Intranasal Vasopressin Treatment in Children with Autism

Study Protocol

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1. PURPOSE OF THE STUDY

a. Brief Summary

We will test the safety and efficacy of intranasal arginine vasopressin (hereafter referred to as vasopressin) treatment for social deficits in children with autism spectrum disorder (ASD). This research also aims to identify those individuals who stand to benefit most from this drug intervention. Vasopressin treatment has enormous potential for enhancing quality of life in people with ASD through improved social cognition and more meaningful social relationships. If efficacious, vasopressin will considerably reduce the emotional and financial burden of ASD on patients, family members, and society.

b. Objectives

Vasopressin is one of the most promising candidate drugs to treat the presently intractable social deficits of ASD. This will be the first large-scale clinical trial to test the efficacy of intranasal vasopressin treatment to enhance social abilities in individuals with ASD. Inclusion of female participants is a strength of this study, because their exclusion, which frequently occurs in ASD treatment studies, may jeopardize optimal testing of effective pharmacotherapies. Evidence from both preclinical and clinical studies has shown that differences in neuropeptide biology and/or pre-treatment social functioning are associated with treatment response outcomes. Our proposed project therefore will also test whether neuropeptide measurements and pre-treatment social functioning contribute to individual differences in vasopressin treatment efficacy.

c. Rationale for Research in Humans

The purpose of the study is to test the tolerability and efficacy of intranasal vasopressin administration in children with ASD.

2. STUDY PROCEDURES

a. Overview and Design

Using a quadruple-blind (participant, care provider, investigator, and outcomes assessor), randomized, placebo-controlled design, we will test the efficacy of 8-week intranasal vasopressin on social functioning in children with ASD (aged 6-17 years). Following a gold-standard Sequential Parallel Comparison Design (SPCD), participants will be randomized into one of three treatment allocations. The treatment allocations are: 1) drug-drug, in which participants will be on active vasopressin for the entire 8-week dosing period, 2) placebo-drug, in which participants will be on placebo for four weeks and then switch to active vasopressin for the remaining four weeks, and 3) placebo-placebo, in which participants will be on placebo for the entire 8-week dosing period. At the completion of the study, participants in the placebo-placebo group will be given the option of switching to active vasopressin for a four-week open-label dosing period. The ratio of participants in the three groups is 1:2:2 (drug-drug: placebo-drug: placebo-placebo). Consent by parents (and assent by participants aged 7-17 years) will be obtained before initiating any study procedures.

After obtaining informed consent, participants will be screened at Visit 1. If the participant so desires, Visit 1 can be split into two shorter clinic visits.

During Visit 1, participants will undergo:

- Medical and psychiatric history assessments
- A physical exam
- ASD diagnostic screening using the Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2) or the Childhood Autism Rating Scale, 2nd Edition (CARS-2), and the Autism Diagnostic Interview-Revised (ADI-R)
- Intellectual ability assessment using the Stanford Binet, 5th Edition or the Wechsler Intelligence Scale for Children, 5th Edition (WISC-V)
- Demographics questionnaire
- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Overt Aggression Scale (OAS) ratings
- The Columbia-Suicide Severity Rating Scale (C-SSRS)
- The Social Responsiveness Scale, 2nd edition (SRS-2)
- Side effects ratings using the Dosage Record and Treatment Emergency Symptom Scale (DOTES)
- Concomitant medications information
- Electrocardiogram (EKG) recordings
- Blood samples (up to 30 mL) for clinical evaluation and safety monitoring (urea nitrogen, sodium, potassium, chloride, CO2, anion gap, glucose, creatinine, calcium, and osmolality)
- Urine samples (up to 5mL) for clinical evaluation and safety monitoring (osmolality; females of childbearing age [12.5 years and older] or females with a history of menstruating will undergo urine pregnancy testing at screening and as needed in later visits)
- Vital signs (including heart rate, blood pressure, and temperature)
- Height and weight measurements

Biological samples for research purposes will also be collected. These samples will be obtained at the same time as the clinical samples mentioned above. These samples will include blood, saliva, buccal cells, and urine.

