Study Product Description

Oxytocin contains the synthesized peptide oxytocin in a solution formulated to promote absorption through the nasal mucosa. There will be two concentrations of spray 80IU and 240IU. The concentration of 80 IU of oxytocin/1ml and each 0.1 ml nasal insulfation delivers 8 IU of oxytocin. The concentration of 240 IU of oxytocin/1ml and each 0.1 ml nasal insulfation delivers 24 IU of oxytocin.

5.1.1 Acquisition

Oxytocin and placebo will be compounded by Triangle Compounding Pharmacy in Cary, NC with full adherence to GMP standards. All the proper documentation regarding these compounded solutions will be submitted to the FDA as an amendment to the current IND (#111604) held by Dr. Linmarie Sikich for sustained intranasal oxytocin treatment in 3-17 year olds with autism.

5.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

Participants or their caregivers will be taught to administer the nasal spray, alternating nostrils, by the study personnel. The caregivers will be provided with suggestions for how to encourage their children to comply with the procedure including setting up a fun routine and providing rewards. The caregivers will also be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit and the dosing (if applicable).

Participants in the study will be randomly assigned to treatment with intranasal oxytocin or placebo. Each insufflation will deliver 8 IU or 24 IU of oxytocin. A maximum of 3 insufflations at a time will be required. Dosing will be flexible between 8 IU/day and 80 IU/day, typically in

two divided doses delivered in the morning and in the afternoon. Doses will increase by 8 IU twice daily (BID) every 15 days until achieving the target dose of 24 IU BID at Month 1. Subsequently doses may be increased in 8 IU BID increments every month until a maximum dose of 40 IU BID is achieved. Doses may be reduced by 8/0 IU BID if safety concerns emerge or if the study physician, caregiver or participant feels a lower dose was more beneficial. Once daily dosing will be permitted for participants who do not tolerate 8 IU BID. Similar titration will be used during open-label treatment. Adherence to treatment will be assessed by caregiver report and weighing returned treatment bottles. See table 3.

Table 3: Oxytocin Flexible Dosing Strategy

Oxytocin/Placeb	**M0**	**W2**	**M1**	**M2**	**M3**	**M4**	**M5**
Open Oxytocin	**M6**	**W28**	**M7**	**M8**	**M9**	**M10**	**M11**
Expected Dose given AM & 3pm	8 IU AM only	8 IU	16 IU	**24 IU**	24-32* IU	24-40* IU	24-40* IU
Total Daily Dose	8 IU	16IU	32 IU	**48 IU**	48 – 64* IU	48 – 80* IU	48 – 80* IU
Yellow Bottle LOW concentration 8 IU/spray	1 spray in AM	1 spray 2x/day	2 sprays 2x/day	None	None if up-titrate, 1-2 sprays 2x/day	None if up-titrate, 1-2 sprays 2x/day	None if up-titrate, 1-2 sprays 2x/day
Blue Bottle HIGH concentration 24 IU/spray	None	None	None	1 spray 2x/day	1 spray 2x/day	1 spray 2x/day	1 spray 2x/day

5.3 Accountability Procedures for the Study Intervention/Investigational Product(s)

Subjects (or there parent/LAR) will be asked to return all previously dispensed product at the next office visit. Staff will return all unused product to the site's investigational pharmacy for destruction.

5.4 Assessment of Subject Compliance with Study Intervention/Investigational Product

Due to the difficulties of measuring compliance with weight, we will utilize a parent completed diary to measure subject compliance with medication treatment. Each daily dose morning and afternoon, will be recorded by the parent/caregiver on this diary.

5.5 Concomitant Medications/Treatments

All concomitant medications will be recorded. Current medications will be defined as any medications taken in the last month or being taken presently. The physician will record any changes to the subjects' psychiatric and non-psychiatric medications from baseline throughout the course of the study. Subjects who are currently being treated with an agent that affects brain serotonin levels, including St. John's Wort will be excluded. If a participant takes one of these agents during the course of the study, he she will be withdrawn by the investigator.

Table 4: SCHEDULE OF EVENTS	Scrn	Double Blind Treatment								Open Treatment							Post Tx f/u
Procedure	1 or 2	M0	M 0.5	M1	M2	M3	M4	M5	M6/T	M 6.5	M7	M8	M9	M10	M11	M12/T	M 18
ADOS-2	X								X							X	
ADI-R	X																
Stanford Binet/Mullen	X																
DSM-V Checklist	X																
Inclusion/Exclusion	X	X															
Demographics		X															
Family Med Hx (NIH Form)	X																
Randomization Form		X															
GUID Acquisition Form																	
GUID Record Form		X															
ConMeds	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Vital Signs	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Med/Psych History (SLAES)	X																
Adverse Effects (SLAES)		X		X	X	X	X	X	X		X	X	X	X	X	X	X
Physical Exam (NIH Form)	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Medical History (NIH Form)	X																
CGI S and I[0]		X		X	X	X	X	X	X		X	X	X	X	X	X	X
Social Skills Therapies		X		X	X	X	X	X	X		X	X	X	X	X	X	X
Psychosocial Therapies		X		X	X	X	X	X	X		X	X	X	X	X	X	X
Laboratory																	
ECG	X								X							X	
Female Reproductive Status	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X
Urine/Serum Pregnancy[1]	X																
Safety labs[2]	X								X							X	
mRNA sample	X								X							X	
Methylation sample	X				X				X			X				X	X
Plasma oxytocin	X				X				X			X				X	X
Whole blood serotonin	X				X				X			X				X	X
Genetic repository sample-subj[3]					X												
Genetic repository –parents[4]					X												
Parent Questionnaires																	
Questionnaire Guidance Form		X		X	X	X	X	X	X		X	X	X	X	X	X	X
Social Opportunity Questionnaire		X		X	X	X	X	X	X		X	X	X	X	X	X	X
ABC		X			X		X		X			X		X		X	X
PDDBI-SV					X		X					X		X			
PDDBI-Full		X							X							X	X
CASI		X							X							X	X
Caregiver Strain		X							X							X	X
Vineland-II (Survey Form)		X				X			X				X			X	X
SRS-2		X				X			X				X			X	X
Subject Completed Questionnaires																	
Reading Mind in Eyes Test[5]		X			X				X			X				X	X
Self-Rating of social function		X							X							X	X
Medication																	
DVD training (med administration)*		X															
Med Administration Form*		X															
Dosing Guide[7]		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Risks Handout		X							X								
Medication Compliance[6]				X	X	X	X	X	X		X	X	X	X	X	X	
Oxytocin Dosing Log (staff)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Medication Diary Dispensed		X		X	X	X	X	X	X		X	X	X	X	X		

0-CGI-S only at baseline, CGI-S and I at all other visits 1-Required at screening but can be done at any time during the course of the study at physician discretion 2-Safety labs to include: glucose (random), CO2, Cl, K, Na, Creatinine, BUN, AST, ALT, urine specific gravity, urine pregnancy in pubertal girls 3- Preferred time to collect, but can be done at any time 4- One sample is requested from each biological parent, can be obtained at any point during the study 5- Assessment will be obtained only in participants who have fluent phrase speech & can define basic feelings and friendship 6- Completed each time the participant returns study medication

7- Dosing guide should be given at each visit (and also phone calls where the dosing has changed) Staff will circle the appropriate column for dosing that the patient should follow

*Must be given at baseline, but also can be given at any other time during the course of the study at study staff discretion

6.1 Screening

Screening Visit (can be divided into two visits, if necessary) ~ approximately 6 hours
Subjects will complete the following procedures and assessments at the Screening visit:

- Provide written informed consent/assent (subject and/or parent/caregiver/LAR)
- Inclusion/Exclusion criteria checklist
- Medical/psych history completed by medical physician using SLAES
- Medical history and family medical history using the NIH-specific form
- Urine or serum pregnancy test for females of childbearing potential
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- ECG (pediatric cardiologist will read/confirm)
- Review of current concomitant medication use
- Physical examination (examination of body systems and measurement of height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, and temperature)
- Safety Labs
- Methylation/mRNA sample (Optional)
- Plasma Oxytocin
- Whole blood serotonin
- Genetics (Optional)
- ADOS-2 and ADI-R
- Stanford Binet (abbreviated-ABIQ) or Mullen
- DSM-V Checklist

Subjects may return for a second Screening visit in cases where assessments could not be completed in one visit (i.e., time constraints, subject noncompliance, etc.).

The first set of Genetics and Plasma Oxytocin samples will be drawn at the Screening visit(s) in conjunction with safety labs to reduce the number of needle pricks during the study. However, the subject may choose to wait to have these samples drawn until their Baseline visits once their eligibility has been confirmed. If the samples have been collected and the subject is determined to be ineligible, the blood samples collected for these purposes will be destroyed.

6. 2 Randomized Double-Blind Treatment Phase: Oxytocin or Placebo (6 months; Month 0-Month 6)

Baseline Visit (Month 0) ~ approximately 3-4 hours
Subjects will complete the following procedures and assessments at the Baseline visit:

At the baseline visit, enrolled subjects will undergo the following procedures:

- Confirmation of Inclusion/Exclusion criteria
- Review all current medications (concomitant medication log)
- Physical examination
- Full vital signs (heart rate, blood pressure, temperature)

- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- Demographics (age, sex, race, ethnicity, date of birth)
- SLAES (monitoring for adverse events)
- Oxytocin Dosing Log
- Social Reciprocity Scale (SRS-2)
- Vineland Adaptive Behavior (Vineland-II Survey Form)
- Caregiver Strain Questionnaire
- Aberrant Behavior Checklist
- PDDBI-Full
- CASI (Sprafkin 2002, Gadow 2009)
- CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET)
- Self-Rating of Social Functioning
- Medication Diary dispensed to caregiver
- Parent Handout (study drug administration instructions)
- Parent Handout (study drug dosing guidelines)
- Parent Handout (risks)
- Randomization
- GUID Record Form
- DVD for administration instructions

Participants or their caregivers will be taught to administer the nasal spray, alternating nostrils, by the study coordinators who will demonstrate medication administration through use of a DVD training module. The caregivers will be provided with suggestions for how to encourage their children to comply with the procedure including setting up a fun routine and providing rewards and will also be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Subjects will be given a 4 week supply of Syntocinon® /placebo nasal spray to take twice a day. Caregivers will be instructed to administer the first dose on the afternoon of the baseline visit, between 12PM and 4PM. Parents will be given a hand out which highlights all the cardiac and anaphylaxis risks. The handout will also include information about how to monitor their child's pulse and what the normal ranges for pulse are based on age. Staff will review handout with parent to ensure that they understand all aspects of the handout.

Month 0.5 Phone Call ~ approximately 10-15 minutes

Between the Baseline and Month 1 visit, the study physician will contact subjects and their parent/guardian or LAR (if applicable) to see how the subject is doing on the medicine and to

monitor for any adverse events. During this phone call, staff will circle the appropriate column for dosing that the patient should follow (if the study physician believes the subject is tolerating the medication and can titrate up to the next dose).

Month 1 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 1 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Medication Diary dispensed to caregiver
- Oxytocin Dosing Log
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 2 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 2 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial TherapyLog
- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET)
- Methylation sample (Optional)
- Plasma Oxytocin
- Whole blood serotonin
- Genetics (Optional)
- Medication Diary dispensed to caregiver

- Oxytocin Dosing Log
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 3 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 3 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Oxytocin dosing log
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Vineland
- SRS-2
- Medication Diary dispensed to caregiver
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 4 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 4 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial TherapyLog
- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Medication Diary dispensed to caregiver
- Oxytocin Dosing Log

- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 5 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 5 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Medication Diary dispensed to caregiver
- Oxytocin Dosing Log
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

6. 3 Open-Label Treatment Phase

Month 6 ~approximately 3-4 hours (start of Open-Label Treatment Phase)

Subjects will complete the following procedures and assessments at the Month 6 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- Vineland
- CSQ
- CASI
- PDDBI-Full
- Questionnaire Guidance Form
- Social Opportunity Questionnaire

- Reading Mind in Eyes Test (RMET)
- Self-Rating of Social Functioning
- SRS-2
- Methylation/mRNA sample (optional)
- Plasma Oxytocin
- Genetics (optional)
- ECG
- Safety Labs
- Whole blood serotonin
- Medication Diary dispensed to caregiver
- Risks Handout
- ADOS-2
- Oxytocin Dosing Log
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 6.5 Phone Call ~ approximately 10-15 minutes

Between the Month 6 and Month 7 visit, the study physician will contact subjects and their parent/guardian or LAR (if applicable) to see how the subject is doing on the medicine and to monitor for any adverse events. During this phone call, staff will circle the appropriate column for dosing that the patient should follow (if the study physician believes the subject is tolerating the medication and can titrate up to the next dose).

Month 7 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 7 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Medication Diary dispensed to caregiver
- Oxytocin Dosing Log
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 8 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 8 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial TherapyLog
- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET)
- Methylation sample (optional)
- Plasma Oxytocin
- Whole blood serotonin
- Genetics (Optional)
- Medication Diary dispensed to caregiver
- Oxytocin Dosing Log
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 9 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 9 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Oxytocin dosing log
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Vineland
- SRS-2

- Medication Diary dispensed to caregiver
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 10 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 10 visit:
Subjects will complete the following procedures and assessments at the Month 4 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial TherapyLog
- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Medication Diary dispensed to caregiver
- Oxytocin Dosing Log
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 11~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the Month 11 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Medication Diary dispensed to caregiver
- Oxytocin Dosing Log

- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered.

Month 12 ~ approximately 3-4 hours (End of Treatment Visit)
Subjects will complete the following procedures and assessments at the Month 12 visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events)
- ADOS-2
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- Vineland
- CSQ
- CASI
- PDDBI-Full
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET)
- Self-Rating of Social Functioning
- SRS-2
- Methylation sample/mRNA (optional)
- Plasma Oxytocin
- Genetics (optional)
- ECG
- Safety Labs
- Whole blood serotonin
- Oxytocin Dosing Log

6. 4 Follow Up Phase

Month 18 ~ approximately 3-4 hours
Subjects will complete the following procedures and assessments at the Follow-Up visit:

- Review all current medications (concomitant medication log)
- Physical examination (including height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, temperature)
- SLAES (monitoring for adverse events)
- CGI-I and CGI-S

- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- Vineland
- CSQ
- CASI
- PDDBI-Full
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET)
- Self-Rating of Social Functioning
- SRS-2
- Methylation sample (optional)
- Plasma Oxytocin
- Genetics (optional)
- Whole blood serotonin

6.5 Early Termination Visit

If a subject terminates from the study early, then all month 6/12 procedures will be completed.

6.6 Unscheduled Visit

Subjects may be asked to come in for an unscheduled visit at any time during the course of the study if the treating physician feels it is necessary to see the subject in person. During these visits, all efforts should be made to obtain the following assessments:

- Vital signs
- Review of concomitant medications
- SLAES (adverse events)
- CGI-S and CGI-I

7 STUDY PROCEDURES/EVALUATIONS

7.1 Study Assessments

Parent Assessments

- *Questionnaire Guidance Form:* This form will give parents guidance on time frames and specific instructions for how to complete the questionnaires. This form was created by study staff with guidance from manuals from the standardized forms listed below.
- *Social Opportunities Questionnaire*: This UNC created form asks parents to rate how frequently their child has the opportunity to interact with different individuals in the community, home, school and daycare/after-school setting. It also asks, of those opportunities that their child has, does their child actually utilze those opportunities to interact with individuals in a social manner.

- *Social Reciprocity Scale-2*: This 65-item rating scale measures the severity of autism spectrum symptoms as they occur in natural social settings (Constantino et al. 2003). Completed by a parent or teacher in just 15 to 20 minutes, the SRS provides a clear picture of a child's social impairments, assessing social awareness, social information processing, capacity for reciprocal social communication, social anxiety/avoidance, and autistic preoccupations and traits. It is appropriate for use with children from 4 to 18 years of age.

The SRS measures impairment on a quantitative scale across a wide range of severity--which is consistent with recent research indicating that autism is best conceptualized as a spectrum condition rather than an all-or-nothing diagnosis. The SRS-R will take the parent/caregiver/LAR approximately 10 minutes to complete.

- *Pervasive Developmental Disorders Behavior Inventory (PDDBI- Full and PDDBI-Screening Version)* - The PDD-BI examines both adaptive and maladaptive behaviors related to autism. It has normative scores for children between 2 and 11 years. For children 12 years and older, the norms (11 years, 11 months) will be used. This should take the caregiver approximately 10 minutes to complete. The screening version is a much shorter 18 item questionnaire.
- *Aberrant Behavior Checklist (ABC)*-The ABC focuses on problem behaviors in five subdomains: irritability, attention, repetitive behaviors, unusual speech, and social withdrawal. This should take the caregiver approximately 10 minutes to complete.
- *Caregiver Strain Questionnaire* (CSQ – Berument): The CSQ will assess family stress and was developed for caregivers of children with developmental disabilities. This should take the caregiver approximately 5 minutes to complete.
- *Medication Diary:* Caregivers will be asked to record each dose given to the child on a written log. Caregivers will be asked to return this at each visit for the study team to review if there are any medication compliance issues.
- *Parent Handout (Risks):* Caregivers will be given an informational handout at baseline (beginning of Randomized Phase) and again at month 6 (beginning of Open Label Phase). This handout will describe possible symptoms to look for.
- *Dosing Guide/Medication Administration Form:* Caregivers will be given an informational handout that describes study drug administration and dosing guidelines. Participants will be instructed to increase the dose per the titration schedule as long as there are no problems and will be strongly encouraged to contact the study coordinator if there are any concerns.
- *Vineland Scales of Adaptive Functioning* (2^{nd} edition, survey form): The VABS-II is a survey administered to a parent or caregiver using a semi-structured interview format, and is organized around four Behavior Domains: Communication, Daily Living Skills, Socialization, and Motor Skills. The VABS-II will take the parent/caregiver/LAR approximately 20-60 (average 30) minutes to complete, depending on the subject's age and level of functioning.
- *Childhood Anxiety Sensitivity Index (CASI):* This scale is designed for measuring anxiety sensitivity (i.e., the belief that anxiety symptoms have negative consequences

Physician/Clinician Assessments

- *Clinical Global Impressions Scale:* Overall psychiatric functioning will be assessed with the severity (CGI-S) and improvement (CGI-I) subscales of the CGI (Guy 1976). CGI-S items are rated from 1 (normal, not ill) to 7 (very severely ill). CGI-I items are rated from 1 (very much improved) to 7 (very much worse).

- *Physical Examination:* A physical examination will include abdominal palpation to assess for liver enlargement, assessing for decreased tone and postural problems, increased drooling and poor orofacial tone, problems holding head up as well, difficulty with coordination of motor actions.
- *Concomitant Medication Log*: At screening, the physician will make a list of any psychiatric medications the subject has taken in the past and are currently taking. Current medications will be defined as any medications taken in the last month or being taken presently. The physician will record any changes to the subjects' psychiatric and non-psychiatric medications from baseline throughout the course of the study.
- *Inclusion/Exclusion:* A checklist will be completed to ensure all inclusion and none of the exclusion criteria are met.
- *DSM-V Checklist for Autism:* The proposed DSM-V criteria for autism will be documented in the form of a checklist for a clinician/physician to complete.
- *Family Medical History:* This form will ask for information about genetic, mental health and medical conditions of relatives of the subject.
- *Medical History:* This form will ask about the subject's medical history such as surgeries, medical procedures etc.
- *Oxytocin Dosing Log:* This log is completed by the physician to record all changes in dosing of oxytocin. It will include start/stop dates of when the dose changes.

Trained Rater/Physician

- *Systematic Longitudinal Adverse Events Scale (SLAES):* systematic elicitation and screening of adverse events will be completed using the SLAES. This will also be used at screening and baseline to obtain a comprehensive psychiatric and medical history of the patient.
- *Female Reproductive Form:* We will record the last menstrual period, document irregular periods and verify continued use of two forms of birth control (if sexually active).
- *Vital Signs:* Vital signs will be measured at each visit and will include heart rate, sitting blood pressure, and temperature.
- *Stanford Binet (5th edition):* The Stanford-Binet intelligence scale is a standardized test that assesses intelligence and cognitive abilities in children and adults aged two to 85+ years. The Stanford-Binet Scale tests intelligence across four areas: verbal reasoning, quantitative reasoning, abstract/visual reasoning, and short-term memory. The areas are covered by 15 subtests, including vocabulary, comprehension, verbal absurdities, pattern analysis, matrices, paper folding and cutting, copying, quantitative, number series, equation building, memory for sentences, memory for digits, memory for objects, and bead memory. The abbreviated IQ (ABIQ) will be used for this study and includes non verbal fluid reasoning and verbal knowledge subtests.
- *Mullen Early Scales of Learning (Mullen):* Is for birth to 68 months and is designed to assess five scales: Gross Motor, Visual Reception, Fine Motor, Expressive Language, and Receptive Language
- *Autism Diagnostic Observation Schedule-2 (ADOS-2):* The ADOS is a semi-structured assessment used to assess and diagnose individuals suspected of having autism of varying ages, developmental levels, and language skills (from no speech to verbally fluent). The ADOS includes four modules, each requiring just 35 to 40 minutes to administer. The individual being evaluated is given just one module, depending on his or her expressive language level and chronological age. The rater will observe social and communication behaviors during various activities in the appropriate module.

- *Autism Diagnostic Interview, Revised (ADI-R):* The ADI-R is a semi-structured, investigator-based interview for caregivers of children and adults for whom autism or pervasive developmental disorders is a possible diagnosis. The revised interview has been reorganized, shortened, modified to be appropriate for children with mental ages from about 18 months into adulthood and linked to ICD-10 and DSM-IV criteria. The detailed interview focuses on early development in social and communication and self-help skills of the child, and takes approximately 2 hours to administer.
- *Demographics:* Information such as age, race, ethnicity, family status and income will be collected.
- *Electrocardiogram:* Trained staff will collect an EKG on each subject and this will be read and confirmed by a pediatric cardiologist.
- *Reading in the Mind's Eye Task:* This computerized task consists of a series of pictures of eyes in which the subject needs to determine which emotion the eyes are expressing.
- *Self-Rating of Social Functioning:* This staff created assessment consists of several questions that asks the participant directly to reflect on their own experience with social skills.
- *DVD Training:* This DVD is designed to show participants how to administer the medication and also will give them guidance on how to complete the questionnaires.

Other

- *GUID Record Form:* This form will record the GUIDs that are obtained for the subject and any parent who is willing to consent to have his/her blood take for the genetic repository at NIMH
- *GUID Acquisition Form:* This form is designed to obtain all the information necessary in order to assign a GUID such as full name, date of birth, city of birth (as it is written on one's birth certificate).
- *Randomization Form:* This form will be used by study staff to enter into the database in order to determine if a subject is high functioning or low functioning for randomization purposes.
- *Social Skills Therapies Log:* This form will record the number of hours in the previous month that the child received social skills therapy.
- *Psychosocial Therapy Log:* This form will record the number of hours in the previous month that the subject received any additional psychosocial therapy such as ABA, equine therapy, speech therapy etc.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

- *Chemistry Panel:* The chemistry panel will including glucose (random, non-fasting), CO2, Cl, K, Na, Creatinine, BUN, AST, ALT. Total blood to be collected is 8.5mL in SST tube.
- *Pregnancy test:* Will be serum completed at screening but can be done at any time throughout the course of the study at the physician's discretion. This will not require any additional blood and will be collected as part of the above chemistry panel.
- *Plasma Oxytocin:* We will assay plasma oxytocin levels using standard radioimmuno assays in Dr. Pedersen's lab in order to describe potential relationships between baseline levels and treatment response. We expect there to be minimal changes in plasma levels with intranasal

administration, since oxytocin is rapidly degraded. Total 7ml of blood drawn (one 7ml lavender top tube).