Whole blood samples for research purposes will be processed and banked. Plasma-borne, DNA, and RNA biomarkers of treatment response will be evaluated. Because limitations to how much blood can be drawn depend on the participant's weight, peripheral blood mononuclear cells (PBMCs) will only be obtained at Visit 1 from participants who meet the weight requirements.

Saliva and buccal cell samples for research purposes will also be collected, to evaluate biomarkers of treatment response. For every sample collection, participants are asked to fast for one hour prior to saliva and buccal cell sample collection. Participants will also be instructed to rinse their mouths prior to saliva and buccal cell collection. This procedure applies for all future visits at which saliva and buccal cell samples are collected. As much as 4 mL of saliva will be collected. For participants who enroll in the

trial after the implementation of COVID-19 protocol changes, saliva and buccal cell samples will not be collected.

Urine samples will also be collected for research purposes, to evaluate biomarkers of treatment response. Urine samples for research purposes will not be collected for participants enrolled after the implementation of the COVID-19 protocol changes.

Participants' tissues, cells, or other materials derived from these biospecimens may be kept for many years and may be used by researchers at Stanford or by researchers at entities outside Stanford in future studies, which are not foreseeable now. They may include research that involves genetic manipulation as well as those that involve induced pluripotent stems cells (iPSCs) and iPSC-derived neurons and organoids.

Prior to Visit 2, inclusion and exclusion criteria (as outlined in section 8h) will be assessed. One to six weeks later, participants will return to the clinic or schedule a Zoom visit for Visit 2 (Baseline and Randomization).

The following information will be obtained at this time point:

- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Dosage Record and Treatment Emergency Symptom Scale (DOTES) ratings
- Concomitant medications information
- Vital signs
- Height and weight measurements

Parent ratings on the following will also be obtained at this time point:

- Social Responsiveness Scale, 2nd edition (SRS-2)
- Wing Subgroup Questionnaire (WSQ)
- Vineland Adaptive Behavior Scales 3: Social Skills and Relationships Domain (VABS-3)
- Repetitive Behaviors Scale – Revised (RBS-R)
- Stanford Social Motivation Scale, also known as the Stanford Social Dimensions Scale (SSDS)
- Spence Children's Anxiety Scale (SCAS)
- Parent Rated Anxiety Scale-ASD (PRAS-ASD)
- Pediatric Quality of Life (PedsQL) Inventory
- Child's Sleep Habits Questionnaire (CSHQ)

We will also perform an optional high-density electroencephalogram (EEG) to detect the participant's brain activity. Resting-state EEG will be recorded from participants comfortably seated with their eyes open during each visit. Participants will be familiarized with all experimental materials prior to recording and provided with an explicit visual schedule to diminish any undue stress associated with novel environments, equipment (e.g., electrode caps), or support staff. A practice cap will also be made available for participants should further familiarization be requested. Data will be collected using a 64-channel Ag/AgCl electrode cap (ANT North America, Madison, WI) and continuously digitized at 500Hz with an asalab Turnkey System (Madison, WI).

Placement of electrodes will conform to the International 10–20 System. MNE-Python will be used to pre-process data and monitor impedances to be less than 10kOhms. Approximately 5 minutes of continuous EEG will be recorded as participants sit with eyes open. Offline processing will also be performed using the MNE neuroimaging suite and includes (but is not limited to) re-referencing to linked-ear mastoids, correction of DC drift, and the removal of eye-blink artifacts.

The EEG, vital signs, and height and weight measurements at this time point will not be collected from participants who enroll in the trial after the COVID-19 protocol changes.

Participants will undergo experimental assessments measuring social behavior and cognition to facilitate the analysis of post-treatment changes in functioning by accounting for any pre-existing individual differences. These assessments will include: the Reading the Mind in the Eyes Test, NEPSY-II Theory of Mind Test, Diagnostic Analysis of Nonverbal Accuracy, 2nd edition: Child Voices (DANVA-2), and Facial Emotion Recognition Test. We will also record participants' eye gaze using eye-tracking technology while the participants view emotional faces (Eye Gaze Assessment).

Participants who enroll in the trial after the COVID-19 protocol changes will complete the social behavior and cognition assessments via Zoom. The Eye Gaze Assessment will no longer be performed.

Participants will then be randomized in a blinded fashion to a treatment condition (vasopressin or placebo). Stratification will be based on gender and age. Participants will receive 32 IU (16 IU BID) of vasopressin or placebo. Parents will be given a dosing schedule that they will be required to follow and sign each day. Participants will continue vasopressin or placebo administration for 4 weeks at home and their parents will be responsible for reporting any adverse events to research staff. Participants will be screened for excessive water intake and will be informed to maintain an average water intake to decrease the potential occurrence of hyponatremia and water intoxication during the treatment period.

One week after starting the nasal spray, research staff will contact families to check in and inquire about side effects.

Two weeks after starting the nasal spray, participants will return to the clinic for Visit 3. For participants who enroll in the trial after the COVID-19 protocol changes, Visit 3 will be conducted remotely, with the physician visit completed via Zoom.

The following will be obtained at this time point:

- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Clinical Global Impressions Scale—Improvement (CGI-I) ratings
- Overt Aggression Scale (OAS) ratings
- Dosage Record and Treatment Emergency Symptom Scale (DOTES) ratings
- Concomitant medications information
- Vital signs
- Height and weight measurements

- Optional PBMC collection
- Social Responsiveness Scale, 2nd edition (SRS-2) ratings

For participants who enroll in the trial after the COVID-19 protocol changes, vital signs and height and weight measurements will not be obtained at Visit 3 unless instructed by the study physician.

Three weeks after starting the nasal spray, research staff will contact families to check in and inquire about side effects.

Four weeks after starting the nasal spray, participants will return to the clinic for Visit 4 or complete a portion of the visit locally through the primary physician's office (an option instituted after the COVID-19 pandemic).

During Visit 4, the following information will be obtained:

- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Clinical Global Impressions Scale – Improvement (CGI-I) ratings
- Overt Aggression Scale (OAS) ratings
- The Columbia-Suicide Severity Rating Scale (C-SSRS)
- Dosage Record and Treatment Emergency Symptom Scale (DOTES) ratings
- Concomitant medications information
- EKG recordings
- Optional EEG recordings
- Vital signs
- Height and weight measurements
- Clinical blood and urine samples
- Research biological samples (e.g., blood, urine, saliva, buccal cells)

Parent ratings on the following will also be obtained:

- Social Responsiveness Scale, 2nd edition (SRS-2)
- Vineland Adaptive Behavior Scales 3: Social Skills and Relationships Domain (VABS-3)
- Repetitive Behaviors Scale – Revised (RBS-R)
- Stanford Social Motivation Scale, also known as the Stanford Social Dimensions Scale (SSDS)
- Spence Children's Anxiety Scale (SCAS)
- Parent Rated Anxiety Scale-ASD (PRAS-ASD)
- Pediatric Quality of Life (PedsQL) Inventory
- Child's Sleep Habits Questionnaire (CSHQ)

For participants who enroll in the trial after the implementation of the COVID-19 protocol changes, the research saliva, buccal cell, and urine samples will not be collected.

If participants choose to complete the blood draw locally through the primary physician's office, research blood samples will not be obtained. Participants who opt to complete the

visit through the primary physician's office must obtain and send to the study team the EKG recording, vital signs, and height and weight measurements.

Participants will undergo experimental assessments including:

- Eye Gaze Assessment
- Reading the Mind in the Eyes Test
- NEPSY-II Theory of Mind Test
- Diagnostic Analysis of Nonverbal Accuracy, 2nd edition (Child Voices)
- Facial Emotion Recognition Test

For participants who enroll in the trial after the COVID-19 protocol changes, the Eye Gaze Assessment will not be performed.