- *Serotonin Levels:* Serum serotonin levels will be measured by aliquoting 200uL of whole blood from the methylation tube. No extra blood is needed for this.
- *Urinalysis:* Only specific gravity is required, however a full urinalysis panel may be run at the discretion of the physician.

7.2.2 Duke Genetics (Optional)

Optional Genetics Study: Dr. Gregory will perform the methylation and mRNA expression studies, For the methylation studies, DNA will be extracted from peripheral blood, bisulfite converted, PCR performed using primers targeted to the bisulfite-converted regions of the CpG islands in the promoter region and third intron of the *OXTR* gene, and the resulting clones sequenced to calculate percentage of methylation at each of the CpG sites. Total RNA will be extracted from blood samples, RNA quality checked and quantitative PCR run after reverse transcription using primers for *OTXR* exon 2 and the *OXT*. We will use already collected samples (after running initial analysis of mRNA expression and methylation) for unspecified genetic analysis and also for other analyses to identify possible unspecified genetic factors that may influence response to oxytocin treatment.

- Total 8.5ml of blood drawn (one 2.5ml PAX gene tube and two 3ml lavender top tubes)

7.2.3. NIMH Genetic Repository (Optional)

Subject: This sample will be obtained only once throughout the course of the study and is optional. It will be collected at the time of another blood draw, so no extra needle stick is needed. The sample will be collected by study staff and then shipped to NIMH's genetic repository for testing. There will be a data sharing agreement in place. Total amount of blood to be drawn is 7.0mL

Biological Parent: Both biological parents (if available) will be asked if they would like to provide a sample for the NIMH genetic repository. If they agree, they will each be asked to sign a different consent form. This sample will be obtained only once throughout the course of the study and is optional. The sample will be collected by study staff and then shipped to NIMH's genetic repository for testing. There will be a data sharing agreement in place. Total amount of blood to be drawn is 7.0mL

8 ASSESSMENTS OF SAFETY

8.1 Adverse Events

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel

during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor.
All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" will be captured on the appropriate source documentaiton. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, DDS, DMD, PA, Nurse Practitioner or DO), 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. Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if it worsens at any time during the study, it will be recorded as an AE.
All AEs must be graded for severity and relationship to study product.
Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity.

- **Mild:** events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate:** events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe:** events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

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

Relationship to Study Treatment: The clinician's assessment of an AE's relationship to test article (study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to study product assessed using the terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Certain: The adverse event and administration of the study drug are related in time, and a direct association can be demonstrated.
- Probably: The adverse event and administration of the study drug are reasonably related in time, and the adverse event is more likely explained by the study drug than other causes
- Possible: The adverse event and administration of the study drug are reasonably related in time, and the adverse event can be explained equally well by causes other than study drug.
- Unrelated: The adverse event is clearly explained by another cause not related to the study drug.

8.2 Serious Adverse Events

Serious Adverse Event (SAE): An SAE is defined as an AE that meets one of the following conditions:

- Death during the period of protocol-defined surveillance
- Life-threatening event (defined as a subject at immediate risk of death at the time of the event)

- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician."

8.3 Unanticipated Problems

We will consider unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4 Reporting Procedures: Serious Adverse Events

Any AE considered serious by the PI or Subinvestigator or which meets the aforementioned criteria must be submitted on an SAE form to the Coordinating Center and the independent medical monitor on the following numbers:

Coordinating Center Fax Line: 919-493-8985
Coordinating Center Phone Number: 919-972-7500
Coordinating Center E-mail: cheryl_alderman@med.unc.edu and lsikich@med.unc.ed
Medical Monitor Email: *eanagnostou@hollandbloorview.ca*
Medical Monitor Phone: 416-753-6005
Medical Monitor Fax: 416753-6046

The study clinician will complete a Serious Adverse Event Form within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the Serious Adverse Event Form and sent by fax within 24 hours of site awareness.
- Serious adverse events other than death and immediately life-threatening events, regardless of relationship, will be reported by fax within 72 hours of site awareness.

Other supporting documentation of the event may be requested and should be provided as soon as possible.

All SAEs will be followed until satisfactory resolution or until the PI or Subinvestigator deems the event to be chronic or the patient to be stable.

8.5 Stopping Procedures

Subjects may be withdrawn for any of the following reasons:

- at the participant or guardian request
- if moderate or severe adverse events occur that cannot be addressed by dose adjustment or addition of concomitant medications
- clinical worsening (much worse or very much worse on CGI-I) at two consecutive visits unless explicitly discussed and agreed with SC and Medical Monitor that participant can continue in the study.
- study noncompliance
- study physician discretion

8.6 Safety Oversight

8.6.1 Independent Medical Monitor

Evdokia Anagnostou, MD has a wealth of experience in pediatric oxytocin administration as she has conducted many of her own trials. She will serve as the independent medical monitor for this study. She will review alarm values of labs and ECGs (to be defined by the medical monitor and the site PIs) and serious adverse events within 6 working days of their reporting and who will review all new treatment emergent adverse effects of moderate or greater severity three times a year prior to DSMB meetings. She will be available to DSMB and to steering committee to discuss concerns about safety related to the 2 above activities.

8.6.2 Data Safety Monitoring Board (DSMB)

The UNC TRACS Data and Safety Monitoring Board (DSMB) will monitor data from all sites. The principle role of the DSMB is to monitor the data from clinical trials to protect the safety of the research participants. To achieve this, the DSMB will: a) establish a quarterly meeting schedule; b) review proposed protocols for safety and validity; c) evaluate recruitment and rate of enrollment in relation to the projected activity; d) monitor the occurrence of adverse events, serious adverse events, and early withdrawals or terminations throughout the course of the study; e) review with a designated research staff member the pattern of the study data; and f) evaluate study outcomes, when available. It will be reviewed by the DSMB 3 times/year.

9 CLINICAL MONITORING

9.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6 and, when appropriate, regulatory guidelines. This section will give a general description of how site monitoring will be conducted. The data monitor will be housed at the Data Center at UNC Chapel Hill. The Data/Regulatory Monitor will monitor the database, with the data manager, in order to assess quality assurance and control issues, and will generate queries from the database. This individual will also monitor each site's regulatory documents for completeness and accuracy (delegation of duties logs, 1572s, FDFs, general correspondence, IRB correspondence, laboratory values etc). He/she will travel to each site at least once per year in Years 1 and 5 and separately twice per site each year for Years 2, 3 and 4 in order to monitor the study coordinators and the case report forms to ensure that data are collected completely and accurately. He/she will ensure that queries are resolved. He/she will also ensure that data is properly and accurately entered into the database.

10 STATISTICAL CONSIDERATIONS

Dr. Hamer and Dr. Johnson will conduct and have ultimate responsibility for all data analysis described below. All statistical computing will be done using the most recent version of SAS. Dr. Johnson will do most of the programming and perform most of the analyses, but Drs. Johnson and Hamer will be jointly responsible for all results. The analyses described below will be performed on the intent-to-treat population. Additional exploratory analyses may be performed on the per protocol population or key subsets of the sample (e.g. those participants without fluent phrase speech). All data will be explored using descriptive statistics and graphical techniques prior to any hypothesis testing. For categorical variables, we will examine frequency distributions and where appropriate contingency tables and histograms. For continuous variables, we will examine frequency distributions and, where appropriate, box-and-whisker plots. When appropriate, we will consider transformation. If necessary due to distributional considerations, we will cautiously consider a change of analysis method to a less parametric one.

10.1 Statistical Analysis

General Modeling: Unless otherwise specified, we will fit a mixed longitudinal model with change from baseline to each post-baseline month for a response variable, functioning (low versus high) as the stratification variable, center as a blocking factor, treatment (oxytocin versus placebo) as the between-subjects factor, month as a within-subjects factor, their interaction, and baseline as a covariate. We will choose between two kinds of models: (1) a random coefficients model treating month continuously with random coefficients for the intercept, slope, and slope squared, and an unstructured covariance matrix among them, and (2) an mixed model with repeated measures (MMRM) treating month categorically and examining unstructured, Toeplitz, AR(1), and compound symmetric covariance structures among months. We will choose among these candidate models using an information criterion (AIC) while remaining *blind to the*

significance of the treatment effect to avoid bias in the choice of model. Distributional assumptions will be examined using residuals. These mixed models could fail to converge or encounter difficulties based on their use of asymptotics. If they do, we will attempt to simplify the models in order to eliminate the problems, and if necessary, move to analyses that assume compound symmetry, but use a Huynh-Feldt correction if compound symmetry fails.

Missing Data: The mixed models used to evaluate the continuous response variables are able to handle moderate amounts of missing data provided they are missing at random. We will examine the missing at random assumption by assessing baseline differences between dropouts and completers, as well as differences in response variables up to the point of premature withdrawal. If the missing at random assumption does not appear to be tenable, we will report the mixed models results but spend additional effort characterizing treatment effect at time of premature withdrawal.

10.2 Sample Size Considerations

We will use a mixed longitudinal model for our primary analyses (see Statistical Analyses below). Since some aspects of the model such as covariance structure are unknown, we performed power and sample size calculations using a more conservative, simplified model corresponding to a two-group t-test on change scores. We plan to analyze two co-primary outcome measures: the ABC-SW, which will provide consistency with pivotal trials of other medications hypothesized to improve ASD social symptoms, and the combined social score, which integrates ABC-SW symptoms with the new Pervasive Developmental Disorders Behavior Inventory-Screening Version SOCDEF raw score (PDDBI-SV, Cohen 2011, also see Outcome Measures) in order to fully capture the range of impairments in reciprocal social behaviors observed in ASD. We will use an alpha of 0.025 to correct for two co-primary outcomes. SOARS-B is powered for Aim 1b to allow independent evaluation of oxytocin efficacy in low and high-functioning youth.
Standard deviations of change scores for the ABC-SW range from ~5 to 9 in several large ASD intervention trials (Shea 2004; McCracken 2002; Owen 2009; Marcus 2009, King 2009; Aman 2009). We consider a between groups difference of 5-7 points in ABC-SW change scores to be clinically meaningful. In our power calculations, we use conservative estimates of 9 points for the SD of ABC-SW change and 5 points for between group differences in ABC-SW changes (differential improvement on ~1/3 of items). To achieve 80% power with an alpha of 0.025 on the ABC-SW, we will require 71 participants in each treatment group within the two strata. Thus, our total required sample for the ABC-SW in Aim 1b is 284. We examined change in the combined social score in 30 3-17 year olds with ASD, 20 treated with aripiprazole and 10 medication-free controls. The mean baseline value was 33.5 (17.2 SD) and the change overall was -10.7 (SD 13.0), with a SD of change in each group of 11.9. Within each treatment group, changes in the combined social score paralleled those in the ABC-SW and PDDBI-SV, but had less variability (Sikich, personal data). We consider a between groups difference in the combined social measure changes of 10-12 to be clinically meaningful. Conservative estimates using a SD of 15 and a between groups difference of 10 in the combined social score changes results in a larger effect size for the combined social measure than for the ABC-SW. Consequently, we should have at least 80% power. A sample size of 300 will allow for 5% attrition between randomization and the first post-randomization visit. Aims 1a, 3 and 4 consider

all participants, resulting in much greater power than for Aim 1b. The power of moderator analyses depends on the distribution of participant characteristics and variability in methylation and mRNA expression observed, which can't be estimated reliably at this time.

11 SUBJECT CONFIDENTIALITY

In order to protect confidentiality, only randomly assigned ID numbers rather than names will appear on clinically sensitive charts, files, and digital data. The key linking the numeric identifier and participant's identity will be maintained on a separate drive that is double password protected and to which only the site PI and coordinator have access. Contact information will be recorded separately in double password protected files with restricted access as above. The code linking the names with the ID numbers will be securely protected with limited access. Medical records will be kept confidential with access granted only to those medical and research professionals directly involved with the study. If any scientific paper based on the data collected for this study is published, no information that could be linked to any single participant will be reported. Confidentiality will be protected to the fullest extent permitted by law. All research personnel have completed HIPPA training for researchers and human ethics training.

The samples for the methylation portion of the study (which will be sent to Simon Gregory's team at Duke) will be single-coded, that is, labeled with a single specific code that does not carry any personal identifiers. Single coding is the current standard used in clinical research and offers additional safeguards to the subject's identifiers compared to the HIPAA authorization. With this method it is possible to trace the samples back to a given subject with the use of the single coding key. The clinical study investigator is responsible for maintaining the coding key. This coding key will be stored separately from the research data. When stored electronically, it will be password protected as it will contain identifying information. The samples will also note the date of collection and visit number.

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Final SOARS-B Protocol, Version 6.0

Dated March 21, 2016

Study of Oxytocin in Autism to improve Reciprocal Social Behaviors
(SOARS-B)

Protocol Number 111604-3

Protocol Version: 6.0

Sponsored by the Eunice Kennedy Shriver Institute for Child Health and Human Development
Under
Cooperative Agreement 1U01HD073984-01

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STATEMENT OF COMPLIANCE

This study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following *(use applicable regulations depending on study location and sponsor requirements; samples follow):*

United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)

ICH E6; 62 Federal Register 25691 (May 9, 1997)

NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

*Site Investigator: _____ (name)

Signed: _____ Date: _____

** The protocol should be signed by the clinical site investigator who is responsible for the day to day study implementation at his/her specific clinical site; i.e., if Investigational New Drug (IND) study, the individual who signs the Form FDA 1572.*

TABLE OF CONTENTS

PROTOCOL SUMMARY

Title:	Study of Oxytocin in Autism to improve Reciprocal Social Behaviors
Synopsis:	This Phase 2 study will evaluate oxytocin as a supplemental treatment for improving social difficulties in individuals with autism.
Objectives:	We will randomly assign 300 individuals between the ages of 3 - 17 years old with an autism spectrum disorder to 24 weeks of treatment with oxytocin or a matched placebo across six sites in the United States. Subsequently all participants will receive open-label oxytocin for 24 additional weeks. Post-treatment assessments will be done ~4 weeks after treatment stops. We will also determine *OXTR* methylation status at baseline, 8 weeks, 24 weeks and 36 weeks to explore potential relationships between *OXTR* methylation and baseline severity of social problems and/or treatment response.
Population:	300 subjects, male or female, ages 3-17 with an autism spectrum disorder
Phase:	2
Number of Sites:	6 treatment sites
Description of Agent or Intervention:	Oxytocin, intranasal (8IU to 80IU daily)

Key Roles

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1 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

There is a tremendous unmet need for accessible treatments that address core symptoms of ASD and are safe for sustained use, especially for the population of nonverbal individuals who require the most intensive lifelong support. We have created the ACE SOARS Network to provide the infrastructure necessary to meet this need on an ongoing basis. Our initial clinical trial, described in this application, is SOARS-B (the Study of Oxytocin in ASD to improve Reciprocal Social Behaviors). SOARS-B will test a very promising potential treatment−intranasal oxytocin−for ASD's fundamental social communication deficits in a large, highly generalizable group of verbal and nonverbal children. SOARS-B will also provide information about the regulation of DNA methylation and transcription of the oxytocin receptor gene (*OXTR*), as well as other genes relevant to oxytocin's CNS activity, as a function of time and in response to oxytocin treatment. These data will fill a key gap in our understanding of oxytocin's role in ASD and its ability to alter epigenetic modifications of *OXTR*.

1.2 Rationale

THE SOCIAL MOTIVATION MODEL OF ASD PATHOPHYSIOLOGY

Difficulties in social orienting are evident across auditory and visual modalities and across the lifespan in ASD (Dawson, Meltzoff et al. 1998; Dawson, Webb et al. 2004; Sasson, Turner-Brown et al. 2008; Whitehouse and Bishop 2008). Electrophysiologic studies have found that individuals with ASD can attend to social stimuli if instructed to do so, but that they do not do so spontaneously (Ceponiene, Lepisto et al. 2003). In contrast, typically developing individuals give preferential attention to social stimuli, processing them faster with greater brain activation in prefrontal cortex than nonsocial stimuli (Goren 1975; Greene, Colich et al. 2011). Youth with ASD show slower processing of social versus nonsocial stimuli and the rate inversely correlates with the magnitude of social impairments (Dawson, Webb et al. 2004). Individuals with ASD also appear to experience reduced relative rewards from interpersonal interactions. In contrast to their typically developing peers, children with ASD prefer looking at pictures of inanimate objects to looking at pictures of people(Sasson, Turner-Brown et al. 2008; Sasson, Elison et al. 2011) They also fail to activate the ventral striatum, which is the center of the brain's reward circuit, in response to social rewards whereas high levels of activation are evoked by social rewards in typically developing children(Scott-Van Zeeland, Dapretto et al. 2010). Further, when presented with social stimuli, children with ASD show reduced rather than increased activity in the prefrontal cortex, which assesses the relative value of a reward (Greene, Colich et al. 2011). The extent of reduction correlates with the severity of social and communication impairments in children with ASD (Dawson, Meltzoff et al. 1998; Ohnishi, Matsuda et al. 2000). These differences, as well as ASD's clinical presentation, have led to the hypothesis that ASD is related to a fundamental impairment in social motivation (Waterhouse, Fein et al. 1996; Dawson, Carver et al. 2002; Grelotti, Gauthier et al. 2002; Dawson, Webb et al. 2005).

THE ROLE OF OXYTOCIN IN SOCIAL BEHAVIOR

Oxytocin is the brain's most abundant neuropeptide. It can act as a classical neurotransmitter, a neuromodulator and a hormone with actions throughout the body (Gimpl and Fahrenholz 2001; Baskerville and Douglas 2010; Veening, de Jong et al. 2010). Oxytocin's half-life in the plasma is 1-2 minutes compared to ~30 minutes in the CSF. Central release of oxytocin is dependent upon CD38 and dramatically stimulates further release of oxytocin (~1000 fold) and increases the number of oxytocin-containing cells in the periventricular nucleus. Together these factors lead to long lasting oxytocin

elevations throughout the brain following acute increases in CSF oxytocin. One of oxytocin's major central actions is to activate the brain's reward circuit by increasing dopamine release from the ventral tegmental area to the ventral striatum, amygdala and hippocampus. Its neuromodulatory actions appear to result primarily from somatodendritic release with binding to oxytocin receptors that are widely distributed throughout the limbic region and prefrontal cortex. Differential species-specific patterns of social behavior appear related to the distribution and density of oxytocin and vasopressin receptors in the brain. Oxytocin can bind vasopressin receptors, although it is unknown to what it extent it does so normally. Oxytocin also can increase expression of oxytocin and vasopressin receptors in the brain although these effects are often sexually dimorphic and highly regulated by hormones and interleukins (Moos, Poulain et al. 1989; Tribollet, Charpak et al. 1989; Miyan, Nabiyouni et al. 2003; Morris and Ludwig 2004; Bales, Plotsky et al. 2007; Bales, van Westerhuyzen et al. 2007; Carter 2007; Carter, Boone et al. 2009). Social isolation and social stress somewhat later, during the post-weaning period, also have sexually dimorphic effects on the number of oxytocin and vasopressin neurons (Bales, Lewis-Reese et al. 2007; Tanaka, Osako et al. 2010).

In animal models including primates, oxytocin has been demonstrated to increase eye contact, social approach, social recognition, social memory, and generosity and to reduce stress responses (Takayanagi, Yoshida et al. 2005; Liu, Lopatina et al. 2008; Insel 2010). Oxytocin also influences social behavior in people(Macdonald and Macdonald 2010). Exogenous oxytocin increases gaze to eye regions, social cognition, social memory, positive communication, empathy, perceptions of trustworthiness, and cooperation within one's own group (Kosfeld, Heinrichs et al. 2005; Zak, Kurzban et al. 2005; Domes, Heinrichs et al. 2007; Zak, Stanton et al. 2007; Baumgartner, Heinrichs et al. 2008; Guastella, Mitchell et al. 2008; Petrovic, Kalisch et al. 2008; Savaskan, Ehrhardt et al. 2008; Unkelbach, Guastella et al. 2008; Di Simplicio, Massey-Chase et al. 2009; Ditzen, Schaer et al. 2009; Keri, Kiss et al. 2009; Rimmele, Hediger et al. 2009; Theodoridou, Rowe et al. 2009; De Dreu, Greer et al. 2010; Fischer-Shofty, Shamay-Tsoory et al. 2010; Guastella, Einfeld et al. 2010). Intranasal oxytocin also reduces cortisol; perceived stress, amygdala activation to threatening social images, and tolerance of ethnic differences (Heinrichs, Baumgartner et al. 2003; Kirsch, Esslinger et al. 2005; Petrovic, Kalisch et al. 2008; De Dreu, Greer et al. 2010; De Dreu, Greer et al. 2011). However, some of these effects, including enhancement of social memories and secure attachment, may be limited to individuals with less robust prosocial behaviors initially (Buchheim, Heinrichs et al. 2009; Bartz, Zaki et al. 2010; Bartz and Piantadosi 2010). Allelic variations in *OXTR* have also been correlated with infant attachment, social auditory processing, empathy and prosocial decision making (Lerer, Levi et al. 2010; Chen, Barth et al. 2011; Tops, van Ijzendoorn et al. 2011).

OXYTOCIN'S POTENTIAL ROLE IN ASD

Multiple variations in the oxytocin signaling system have been associated with ASD relative to controls. Allelic variations in the CD38 gene, which is required for central release of oxytocin, and in *OXTR* have been identified, some of which also have been correlated with cognition and functioning in individuals with ASD (Green, Fein et al. 2001; Wu, Jia et al. 2005; Jacob, Brune et al. 2007; Yrigollen, Han et al. 2008; Andari, Duhamel et al. 2010; Lerer, Levi et al. 2010; Liu, Kawamura et al. 2010; Munesue, Yokoyama et al. 2010; Tansey, Brookes et al. 2010; Wermter, Kamp-Becker et al. 2010; Campbell, Datta et al. 2011; Riebold, Mankuta et al. 2011). Modestly greater (~ 20-40%) *OXTR* methylation relative to controls has been found in two small, independent ASD samples (Gregory, Connelly et al. 2009). Reduced plasma oxytocin and CD38 mRNA expression have also been reported in ASD (Modahl, Green et al. 1998; Jin, Liu et al. 2007).

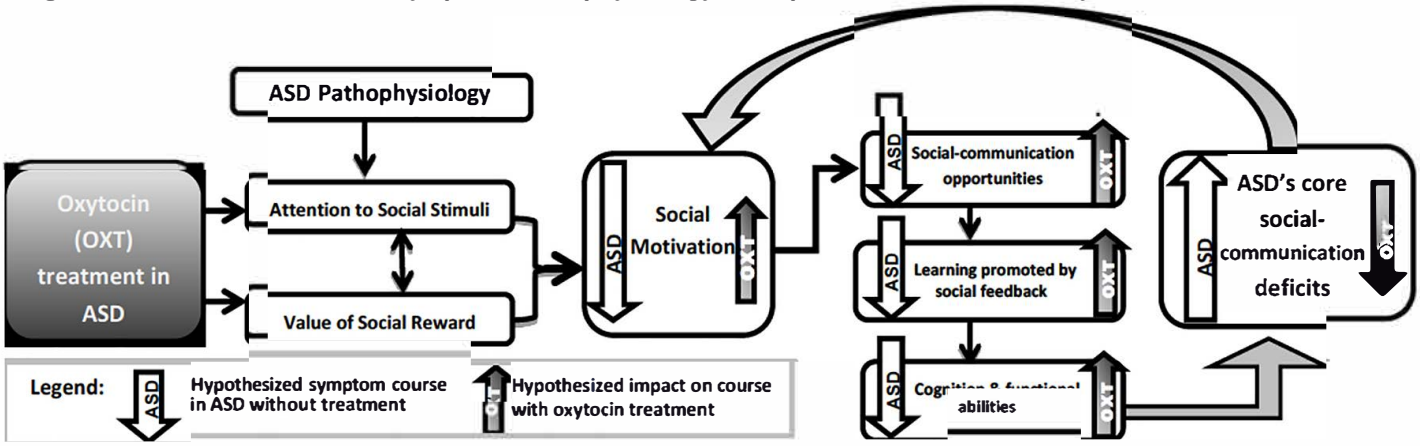
Oxytocin has shown promise for modifying social behavior in mouse models for ASD and in people with ASD and related disorders. In the *Otxr* null mouse, a single dose of oxytocin improved social

deficits, reduced aggression and reduced vulnerability to drug-induced seizures, apparently acting through the vasopressin 1A receptor. Further, in these mice, repeated doses of oxytocin improved reversal learning, indicating enhanced cognitive flexibility (Sala, Braida et al. 2011) . A single dose of oxytocin also improved social deficits in *Cd38* null mice (Higashida, Lopatina et al. 2010). In Fragile X, which is frequently comorbid with ASD, oxytocin improved gaze avoidance and reduced cortisol elevations elicited by social interaction (Hall, Lightbody et al. 2012). In high functioning individuals with ASD, single doses of oxytocin enhanced attention to faces and eyes, visual and auditory affect recognition, the ability to distinguish whether others were being cooperative, and preference for interacting with receptive individuals (Hollander, Bartz et al. 2007; Andari, Duhamel et al. 2010; Guastella, Einfeld et al. 2010). Oxytocin challenge also reduced self-reported compulsive and repetitive behaviors(Hollander, Phillips et al. 2003). Sustained oxytocin treatment has been reported to improve ASD symptoms in one child (Munesue, Yokoyama et al. 2010)). In the only pilot study of sustained oxytocin treatment currently completed, affect recognition accuracy improved three times as much with 6 weeks of oxytocin treatment as had been reported in Andari's single dose study (Anagoustou in preparation, network preliminary data). An 8-week blinded study of oxytocin in 24 children with ASD has been completed at UNC.

Our Working Model

The above evidence regarding impairments in social orienting and social reward in ASD, oxytocin's role in social orienting and social motivation, and variability in various aspects of oxytocin signaling among people with ASD, together with extensive preliminary evidence that supplemental oxytocin can impact social behaviors in ASD, have led to the formulation of our working model (Figure 1). We postulate that ASD pathophysiology acts to fundamentally alter social orienting and decrease the relative value ascribed to social rewards resulting in limited social motivation. Reduced social motivation leads to a vicious cycle of reduced social opportunities, reduced learning from social feedback, reduced skills and functional abilities and worsening of ASD's core social communication symptoms, which further reduces social motivation. We hypothesize that sustained intranasal oxytocin treatment will positively modify the earliest parts of this cycle – social orienting and the value of social rewards – and disrupt the cycle leading to improvements in the core social and communication impairments of ASD.

Figure 1: Model of ASD Social Symptom Pathophysiology & Proposed Mechanism of Oxytocin Treatment



1.3 Potential Risks

There is limited information about the safety of sustained use of oxytocin, although more information is emerging on a daily basis. Indeed, the objective of specific Aim 2 is to provide such information, gathered prospectively in a systematically elicited fashion.

Oxytocin has been reported to exert a large number of effects in the body and the brain (Gimpl & Fahrenholz 2001). The classic physiological effects of oxytocin in humans as well as all placental mammals are uterine contractions and milk ejection. These are conclusively known to occur only under the hormonal conditions at the end of pregnancy and postpartum when oxytocin receptors proliferate in the uterus and mammary tissue and lactogenesis occurs. Theoretically, oxytocin administration could cause uterine contractions during phases of the menstrual cycle when estrogen levels are relatively high or in women who are receiving estrogen. This, however, has never been demonstrated or reported. IV administration of Oxytocin is FDA approved for induction or facilitation of labor and postpartum contraction of the uterus. Intranasal oxytocin spray is also available in other countries to facilitate nursing in new mothers. Intranasal oxytocin also was FDA approved as a lactation aid in the United States until 1995 when it was withdrawn from the US market by the company that manufactured it, Novartis. This was a business decision and the FDA has confirmed no safety concerns were involved in this decision.

A number of adverse effects have been reported in the context of these two clinical applications, primarily with intravenous administration of oxytocin, which include anaphylactic reaction, hypertension, hypotension, cardiac arrhythmias, nausea and vomiting, afibrinogenemia and associated bleeding and, in the context of prolonged intravenous administration of oxytocin, hyponatremia. There has also been at least one report of life-threatening anaphylaxis during surgery when multiple drugs were being intravenously infused simultaneously (D. Pant, et al, 2009). Symptoms that may result from anaphylaxis include generalized hives, itchiness, or flushing; crampy abdominal pain, diarrhea, and vomiting; a feeling of anxiety and impending doom; swelling of the lips, tongue, throat resulting in shortness of breath, wheezes or stridor, and low oxygen; a drop in blood pressure that may result in a feeling of lightheadedness and loss of consciousness; reduced muscle tone with possible loss of bladder control; and, most seriously, coronary artery spasm may occur with subsequent myocardial infarction or cardiac dysrhythmia and possible death. There is also a case report of psychosis occurring during oxytocin treatment of a man with obsessive compulsive disorder (Ansseau, Legros et al. 1987), although there are two completed pilot studies in schizophrenia that have reported reduction of psychotic symptoms(Feifel, Macdonald et al. 2010) – using a TDD of 40 IU during week 1, followed by a TDD of 80 IU for 2 weeks in 16 people; (Pedersen, Gibson et al. 2011) using TDD 48 IU for 2 weeks in 7 people) with no significant adverse events. There are a limited number of published, sustained oxytocin treatment studies in adults with other diseases (Denboer and Westenberg 1992) (Epperson, McDougle et al. 1996; Epperson, McDougle et al. 1996)(Den Boer 1992, Epperson 1996a, Epperson 1996b, none of which report significant adverse effects.

Evidence for safety of sustained intranasal oxytocin treatment in ASD

Placebo controlled safety data of sustained intranasal oxytocin treatment in people with ASD is available from one adult study (Anagnostou, Soorya et al. 2012) and two currently unpublished trials: the Anagnostou adult study (10 on TDD 48 IU oxytocin for 6 weeks, 9 on placebo), a trial conducted by Adam Guastella, personal communication 2012 (26 teens on total daily dose [TDD] 24 IU oxytocin for 8 weeks, 24 on placebo), and the UNC Autism Speaks pilot trial in 3-17 year olds (12 on oxytocin with TDD ranging between 4 IU and 64 IU for 8 weeks and 13 on placebo) and 24 on open label oxytocin TDD up to 48 IU for 8 weeks.

Adverse events observed during the double blind and open-label phases of the UNC oxytocin pilot study are shown in Table 1. Pink shading indicates events that were numerically more common in the oxytocin group than the placebo group. Green shading indicates events that were numerically more common in the placebo group. We also have been able to access the raw adverse event data from the two non UNC trials and have aggregated it to provide adverse event data on 48 individuals with ASD exposed to oxytocin for at least 6 weeks and 46 individuals with ASD treated with placebo. This aggregation, shown in Table 2, found low rates of adverse events in both oxytocin and placebo groups and no signal for greater adverse events with oxytocin treatment. None of these studies identified any significant or systematic changes in electrolytes or vital signs. The apparently higher rates in the UNC Pilot study probably reflect differences in the methods of assessing adverse events. The UNC pilot study used systematic elicitation of adverse events (same instrument as proposed for SOARS-B trial) and the other studies used spontaneous report or much more limited questionnaires.

Table 1. Treatment Emergent Adverse Effects in UNC Pilot study of children with ASD (subjects ages 3 -17 years old)

Events of Moderate or Severe Intensity (Treatment Emergent)	8 Weeks OXYTOCIN Emergent by week 8	8 Weeks PLACEBO Emergent by week 8	16 Weeks OXYTOCIN Emergent b/w weeks 8-16	8 Weeks PLACEBO + 8 Weeks OXYTOCIN Emergent b/w weeks 8-16
Diarrhea	2 (16.7%)	0	0	0
Allergies	1 (8.3%)	0	1 (9.1%)	1 (7.7%)
Mood Lability	1 (8.3%)	1 (7.7%)	1 (9.1%)	3 (23.1%)
Weight Increased	1 (8.3%)	0	0	0
Insomnia, Initial	3 (25.0%)	2 (15.4%)	0	0
Insomnia, Mid/ Terminal	3 (25.0%)	2 (15.4%)	1 (9.1%)	0
Agitation	2 (16.7%)	1 (7.7%)	1 (9.1%)	1 (7.7%)
Aggression or Hostility	1 (8.3%)	4 (30.8%)	1 (9.1%)	1 (7.7%)
Anger/ Irritability	1 (8.3%)	3 (23.1%)	1 (9.1%)	3 (23.1%)
Oppositional	2 (16.7%)	3 (23.1%)	1 (9.1%)	2 (15.4%)
Low frustration tolerance	1 (8.3%)	2 (15.4%)	0	1 (7.7%)
↓ Attention/ Concentration	1 (8.3%)	2 (15.4%)	0	0
Accidental Injury	1 (8.3%)	2 (15.4%)	0	0
Hyperactivity	0	1 (7.7%)	0	0
Apathy	0	0	0	1 (7.7%)
Rituals/ Repetitive Behaviors	0	0	0	1 (7.7%)

Table 2. Aggregated adverse events with sustained intranasal oxytocin treatment in ASD

Adverse Events	6-8 Weeks Oxytocin (n=48)	Placebo (n=46)
Increased Allergies	6%	2%
Nausea	6%	2%
Insomnia	6%	4%
Vomiting	4%	2%
Shortness of Breath	4%	2%
Agitation	2%	0
Absence Seizure	8%	17%
Increased Thirst	8%	11%
Increased Urination	2%	9%
Aggression	2%	9%
Fatigue	0	4%
Diarrhea	4%	7%
Lightheaded	4%	7%
Oppositionality	4%	7%
Mood Lability	0	2%
Cough	0	2%
Rash	0	2%
Increased anxiety/Panic attack	0	2%
Increased social withdrawal	0	2%
Increased tTics	0	2%
Increased Appetite	0	2%
Decreased Appetite	6%	7%
Headache	6%	7%
Irritability	2%	2%
Decreased Attention	2%	2%
Increased Restlessness	2%	2%
Disinhibition	2%	2%
Low Frustration Tolerance	2%	2%
Increased Crying	2%	2%

There is also preliminary safety data regarding even more extended oxytocin treatment for up to 6 months in children with ASD available by personal communication from an ongoing trial funded by the US Department of Defense being conducted by Evdokia Anagnostou in 11 youth 10-17 years treated for up to 6 months treated with oxytocin. There have been no significant lab abnormalities in these participants. Three (14%) have shown increases in irritability or mood lability and one has had increased allergy symptoms.

Blood Draw Risks:

During laboratory blood draws the risks involved are pain, bruising, and rarely, infection at the location where the blood was taken. This risk will be minimized by having a trained professional take each participant's blood. We will attempt to avoid these risks by using aseptic techniques, and applying pressure after the phlebotomy. A local anesthetic may be used to reduce associated pain if

the participant wishes to use it. If a subject becomes nervous or agitated about the phlebotomy procedures, we will utilize an anti-anxiety medication to calm subjects who may be nervous.

Psychological/Psychiatric Risk:
This patient population can be at risk for worsening of psychiatric/psychological symptoms that may be attributed to their mental illness alone, medication non-adherence, or a number of other causes that may not be related to this study. The Principal Investigator will use clinical judgment to assess each treatment emergent adverse event and determine intensity and relatedness to the study. In cases of worsening of symptoms patients may require inpatient hospitalization. If the study doctor feels that the subject is at serious risk for hurting themselves or others, he/she can ask a judge to allow the participant to be hospitalized against his/her will. Subjects may become frustrated during study procedures. Research staff will work to ensure that all directions and questions are easy to understand for subjects who have intellectual difficulties. Subjects will be allowed breaks, if needed.

Since this is a large trial of oxytocin that will require patient involvement for up to 52 weeks (1 year), it is suggested that a subject's psychiatric care will be assumed by the study physician. However, this can be determined by site as some institutions have different regulations surrounding psychiatric care and research participation.

Confidentiality Risk
The potential indirect risks are related to loss of confidentiality and could include someone such as an insurance agency or employer learning that the participant has a serious mental illness with resulting potential stigmatization. It is also possible that public knowledge of this diagnosis or its documentation in the medical record could lead to inability to obtain insurance coverage at reasonable rates in the future.

Additional risk for biomarker/genetics portion:
There are no additional needle sticks for this portion of the study. However, the additive in the DNA collection tube can be irritating if it contacts the skin so special care will be taken so that, during the blood draw, the additive in the tube doesn't flow out into the collection needle.

2 OBJECTIVES

The ACE SOARS Network's immediate goals are to translate these exciting findings regarding oxytocin's neurobehavioral effects into an evidence-based, widely accessible intervention for ASD's fundamental impairments in reciprocal social behaviors and to identify factors that differentially influence response to oxytocin treatment in ASD. Our central hypothesis is that intranasal oxytocin will partially reverse the early pathophysiologic alterations in social orienting and the salience of social rewards present in ASD, which lead to decreased social motivation and ultimately to ASD's core social communication impairments, thereby enhancing reciprocal social behaviors (see Figure 1, in *Impact section*). Sustained improvements in social motivation and social reciprocity are expected to facilitate communication and learning and ultimately improve functioning. We will accomplish our immediate goals by conducting SOARS-B, a large (n=300) randomized, double-blind trial of sustained (24 week treatment with intranasal oxytocin in children 3-17 years old with ASD and examining clinical and biological factors that may predict or enhance response. Regardless of SOARS-B's outcome, its results will significantly impact the care of people with ASD by definitively testing a very promising

translational treatment strategy in a highly generalizable sample. The moderator analysis is likely to support treatment personalization. The exploratory epigenetic studies will enhance understanding of the regulation of key biological pathways in ASD and facilitate development of future treatments.

2.1 Specific Aims

Specific Aim 1: **Determine the efficacy of intranasal oxytocin treatment in children with ASD.**
Analysis 1a: Compare oxytocin treatment to placebo for improving reciprocal social behaviors.
Hypothesis: oxytocin will reduce maladaptive social behaviors and increase prosocial behaviors.
Analysis 1b: Compare oxytocin treatment to placebo for improving reciprocal social behaviors separately in subgroups of children who have significant language impairment and intellectual disability and those whose language and intellectual abilities are in the normal range at baseline.
Hypothesis: oxytocin's actions on the social reward system are unrelated to verbal or cognitive abilities.
Analysis 1c: Compare the treatments' enhancement of social motivation, cognitive skills and functioning. *Hypothesis: oxytocin will increase social motivation, improving acquisition of cognitive and functional skills.*

Specific Aim 2: Provide information about the safety and tolerability of intranasal oxytocin.
Analysis 2a: Compare incidence and severity of treatment emergent adverse effects, clinically significant laboratory and electrocardiogram changes, serious adverse events and treatment discontinuation due to tolerability issues over 24 weeks in the oxytocin and placebo groups.
Analysis 2b: Tabulate these safety indicators during 24 weeks of open oxytocin treatment

Specific Aim 3: Identify clinical or biological traits that preferentially influence response to oxytocin.
Analysis 3a: Determine if baseline age influences behavioral changes more in the oxytocin group.
Hypothesis: the magnitude of changes observed depends on both intervention efficacy and brain plasticity.
Analysis 3b: Determine if baseline *OXTR* methylation levels predict level of behavioral improvement.
Working hypothesis: greater OXTR methylation decreases oxytocin receptor density and increases the amount of oxytocin required to bind available receptors and elicit optimal reciprocal social behaviors.
Analysis 3c: Determine if other baseline traits or intervening experiences interact preferentially with oxytocin

Exploratory Aim 4: Describe the changes in *OXTR* methylation and mRNA expression of genes related to oxytocin signaling occurring over 24 weeks with oxytocin and placebo treatment.
Rationale: understanding the regulation of oxytocin signaling will facilitate development of novel treatments.

2.2 Study Outcome Measures

2.2.1 Primary Outcome Measures

Our primary outcome is reciprocal social behaviors, which we will assess using two co-primary measures. The first measure is the ABC-SW subscale, which is being used in other clinical trials focusing on the core social and communication symptoms of autism. The other measure is the

Sociability Factor (SF), a combined measure derived from 13 items of the ABC-SW, which primarily captures aloof, and avoidant behaviors, and the Pervasive Developmental Disorders Behavior Inventory-Screening Version (PDDBI-SV, Cohen 2011), a recently developed measure which assesses both maladaptive social problems and social skills. The PDDBI-SV is comprised of 18 items included in the older, more comprehensive Pervasive Developmental Disorders Behavior Inventory (PDD-BI, Cohen 2003). The PDDBI-SV assesses both social impairments typically associated with the active but odd subtype of ASD and development of pro-social skills that are integral to improved reciprocal social behavior. The PDDBI-SV results in a raw SOCDEF score, which is the sum of the reverse-scored social skills and the regularly-scored problems, and a SOCDEF T score. In marked contrast to the PDD-BI's age-based standard scores (for children 1.5 and 12.5 years old), the PDDBI-SV SOCDEF score does *not* change with age in individuals with ASD. The PDDBI-SV has been validated in original PDD-BI development sample of 311 children 1-17 years old and a recently-acquired sample that includes 145 youth between 13 and 15 years old, 90 between 16 and 19 years, and 59 between 20 and 40 years (Cohen 2011, personal communication). The sociability factor SF will be calculated by discarding the three lethargy items from the ABC-SW (questions 3, 32, and 53, which all assess reduced physical movement), summing the 13 remaining items of the ABC-SW subscale, and adding it to the PDDBI-SV raw SOCDEF score. The sociability factor SF is derived from two well-validated instruments and has face validity for capturing the full range of impairments in reciprocal social behaviors observed in ASD. We chose not to use the Social Responsiveness Scale (SRS), which was developed to provide a quantitative measure of social impairments typically observed in ASD in children 3-18 years old, because it has been demonstrated to be quite stable over time (Constantino, Przybeck et al. 2000; Constantino 2009) and no intervention studies have clearly demonstrated its sensitivity to change.

2.2.2 Secondary Outcome Measures

Secondary Outcome Measures: We will use the SRS Social Motivation subscale to assess oxytocin's hypothesized mechanism of action. Cognitive skills will be assessed using the Stanford Binet-5th Edition (SB-5) (Roid 2003). If a child cannot complete the routing tests on the SB-5, they will be assessed using the Mullen. Functional skills including communication will be assessed using the standard score of the Vineland 2 adaptive behavior composite and all its subscales, the Caregiver Strain Questionnaire total and subscale scores (Brannan, Heflinger et al. 1997) and, in verbally fluent youth, a 7 item questionnaire being piloted to assess participant satisfaction with their social relationships, which is included in the appendix. The specific components of these measures tested in Aim 1c are SRS Social Motivation subscale, the NV IQ or Mullen Early Learning Composite standard score, and the Vineland Adaptive Behavior Composite. Other subscales of the ABC, Caregiver Strain Questionnaire and SRS measures will be analyzed in an exploratory way.

2.2.3 Additional Exploratory Outcome Measures

Additional Clinical Assessments: In order to provide a comprehensive assessment of the clinical features of children in the trial we will also perform several other assessments. The Clinical Global Impressions – Improvement (CGI-I) score, which is routinely used in pharmacologic clinical trials, will capture the study physician's global impression of response. We will also incorporate a continuous visual analog scale of change in three key areas of overall functioning (similar basis for assessment as CGI-I), social communicative functioning, and repetitive behaviors and restricted

interests that is rated with reference to the individual participant's baseline characteristics. We will also incorporate the Reading the Mind in the Eyes Task (Baron-Cohen, Wheelwright et al. 2001) into our analysis, which has been shown to be sensitive to both single and sustained doses of oxytocin (Domes, Heinrichs et al. 2007). However, its use is restricted to high-functioning individuals who can verbally identify specific emotions. We will also obtain the other SRS-2 subscales to specifically assess its sensitivity to change in comparison to other outcomes. All concomitant medications and behavioral or alternative medication therapies will be recorded at each visit. Assessment of treatment-emergent adverse effects will utilize the systematic longitudinal adverse effects scale developed by Sikich and described in the oxytocin treatment section. We will also determine the reason for premature discontinuation of treatment and physician, caregiver judgment regarding the child's treatment group.

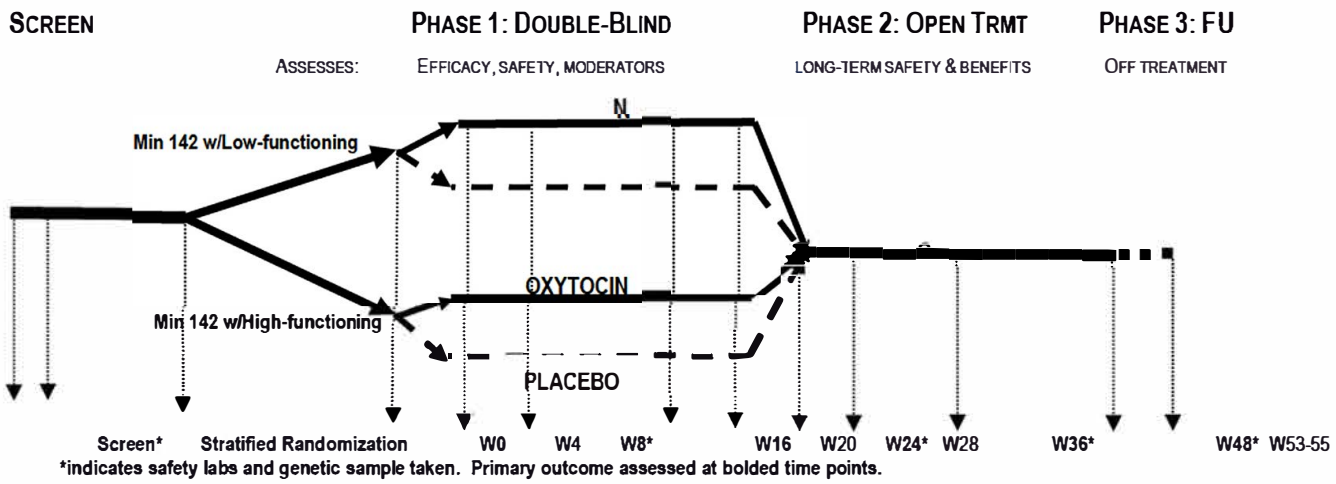
Biologic Outcome Measures: We will obtain blood, urine and vital signs from participants at regular intervals in order to assess the safety of oxytocin. In addition, we obtain blood from participants at screening and weeks 8, 24 and 36 that will used to assess oxytocin levels, *OTXR* methylation status and to assess mRNA expression. We will obtain saliva at the same time points to perform salivary oxytocin levels. We will also determine whole blood serotonin at screening, weeks 8 and 24.