At this time point, participants who were in the placebo group will either continue on blinded placebo or be switched in a blinded manner to the active vasopressin for the remaining 4 weeks. Participants who were taking the active vasopressin will continue taking the drug in a blinded fashion for the remainder of the study.

Five weeks after starting the nasal spray, research staff will contact families to check in and inquire about side effects.

Six weeks after starting the nasal spray, participants will return to the clinic for Visit 5. For participants who enroll in the trial after the COVID-19 protocol changes, Visit 5 will be conducted remotely, with the physician visit completed via Zoom.

The following information will be obtained at this time point:

- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Clinical Global Impressions Scale – Improvement (CGI-I) ratings
- Overt Aggression Scale (OAS) ratings
- Dosage Record and Treatment Emergency Symptom Scale (DOTES) ratings
- Concomitant medications information
- Vital signs
- Height and weight measurements
- Optional PBMC collection
- Social Responsiveness Scale, 2nd edition (SRS-2) ratings

For participants who enroll in the trial after the COVID-19 protocol changes, vital signs and height and weight measurements will not be obtained at Visit 5 unless instructed by the study physician. If the required blood volume for optional PBMC collection was not obtained at Visit 3 due to the participant's weight, additional blood samples may be obtained at Visit 5. As much as 30 mL of blood will be collected.

Seven weeks after starting the nasal spray, research staff will contact families to check in and inquire about side effects.

Eight weeks after starting the nasal spray, participants will return to the clinic for Visit 6 or complete a portion of the visit locally through the primary physician's office (an option instituted after the COVID-19 pandemic).

The following information will be obtained at this time point:

- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Clinical Global Impressions Scale – Improvement (CGI-I) ratings
- Overt Aggression Scale (OAS) ratings
- The Columbia-Suicide Severity Rating Scale (C-SSRS)
- Dosage Record and Treatment Emergency Symptom Scale (DOTES) ratings
- Concomitant medications information
- EKG recordings
- Optional EEG recordings
- Vital signs
- Height and weight measurements
- Clinical blood and urine samples
- Research blood, urine, saliva, and buccal cell samples

Parent ratings on the following will also be obtained:

- Social Responsiveness Scale, 2nd edition (SRS-2)
- Vineland Adaptive Behavior Scales 3: Social Skills and Relationships Domain (VABS-3)
- Repetitive Behaviors Scale – Revised (RBS-R)
- Stanford Social Motivation Scale, also known as the Stanford Social Dimensions Scale (SSDS)
- Spence Children's Anxiety Scale (SCAS)
- Parent Rated Anxiety Scale-ASD (PRAS-ASD)
- Pediatric Quality of Life (PedsQL) Inventory
- Child's Sleep Habits Questionnaire (CSHQ)

For participants who enroll in the trial after the implementation of the COVID-19 protocol changes, saliva, urine, and buccal cell samples will not be collected.

If the participant chooses to complete the blood draw locally through the primary physician's office, research blood samples will not be obtained. Participants who opt to complete the visit locally must also obtain and send to the study team the EKG recording, vital signs, and height and weight measurements obtained through the primary physician's office.

Participants will undergo experimental assessments including:

- Eye Gaze Assessment
- Reading the Mind in the Eyes Test
- NEPSY-II Theory of Mind Test
- Diagnostic Analysis of Nonverbal Accuracy, 2nd edition (Child Voices)
- Facial Emotion Recognition Test

For participants who enroll in the trial after the COVID-19 protocol changes, the Eye Gaze Assessment will not be performed.

After completion of the blinded 8-week treatment period, participants will be informed of their treatment group. Participants in the placebo-placebo group will have the option of participating in a 4-week open-label vasopressin treatment extension period.

One week after starting open-label vasopressin treatment, research staff will contact families in this open-label extension period to check in and inquire about side effects.

Two weeks after starting open-label vasopressin treatment, participants in this open-label extension period will return to the clinic for Visit 7. Participants who enroll in the trial after the COVID-19 protocol changes will complete Visit 7 remotely, with the physician visit completed via Zoom.