Potential interacting factors: We will assess potential environmental factors that may interact with oxytocin treatment through caregiver reports. We are particularly interested in social skills treatments, interventions the child receives outside of the school setting and social opportunities outside of school settings for interactions with peers, adults who are not caregivers/treatment providers, and caregivers/treatment providers. We will also examine potential interactions between antipsychotic treatment, stimulant treatment, α-adrenergic treatment and oxytocin treatment.

3 Study Design

SOARS-B is a large (n =300) randomized, placebo-controlled trial of sustained (24 week), flexible dose intranasal oxytocin treatment in children 3 to 17 years old with ASD. The primary aim is to determine the efficacy of oxytocin for improving reciprocal social behaviors in the entire sample and the low- and high-functioning subgroups. Social motivation, cognitive skills and functional behaviors will be secondary outcomes. We will examine safety during the double-blind phase and the 24 week open extension phase. The second major aim of the study is to identify whether participant baseline clinical and biological characteristics such as age and extent of OXTR methylation preferentially influence response to supplemental oxytocin. In post-hoc exploratory analyses, we will examine the influence of intervening processes, such as concomitant antipsychotic or social skills treatments that are likely to be randomly distributed between the oxytocin and placebo groups, on oxytocin response. Finally, participants will be assessed approximately 4 weeks after the last dose of oxytocin (~week 53-55)to assess safety and follow up on any unresolved/continuing adverse events. The target dose will be 48/0 IU total daily dose (TDD), which will typically be achieved by week 8 during the blinded phase and week 28 in the open label phase according to a recommended titration schedule. However, doses will be flexible with slowing of the titration, alteration of the dosing administration times, reduction of dose and increases beyond the target dose (after 7 weeks of target dose treatment) allowed according to specific guidelines.

Figure 2: SOARS-B Study Design



4 STUDY ENROLLMENT AND WITHDRAWAL

4.1 Subject Inclusion Criteria

Be between the ages of 3 years 0 months and 17 years 11 months at the time of randomization
Be diagnosed by clinician experienced in assessment of ASD with an autism spectrum disorder using DSM-5-TR criteria
Have a clinical diagnosis of ASD confirmed either using the Autism Diagnostic Observation Scale-2 (ADOS-2) or the Autism Diagnostic Interview-Revised (ADI-R, Rutter, 2003). For subjects who do not meet criteria on either, but the clinician still believes to have ASD, those individuals may be included if the SC agrees.
Have a guardian who is able to provide informed consent
If cognitively able, subject must be able to provide informed assent/consent

4.2 Subject Exclusion Criteria

- Have a known diagnosis of Rett Syndrome or Childhood Disintegrative Disorder
- Have marked sensory impairment such as deafness or blindness. Legal blindness is defined as a visual acuity of 20/200 or less and central visual field of 20 degrees or less with both eyes open. Profound deafness is defined as a flat audiogram.
- Have active cardiovascular disease or renal disease that is not controlled by medication
- Subjects who are pregnant, lactating, or who refuse to practice contraception if sexually active
- Subjects who have had changes in allied health therapies, behavioral or educational interventions within the two months prior to randomization other than those associated with school holidays
- Subjects who have had changes in psychiatric medications within 4 weeks of randomization

- Subjects who have had previous treatment with chronic intranasal oxytocin (daily dosing more than 1 month)Subjects who have caretakers who are unable to speak English, be consistently present at visits to report on symptoms, or are otherwise judged as unable to comply with the protocol by the data collection site team
- Subjects with active seizures within the 6 months preceding screening or baseline.

4.3 Rationale for Inclusion and Exclusion Criteria

Frequently when large trials are done with a very specific subpopulation, no further studies are done to explore response in other subgroups because of the large cost of such trials and public frustration with studies perceived as duplicative and incremental. However, families and clinicians caring for individuals from untested subgroups often struggle to make treatment decisions without any specific evidence regarding efficacy or safety. Therefore, we have made the entry criteria for participation in this trial as broad and generalizable as possible. We will conduct secondary post-hoc analyses of various clinical and biological factors that might typically be excluded (eg. concomitant antipsychotic treatment, Fragile X) instead. This approach will provide the greatest amount of clinically relevant information regarding sustained oxytocin treatment in shortest amount of time possible.

We also will allow psychotropic medications with strong evidence of efficacy for treating problem behaviors or psychiatric disorders frequently associated with ASD (specifically antipsychotics, anticonvulsants, stimulants, and other medications approved for ADHD) to increase generalizability. We will allow agents that influence serotonergic neurotransmission or specifically impair reward pathways in the brain (eg. naltrexone) in order to make the study results more generalizable to the actual autism population, ensure a diverse sample and increase recruitment. While there is minimal evidence to support efficacy in ASD symptoms, the truth is that community practitioners are prescribing these agents to children and adolescents with ASD.
We chose not to require a minimal baseline score on the Aberrant Behavior Checklist –social withdrawal subscale (ABC-SW,(Aman, Singh et al. 1985)) due to concerns this would exclude many people with ASD, even though such a threshold would increase comparability with an ongoing trial also targeting ASD's core social behaviors and might increase the probability of observing ABC-SW changes. Also, diagnosis of ASD requires significantly impaired reciprocal social behavior.

We are including very young children because we believe they are likely to demonstrate significantly greater functional improvements than older children are as a result of increased intervention services and greater brain plasticity. We believe that the careful titration schedule and close adverse effect monitoring, along with a dosing administration handout that staff will provide to participants) will provide sufficient safety for this vulnerable population. Further, the prospective, systematically elicited information about safety in young children gained in this study will inform the design of future trials and off label use in young children.

We are including children with low cognitive functioning because there is no rationale for believing that they would be less likely to respond to oxytocin treatment. Such children are seldom included in clinical trials and desperately need effective treatments to reduce their suffering. We expect that we will still be able to measure social reciprocity which will be valuable in studying the effects of oxytocin on children with autism of varying cognitive functioning.

We will exclude subjects with non-English speaking caretakers because our staff is not qualified (i.e. they are not fluent in other languages) to explain the study to non-English speaking subjects. In addition, many of the pencil and paper assessments used to diagnose the included diagnoses have not been normed with non-English speaking populations. Thus, the measures may not be as appropriate, valid, and/or reliable for non-English speaking subjects.

We are excluding children who are deaf and blind because part of the outcome measures are child rated (ie: reading in the mind's eye and self-rating of social functioning). Children who are blind and/or deaf would not have reliable testing for these measures and therefore invalidate that data during analysis. If it is unclear if a subject is blind or deaf, we will refer them to an external provider for further testing prior to enrolling the subject.

We will exclude individuals who have uncontrolled seizures (ie: seizure activity within the past 6 months prior to baseline or screening). This is an added safety measure to ensure that any individuals with seizures have them properly under control prior to randomization.

4.4 Strategies for Recruitment and Retention

Before the study is initiated, it will be approved by the Institutional Review Board (IRB) at each treatment site and the Network DSMB. All of the subjects and their parent/guardian will provide consent/assent for participation in this study. In addition to the informed consent requirements, per the Privacy Rule (HIPAA) regulations, research participants will provide a written "authorization" to use their Protected Health Information (PHI) in connection with research. In requesting an authorization from potential participants, investigators will specify how the information will be used and how the privacy of that information will be protected. The researcher obtaining Informed Consent will thoroughly review the consent form with the child and his or her parents, including study procedures and potential risks and benefits of study participation. The child and parents will be encouraged to ask questions throughout the process. It will be emphasized that research is voluntary and that the subject can opt out of this project at any time without jeopardizing his/her treatment. Participants who turn 18 during their participation in the study and who have the cognitive capacity to read and understand the consent form, and whose parents do not have legal guardianship of them, while participating in this study will be re-approached by one of the physician investigators. The investigator will continue the informed consent process acknowledging that the individual is now legally an adult and will ask the participant if they voluntarily wish to continue their participation in the study. If he/she does, the participant will be asked to sign the informed consent document and a new HIPAA authorization. If the individual does not wish to continue participation they will be withdrawn from study treatment just as any other individual who chooses to withdraw consent from participation.

This study will be registered in ClinicalTrials.gov no later than 21 days after the first subject is enrolled. Summary results information (including adverse events) will be reported no later than 1 year after the completion date for the study. Grant and progress report forms shall include a certification that the investigators have made all required submissions to ClinicalTrials.gov.

4.5 Treatment Assignment Procedures

4.5.1 Randomization Procedures

We expect to screen ~ 400 children in order to randomize 300. Individuals, who meet all inclusion criteria and none of the exclusion criteria, will be categorized by the data base according to the following criteria to determine if they belong in the high or low functioning stratum. Using a centralized, randomization scheme with permuted blocks of 4 or 6, stratified by functional status, the database will randomly assign the treatment which will be communicated to the site investigational pharmacy. Enrollment within the two strata will be monitored to ensure that there are at least 142 participants in each. Randomization will be further stratified by age so that at least 21% of the participants within each stratum fall into each of the specified age groups 3-6, 7-11, 12-17. The minimal representation of these age groups should ensure that the potential moderating effects of age can be fully assessed. We will not stratify by age due to the potential for very small cell sizes with large numbers of strata.

If a functional or age subgroup appears to be under represented after 50%, 75% and 90% of participants are randomized, recruitment efforts will be focused on the under-represented subgroups. Children will be considered low-functioning if they have significant language impairment and intellectual disability (NV IQ or Mullen Early Learning Composite Standard Score < 70). We define significant language impairment in participants 72 months or older, as having a lack of fluent phrase speech reflected by use of ADOS Module 1 or 2. In participants 5 years, 8 months or younger, significant language impairment will be defined as lack of phrase speech *and* Mullen receptive language subtest score more than 1 SD below the mean for age. Participants will be considered as high-functioning if they have nonverbal IQ ≥ 70 and significant language skills, which we define as fluent phrase speech reflected by use of ADOS Module 3 or 4, *or*, if 5 years, 8 months or younger, Mullen receptive language subtest score within 1 SD of the mean for age or higher. Participants who fail to meet both the language and cognitive criteria for low- or high-functioning will be stratified according to their language abilities, which more strongly predict long-term outcome (Venter, Lord et al. 1992; Szatmari, Bryson et al. 2003; Billstedt, Gillberg et al. 2005; Sallows and Graupner 2005).

Randomization Method: We will randomize centrally, using our data management system. The UNC Data Team is familiar with this process as they have done this in our multicenter IMPACT trial. When a site needs to randomize a subject, after baseline is assessed, the coordinator opens the Randomization form. This form uses already assessed strata to randomize from the appropriate stratum. We will not stratify by site. We felt that both functional status and age group were more likely to be related to the outcome than site, and there are limits to how many strata we can have. We will stratify by functional status (high or low) and age group (3 age groups), producing 6 strata. The unblinded statistician will generate a randomization plan for each of the 6 strata, using a permuted block algorithm with randomly selected block sizes of 2 and 4, using SAS. The table will be exported to an Excel file and sent to the data manager. This can be done without having the lead statistician ever actually see the randomization plan. (He/she will debug the program and when debugged, run it with a new seed for the random number generator without seeing the results.) The database manager will import the randomization plan as a table in Access. The Randomization form

will use Visual Basic code and the values of the strata variables to enter the correct subset of the table and choose the first unused treatment. It will generate an ID number and send an email to the appropriate pharmacy, where drug will be assigned.

4.5.2 Masking Procedures

The spray and a matched placebo solution containing all of the same ingredients except for oxytocin will be packaged in indistinguishable nasal administration bottles.

5 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

5.1 Study Product Description

Oxytocin contains the synthesized peptide oxytocin in a solution formulated to promote absorption through the nasal mucosa.

5.1.1 Acquisition

All the proper documentation regarding how the drug and placebo is compounded/manufactured will be submitted to the FDA as an amendment to the current IND (#111604) held by Dr. Linmarie Sikich for sustained intranasal oxytocin treatment in 3-17 year olds with autism.

5.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

Participants in the study will be randomly assigned to treatment with intranasal oxytocin or placebo. Participants or their caregivers will be taught to administer the nasal spray, alternating nostrils, by the study personnel. The caregivers will be provided with suggestions for how to encourage their children to comply with the procedure including setting up a fun routine and providing rewards. The caregivers will also be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit and the dosing (if applicable). **The target dose for the study is 48/0 IU total daily dose (TDD).** The target dose will begin at the week 8/28 visit, unless issues with tolerance or compliance slow the recommended titration schedule. **Every effort will be made to achieve the target dose of 48/0 IU TDD** unless it is clear that the child cannot tolerate it.

5.2.1 Flexible Dose:

In the blinded phase, dosing will be flexible between 8/0 IU TDD and, after the target dose (48 IU TDD) has been maintained for ~ 7-8 weeks, up to 80/0 IU TDD, to allow one to optimize dosing for the individual child in cases of intolerance or inadequate response. Doses will typically be given in two equal amounts in the AM and the PM, with the maximal dose at any one time being 48/0 IU. If doses are uneven the larger dose should be given in the AM.
In the open label phase, dosing will be flexible between 24 IU TDD and, after the target dose (48 IU TDD) has been maintained for ~7-8 weeks, the dose can be increased to 72 TDD at week 36. The

preferences is for24 IU TDD to be given in the AM, 48 IU TDD should be split equally between morning and PM dosing and 72 IU TDD should be split as 48 IU in the AM and 24 IU in the PM.

Suggested Titration to the Target Dose

Blinded Phase: In the double-blind phase, titration to the target dose is expected to take 8 weeks and follow the suggested table below unless there are issues with tolerance or compliance. Dosing will begin at 8/0 IU in the AM at baseline. Typically, the dose will increase to 8/0 IU twice daily (BID) (16/0 IU TDD) at week 2. Dose will then be increased by 8/0 IU twice daily (BID) at weeks 4 and 8 with TDDs of 32/0 IU and 48/0 IU respectively. During the initial titration period (baseline to week 8), dosing increases should never occur before the time point specified in the schedule. Specifically one could not go to a TDD of 16/0 IU prior to week 2, or 32/0 IU prior to week 4 or 48/0 IU prior to week 8.

Week 0	Week 2	Week 4	Week 8
8 IU qD (8 IU TDD)	8 IU BID (16 IU TDD)	16 IU BID (32 IU TDD)	24 IU BID (48 IU TDD)

Open Label Phase: In the open-label phase, from week 24 to week 28, the dose will be 24IU in AM for a TDD of 24 IU. From week 28-36 the dose will be 48 IU TDD (ie. 24 IU BID). At week 36 the dose can be increased to 72 IU TDD (ie. 48 IU in the morning and 24 IU in the afternoon).

Week 24	Week 28
24 IU qAM (24 IU TDD)	24 IU BID (48 IU TDD)

5.2.3 Dose Deviations from Titration Schedule

Maintaining pre-target dose:

In the blinded phase (week 0-24) If the child is having difficulty tolerating the medication or administration of the nasal spray during the initial dose titration phase, the clinician may choose to hold the dose at weeks 2, 4 or 8 rather than increasing per the titration suggestions. If dose is held, it may then be increased by 8/0 IU 1-2 x/day after assessment of the participant (phone/email or in person) between scheduled visits if desired in order to achieve the target dose of 24/0 IU twice daily or 48/0 IU total daily dose as close to the week 8 visit as possible.

In the open-label phase if the child is having difficulty tolerating the medication, the clinician may choose to hold the dose at 24 IU qD at week 28, 32, 36, 40, or 44.

Dosing Reductions: All dosing reductions should be discussed with the site PI within 2 business days and reported to the lead site for discussion with other treatment site PIs.

Mandatory Dose Reductions: The dose must be reduced to the highest previously well tolerated dose *if any of the following criteria* are met AND the treating clinician is

comfortable with the decrease. The dose may be reduced between appointments in the first two cases.

Clinician judges an AE to require immediate reduction of dose OR
CGI-I score is 6 or 7 OR
Two consecutive CGI-I scores at in person visits are worse than the preceding two CGI-I scores.

If any of the three criteria are met, but the treating clinician is not comfortable with the decrease, the dose will be maintained until discussed with the all treating site PIs. The majority of the treating site PIs may determine that a mandatory decrease should be made.

Optional Dose Reductions:

In the blinded phase the dose may be reduced by 8/0 IU once or twice a day at any time at the clinician's discretion due to a clinically significant adverse event, poor tolerability of dosing, parent request. The dose should not be subsequently increased without reassessing the participant by phone/email or in person. The dose can be increased between scheduled visits after clinical reassessment of the participant (phone, email or in person).SLAES and CGI-I to be completed at physician discretion. If SLAES is not completed, then adverse events must be assessed and documented.

In the open-label phase the dose may be reduced in 24 IU increments to a minimum dose of 24 IU every other day.

Dosing Increases Above Target Dose:

In the blinded phase after the target dose has been maintained for at least 7 weeks the dose may be increased by 16/0 IU TDD only at each of the subsequent visits. Thus, at week 16, the maximal possible dose will be 64/0 IU TDD. However, at week 20 the dose could be increased to either 64/0 IU TDD if there had been no prior increase at week 16 or, could be increased to 80/0 IU TDD if the dose had been increased at week 16.

In the open-label phase after the target dose has been maintained for ~ 7-8 weeks then the dose can be increased (at week 36) to 72 IU TDD.

Applies to both phases: Increases past the target dose are NOT required and may ONLY be made if the following conditions are met

- No evidence of clinically significant adverse events
- Parents agree
- Clinician agrees

5.2.4 Weaning Dose at Completion of Trial

For 72 IU TDD at week 48 we would reduce dose to 24 IU BID for 1 week then 24 qAM and then 24 IU every other day in the morning for a week then stop.

For 48 IU TDD at week 48 we would reduce to 24 IU every morning for a week and then reduce to 24 IU every other day for 1 week then stop.
For 24 IU TDD at week 48 we would reduce to 24 IU every other day for 1 week then stop.
For anything less than 24 IU TDD, no down titration is required

For early terminations, attempts should be made to follow down titration as listed above. It will not be considered a deviation if families are non-compliant.

At week 53-55 a phone/email visit should be completed to review the SLAES.

5.2.5 Interrupting Dosing

Administration of oxytocin may be interrupted on rare occasions due to other clinical conditions that temporarily prevent nasal administration, poor compliance with administration directions, or significant adverse events or acute worsening of symptoms or functioning. The treating clinician may restart treatment at the same or a lower dose after assessment of the participant (including email/phone with participant's caregiver). The lead site should be informed of interrupted dosing lasting more than 1week.

Table 3: Possible Dosing Options

DOSE NAME	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
Total Morning Dose	8	8	16	16	24	32	16	24	24	32	40	24	32	40	48	32	40	32	40	40	48	40
Total Afternoon Dose	0	8	0	8	0	0	16	8	16	8	0	24	16	8	0	24	16	32	24	32	24	40
Total Daily Dose	8	16	16	24	24	32	32	32	40	40	40	48	48	48	48	56	56	64	64	72	72	80

5.3 Accountability Procedures for the Study Intervention/Investigational Product(s)

Subjects (or their parent/LAR) will be asked to return all previously dispensed product at the next office visit. Staff will return all unused product to the site's investigational pharmacy for destruction. Sites should make all attempts to collect investigational product after the final phone/email contact even though subjects will not be returning to the clinic for an in person visit.

5.4 Assessment of Subject Compliance with Study Intervention/Investigational Product

Due to the difficulties of measuring compliance with weight, we will utilize a parent completed diary to measure subject compliance with medication treatment. Each daily dose morning and afternoon, will be recorded by the parent/caregiver on this diary.

5.5 Blinding/Unblinding

Due to the short half-life of oxytocin (estimated 3-15 minutes), and the lack of any treatment for overdose, unblinding will not be permitted in this study. The knowledge of treatment assignment will not alter the subject's immediate management, so therefore unblinding is not deemed appropriate for this study. Instead, in the case of a medical emergency, unexpected serious adverse reaction or clinician judgment, the study medication may be interrupted. Study medication may be interrupted (temporarily discontinued) at the discretion of any treating physician at a site at any time without prior approval from the site PI or Steering Committee (SC). In the case when the treatment is interrupted, the subject may be re-challenged if the case is discussed and approved by the SC.

5.6 Concomitant Medications/Treatments

All concomitant medications will be recorded. PRNs will not be recorded unless: they are taken for a period of 2 weeks or more AND they are taken more than 57% of the time (ie: 9 out of 15 days) OR the clinician feels there is a compelling reason to document them. The physician will record any changes to the subjects' psychiatric and non-psychiatric medications from baseline throughout the course of the study.

6.0 Schedule of Events	Scrn		Double Blind Treatment							Open Treatment			FU
Procedure		W0	W2	W4	W8	W12	W16	W20	W24/T	W28	W36	W48	W53-55
ADOS-2	X								X[7]				
ADI-R	X[7]												
Stanford Binet/Mullen	X								X				
DSM-V Checklist	X												
Inclusion/Exclusion	X	X											
Demographics		X											
Family Med Hx (N H Form)	X												
Randomization Form		X											
GUID Acquisition Form/GU D Record		X											
Con Meds	X	X		X	X		X	X	X	X	X	X	
Vital Signs	X	X		X	X		X	X	X	X	X	X	
Med/Psych History (SLAES)	X	X											
Adverse Effects (SLAES/Suicidality)	X	X		X	X		X	X	X	X	X	X	X
Full Physical Exam (NIH Form)	X												
Focused Clinical Physical Exam		X		X	X		X	X	X	X	X	X	
Medical History (N H Form)	X												
CGI S and I[0]		X		X	X		X	X	X	X	X	X	
VAS Change (Clinician and Coord)		X		X	X		X	X	X	X	X	X	
Social Skills Therapies		X		X	X		X	X	X	X	X	X	
Psychosocial Therapies		X		X	X		X	X	X	X	X	X	
Laboratory													
ECG	X								X				
Female Reproductive Status	X	X		X	X		X	X	X	X	X	X	
Urine/Serum Pregnancy[1]	X												
Safety labs[2]	X								X		X	X[7]	
mRNA sample	X	X**			X				X		X		
Methylation sample	X	X**			X				X		X		
Plasma/salivary oxytocin	X	X**			X				X		X		
Whole blood serotonin	X				X				X				
Parent Questionnaires													
Questionnaire Guidance Form		X		X	X	X	X	X	X	X	X	X	
Social Opportunity Questionnaire		X		X	X	X	X	X	X	X	X	X	
ABC		X		X	X	X	X	X	X	X	X	X	
PDDBI-SV		X		X	X	X	X	X	X	X	X	X	
CASI-5		X							X			X	
Caregiver Strain		X							X			X	
Vineland-II (Survey Form)		X							X			X	
SRS-2		X				X			X		X	X	
Subject Completed Questionnaires													
Reading Mind in Eyes Test[3]		X			X				X			X	
Self-Rating of social function		X							X			X	
Medication													
Oxytocin Administration Instructions*		X											
SOARS-B Parent Dose Sheet[5]		X	X	X	X		X	X	X	X	X	X	
Risks Handout		X							X				
Medication Compliance[4]				X	X		X	X	X	X	X	X	X
Oxytocin Dosing Log (staff)		X	X	X	X		X	X	X	X	X	X	X
Medication Diary Dispensed		X		X	X		X	X	X	X	X	X	

0-CGI-S only at baseline, CGI-I and CGI-S at most other time points (office visits)
1-Required at screening but can be done at any time during the course of the study at physician discretion Must have a negative pregnancy test within two weeks of baseline
2-Safety labs to include: glucose (random), CO2, Cl, K, Na, Creatinine, BUN, AST, ALT, full urinalysis, urine pregnancy in pubertal girls Screening safety labs must be repeated if more than 12 months between screening and baseline
3- Assessment will be obtained only in participants who have fluent phrase speech & can define basic feelings and friendship
4- Completed each time the participant returns study medication
5- The appropriate SOARS-B Parent Dose Sheet should be given at each time dose changes to parents
6-Stanford Binet or Mullen may be completed at physician discretion
7- At physician discretion
*Must be given at baseline, but also can be given at any other time during the course of the study at study staff discretion
**Optional redraw of mRNA, plasma/salivary oxytocin, and methylation at baseline for those who may have had a lengthy time between screening and baseline (not safety labs)
Procedures for visits are allowed to be completed on separate days, however, subjects may not be dispensed open label medication until all week 24 procedures are completed

6.1 Screening

NOTE: At any point in time, visits may be referred to as months or weeks. For the purposes of this protocol 1 month = 4 weeks (28 days).

Screening Visit (can be divided into multiple visits, if necessary) ~ approximately 6 hours

Subjects will complete the following procedures and assessments at the Screening visit:

- Provide written informed consent/assent (subject and/or parent/caregiver/LAR)
- Inclusion/Exclusion criteria checklist
- Medical/psych history completed by medical physician using SLAES and suicidality assessment
- Medical history and family medical history using the NIH-specific form
- Urine or serum pregnancy test for females of childbearing potential
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- ECG (pediatric cardiologist will read/confirm)
- Review of current concomitant medication use
- Full Physical examination (using NIH form including examination of body systems, head circumference and measurement of height and weight on standard scales)
- Full vital signs (heart rate, blood pressure, and temperature)
- Safety Labs (to the extent possible, blood samples will be obtained at approximately the same time of day and under non-fasting conditions)
- Genetics (Methylation/mRNA sample)
- Plasma/salivary Oxytocin (ideally drawn between 2pm and 6pm)
- Whole blood serotonin
- ADOS-2 (must be completed prior to randomization) and ADI-R (optional at physician discretion)
- Stanford Binet (abbreviated-ABIQ) or Mullen.
- For randomization purposes: The Mullen receptive language subscale MUST be completed on subjects who are 5 years, 8 months and younger if the Stanford Binet is completed)
- DSM-5 Checklist

Subjects may return for multiple screening visits in cases where assessments could not be completed in one visit (i.e., time constraints, subject noncompliance, etc.).

The first set of Genetics and Plasma Oxytocin samples will be drawn at the Screening visit(s) in conjunction with safety labs to reduce the number of needle pricks during the study. However, the subject may choose to wait to have these samples drawn until their Baseline visits once their eligibility has been confirmed. If the samples have been collected and the subject is determined to be ineligible, the blood samples collected for these purposes will be destroyed. If there is more than 12 months between screen and baseline then safety labs will have to be re-drawn to ensure safety of the participant.

6. 2 Randomized Double-Blind Treatment Phase: Oxytocin or Placebo (24 weeks; Week 0-Week 24)

Baseline Visit (Week 0) ~ approximately 3-4 hours

Subjects will complete the following procedures and assessments at the Baseline visit:

- Confirmation of Inclusion/Exclusion criteria
- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination

- Full vital signs (heart rate, blood pressure, temperature)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- Females of childbearing potential must have had a negative pregnancy test (either urine or serum) within 2 weeks of baseline visit
- Demographics (age, sex, race, ethnicity, date of birth)
- SLAES (monitoring for adverse events) and suicidality assessment
- Oxytocin Dosing Log
- Social Reciprocity Scale (SRS-2)
- Vineland Adaptive Behavior (Vineland-II Survey Form)
- Caregiver Strain Questionnaire
- Aberrant Behavior Checklist
- PDDBI-SV
- CASI-5(Sprafkin, Gadow et al. 2002; Gadow and Sprafkin 2009)
- CGI-S and baseline description of overall functioning
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET), if cognitively able
- Self-Rating of Social Functioning, if cognitively able
- Medication Diary dispensed to caregiver
- Parent Handout (study drug administration instructions)
- Parent Handout (study drug dosing guidelines)
- Parent Handout (risks)
- Randomization
- GUID Record Form
- For randomization purposes: The Mullen receptive language subscale MUST be completed on subjects who are 5 years, 8 months and younger if the Stanford Binet was completed, this must be done prior to randomization in the database)
- Visual Analogue Scale of Change (Clinician), baseline description of functioning only
- OPTIONAL redraw of mRNA, plasma/salivary oxytocin, and methylation for those subjects who may have had a lengthy time between screening and baseline appointments
- Participants or their caregivers will be taught to administer the nasal spray, alternating nostrils, by the study coordinators who will demonstrate medication administration. The caregivers will be provided with suggestions for how to encourage their children to comply with the procedure including setting up a fun routine and providing rewards.
- Caregivers will also be provided with a dosing handout (Parent Dose Sheet) to describe how many sprays of which bottle to take daily.

- Medication Diary Dispensed. This monitors subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable). The caregiver will also be asked to complete a medication diary to indicate when doses were administered. Subjects will be given enough oxytocin/placebo nasal spray to last them until their next visit. They will take it

once or twice a day. Caregivers will be instructed to administer the first dose on the afternoon of the baseline visit, between 12PM and 4PM.

- Parents will be given a hand out which highlights all the cardiac and anaphylaxis risks. The handout will also include information about how to monitor their child's pulse and what the normal ranges for pulse are based on age. Staff will review handout with parent to ensure that they understand all aspects of the handout.

Week 2 Email-10 minutes
Between week 0 and week 4 (at around week 2) staff will send the new dose schedule to the family to titrate the dose up. The families will be instructed to contact the study staff at any time if they feel that their child cannot tolerate the next dose.

Week 4 ~ approximately 1-2 hours
Subjects will complete the following procedures and assessments at the week 4 visit:

- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination
- Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Oxytocin Dosing Log
- Medication Diary dispensed to caregiver. The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable).
- Caregivers will also be given the Parent Dose Sheet that describes how many sprays to take of the study medication.
- Caregivers will be given a supply of medication

Week 8 ~ approximately 1-2 hours
Subjects will complete the following procedures and assessments at the week 8 visit:

- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination
- Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)

- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET), if cognitively able
- Genetics (Methylation/mRNA sample)
- Plasma/salivary Oxytocin (ideally drawn between 2pm and 6pm)
- Whole blood serotonin
- Oxytocin Dosing Log
- Medication Diary dispensed to caregiver (provide 2 months' supply of diaries). The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable).
- Caregivers will also be given the Parent Dose Sheet that describes how many sprays to take of the study medication.
- Caregivers will be given a supply of medication (to last until week 16 visit)

Week 12 ~ approximately 30 minutes-
Subjects will complete the following assessments at the week 12 visit:

- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- SRS-2

Week 16 ~ approximately 1-2 hours
Subjects will complete the following procedures and assessments at the week 16 visit:

- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination
- Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)

- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Oxytocin Dosing Log
- Medication Diary dispensed to caregiver. The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable).
- Caregivers will be given enough medication to last until the next visit.
- Caregivers will also be given the Parent Dose Sheet that describes how many sprays to take of the study medication.

Week 20 ~ approximately 1-2 hours

- Subjects will complete the following procedures and assessments at the week 20 visit:
- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination
- Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)
- Aberrant Behavior Checklist
- PDDBI-SV
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Oxytocin Dosing Log
- Medication Diary dispensed to caregiver. The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable).
- Caregivers will be given enough supply of medication to last until the next visit.
- Caregivers will also be given the Parent Dose Sheet that describes how many sprays to take of the study medication.

6. 3 Open-Label Treatment Phase

Week 24 ~approximately 3-4 hours (end of double-blind, start of Open-Label Treatment Phase)
Subjects will complete the following procedures and assessments at the week 24 visit:

- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination
- Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- Vineland
- CSQ
- CASI-5
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET), if cognitively able
- Self-Rating of Social Functioning, if cognitively able
- SRS-2
- Genetics (Methylation/mRNA) sample
- Plasma/salivary Oxytocin (ideally drawn between 2pm and 6pm)
- ECG
- Safety Labs
- Whole blood serotonin
- Risks Handout
- ADOS-2 (optional measure at site discretion)
- Oxytocin Dosing Log
- Stanford Binet/Mullen
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)
- Caregivers will be given enough supply of medication to last until their next visit
- Caregivers will also be given the Parent Dose Sheet that describes how many sprays to take of the study medication.
- Medication Diary dispensed to caregiver The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable).

Week 28 ~ approximately 1-2 hours (dose increased to target dose unless not clinically indicated)
Subjects will complete the following procedures and assessments at the week 28 visit:

- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)

- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)
- Aberrant Behavior Checklist
- PDDBI-SV
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Oxytocin Dosing Log
- Caregivers will be given enough supply of medication to last until their next office visit.
- Caregivers will also be given the Parent Dose Sheet that describes how many sprays to take of the study medication.
- Medication Diary dispensed to caregiver (provide 2 months' supply of diaries). The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable).

Week 36 ~ approximately 1-2 hours

Subjects will complete the following procedures and assessments at the week 36 visit:

- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination
- Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)
- Aberrant Behavior Checklist
- PDDBI-SV
- Oxytocin dosing log
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- SRS-2
- Safety Labs (blood and urine)
- Methylation sample
- mRNA sample
- Plasma/salivary oxytocin (ideally drawn between 2pm and 6pm)

- Medication Diary dispensed to caregiver (provide with 3 months' supply of diaries)
- The caregivers will be provided with a dosing handout to monitor subjects' weekly dosing to ensure that participants are reaching their optimal dose. The study staff will fill in the dates at each visit, as well as the dosing (if applicable).
- Caregivers will be given enough supply of medication to last until their next visit.
- Caregivers will also be given the Parent Dose Sheet that describes how many sprays to take of the study medication.
- For individuals participating in the PK study, there is no need for an additional tube for the regular plasma oxytocin levels. The T0 in the PK study serves as the plasma OT levels for this visit.

Week 48 ~ approximately 3-4 hours (End of Treatment Visit)
Subjects will complete the following procedures and assessments at the week 48 visit:

- Review all current medications (concomitant medication log)
- Focused Clinical Physical examination
- Full vital signs (heart rate, blood pressure, temperature, height and weight on standard scales)
- Females of childbearing potential will be asked to confirm the date of their last menstrual cycle and confirm contraception use if sexually active
- SLAES (monitoring for adverse events) and suicidality assessment
- CGI-I and CGI-S
- Social Skills Therapy Log
- Psychosocial Therapy Log
- Aberrant Behavior Checklist
- Vineland
- CSQ
- CASI-5
- PDDBI-SV
- Questionnaire Guidance Form
- Social Opportunity Questionnaire
- Reading Mind in Eyes Test (RMET), if cognitively able
- Self-Rating of Social Functioning, if cognitively able
- SRS-2
- Plasma/salivary Oxytocin (ideally drawn between 2pm and 6pm)
- Safety Labs (optional at physician discretion)
- Oxytocin Dosing Log
- Visual Analogue Scale of Change (Clinician)
- Visual Analogue Scale of Change (Coordinator)

Week 53-55- OFF treatment follow-up

This visit is to be completed shortly after the last dose of study medication. This may be completed via phone or email.

- SLAES (monitoring for adverse events)

- All attempts will be made by study staff (and documented) to obtain all dispensed bottles of medication

PK Visits (Optional)

In order to assess the PK (pharmacokinetic) properties of oxytocin, a select group of subjects who participate in the SOARS-B trial will be asked to participate in the PK study. Study staff will select the participants based on a) their willingness to participate and b) their ability to tolerate the study procedures. A total of 18 subjects will be enrolled across all 6 sites for the week 32 samples. There will be 6 subjects in each of the following age groups: 3-6, 7-12 and 13 -17 years old. Each site will be asked to enroll one subject from each of the age groups. There will be a separate consent form for this optional study.

Target Dose PK sample (48 IU TDD)

This visit may occur any time after week 32 (48 IU TDD)
This visit can be completed at any visit after week 32 or at an unscheduled visit
There will be a target of 6 participants in each age cohort total
if possible please also obtain vitals and PDDBI-SV and ABC.

Procedures are as follows:

- IV placement by study staff (saline solution only)
- Plasma oxytocin draw at T = 0 (time 0, immediately after insertion)
- Administration of intranasal oxytocin (target dose = 24IU but will be whatever dose the subject is currently prescribed to as part of the SOARS-B protocol)
- Plasma oxytocin draw at T = 15 minutes
- Plasma oxytocin draw at T = 30 minutes
- Plasma oxytocin draw at T = 45 minutes
- Plasma oxytocin draw at T = 60 minutes
- If feasible plasma oxytocin draw at T= 2-7 hours post dose
- Total blood volume = 42 mL (7mL per draw times 6 draws)

Max Open Label Dose PK sample (72 IU TDD)

This visit may occur any time after the highest dose has been achieved (at an unscheduled visit or regularly scheduled visit)
The target is to have 2-6 participants receiving the 72 IU total dose, (at all sites) regardless of age
If this is not occurring at a regularly scheduled visit, please also obtain vitals and PDDBI-SV and ABC.

Procedures are as follows:

- IV placement by study staff (saline solution only)
- Plasma oxytocin draw at T = 0 (time 0, immediately after insertion)

- Administration of intranasal oxytocin (target dose = 40IU BID)
- Plasma oxytocin draw at T = 15 minutes
- Plasma oxytocin draw at T = 30 minutes
- Plasma oxytocin draw at T = 45 minutes
- Plasma oxytocin draw at T = 60 minutes
- If feasible plasma oxytocin draw at T= 2-7 hours post dose
- Total blood volume = 42 mL (7mL per draw times 6 draws)

Additional Risks for IV

Most of the risks for IV insertion are the same as for venipuncture (bruising, pain, possible infection). Complications of gaining IV may include infiltration, hematoma, an air embolism, phlebitis, and intra-arterial injection. Infiltration is caused by the infusion of fluid outside the vesicles (vein), into the surrounding soft tissue. This is generally caused by poor placement of a needle outside of the vessel lumen. Clinically, you will notice swelling of the soft tissue surrounding the IV, and the skin will feel cool, firm, and pale. IV fluids are not dangerous to the surrounding soft tissues however certain medications can be toxic. We will not be administering any medications only IV fluids therefore this will not be an issue in our study. A hematoma occurs when there is leakage of blood from the vessel into the surrounding soft tissue. This can occur when an IV catheter passes through more than one wall of a vessel or if pressure is not applied to the IV site when the catheter is removed. A hematoma can be controlled with direct pressure and will resolve over the course of 2 weeks. An air embolism occurs as a result of a large volume of air entering the patient's vein via the IV administration set. Air embolisms are rare and easily prevented by making sure that all the air bubbles are out of the IV tubing before placement. Phlebitis is inflammation of the vein which occurs due to the pH of the agent being administered during the administration of the IV, since we are not placing any medications into the IV we will not be at risk for this complication. An intra-arterial injection occurs very rarely. This can easily be prevented, by making sure that the needle is inserted in a vein. An experienced phlebotomist would not have an issue with the placement. If an artery is cannulated, there will be a pumping of bright red blood back into the angiocath, which would not be seen when you cannulate a vein. In the case of intraarterial injection, it is the intravenous drugs which pose severe problems, rather than the IV solution and since we will not be placing any drugs into the IV this will not be an issue for our study. In order to minimize these risks, we will ensure that all study staff that place the IV are well trained in proper procedures and use aseptic procedures.

6.5 Early Termination Visit

If a subject terminates from the study early, then the following procedures should be completed as close as possible to the last dose. These procedures may be completed over the phone/email or in an office visit:

- SLAES

- Suicidality assessment
- CGI-I/S
- Con Meds
- Psychosocial/Social Skills Therapy Logs
- Vitals (only if in office)
- Self-Rating of Social Functioning (only if in office)
- RMET (only if in office).

The questionnaires from the week 24 visit should be sent to the families and completed as close to the last dose taken as possible. If in open-label phase send week 48 questionnaires.
Any subject who is not able to return to the clinic or available for a phone call for an early termination visit within 2 weeks of their last dose will be considered lost to follow up no ET visit is required. All efforts should be made to retrieve the medication bottles from the families either mailed back or in person.

6.6 Unscheduled Visit or Re-Evaluation Visit

Subjects may be asked to come in for an unscheduled visit at any time during the course of the study if the treating physician feels it is necessary to see the subject in person or to complete a PK visit. Subjects will be instructed to contact the site between scheduled visits if there are any issues that need to be addressed.

Subjects may also be re-evaluated with appropriate assessments completed for any of the following reasons:

- If the subject's dose is mandatorily reduced, then these assessments should be completed 2-4 weeks after the dose has been reduced.
- If the subject has had a CGI-I score of 6 or 7, then the assessments should be completed two-four weeks later at physician discretion.

During these visits, all efforts should be made to obtain the following assessments:

- Vital signs (only if in person visit)
- Review of concomitant medications
- SLAES (adverse events) and suicidality assessment
- CGI-S and CGI-I
- Focused Clinical Physical Exam (only if in person visit)
- If physician feels it is indicated, safety labs or ECG may be obtained.

Unscheduled Assessments

If the site learns that a participant's treatment has been interrupted for 1 week or longer, an unscheduled visit should be performed.

7 STUDY PROCEDURES/EVALUATIONS

7.1 Study Assessments

Parent Assessments

Questionnaire Guidance Form: This form will give parents guidance on time frames and specific instructions for how to complete the questionnaires. This form was created by study staff with guidance from manuals from the standardized forms listed below.

Social Opportunities Questionnaire: This created form asks parents to rate how frequently their child has the opportunity to interact with different individuals in the community, home, school and daycare/after-school setting. It also asks, of those opportunities that their child has, does their child actually utilize those opportunities to interact with individuals in a social manner.

Social Reciprocity Scale-2: This 65-item rating scale measures the severity of autism spectrum symptoms as they occur in natural social settings (Constantino, Davis et al. 2003). Completed by a parent or teacher in just 15 to 20 minutes, the SRS provides a clear picture of a child's social impairments, assessing social awareness, social information processing, capacity for reciprocal social communication, social anxiety/avoidance, and autistic preoccupations and traits. It is appropriate for use with children from 4 to 18 years of age. The SRS measures impairment on a quantitative scale across a wide range of severity--which is consistent with recent research indicating that autism is best conceptualized as a spectrum condition rather than an all-or-nothing diagnosis. The SRS-R will take the parent/caregiver/LAR approximately 10 minutes to complete.

Pervasive Developmental Disorders Behavior Inventory (PDDBI-Screening Version) - The PDDBI-SV examines both adaptive and maladaptive behaviors related to social behaviors in autism. It is a parent/caregiver questionnaire and has 18 items. It takes about 5-10 minutes to administer.

Aberrant Behavior Checklist (ABC)-The ABC focuses on problem behaviors in five subdomains: irritability, attention, repetitive behaviors, unusual speech, and social withdrawal. This should take the caregiver approximately 10 minutes to complete.

Caregiver Strain Questionnaire (CSQ – Berument): The CSQ will assess family stress and was developed for caregivers of children with developmental disabilities. This should take the caregiver approximately 5 minutes to complete.

Medication Diary: Caregivers will be asked to record each dose given to the child on a written log. Caregivers will be asked to return this at each visit for the study team to review if there are any medication compliance issues.

Parent Handout (Risks): Caregivers will be given an informational handout at baseline (beginning of Randomized Phase) and again at week 24 (beginning of Open Label Phase). This handout will describe possible symptoms to look for.

Dosing Guide/Medication Administration Form: Caregivers will be given an informational handout that describes study drug administration and dosing guidelines. Participants will be instructed to increase the dose per the titration schedule as long as there are no problems and will be strongly encouraged to contact the study coordinator if there are any concerns.

Vineland Scales of Adaptive Functioning (2nd edition, Parent/Caregiver Rating Form): The VABS-II is a survey administered to a parent or caregiver in a questionnaire format and is organized around four Behavior Domains: Communication, Daily Living Skills, Socialization, and Motor Skills. The

VABS-II will take the parent/caregiver/LAR approximately 20-60 (average 30) minutes to complete, depending on the subject's age and level of functioning. For the purposes of this study, we will not be assessing maladaptive behaviors.

Childhood Anxiety Sensitivity Index (CASI-5): This scale is designed for measuring anxiety sensitivity (i.e., the belief that anxiety symptoms have negative consequences. There will be one form that is for children ages 3-4 and a different one for ages 5-17.

Physician/Clinician Assessments

Clinical Global Impressions Scale: Overall psychiatric functioning will be assessed with the severity (CGI-S) and improvement (CGI-I) subscales of the CGI (Guy 1976). CGI-S items are rated from 1 (normal, not ill) to 7 (very severely ill). CGI-I items are rated from 1 (very much improved) to 7 (very much worse).

Physical Examination: A physical examination can include, but is not limited to, abdominal palpation to assess for liver enlargement, assessing for decreased tone and postural problems, increased drooling and poor orofacial tone, problems holding head up as well, difficulty with coordination of motor actions. A more thorough exam will be done at screening and a more brief/clinician focused one completed at subsequent in person visit.

Concomitant Medication Log: At screening, the physician will make a list of any medications the subject is currently taking (ie: on the day of screening). The physician will record any changes to the subjects' psychiatric and non-psychiatric medications from screening throughout the course of the study. PRNs will not be recorded unless:

> they are taken for a period of 2 weeks or more AND they are taken more than 57% of the time (ie: 9 out of 15 days)
>
> OR
>
> the clinician feels there is a compelling reason to document them in which case this can be documented in a note to file. Example: a PRN that is used to address an AE may be noted directly on the SLAES form for clinician reference in the future, but will not be data entered.

Inclusion/Exclusion: A checklist will be completed to ensure all inclusion and none of the exclusion criteria are met.

DSM-V Checklist for Autism: The proposed DSM-V criteria for autism will be documented in the form of a checklist for a clinician/physician to complete.

Family Medical History: This form will ask for information about genetic, mental health and medical conditions of relatives of the subject.

Medical History: This form will ask about the subject's medical history such as surgeries, medical procedures etc.

Oxytocin Dosing Log: This log is completed by the physician to record all changes in dosing of oxytocin. It will include start/stop dates of when the dose changes.