The following information will be obtained at this time point:

- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Clinical Global Impressions Scale – Improvement (CGI-I) ratings
- Overt Aggression Scale (OAS) ratings
- Dosage Record and Treatment Emergency Symptom Scale (DOTES) ratings
- Concomitant medication information
- Vital signs
- Height and weight measurements
- Optional PBMC collection
- Social Responsiveness Scale, 2nd edition (SRS-2) ratings

For participants who enroll in the trial after the COVID-19 protocol changes, vital signs and height and weight measurements will not be obtained at Visit 7 unless instructed by the study physician.

Three weeks after starting open-label vasopressin treatment, research staff will contact families in the open-label period to check in and inquire about side effects.

Four weeks after starting open-label vasopressin treatment, participants in the open-label phase will return to the clinic for Visit 8 or complete this visit locally through the primary physician's office (an option instituted after the COVID-19 pandemic).

The following information will be obtained at this time point:

- Clinical Global Impressions Scale – Severity (CGI-S) ratings
- Clinical Global Impressions Scale – Improvement (CGI-I) ratings
- Overt Aggression Scale (OAS) ratings
- The Columbia-Suicide Severity Rating Scale (C-SSRS)
- Dosage Record and Treatment Emergency Symptom Scale (DOTES) ratings
- Concomitant medication information
- EKG recordings
- Optional EEG recordings

- Vital signs
- Height and weight measurements
- Clinical blood and urine samples
- Research blood, urine, saliva, and buccal cell samples

Parent ratings will be obtained on the following at this time point:

- Social Responsiveness Scale, 2nd edition (SRS-2)
- Vineland Adaptive Behavior Scales 3: Social Skills and Relationships Domain (VABS-3)
- Repetitive Behaviors Scale – Revised (RBS-R)
- Stanford Social Motivation Scale, also known as the Stanford Social Dimensions Scale (SSDS)
- Spence Children's Anxiety Scale (SCAS)
- Parent Rated Anxiety Scale-ASD (PRAS-ASD)
- Pediatric Quality of Life (PedsQL) Inventory
- Child's Sleep Habits Questionnaire (CSHQ)

For participants who enroll in the trial after the implementation of the COVID-19 protocol changes, saliva, buccal cell, and urine samples will not be collected. If the participant chooses to complete the blood draw locally through the primary physician's office, research blood samples will not be obtained. Participants who opt to complete the visit locally must send to the study team the EKG recording, vital signs, and height and weight measurements obtained through the primary physician's office.

Participants will undergo experimental assessments including:

- Eye Gaze Assessment
- Reading the Mind in the Eyes Test
- NEPSY-II Theory of Mind Test
- Diagnostic Analysis of Nonverbal Accuracy, 2nd edition (Child Voices)
- Facial Emotion Recognition Test

For participants who enroll in the trial after the COVID-19 protocol changes, the Eye Gaze Assessment will not be performed.

For participants in all 3 treatment allocation groups, research staff will contact families 4 weeks after completion of the last vasopressin dose to ask them to complete the Social Responsiveness Scale, 2nd edition.

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b. Procedure Risks

Given that this research is some of the first to determine the effects of intranasal vasopressin for the treatment of social deficits in children with ASD, we have chosen doses and a treatment duration that we believe will prove both safe and effective. Participants will receive 32 IU vasopressin (16 IU bid). If severe side effects occur, the study physician may lower the dose or instruct the participant to discontinue the nasal spray.