Trained Rater/Physician

Systematic Longitudinal Adverse Events Scale (SLAES): systematic elicitation and screening of adverse events will be completed using the SLAES. This will also be used at screening and baseline to obtain a comprehensive psychiatric and medical history of the patient.

Suicidality Assessment: The physician will use clinical judgment to determine if the participant understands the concepts of death and making one's self die or hurt. If the participant is deemed able to understand these concepts they will be asked if they have had any thoughts about wanting to die, wanting to hurt themselves, wanting to kill themselves and if s/he has done anything to hurt himself/herself so he/she would die or have done anything to hurt himself/herself for any other reason. If the participant is deemed not to understand these concepts, his/her caregiver will be queried by asking whether the child has said or done anything that makes the parent think the child wanted to die, to hurt himself/herself or to kill himself/herself and asking about suicidal behaviors. If any are endorsed, the caregiver will also be asked if this is a significant change in severity or frequency from the participant's baseline. The clinician will determine whether the behaviors clearly do not appear stereotypic, might be stereotypic and are clearly stereotypic (e.g. chronic repeated head banging or self biting).

Female Reproductive Form: We will record the last menstrual period, document irregular periods and verify continued use of two forms of birth control (if sexually active).

Vital Signs: Vital signs will be measured at each visit and will include heart rate, sitting blood pressure, and temperature.

Stanford Binet (5th edition): The Stanford-Binet intelligence scale is a standardized test that assesses intelligence and cognitive abilities in children and adults aged two to 85+ years. The Stanford-Binet Scale tests intelligence across four areas: verbal reasoning, quantitative reasoning, abstract/visual reasoning, and short-term memory. The areas are covered by 15 subtests, including vocabulary, comprehension, verbal absurdities, pattern analysis, matrices, paper folding and cutting, copying, quantitative, number series, equation building, memory for sentences, memory for digits, memory for objects, and bead memory. The abbreviated IQ (ABIQ) will be used for this study and includes non-verbal fluid reasoning and verbal knowledge subtests.

Mullen Early Scales of Learning (Mullen): Is for birth to 68 months and is designed to assess five scales: Gross Motor, Visual Reception, Fine Motor, Expressive Language, and Receptive Language. We will not be using the Gross Motor subtest for this study since all our participants are over 36 months. If children younger than 5 years 9 months are assessed with the ADOS Module 1 or 2, the receptive language subscale of the Mullen must be administered to determine their functional status even if the Stanford Binet has been completed.

Autism Diagnostic Observation Schedule-2 (ADOS-2): The ADOS is a semi-structured assessment used to assess and diagnose individuals suspected of having autism of varying ages, developmental levels, and language skills (from no speech to verbally fluent). The ADOS includes four modules, each requiring just 35 to 40 minutes to administer. The individual being evaluated is given just one module, depending on his or her expressive language level and chronological age. The rater will observe social and communication behaviors during various activities in the appropriate module.

Autism Diagnostic Interview, Revised (ADI-R): The ADI-R is a semi-structured, investigator-based interview for caregivers of children and adults for whom autism or pervasive developmental disorders is a possible diagnosis. The revised interview has been reorganized, shortened, modified to be appropriate for children with mental ages from about 18 months into adulthood and linked to ICD-10 and DSM-IV criteria. The detailed interview focuses on early development in social and communication and self-help skills of the child, and takes approximately 2 hours to administer.

Demographics: Information such as age, race, ethnicity, family status and income will be collected.

Electrocardiogram: Trained staff will collect an EKG on each subject and this will be read and confirmed by a pediatric cardiologist.

Reading in the Mind's Eye Task: This computerized task consists of a series of pictures of eyes in which the subject needs to determine which emotion the eyes are expressing.

Self-Rating of Social Functioning: This staff created assessment consists of several questions that asks the participant directly to reflect on their own experience with social skills.

Visual Analogue Scale of Change (Clinician and Coordinator): Both the treating clinician and study coordinator (separately) will place a vertical line across a continuous 18 cm horizontal line. No change will be reflected at the midpoint of the line, extreme worsening at the leftmost side and extremely better at the right most side. Three areas will be assessed: overall functioning similar to the CGI-I, social-communicative functioning and repetitive behaviors/restricted interests. In contrast to the CGI-I, the changes will be assessed after carefully considering the individual's functioning in each of the domains at baseline as briefly described by the clinician. The outcome measure will be the distance in cm from the left end of the line to the vertical mark.

Other

- *GUID Record Form:* This form will record the GUIDs that are obtained for the subject and any parent who is willing to consent to have his/her blood take for the genetic repository at NIMH
- *GUID Acquisition Form:* This form is designed to obtain all the information necessary in order to assign a GUID such as full name, date of birth, city of birth (as it is written on one's birth certificate).
- *Randomization Form:* This form will be used by study staff to enter into the database in order to determine if a subject is high functioning or low functioning for randomization purposes.
- *Social Skills Therapies Log:* This form will record the number of hours in the previous month that the child received social skills therapy.
- *Psychosocial Therapy Log:* This form will record the number of hours in the previous month that the subject received any additional psychosocial therapy such as ABA, equine therapy, speech therapy etc.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

Chemistry Panel: The chemistry panel will include glucose (random, non-fasting), CO2, Cl, K, Na, Creatinine, BUN, AST, ALT at Screening, week 24, and week 24. Total blood to be collected is 8.5mL in SST tube.

Pregnancy test: Will be serum or urine completed at screening but can be done at any time throughout the course of the study at the physician's discretion. Additionally, for females of childbearing potential, they must have had a negative pregnancy test (urine or serum) within 2 weeks of randomization. This can be done by an onsite urine dipstick pregnancy test either at screening or baseline (prior to randomization). This will not require any additional blood and will be collected as part of the above chemistry panel.

Plasma Oxytocin: We will assay plasma oxytocin levels using standard radioimmuno assays in Dr. Pedersen's lab in order to describe potential relationships between baseline levels and treatment response. We expect there to be minimal changes in plasma levels with intranasal administration, since oxytocin is rapidly degraded. Total 7ml of blood drawn (one 7ml lavender top tube).

Serotonin Levels: Serum serotonin levels will be measured by aliquoting 200uL of whole blood from the methylation tube. No extra blood is needed for this.

Urinalysis: A full urinalysis panel is required including at least the following: specific gravity, pH, color/appearance, protein, glucose, leukocyte esterace, occult blood).

Salivary Oxytocin: Saliva will be collected from subjects. Subjects will be asked to hold a children's salivary swab in their mouths under the tongue (like a thermometer) for 1.5 min.

7.2.2 Duke Genetics

Genetics Study: Dr. Gregory will perform the methylation and mRNA expression studies, For the methylation studies, DNA will be extracted from peripheral blood, bisulfite converted, PCR performed using primers targeted to the bisulfite-converted regions of the CpG islands in the promoter region and third intron of the *OXTR* gene, and the resulting clones sequenced to calculate percentage of methylation at each of the CpG sites. Total RNA will be extracted from blood samples, RNA quality checked and quantitative PCR run after reverse transcription using primers for *OTXR* exon 2 and the *OXT*. We will use already collected samples (after running initial analysis of mRNA expression and methylation) for unspecified genetic analysis and also for other analyses to identify possible unspecified genetic factors that may influence response to oxytocin treatment. Total 8.5ml of blood drawn (one 2.5ml PAX gene tube and two 3ml lavender top tubes)

8 ASSESSMENTS OF SAFETY

8.1 Adverse Events

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding that is not directly obtained for purposes of the study), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor.

All AEs will be captured on the appropriate source documentation, the Systematic Longitudinal Adverse Event Scale (SLAES).Information to be collected includes event description and clinician's assessment of severity.

8.1.1 Treatment Emergent Adverse Events

In this trial we are documenting all AE's, but analysis will focus only on Treatment Emergent AE's (TEAE's). Medical and behavioral conditions that are present at screening and/or baseline will only be considered treatment emergent adverse events if their severity increases significantly after the participant has taken at least one dose of study treatment. Intermittent conditions such as seasonal allergies will only be considered TEAEs, if the severity or frequency is significantly greater than in the previous two years. Only TEAEs will be considered in the adverse event safety analyses.

If a TEAE occurs, its relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, DDS, DMD, PA, Nurse, Nurse Practitioner or DO), and resolution/stabilization will be coded at the resolution of the event or end of the subject's participation in the study. All TEAEs occurring while on study must be documented appropriately regardless of relationship. All TEAEs will be followed to adequate resolution. If a TEAE is a severe adverse event rating (3) and an Unexpected Problem (UP-no prior history of issue/not commonly seen in autistic patients and not described as a risk of the treatment), the UP form must be completed. If a TEAE is an SAE the the SAE form and SLAES (unscheduled visit) must be completed.

Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity.

- **Mild:** events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate:** events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe:** events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- **Life-threatening**: Substantial risk dying or requires intervention to prevent death
- **Death related to AE:** Subject died as a result of the event, self-explanatory

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

Relationship to Study Treatment: The clinician's assessment of an AE's relationship to test article (study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to study product assessed using the terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Yes (certain)**: The adverse event and administration of the study drug are related in time, and a direct association can be demonstrated.
- **Likely (probably)**: The adverse event and administration of the study drug are reasonably related in time, and the adverse event is more likely explained by the study drug than other causes
- **Possible**: The adverse event and administration of the study drug are reasonably related in time, and the adverse event can be explained equally well by causes other than study drug.
- **No (unrelated)**: The adverse event is clearly explained by another cause not related to the study drug.

Pattern Definition: This is to help the clinician define the pattern of the AE and give some clarity to timing of an event.

- **Isolated**- Occurs a single time
- **Intermittent**- An AE that stops and starts with clear defined points (eg. seasonal allergy)
- **Continuous**- ongoing AE (eg. migraines)

Change/Outcome-This should be captured in order to help the clinician make a clear decision on a severity rating and better follow the AE through time.

- **No change** from last AE report
- **Worsening** from the last AE report
- **Improving** from the last AE report
- **Recovered** from the last AE report

Action Taken-The action taken after the report of an AE can be captured by the definitions below:

- O = None
- M = Monitoring
- CM = Conmed/Rx
- D = Decrease study med
- UP = Increase study med
- S = Interrupt study med
- W = Withdrawal from study

8.1.2 Suicidality Assessment

Suicidality assessments will be completed for each participant at each visit except weeks 2 and 26. The physician will use clinical judgment to determine if the participant understands the concepts of death and making one's self die or hurt. If the participant is deemed able to understand these concepts they will be asked if they have had any thoughts about wanting to die, wanting to hurt themselves, wanting to kill themselves and if s/he has done anything to hurt himself/herself so he/she would die or have done anything to hurt himself/herself for any other reason. If the participant is deemed not to understand these concepts, his/her caregiver will be queried with the thought questions replied by saying or doing anything that makes the parent think the child wanted to die, to hurt himself/herself or to kill himself/herself and asking about suicidal behaviors.

The caregiver will also be asked if this is a significant change in severity or frequency from the participant's baseline.

The clinician will discuss any self-injurious behaviors with the steering committee describing the events, the participant's prior history if any of similar behaviors or suicidal ideation, and the caregiver's perception of any change in severity or frequency of the behaviors. The steering committee will then determine whether the behaviors clearly do not appear stereotypic, might be stereotypic and are clearly stereotypic (e.g. chronic repeated head banging or self biting). These cases will also be reviewed by the medical monitor and DSMB.

8.2 Reportable Events

8.2.1 Serious Adverse Event (SAE):

An SAE is defined as an AE that meets one of the following conditions:

- Death during the period of protocol-defined surveillance
- Life-threatening event (defined as a subject at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, requires medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room, blood dyscrasias or new onset of convulsions that do not result in inpatient hospitalization.

All SAEs will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician.

8.2.2 Unanticipated Problems (UP)

We will consider unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2.3 Pregnancy

In the case of a pregnancy of either the subject (female) or the subject's partner (for male subjects), sites/investigators should follow the pregnancy until completing (either termination or birth). The baby should also be followed until he/she is 1 month old to assess and record any medical complications. All this information should be sent to the Coordinating Center via the above referenced contact information. The initial report (on the appropriate source document) should be to the coordinating center within 7 days of the site's awareness.

8.2.4. Serious Suicidal Ideation

In the case of serious intentional self-harm/serious suicidal ideation, the physician/clinician should take necessary steps to ensure the safety of the subject including involuntary hospitalization (if needed). This should be reported (on the appropriate source document) to the coordinating center within 7 days of the site's awareness.

8.2.5. Alert Lab/ECG values

Alert values for laboratory tests (clinical/safety labs) and for ECGs will be determined by the site's treating physician and/or PI for each individual study subject. For the DSMB reports, the PIs will decide on specific values for each of the clinical labs and ECGs. In the event that a site has a lab/ECG value that is considered to alarming by either the site treating physician or site PI, this must be reported (on the appropriate source document) to the coordinating center with 7 days of the site's awareness.

8.3 Reporting Procedures

8.3.1 Treatment Sites Reporting to Duke

Any event that is considered reportable based on section 8.2 by the PI or Subinvestigator or which meets the aforementioned criteria must be submitted to the Coordinating Center. It should be reported on the following numbers. It is preferable to have a written report (fax or email) for documentation purposes.

Coordinating Center E-mail: cheryl.alderman@duke.edu and linmarie.sikich@duke.edu

The study clinician will complete and submit the appropriate reporting form within the following timelines:

- SAEs that are deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the Serious Adverse Event Form and sent by fax within 2 business days of site awareness.

- SAEs other than death and immediately life-threatening events, regardless of relationship, will be reported by fax within 7 business days of site awareness.
- UPs Form within 7 business days of the site's awareness of the event.
- Pregnancies within 7 business days of the site's awareness
- Serious Suicidal Ideation within 7 business days of the site's awareness
- Alert lab or ECG values within 7 business days of the site's awareness
- Decisions to reduce dose within 7 business days.
- Interrupted dosing lasting more than 7 days within 7 business days of site's awareness.
- Requests to delay mandatory reductions in dose within 5 business days.
- Participants with a CGI-I of 6 or 7 within 7 business days of the assessment.

Other supporting documentation of the event may be requested and should be provided as soon as possible. Sites should report SAEs, UPs and other reportable events to their local IRB's per the local IRB's guidelines and procedures. This may differ between sites.

8.3.2 Duke Reporting to Other Regulatory Bodies

Medical Monitor: Duke will report all SAEs, UPs and other reportable events (section 8.2) to the medical monitor within 14 days and receive acknowledgment that she has reviewed them.

DSMB: Duke will report SAEs, UPs and other reportable events (section 8.2) that are considered possibly related to study treatment to the DSMB within 14 days of being made aware of their occurrence.

FDA: To be reportable to the FDA, the event must meet 3 criteria. The investigator or the holder of the IND may make the determination if the event meets all three of the following criteria.

- Suspected Adverse Reaction: meaning there is a reasonable probability that the drug caused the event. Meaning there is evidence to suggest a causal relationship between the drug and the adverse event.
- Serious (use definition of serious in section 8.2.1)
- Unexpected (use definition of unanticipated in section 8.2.2)

Timelines for reporting to the FDA:

- Unanticipated fatal or life threatening adverse events (7 days)
- Unanticipated non-fatal/non-life threatening (14 days)

Steering Committee: Duke will report all SAEs/UPs/other reportable events (section 8.2) to the steering committee within 14 business days of Duke's awareness.

Duke's local IRB: The coordinating center is responsible for reporting unanticipated problems to their local IRB as their local IRB has direct oversight of Duke and indirect oversight of other sites.

The timelines for reporting unanticipated problems to the IRB are:

- Unanticipated Problems that are serious adverse events should be reported to the IRB within one (1) week of the investigator becoming aware of the event.
- Any other Unanticipated Problem should be reported to the IRB within two (2) weeks of the investigator becoming aware of the problem.
- Other reportable events (section 8.2): during annual renewal

8.5 Individual Stopping Procedures

If a subject is worsening clinically (they have CGI-I score of 6-much worse or 7-very much worse on CGI-I, there has to be a return visit/phone call within 1-2 weeks to revisit/re-evaluate with CGI-I. If the subject still has 6 or 7, then case must be discussed with SC and Medical Monitor within 10 business days to get approval for the subject to continue in the trial.

Subjects **may be withdrawn** for any of the following reasons:

- If clinically significant and/or personally intolerable moderate or severe adverse events occur that cannot be addressed by dose adjustment or addition of concomitant medications (these can be discussed with SC and Medical Monitor. Investigators are encouraged to try dose reduction or addition of concomitant medications prior to withdrawal of participants).
- If a subject is worsening clinically (they have 6 much worse or 7 very much worse on CGI-I), there has to be a return visit/phone call within 1-2 weeks to revisit/re-evaluate with CGI-I. If the subject still has 6 or 7, then case must be discussed with SC and Medical Monitor to get approval for participation to continue.
- Study noncompliance
- Study physician discretion

Subjects **must be withdrawn** for the following reasons:

- At the participant or guardian request
- If the medical monitor feels worsening of their clinical condition or life threatening adverse events are likely to be related to the study treatment or procedures.

8.6 Safety Oversight

8.6.1 Independent Medical Monitor

[redacted] has a wealth of experience in pediatric oxytocin administration as she has conducted many of her own trials. She will serve as the independent medical monitor for this study. She will review alarm values of labs and ECGs as defined by site treating physician and/or site PI, UPs, SAEs, pregnancies, serious suicidal ideation. and serious adverse events within 14 working days of their reporting. She will also review all new treatment emergent adverse effects of moderate or greater severity two times a year prior to DSMB meetings. She will be available to DSMB and to steering committee to discuss concerns about safety related to the 2 above activities. She will also discuss any case in which an individual participant has significant worsening reflected by a CGI-I of 6 or 7 that has not improved over the course of 8 weeks and/or with dose reduction.

8.6.2 Data Safety Monitoring Board (DSMB)

The UNC TRACS Data and Safety Monitoring Board (DSMB) will monitor data from all sites. The principle role of the DSMB is to monitor the data from clinical trials to protect the safety of the research participants. To achieve this, the DSMB will: a) establish a 2 times/year schedule; b) review proposed protocols for safety and validity; c) evaluate recruitment and rate of enrollment in relation to the projected activity; d) monitor the occurrence of adverse events, serious adverse

events, and early withdrawals or terminations throughout the course of the study; e) review with a designated research staff member the pattern of the study data; and f) evaluate study outcomes, when available. It will be reviewed by the DSMB 2 times/year.

8.6.3 Overall Study Stopping Criteria

At 26 week intervals, the study biostatistician will test whether there are significant differences between those on active treatment and those on placebo for Severe and Life Threatening/Fatal adverse events. The statistical analysis method will be a Fisher Exact Test. The significance level considered significant for these tests will be 0.05 without correction for multiple comparisons. If there are any significant differences that indicate greater risk for active treatment, further enrollment will be stopped, but participants currently being treated will continue. These results will be provided to the DSMB along with the summary report of enrollment and other safety data at their next regularly scheduled meeting. If the DSMB will evaluate the apparently increased risk and work with the SOARS Steering committee as necessary to develop a mutually acceptable plan for addressing the apparently increased risk. Such decisions might involve closing the study entirely, changing dosing or implementing additional monitoring.

8.6.4 Cardiac Monitoring Plan

Based on the patient's age, gender, and height percentile, we have adapted blood pressure parameters where blood pressures above or below the set threshold will prompt the PI to consider doing an EKG. These parameters were derived from the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents(Falkner, Daniels et al. 2004). We have likewise adapted parameters from Pediatric cardiology for practitioners(Park 2008) and Normal ECG standards for infants and children (Davignon, Rautaharju et al. 1979)to establish heart rate guidelines where patients whose heart rate is above or below these thresholds will be considered for an EKG at every visit. Finally, we will incorporate clinical signs and symptoms of cardiac risk into our assessment with the vital sign measurement and specifically query for the presence of syncope, dizziness, palpitations, shortness of breath, and bradycardia or tachycardia. The clinical parameters will be incorporated into the SLAES. The specific parameters for heart rate, blood pressure are located in Appendix A. We believe, given the low cardiac risk of oxytocin, that careful monitoring of vital signs and clinical signs and symptoms at every visit will be adequate to ensure the safety of our patients.

9 CLINICAL MONITORING

9.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6 and, when appropriate, regulatory guidelines. This section will give a general description of how site monitoring will be conducted. The data monitor will be housed at the Data Center at UNC Chapel Hill. The Data/Regulatory Monitor will monitor the database, with the data manager, in order to assess quality assurance and control issues, and will generate queries from the database. This individual will also monitor each site's regulatory documents for

completeness and accuracy (delegation of duties logs, 1572s, FDFs, general correspondence, IRB correspondence, laboratory values etc). He/she will travel to each site in order to monitor the study coordinators and the case report forms to ensure that data are collected completely and accurately. Specifics of site monitoring will be included in the separate data monitoring plan. He/she will ensure that queries are resolved. He/she will also ensure that data is properly and accurately entered into the database.

10 STATISTICAL CONSIDERATIONS

The overall statistician or his/her designee will conduct and have ultimate responsibility for all data analysis described below. All statistical computing will be done using the most recent version of SAS. The lead statistician will do the programming and perform the analyses. The analyses described below will be performed on the intent-to-treat population. Additional exploratory analyses may be performed on the per protocol population or key subsets of the sample (e.g. those participants without fluent phrase speech). All data will be explored using descriptive statistics and graphical techniques prior to any hypothesis testing. For categorical variables, we will examine frequency distributions and where appropriate contingency tables and histograms. For continuous variables, we will examine frequency distributions and, where appropriate, box-and-whisker plots. When appropriate, we will consider transformation. If necessary due to distributional considerations, we will cautiously consider a change of analysis method to a less parametric one.

10.1 Statistical Analysis (Please also see SAP)

General Modeling: Unless otherwise specified, we will fit a mixed longitudinal model with change from baseline to each post-baseline month (4 weeks) for a response variable, functioning (low versus high) as the stratification variable, center as a blocking factor, treatment (oxytocin versus placebo) as the between-subjects factor, week as a within-subjects factor, their interaction, and baseline as a covariate. The primary model used will be a mixed model with repeated measures (MMRM), treating week categorically and using an autoregressive (with a lag of 1) covariance structure. As a secondary sensitivity analysis, we will fit a random coefficients model treating week continuously with random coefficients for the intercept, slope, and slope squared, with an unstructured structure among the random coefficients. The primary hypothesis test of interest will be a test of treatment effect at week 24. Distributional assumptions will be examined using residuals. These mixed models could fail to converge or encounter difficulties based on their use of asymptotics. If they do, we will attempt to simplify the models in order to eliminate the problems, and if necessary, move to analyses that assume compound symmetry, but use a Huynh-Feldt correction if compound symmetry fails.

Missing Data: The mixed models used to evaluate the continuous response variables are able to handle moderate amounts of missing data provided they are missing at random. We will examine the missing at random assumption by assessing baseline differences between dropouts and completers, as well as differences in response variables up to the point of premature withdrawal. If the missing at random assumption does not appear to be tenable, we will report the mixed models results but spend additional effort characterizing treatment effect at time of premature withdrawal or the effect of not including participants with significant missing data in supplementary analyses.