Safety monitoring: A medical and psychiatric history will be obtained, and a physical examination conducted during the screening phase, prior to randomization. All participants will be in good general medical health. Additional evaluations will be conducted at later visits. Seizure history will be reviewed carefully during the screening visit and, as stated in the protocol's exclusion criteria, children with seizure disorder, or a history of seizure-like activity, will be excluded. Drug side effects will be evaluated with the Dosage Record and Treatment Emergent Symptom Scale at each visit. The Dosage Record and Treatment Emergency Symptom Scale is a general rating scale published by the Early Clinical Drug Evaluation Unit of the National Institute of Mental Health. The scale has been widely used clinically for children and adults to assess many central nervous system side effects as well as some behavioral side effects. We have modified the original version of the Dosage Record and Treatment Emergency Symptom Scale to include specific questions related to potential side effects that might be related to vasopressin, such as hyponatremia/water intoxication. Vital signs (heart rate/blood pressure and temperature) will be assessed at each visit. Height and weight will also be assessed at each visit. In order to increase safety monitoring, blood and urine safety measurement will be conducted during the screening phase and at Visits 3, 4, 5, and 6. Clinical blood work will consist of a basic metabolic panel of blood urea nitrogen, sodium, potassium, chloride, CO2, anion gap, glucose, creatinine, calcium). Osmolality (urine, blood) will also be assessed. In order to determine the impact of vasopressin on heart function, EKG recordings will be obtained at screening and Visits 3, 4, 5, and 6.

After treating more than 40 pediatric participants with vasopressin using a conservative monitoring plan, we have concluded that the drug has been well tolerated with minimal side effects. Therefore, we have decreased the number of safety monitoring points during this trial, which will allow us to collect fewer venous blood samples, urine samples, and EKGs. We received Stanford Institutional Review Board (IRB) approval on 01FEB2019 for these changes to the frequency of safety monitoring points. We also received approval for this change from the Food and Drug Administration (FDA) and the National Institute of Child Health and Human Development. We will keep the safety blood draws and EKGs performed at Visit 1 (screening), Visit 2 (baseline), Visit 4 (4 weeks after baseline), Visit 6 (8 weeks after baseline), and Visit 8 (12 weeks after baseline for participants in the open-label extension phase). We have removed the safety blood draws and EKGs performed at Visit 3 (2 weeks after baseline), Visit 5 (6 weeks after baseline), and Visit 7 (10 weeks after baseline for participants in the open-label extension phase).

For participants who enroll in the trial after the COVID-19 protocol changes, vital signs and height and weight measurements, EKG readings, and blood and urine safety measurement will be obtained at screening and Visits 4 and 6.

In light of the recent evidence linking psychotropic medications to suicidal behaviors, closely monitoring the mental well-being of individuals taking these psychotropic medications is important. Therefore, assessment of suicidal ideation/behaviors will be completed during Visit 1 and will be monitored during the trial at Visits 4, 6, and 8 (if applicable).

Finally, based on preclinical animal studies, some evidence to suggests that vasopressin may be involved in the expression of aggressive behavior. Although the relationship between vasopressin and aggression in humans is currently unknown, we will take every precaution to assess such behavioral changes throughout the study. The Overt Aggression Scale will be administered at screening and Visits 3, 4, 5, and 6. The Overt Aggression Scale is designed to assess observable aggressive or violent behavior and has been used in children with different neuropsychiatric disorders. Identical safety measures as outlined above will be performed during the optional 4-week open-label extension study.

Procedures for handling adverse events: Outpatient dosing phase: For any adverse events, families will be instructed to contact the research coordinator directly during business hours, and through the Stanford page operator after hours and during weekends/holidays. (A project coordinator will be available 24 hours per day/7 days per week to respond to safety concerns.) If the adverse event is life threatening, participants will be asked to call 911 or to visit the nearest emergency room. If the adverse event is not life threatening, the study coordinator will contact the study physician who will contact the family directly and inquire about the adverse event. If the study physician judges the side effects as minor or benign, a decision will be made as to whether the participant will continue in the study. If side effects are judged to be severe and potentially related to vasopressin, the participants will be asked to stop the medication immediately and invited to come to the clinic within 24 hours for a direct evaluation and potential termination of their participation in the study. If the study physician ascertains that the adverse event requires immediate medical attention, parents will be instructed to contact 911 or go to the nearest emergency room. The study physician will also have the ability to break the blind if doing so is in the best interest of the participant's health.

Any serious adverse events will be reported to the Data and Safety Monitoring Board and Stanford IRB within 24 hours and to the FDA within 7 calendar days.

c. Use of Deception in the Study

No deception will be used.