10.2 Sample Size Considerations

We will use a mixed longitudinal model for our primary analyses (see Statistical Analyses below). Since some aspects of the model such as covariance structure are unknown, we performed power and sample size calculations using a more conservative, simplified model corresponding to a two-group t-test on change scores. We plan to analyze two co-primary outcome measures: the ABC-SW, which will provide consistency with pivotal trials of other medications hypothesized to improve ASD social symptoms, and the combined social score, which integrates ABC-SW symptoms with the new Pervasive Developmental Disorders Behavior Inventory-Screening Version SOCDEF raw score (PDDBI-SV, Cohen 2011, also see Outcome Measures) in order to fully capture the range of impairments in reciprocal social behaviors observed in ASD. We will use an alpha of 0.025 to correct for two co-primary outcomes. SOARS-B is powered for Aim 1b to allow independent evaluation of oxytocin efficacy in low and high-functioning youth.

Standard deviations of change scores for the ABC-SW range from ~5 to 9 in several large ASD intervention trials (McCracken, McGough et al. 2002; Shea, Turgay et al. 2004; Aman, McDougle et al. 2009; King, Hollander et al. 2009; Marcus, Owen et al. 2009; Owen, Sikich et al. 2009). We consider a between groups difference of 5-7 points in ABC-SW change scores to be clinically meaningful. In our power calculations, we use conservative estimates of 9 points for the SD of ABC-SW change and 5 points for between group differences in ABC-SW changes (differential improvement on ~1/3 of items). To achieve 80% power with an alpha of 0.025 on the ABC-SW, we will require 71 participants in each treatment group within the two strata. Thus, our total required sample for the ABC-SW in Aim 1b is 284. We examined change in the combined social score in 30 3-17 year olds with ASD, 20 treated with aripiprazole and 10 medication-free controls. The mean baseline value was 33.5 (17.2 SD) and the change overall was -10.7 (SD 13.0), with a SD of change in each group of 11.9. Within each treatment group, changes in the combined social score paralleled those in the ABC-SW and PDDBI-SV, but had less variability (Sikich, personal data). We consider a between groups difference in the combined social measure changes of 10-12 to be clinically meaningful. Conservative estimates using a SD of 15 and a between groups difference of 10 in the combined social score changes results in a larger effect size for the combined social measure than for the ABC-SW. Consequently, we should have at least 80% power. A sample size of 300 will allow for 5% attrition between randomization and the first post-randomization visit. On 3/28/2016 we reduced the attrition rate to 1% based on attrition rate observed to date which gives a final sample size of 290. Aims 1a, 3 and 4 consider all participants, resulting in much greater power than for Aim 1b. The power of moderator analyses depends on the distribution of participant characteristics and variability in methylation and mRNA expression observed, which can't be estimated reliably at this time.

11 SUBJECT CONFIDENTIALITY

In order to protect confidentiality, only randomly assigned ID numbers rather than names will appear on clinically sensitive charts, files, and digital data. The key linking the numeric identifier and participant's identity will be maintained on a separate drive that is double password protected and to which only the site PI and coordinator have access. Contact information will be recorded separately in double password protected files with restricted access as above. The code linking the names with

the ID numbers will be securely protected with limited access. Medical records will be kept confidential with access granted only to those medical and research professionals directly involved with the study. If any scientific paper based on the data collected for this study is published, no information that could be linked to any single participant will be reported. Confidentiality will be protected to the fullest extent permitted by law. All research personnel have completed HIPPA training for researchers and human ethics training.

The samples for the methylation portion of the study (which will be sent to Simon Gregory's team at Duke) will be single-coded, that is, labeled with a single specific code that does not carry any personal identifiers. Single coding is the current standard used in clinical research and offers additional safeguards to the subject's identifiers compared to the HIPAA authorization. With this method it is possible to trace the samples back to a given subject with the use of the single coding key. The clinical study investigator is responsible for maintaining the coding key. This coding key will be stored separately from the research data. When stored electronically, it will be password protected as it will contain identifying information. The samples will also note the date of collection and visit number.

13 APPENDIX A (Clinical Parameters for Cardiac Monitoring)

BLOOD PRESSURE PARAMETERS FOR GIRLS

AGE	MIN (5th % height)	MIN (95th % height)	MAX (5th % height)	MAX (95th % height)
3	86/47	93/51	104/65	110/69
4	88/50	94/54	105/68	112/72
5	89/52	96/56	107/70	113/74
6	91/54	98/58	108/72	115/76
7	93/55	99/59	110/73	116/77
8	95/57	101/60	112/75	118/78
9	96/58	103/61	114/76	120/79
10	98/59	105/62	116/77	122/80
11	100/60	107/63	118/78	124/81
12	102/61	109/64	119/79	126/82
13	104/62	110/65	121/80	128/83
14	106/63	112/66	123/81	129/84
15	107/64	113/67	124/82	131/85
16	108/64	114/68	125/82	132/86
17	108/64	115/68	125/82	132/86

BLOOD PRESSURE PARAMTERS FOR BOYS

AGE	MIN (5th % height)	MIN (95th % height)	MAX (5th % height)	MAX (95th % height)
3	86/44	95/48	104/63	113/67
4	88/47	97/52	106/66	115/71
5	90/50	98/55	108/69	116/74
6	91/53	100/57	109/72	117/76
7	92/55	101/59	110/74	119/78
8	94/56	102/61	111/75	120/80
9	95/57	104/62	113/76	121/81
10	97/58	106/63	115/77	123/82
11	99/59	107/63	117/78	125/82
12	101/59	110/64	119/78	127/83
13	104/60	112/64	121/79	130/83
14	106/60	115/65	124/80	132/84
15	109/61	117/66	126/81	135/85
16	111/63	120/67	129/82	137/87
17	114/65	122/70	131/84	140/89

Adapted from National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents: The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents(Falkner, Daniels et al. 2004).

HEART RATE PARAMETERS

AGE	MIN	MAX
1-3	90	150
4-5	65	135
6-8	60	130
9-11	60	110
12-16	60	110
>16	60	100

Adapted from Pediatric cardiology for practitioners(Park 2008) and Normal ECG standards for infants and children(Davignon, Rautaharju et al. 1979).

14 SUMMARY OF PROTOCOL CHANGES

Summary of Changes (Protocol version date 08 March 2013):

- Removal of whole blood serotonin collection at Months 8, 12, and 18
- Addition of salivary oxytocin collection at same time points as plasma oxytocin collection (Screening, Month 2, Month 6, Month 8, Month 12 and Month 18) (added to schedule of events and in visit descriptions)
- Addition of intelligence tests (Stanford-Binet 5/Mullen at Months 6, 12, 18 (added to Schedule of Events and in visit descriptions)
- Clarified scoring procedures on the Mullen assessment regarding when to calculate mental age for participants
- Revised Oxytocin Flexible Dosing Strategy (Table 3)
- Updated/clarified definitions and recording requirements for concomitant medications. Made clarifications regarding the documentation of PRNs
- Added specific list of prohibited meds (ones that affect brain serotonin levels)
- Added an additional reportable event section:
    - serious intentional self-harm/serious suicidal ideation
    - Pregnancy of subject or a subject's sexual partner
- Revised language regarding study stopping criteria:
- Added language regarding Overall Study Stopping Criteria per DSMB request
- Changed "if moderate or severe adverse events occur that cannot be addressed by dose adjustment or addition of concomitant medications" to "if moderate or severe adverse events occur that cannot be addressed by dose adjustment or addition of concomitant medications (these can be discussed with SC and Medical Monitor. Investigators are encouraged to try dose reduction or addition of concomitant medications)."
- Changed "clinical worsening (much worse or very much worse on CGI-I) at two consecutive visits unless explicitly discussed and agreed with SC and Medical Monitor that participant can continue in the study" to "If a subject is worsening clinically (they have score of 6 – much worse or 7 – very much worse on CGI-I, there has to be a return visit/phone call within 1-2 weeks to re-

visit/re-evaluate with CGI-I. If the subject still has 6 or 7, then case must be discussed with SC and Medical Monitor to get approval for participation to continue."

- Changed reporting rule requirements for UPs,SAEs and other reportable events
- Specified UNC's responsibilities for reporting to specific authorities
- Specified reporting requirements for other site
- Added 2 new categories for severity of an adverse event (to be consistent with NC TraCS DSMB
- **Life-threatening**: Substantial risk dying or requires intervention to prevent death
- **Death related to AE:** Subject died as a result of the event, self-explanatory
- Made DSMB section consistent:
- DSMB will meet and review the study 3x/year instead of quarterly as previously indicated
- Clarified definition of Serious Adverse Event
- Updated Tables 1 & 2 regarding Adverse Events
- Updated section regarding randomization method:
- We will randomize centrally, using our data management system. We currently do just that in our multicenter IMPACT trial. When a site needs to randomize a subject, after baseline is assessed, the coordinator opens the Randomization form. This form uses already assessed strata to randomize from the appropriate stratum. We will not stratify by site. We felt that both functional status and age group were more likely to be related to the outcome than site, and there are limits to how many strata we can have. We will stratify by functional status (high or low) and age group (3 age groups), producing 6 strata. Dr. Hamer will generate a randomization plan for each of the 6 strata, using a permuted block algorithm with randomly selected block sizes of 2 and 4, using SAS. The table will be exported to an Excel file and sent to the data manager, Ms. Scheer. This can be done without having Dr. Hamer ever actually see the randomization plan. (He will debug the program and when debugged, run it with a new seed for the random number generator without seeing the results.) Ms. Scheer will import the randomization plan as a table in Access. The Randomization form will use Visual Basic code and the values of the strata variables to enter the correct subset of the table and choose the first unused treatment. It will generate an ID number and send an email to the appropriate pharmacy, where drug will be assigned.

Summary of Changes (Protocol version date March 21, 2013,v. 3.0):

- Changed dearth of safety information to limited safety information given new data emerging, Added clarifying text to the evidence for safety of sustained intranasal oxytocin treatment in ASD.
- Introduced nomenclature of "sociability factor (SF)" to replace prior references to combined social score. There is no change in how it is derived.
- Clarified the key secondary outcomes analyzed in aim 1c.
- Clarified the exploratory measures including the calibrated severity score from the ADOS and the reading the mind in the eyes test.
- Added examination of potential interactions with defined classes of concomitant medications.

- Clarified procedures for flexible dosing and doses labeled by letter.
- Added suicidality assessments to schedule of visits and provided section describing how assessments would be done.

Summary of Changes (Protocol version date: Jan 16, 2014)

- Define deafness and blindness
- Duke genetics testing is now NOT optional, but the NIMH samples ARE still optional. Because the Duke samples (mRNA/methylation) are part of our specific/exploratory aims, we did not want to dilute the sample size. With genetics you run the risk of not having enough power because the sample size is too small, so we felt it was important to require this sample to ensure it is collected from all subjects. We will more likely be able to see an effect with a larger sample.
- Changed citations and fixed the reference section as they appear in the text using Endnote.
- Deleted the previous reference list and created an Endnote automatically updated version\
- Added clarification that screening labs must be repeated if there is more than 12 weeks between the screening and baseline visits.
- Change the CASI-4R to the CASI-V version
- Taking over psychiatric care for subjects is now optional. This will be determined by site.
- Clarified that unblinding will not be permitted in this protocol. However, subject's may have treatment interrupted (temporarily discontinued) by treating clinician and the subject may be re-challenged if it is discussed and approved by the SC.
- Addition of a more specific cardiac monitoring plan where heart rate, blood pressure and pulse will be monitored at every visit in addition to clinical paremeters in the SLAES. Syncope (fainting) is added as a separate line item in the SLAES.
- Added optional PK protocol (section 12)
- Move methylation sample and plasma/salivary OT sample from month 8 (week 32) to week 36 (month 9) to make it coincide with PK sample for those individuals participating in the optional PK study (ie: eliminating an additional blood draw for those who are participating in the PK study).
- Updated table of contents
- Clarified in randomization procedures that we will stratify both by functional status and by age group.
- The ABC and PDDBI-SV will be added to all in office visits. The schedule of events and visit descriptions have been edited to reflect this change.
- Section 10.1 changes made in statistical analysis general modeling section.
- Changed all Month to weeks (example, month 1 will now be week 4)
- Removed information about where the drug will be manufactured/compounded as this information will be directly in the IND application to the FDA and not in the protocol.
- Deleted information about specific dosing concentrations (Tables 3a and 3b).
- Added a table that specified total daily dose (broken up into morning and afternoon doses). Does not specify number of sprays/concentration of each dose.

- Removed ADOS calibrated severity score as a potential exploratory outcome measure
- Removed ADOS from week 48
- Increased the number of mLs needed for NIMH optional genetic sample
- Clarified prohibited benzodiazepines in prohibited meds section of protocol.
- Removed medication administration training video for parents
- Added more specific criteria for increasing and decreasing dose:
    - **Increases in Dose:** Doses may increase if all three of the following criteria are met:
        - CGI-I is not a 1 or a 2
        - No evidence of clinically significant adverse events
        - Parents agree
    - **Decreases in Dose:** Doses must be decreased if any of the following criteria are met:
        - Significant AEs persist for at least 2 weeks or clinician judges it requires immediate adjustment of dose
        - OR
        - Clinician, parent, participant felt they were doing better on lower dose
        - OR
        - CGI score of 6 or 7

Summary of Changes (Protocol version date: March 8, 2014)

- Clarified information concerning dosing (added two sentences).
- Added missed X marks in CGI-I and SLAES in check boxes in table 6 for week 2 and 26.
- Added further clarification to exclusion criteria for chronic oxytocin treatment (added that chronic is daily intranasal oxytocin treatment of more than 1 month)
- Clarified an ideal time for plasma/salivary oxytocin collection
- Clarified details on the specific salivary oxytocin collection procedure
- Editorial changes removing bullet points and double words.

Summary of Changes (Protocol version date: July 25 2014)

- Added cyberball task at baseline, week 8, week 24, week 32, week 48 and week 72
- Suggested time for NIMH sample of subject to be drawn is week 36 due to the volume of blood collected at other visits.
- Safety labs must be re-drawn if has been greater than 12 months between screening and baseline
- Optional re-draw at baseline of plasma oxytocin, methylation and salivary oxytocin (prior to first dose of study drug) in order for these levels to be an accurate reflection of baseline levels (for subjects who have had a lengthy time between screening and baseline)
- Clarifying that the T0 draw for the PK study is the same as the regular plasma oxytocin level at weeks 36 and 44. Therefore, those participating in the PK study do not ALSO need an additional tube for the plasma oxytocin levels.
- Change inclusion criteria regarding ADOS and ADI-R. Now subjects must have a clinical diagnosis of ASD confirmed either using the Autism Diagnostic Observation Scale (ADOS, Lord et al., 2001) or the Autism Diagnostic Interview-Revised (ADI-R, Rutter, 2003). For subjects

who do not meet criteria on either, but the clinician still believes to have ASD, those individuals may be included if the SC agrees.
- Clarified that dosing my only be decreased by 8IU qD increments and increased by 8IU BID increments.
- Dose decrease criteria clarified. Instead of clinical worsening, the criteria now states that dose may be decreased if there are two consecutive CGI-I scores are worse that the preceding two CGI-I scores.

Summary of Changes (Protocol version date: November 17, 2014)
- Methylation and mRNA will be collected at screening, baseline (optional for those who have had lengthy screening periods), week 8, week 24 and week 36
- Stanford Binet and Mullen must be completed within 1 month of baseline (can be completed at screening but must be repeated if not within 1 month of randomization).
- Clarification on prohibited meds: Remeron (mirtazapine) is allowed but tricyclics/tetracyclic antidepressants are now prohibited.
- Clarified that for the Vineland-II, we will be using the parent/caregiver rating form instead of the survey/interview format and we will not be doing the maladaptive behaviors.
- Pregnancy test (urine or serum) for females of childbearing potential must be confirmed as negative within 2 weeks of baseline appointment. This can either be done at a screening visit or can be done at baseline prior to randomization (urine dipstick test).
- Preferred time to collect NIMH repository sample from subject is now at week 48. This is due to the fact that week 48 now has the least amount of blood drawn due to changes in methylation/mRNA sample collection.
- Removed the PDDBI-Full and instead it will be replaced with the much shorter PDDBI-SV and also removed reference to PDDBI-full in the exploratory outcome measures section of the protocol.
- Clarified in the schedule of events superscript 0 beside CGI-S/I. CGI-S at baseline and CGI-S and CGI-I at all other time points.
- Deleted reference to Week 44 regular plasma OT sample in the PK protocol section as it was added erroneously in the previous version.
- Clarified that we will not be doing the gross motor subtest of the Mullen
- Revised dose decreasing criteria
- Clarified in several locations throughout the protocol that there will now be 6 sites instead of 5 sites.
- Added clarification that cyberball task is an additional outcome measure in section 2.2.3.
- Removed estimates of times/year that the monitor will travel to each site. This may be variable depending on the rate of enrollment at each site.
- DSMB will now meet 2 times/year
- The medical monitor will now review all new treatment emergent adverse effects of moderate or greater severity two times a year in order to coincide with the DSMB meetings.

- Alert values for labs and EKGs are determined by site PI and/or site treating physician
- Administrative change: rearranged reportable events section of the protocol (changed section numbers) to be more clear/concise and also added alert lab/EKG values as considered reportable events and must be reported to UNC within 7 days of the sites awareness.
- Addition of a visual analogue scale for treating physician and coordinator to complete at week 0, 12, 24, 36, 48 and 72.
- Made additional clarification in the schedule of events about a previous modification regarding labs. Safety labs (glucose, CO2, Cl, K, Na, Creatinine, BUN, AST, ALT, urine specific gravity and pregnancy test) must be redrawn at baseline if more than 12 months has elapsed between screening and baseline visits. Methylation, plasma/salivary oxytocin has an optional re-draw at baseline if there has been a lengthy time between screening and baseline visits.

Summary of Protocol Changes (Version Date: March 2, 2015)

- Clarified the target dose and the efforts to achieve the target dose if at all possible.
- Increased the requirement for duration of treatment with the target dose (from 4 weeks to 7 weeks) to ensure there was full opportunity to evaluate the target dose. Clarified the rules for maintaining a lower dose than recommended by the titration schedule, timing of subsequent dose increases during the titration phase, mandatory and optional dose reductions and increases above the target dose.
- Increased the total dose given at one time from 40/0 IU (1 high dose insufflation and 2 low dose insufflations) to 48/0 IU (2 high dose insufflations) to allow achievement of the total daily target dose with a single administration time.
- Clarified procedures related to interrupting dosing in order to capture primary outcome measures and safety data very close to the time of the last study treatment to increase interpretability of data.
- Added two additional possible doses in schedule (48 IU AM/0 IU PM and 48 IU AM/24 IU PM) as the maximum dose at any time can be 48 IU
- mRNA was initially overlooked and will be part of optional re-draw at baseline which is now reflected in section 6.2 and schedule of events.
- Clarified wording in the Schedule of events.
- Add full urinalysis at all time points when specific gravity is already analyzed.
- Clarified early termination procedures, need to obtain data within 2 weeks of stopping treatment. Specified those ending in blinded phase would complete week 24 procedures and those ending in open label phase would complete week 48 procedures.
- Clarified interrupted treatment procedures
- Add clarification that follow up safety measures may be added based on physician judgment (such as additional unscheduled labs, ECGs, visits etc) in section 6.7
- Changed timing of visual analog scale to correspond with all post-baseline in person scheduled visits (all visits except 2 and 26)

- Clarified that the visual analogue scale (VAS) will be called the VAS of change (not improvement) and will have three sections: overall functioning, repetitive behavior, and social-communicative functioning. Additionally clarified that only the clinician will complete the baseline description of functioning (not the coordinator) and added the VAS to discussion of additional outcome measures.
- Clarified that the Mullen receptive language subtest needs to be done in all children who require ADOS testing with a Module 1 or 2 who are 5 years 8 months old or younger to determine functional strata.
- A focused clinical exam will be done at all post screening visits (instead of full NIMH physical exam form which combines aspects of physical and medical history) and clarified in assessments section that height/weight will be obtained as part of vital signs
- Clarified that medical monitor will review significant and persistent worsening of functioning (CGI-I of 6 or 7) and require the individual participant stop the study.
- Clarifying procedures for re-evaluation visit (if dose is decreased or CGI-I score is 6 or 7).
- Clarifying procedures for when a subject must be withdrawn versus when a subject may be withdrawn.
- Administrative and editorial changes throughout.

Summary of Protocol Changes (Version Date: May 8, 2015; Protocol Version 5.0)
- Revised inclusion/exclusion criteria to allow for all concomitant psychiatric medication that has been stable for 4 weeks prior to baseline appointment.
- Clarified that individual has to be diagnosed with an autism spectrum disorder using DSM-V criteria.
- Removed prohibited medications section

Summary of Protocol Changes (Version Date: June 4, 2015; Protocol Version 5.1)
- Overall PI (Dr. Sikich) is no longer affiliated with UNC-Chapel Hill. New affiliation is with Duke University. Duke University will now be lead site under direction of overall PI (Sikich). Various changes made to clarify this (phone number, email addresses, etc).

Summary of Protocol Changes (Version Date 3/21/2016; Protocol Version 6.0)
- Adding additional exclusion criteria: subjects cannot have active seizures within the 6 months prior to screening or baseline.
- Remove Dr. Hamer as lead statistician.
- Russ Dean is now the PI at the UNC-Data Center
- Clarified that visit procedures may occur on separate days as needed, but open label study drug administration may not occur until all week 24 procedures have been completed.
- Remove cyberball task entirely
- Remove ADI-R at screening, this will be completed at physician discretion only

- Removed the requirement for the IQ testing (Mullen/SB) to be completed within 1 month of the baseline visit. This does not have to be repeated at baseline if it has been more than 1 month between screening and baseline.
- Replace week 2 phone call/email so that the study team only sends the Parent Dose Sheet (schedule B)
- Changed week 12 to only be questionnaires, no in office visit
- Removed ADOS week 24, this may be completed at the physician's/site's discretion
- Remove week 26 phone call/email
- Removed week 32, 40 and 44 visits where dose is not expected to change. Subjects will be instructed to contact the site if there are any issues between scheduled visits. Subjects may always have the ability for an unscheduled visit as needed.
- Week 36 visit
    - Remove VABS and RMET
    - Added safety lab collection (blood/urine)
- Remove the following procedures at week 48:
    - Removed EKG
    - Removed the blood draw, urine and saliva collection at week 48 (plasma oxytocin, safety labs, salivary oxytocin) at week 48, it is the physician's discretion if they want to assess safety labs (blood and urine) only
- Removed week 72 visit
- Added down titration schedule at the end of the study or if a subject early terminates and agrees to follow the down titration schedule
- Added a follow up phone call (SLAES) to be completed after study drug has been stopped at week 53-55
- Shorten early termination visit. No blood/saliva/EKG/ADOS or IQ testing is required at an E/T visit.
- Made general clarifications and better wording surrounding the dosing. Major changes include:
    - For optional dose reductions, clarified that the SLAES does not need to be completed, but adverse events do need to be assessed and documented.
    - Changed criteria for increasing above the target dose: removed criteria that CGI-I cannot be a 1. If the CGI-I is a 1, but the clinician still feels there is room for improvement, then the dose may still be increased above target.
    - Change dosing scheme in open label phase:
        - Week 24-28 = 24 IU TDD
        - Week 28-36 = 48 IU TDD
        - Week 36-48 = 72 IU TDD
- General clarifications were made regarding wording surrounding the Parent Dose Sheet and medication diary. No procedures were changed.
- It was clarified that subjects will be given enough study drug to last until their next scheduled visit.

- Clarified that PK visits may occur at any time starting at week 36 or later and clarified the suggested dosing for each of the visits.
- Decreased overall samples size to 290 due to lower than expected attrition rates.
- Adding more wording to clarify definitions for relatedness: Yes= related, likely = probably, no = unrelated
- Added additional definitions surrounding adverse event monitoring related to the SLAES:
    - Pattern Definition: This is to help the clinician define the pattern of the AE and give some clarity to timing of an event.
    - Change/Outcome-This should be captured in order to help the clinician make a clear decision on a severity rating and better follow the AE through time.
    - Action Take-The action taken after the report of an AE can be captured by the definitions below:

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HIGH LEVEL SUMMARY OF PROTOCOL CHANGES

Changes were made to the protocol ten times, including six times prior to randomizing participants in December 2014. Early changes were made primarily at the request of NICHD reviewers and regulatory bodies including the DSMB, IRB's at various sites, and the FDA. Changes to the visit schedule and outcome measures were made primarily as a result of unexpected funding decreases and delays and costs in securing study drug. In this summary, changes are presented by type and reason.

In the detailed summary copied from the final version of the protocol, changes are organized by protocol version.

Safety Procedure Changes:

- Added specific list of prohibited meds that might affect serotonin levels
- Expanded reportable events to include intentional self-harm/serious suicidal ideation and pregnancy
- Added criteria for clinical worsening that would lead to dose decrease
- Added blood pressure parameters and ECG at request of a site IRB
- Eliminated safety labs at week 48, instead obtaining them at week 36
- Greater specification of procedures and criteria for stopping the overall study and withdrawing an individual participant due to safety concerns.
- Required discussion of moderate and severe adverse events with Steering Committee (SC) and Medical Monitor (MM)
- Frequency of DSMB meetings reduced from quarterly to twice annually
- Clarified lead site and other treatment sites reporting requirements
-

Schedule of Events:

- Changed from months to weeks for greater precision of time points
- Eliminated in-person visits at Weeks 2 and 26
- Eliminated in-person visits at Weeks 32, 40, and 44
- Eliminated Month18/Week 72 follow-up due to lack of funding and time, replaced with AE follow-up approximately 4 weeks after treatment is stopped
- Added ABC-SW and PPD-BI SV to each in-person visit
- Switched collection of genetic measures and biological samples in open-label treatment from w32 to w36 to provide additional information about time course of biological measures
- Allowed for up to 18 months between screening and baseline with specification of measures that needed to be repeated due to unanticipated delay in access to study drug once screening was opened (~ summer 2013)
- Minimized the number of visits during open label treatment to weeks 28, 36, 48

Outcome Measures:

- Initially planned to have two co-primary outcomes, the ABC-SW and a combined measure later termed the Sociability Factor, later discontinued this in the final SAP though change was not formally made in protocol.

- At one point added the cyberball social exclusion task as an objective measure that had shown sensitivity to change with oxytocin in single dose studies; later eliminated it because we were not able to adapt for use with younger children, individuals with intellectual disability or a racially diverse sample with automated scoring.
- Removed w24 ADOS due to financial constraints and lack of evidence of change previously
- Required collection of study specific genetic measures but not NIMH repository samples
- Added optional PK sample at target and maximal dose per FDA request
- Added cognitive testing as an outcome at week 24 (after initially also including week 48 and 72)
- Added salivary oxytocin collection at same times as plasma oxytocin collection
- Specified key secondary outcomes for analysis 1c
- Removed PDD-BI full version and switched to 18 item PDD-BI-SV to reduce participant burden
- Switched from Vineland Interview version to Vineland caregiver-report version to reduce burden on staff and participants.

Procedural Changes:

- Revised oxytocin dosing schedule to allow more flexibility during double-blind treatment
- Developed specific criteria for increasing oxytocin above target dose
- Developed detailed procedures for increasing or reducing oxytocin dose outside of recommended titration schedule.
- Specified use of a centrally administered randomization only included age group and verbal status as strata and did not include site to minimize uneven cells across full study.
- Made DSM-5 criteria primary inclusion criteria to informed by ADI or ADOS and Steering committee discussion if DSM-5 criteria were positive but ADI/ADOS were not
- Changes to the statistical analysis plan were made both in the protocol and noting should refer to SAP for final plan
- Changed randomization target from 300 to 290 because attrition was lower than expected and the grant funding period was about to expire

Personnel Changes:

- Data Center PI, Dr. Hamer died. He was replaced by Russ Dean. However, he lacked the statistical expertise to perform the analyses so that eventually Duke Clinical Research Institute statistical staff under the direction of Dr. Sheng Luo performed the primary analyses that are the subject of this report
- Lead PI Sikich switched institutions from UNC to Duke in August 2015
- Treatment site PI's Veenstra-Van der Weele, King and Sanders left their original sites during the course of the study. Dr. Veenstra-Van der Weele then headed a new 6th site at Columbia University.

DETAILED, TEMPORAL SUMMARY OF PROTOCOL CHANGES FROM V6.O

Summary of Changes (Protocol version date 08 March 2013):

- Removal of whole blood serotonin collection at Months 8, 12, and 18
- Addition of salivary oxytocin collection at same time points as plasma oxytocin collection (Screening, Month 2, Month 6, Month 8, Month 12 and Month 18) (added to schedule of events and in visit descriptions)
- Addition of intelligence tests (Stanford-Binet 5/Mullen at Months 6, 12, 18 (added to Schedule of Events and in visit descriptions)
- Clarified scoring procedures on the Mullen assessment regarding when to calculate mental age for participants
- Revised Oxytocin Flexible Dosing Strategy (Table 3)
- Updated/clarified definitions and recording requirements for concomitant medications. Made clarifications regarding the documentation of PRNs
- Added specific list of prohibited meds (ones that affect brain serotonin levels)
- Added an additional reportable event section:
    - serious intentional self-harm/serious suicidal ideation
    - Pregnancy of subject or a subject's sexual partner
- Revised language regarding study stopping criteria:
    - Added language regarding Overall Study Stopping Criteria per DSMB request
    - Changed "if moderate or severe adverse events occur that cannot be addressed by dose adjustment or addition of concomitant medications" to "if moderate or severe adverse events occur that cannot be addressed by dose adjustment or addition of concomitant medications (these can be discussed with SC and Medical Monitor. Investigators are encouraged to try dose reduction or addition of concomitant medications)."
    - Changed "clinical worsening (much worse or very much worse on CGI-I) at two consecutive visits unless explicitly discussed and agreed with SC and Medical Monitor that participant can continue in the study" to "If a subject is worsening clinically (they have score of 6 – much worse or 7 – very much worse on CGI-I, there has to be a return visit/phone call within 1-2 weeks to re-visit/re-evaluate with CGI-I. If the subject still has 6 or 7, then case must be discussed with SC and Medical Monitor to get approval for participation to continue."
    - Changed reporting rule requirements for UPs,SAEs and other reportable events
    - Specified UNC's responsibilities for reporting to specific authorities
    - Specified reporting requirements for other site
- Added 2 new categories for severity of an adverse event (to be consistent with NC TraCS DSMB
    - **Life-threatening**: Substantial risk dying or requires intervention to prevent death
    - **Death related to AE:** Subject died as a result of the event, self-explanatory
- Made DSMB section consistent:
    - DSMB will meet and review the study 3x/year instead of quarterly as previously indicated
- Clarified definition of Serious Adverse Event
- Updated Tables 1 & 2 regarding Adverse Events
- Updated section regarding randomization method:
    - We will randomize centrally, using our data management system. We currently do just that in our multicenter IMPACT trial. When a site needs to randomize a subject, after

baseline is assessed, the coordinator opens the Randomization form. This form uses already assessed strata to randomize from the appropriate stratum. We will not stratify by site. We felt that both functional status and age group were more likely to be related to the outcome than site, and there are limits to how many strata we can have. We will stratify by functional status (high or low) and age group (3 age groups), producing 6 strata. Dr. Hamer will generate a randomization plan for each of the 6 strata, using a permuted block algorithm with randomly selected block sizes of 2 and 4, using SAS. The table will be exported to an Excel file and sent to the data manager, Ms. Scheer. This can be done without having Dr. Hamer ever actually see the randomization plan. (He will debug the program and when debugged, run it with a new seed for the random number generator without seeing the results.) Ms. Scheer will import the randomization plan as a table in Access. The Randomization form will use Visual Basic code and the values of the strata variables to enter the correct subset of the table and choose the first unused treatment. It will generate an ID number and send an email to the appropriate pharmacy, where drug will be assigned.

Summary of Changes (Protocol version date March21, 2013 v.3.0):

- Changed dearth of safety information to limited safety information given new data emerging, Added clarifying text to the evidence for safety of sustained intranasal oxytocin treatment in ASD.
- Introduced nomenclature of "sociability factor (SF)" to replace prior references to combined social score. There is no change in how it is derived.
- Clarified the key secondary outcomes analyzed in aim 1c.
- Clarified the exploratory measures including the calibrated severity score from the ADOS and the reading the mind in the eyes test.
- Added examination of potential interactions with defined classes of concomitant medications.
- Clarified procedures for flexible dosing and doses labeled by letter.
- Added suicidality assessments to schedule of visits and provided section describing how assessments would be done.

Summary of Changes (Protocol version date: Jan 16 2014)

- Define deafness and blindness
- Duke genetics testing is now NOT optional, but the NIMH samples ARE still optional. Because the Duke samples (mRNA/methylation) are part of our specific/exploratory aims, we did not want to dilute the sample size. With genetics you run the risk of not having enough power because the sample size is too small, so we felt it was important to require this sample to ensure it is collected from all subjects. We will more likely be able to see an effect with a larger sample.
- Changed citations and fixed the reference section as they appear in the text using Endnote.
- Deleted the previous reference list and created an Endnote automatically updated version\
- Added clarification that screening labs must be repeated if there is more than 12 weeks between the screening and baseline visits.
- Change the CASI-4R to the CASI-V version
- Taking over psychiatric care for subjects is now optional. This will be determined by site.
- Clarified that unblinding will not be permitted in this protocol. However, subject's may have treatment interrupted (temporarily discontinued) by treating clinician and the subject may be re-challenged if it is discussed and approved by the SC.

- Addition of a more specific cardiac monitoring plan where heart rate, blood pressure and pulse will be monitored at every visit in addition to clinical paremeters in the SLAES. Syncope (fainting) is added as a separate line item in the SLAES.
- Added optional PK protocol (section 12)
- Move methylation sample and plasma/salivary OT sample from month 8 (week 32) to week 36 (month 9) to make it coincide with PK sample for those individuals participating in the optional PK study (ie: eliminating an additional blood draw for those who are participating in the PK study).
- Updated table of contents
- Clarified in randomization procedures that we will stratify both by functional status and by age group.
- The ABC and PDDBI-SV will be added to all in office visits. The schedule of events and visit descriptions have been edited to reflect this change.
- Section 10.1 changes made in statistical analysis general modeling section.
- Changed all Month to weeks (example, month 1 will now be week 4)
- Removed information about where the drug will be manufactured/compounded as this information will be directly in the IND application to the FDA and not in the protocol.
- Deleted information about specific dosing concentrations (Tables 3a and 3b).
- Added a table that specified total daily dose (broken up into morning and afternoon doses). Does not specify number of sprays/concentration of each dose.
- Removed ADOS calibrated severity score as a potential exploratory outcome measure
- Removed ADOS from week 48
- Increased the number of mLs needed for NIMH optional genetic sample
- Clarified prohibited benzodiazepines in prohibited meds section of protocol.
- Removed medication administration training video for parents
- Added more specific criteria for increasing and decreasing dose:
  Increases in Dose: Doses may increase if all three of the following criteria are met:
  - CGI-I is not a 1 or a 2
  - No evidence of clinically significant adverse events
  - Parents agree

  Decreases in Dose: Doses must be decreased if any of the following criteria are met:
  - Significant AEs persist for at least 2 weeks or clinician judges it requires immediate adjustment of dose
    OR
  - Clinician, parent, participant felt they were doing better on lower dose
    OR
  - CGI score of 6 or 7

Summary of Changes (Protocol version date: March 8, 2014)

- Clarified information concerning dosing (added two sentences).
- Added missed X marks in CGI-I and SLAES in check boxes in table 6 for week 2 and 26.
- Added further clarification to exclusion criteria for chronic oxytocin treatment (added that chronic is daily intranasal oxytocin treatment of more than 1 month)
- Clarified an ideal time for plasma/salivary oxytocin collection
- Clarified details on the specific salivary oxytocin collection procedure

- Editorial changes removing bullet points and double words.

Summary of Changes (Protocol version date: July 25, 2014)

- Added cyberball task at baseline, week 8, week 24, week 32, week 48 and week 72
- Suggested time for NIMH sample of subject to be drawn is week 36 due to the volume of blood collected at other visits.
- Safety labs must be re-drawn if has been greater than 12 months between screening and baseline
- Optional re-draw at baseline of plasma oxytocin, methylation and salivary oxytocin (prior to first dose of study drug) in order for these levels to be an accurate reflection of baseline levels (for subjects who have had a lengthy time between screening and baseline)
- Clarifying that the T0 draw for the PK study is the same as the regular plasma oxytocin level at weeks 36 and 44. Therefore, those participating in the PK study do not ALSO need an additional tube for the plasma oxytocin levels.
- Change inclusion criteria regarding ADOS and ADI-R. Now subjects must have a clinical diagnosis of ASD confirmed either using the Autism Diagnostic Observation Scale (ADOS, Lord et al., 2001) or the Autism Diagnostic Interview-Revised (ADI-R, Rutter, 2003). For subjects who do not meet criteria on either, but the clinician still believes to have ASD, those individuals may be included if the SC agrees.
- Clarified that dosing my only be decreased by 8IU qD increments and increased by 8IU BID increments.
- Dose decrease criteria clarified. Instead of clinical worsening, the criteria now states that dose may be decreased if there are two consecutive CGI-I scores are worse that the preceding two CGI-I scores.

Summary of Changes (Protocol version date: November 17, 2014)

- Methylation and mRNA will be collected at screening, baseline (optional for those who have had lengthy screening periods), week 8, week 24 and week 36
- Stanford Binet and Mullen must be completed within 1 month of baseline (can be completed at screening but must be repeated if not within 1 month of randomization).
- Clarification on prohibited meds: Remeron (mirtazapine) is allowed but tricyclics/tetracyclic antidepressants are now prohibited.
- Clarified that for the Vineland-II, we will be using the parent/caregiver rating form instead of the survey/interview format and we will not be doing the maladaptive behaviors..
- Pregnancy test (urine or serum) for females of childbearing potential must be confirmed as negative within 2 weeks of baseline appointment. This can either be done at a screening visit or can be done at baseline prior to randomization (urine dipstick test).
- Preferred time to collect NIMH repository sample from subject is now at week 48. This is due to the fact that week 48 now has the least amount of blood drawn due to changes in methylation/mRNA sample collection.
- Removed the PDDBI-Full and instead it will be replaced with the much shorter PDDBI-SV and also removed reference to PDDBI-full in the exploratory outcome measures section of the protocol.
- Clarified in the schedule of events superscript 0 beside CGI-S/I. CGI-S at baseline and CGI-S and CGI-I at all other time points.
- Deleted reference to Week 44 regular plasma OT sample in the PK protocol section as it was added erroneously in the previous version.
- Clarified that we will not be doing the gross motor subtest of the Mullen
- Revised dose decreasing criteria

- Clarified in several locations throughout the protocol that there will now be 6 sites instead of 5 sites.
- Added clarification that cyberball task is an additional outcome measure in section 2.2.3.
- Removed estimates of times/year that the monitor will travel to each site. This may be variable depending on the rate of enrollment at each site.
- DSMB will now meet 2 times/year
- The medical monitor will now review all new treatment emergent adverse effects of moderate or greater severity two times a year in order to coincide with the DSMB meetings.
- Alert values for labs and EKGs are determined by site PI and/or site treating physician
- Administrative change: rearranged reportable events section of the protocol (changed section numbers) to be more clear/concise and also added alert lab/EKG values as considered reportable events and must be reported to UNC within 7 days of the sites awareness.
- Addition of a visual analogue scale for treating physician and coordinator to complete at week 0, 12, 24, 36, 48 and 72.
- Made additional clarification in the schedule of events about a previous modification regarding labs. Safety labs (glucose, CO2, Cl, K, Na, Creatinine, BUN, AST, ALT, urine specific gravity and pregnancy test) must be redrawn at baseline if more than 12 months has elapsed between screening and baseline visits. Methylation, plasma/salivary oxytocin has an optional re-draw at baseline if there has been a lengthy time between screening and baseline visits.

Summary of Protocol Changes (Version Date: March 2, 2015)

- Clarified the target dose and the efforts to achieve the target dose if at all possible.
- Increased the requirement for duration of treatment with the target dose (from 4 weeks to 7 weeks) to ensure there was full opportunity to evaluate the target dose. Clarified the rules for maintaining a lower dose than recommended by the titration schedule, timing of subsequent dose increases during the titration phase, mandatory and optional dose reductions and increases above the target dose.
- Increased the total dose given at one time from 40/0 IU (1 high dose insufflation and 2 low dose insufflations) to 48/0 IU (2 high dose insufflations) to allow achievement of the total daily target dose with a single administration time.
- Clarified procedures related to interrupting dosing in order to capture primary outcome measures and safety data very close to the time of the last study treatment to increase interpretability of data.
- Added two additional possible doses in schedule (48 IU AM/0 IU PM and 48 IU AM/24 IU PM) as the maximum dose at any time can be 48 IU
- mRNA was initially overlooked and will be part of optional re-draw at baseline which is now reflected in section 6.2 and schedule of events.
- Clarified wording in the Schedule of events.
- Add full urinalysis at all time points when specific gravity is already analyzed.
- Clarified early termination procedures, need to obtain data within 2 weeks of stopping treatment. Specified those ending in blinded phase would complete week 24 procedures and those ending in open label phase would complete week 48 procedures.
- Clarified interrupted treatment procedures
- Add clarification that follow up safety measures may be added based on physician judgment (such as additional unscheduled labs, ECGs, visits etc) in section 6.7

- Changed timing of visual analog scale to correspond with all post-baseline in person scheduled visits (all visits except 2 and 26)
- Clarified that the visual analogue scale (VAS) will be called the VAS of change (not improvement) and will have three sections: overall functioning, repetitive behavior, and social-communicative functioning. Additionally clarified that only the clinician will complete the baseline description of functioning (not the coordinator) and added the VAS to discussion of additional outcome measures.
- Clarified that the Mullen receptive language subtest needs to be done in all children who require ADOS testing with a Module 1 or 2 who are 5 years 8 months old or younger to determine functional strata.
- A focused clinical exam will be done at all post screening visits (instead of full NIMH physical exam form which combines aspects of physical and medical history) and clarified in assessments section that height/weight will be obtained as part of vital signs
- Clarified that medical monitor will review significant and persistent worsening of functioning (CGI-I of 6 or 7) and require the individual participant stop the study.
- Clarifying procedures for re-evaluation visit (if dose is decreased or CGI-I score is 6 or 7).
- Clarifying procedures for when a subject must be withdrawn versus when a subject may be withdrawn.
- Administrative and editorial changes throughout.

Summary of Protocol Changes (Version Date: May 8, 2015; Protocol Version 5.0)

- Revised inclusion/exclusion criteria to allow for all concomitant psychiatric medication that has been stable for 4 weeks prior to baseline appointment.
- Clarified that individual has to be diagnosed with an autism spectrum disorder using DSM-V criteria.
- Removed prohibited medications section

Summary of Protocol Changes (Version Date: June 4, 2015; Protocol Version 5.1)

- Overall PI (Dr. Sikich) is no longer affiliated with UNC-Chapel Hill. New affiliation is with Duke University. Duke University will now be lead site under direction of overall PI (Sikich). Various changes made to clarify this (phone number, email addresses, etc).

Summary of Protocol Changes (Version Date 3/21/2016; Protocol Version 6.0- FINAL Version)

- Adding additional exclusion criteria: subjects cannot have active seizures within the 6 months prior to screening or baseline.
- Remove Dr. Hamer as lead statistician.
- Russ Dean is now the PI at the UNC-Data Center
- Clarified that visit procedures may occur on separate days as needed, but open label study drug administration may not occur until all week 24 procedures have been completed.
- Remove cyberball task entirely
- Remove ADI-R at screening, this will be completed at physician discretion only
- Removed the requirement for the IQ testing (Mullen/SB) to be completed within 1 month of the baseline visit. This does not have to be repeated at baseline if it has been more than 1 month between screening and baseline.

- Replace week 2 phone call/email so that the study team only sends the Parent Dose Sheet (schedule B)
- Changed week 12 to only be questionnaires, no in office visit
- Removed ADOS week 24, this may be completed at the physician's/site's discretion
- Remove week 26 phone call/email
- Removed week 32, 40 and 44 visits where dose is not expected to change. Subjects will be instructed to contact the site if there are any issues between scheduled visits. Subjects may always have the ability for an unscheduled visit as needed.
- Week 36 visit
  - Remove VABS and RMET
  - Added safety lab collection (blood/urine)
- Remove the following procedures at week 48:
  - Removed EKG
  - Removed the blood draw, urine and saliva collection at week 48 (plasma oxytocin, safety labs, salivary oxytocin) at week 48, it is the physician's discretion if they want to assess safety labs (blood and urine) only
- Removed week 72 visit
- Added down titration schedule at the end of the study or if a subject early terminates and agrees to follow the down titration schedule
- Added a follow up phone call (SLAES) to be completed after study drug has been stopped at week 53-55
- Shorten early termination visit. No blood/saliva/EKG/ADOS or IQ testing is required at an E/T visit.
- Made general clarifications and better wording surrounding the dosing. Major changes include:
  - For optional dose reductions, clarified that the SLAES does not need to be completed, but adverse events do need to be assessed and documented.
  - Changed criteria for increasing above the target dose: removed criteria that CGI-I cannot be a 1. If the CGI-I is a 1, but the clinician still feels there is room for improvement, then the dose may still be increased above target.
  - Change dosing scheme in open label phase:
    - Week 24-28 = 24 IU TDD
    - Week 28-36 = 48 IU TDD
    - Week 36-48 = 72 IU TDD
- General clarifications were made regarding wording surrounding the Parent Dose Sheet and medication diary. No procedures were changed.
- It was clarified that subjects will be given enough study drug to last until their next scheduled visit.
- Clarified that PK visits may occur at any time starting at week 36 or later and clarified the suggested dosing for each of the visits.
- Decreased overall samples size to 290 due to lower than expected attrition rates.
- Adding more wording to clarify definitions for relatedness: Yes= related, likely = probably, no = unrelated
- Added additional definitions surrounding adverse event monitoring related to the SLAES:
  - Pattern Definition: This is to help the clinician define the pattern of the AE and give some clarity to timing of an event.
  - Change/Outcome-This should be captured in order to help the clinician make a clear decision on a severity rating and better follow the AE through time.
  - Action Taken-The action taken after the report of an AE can be captured by the definitions below: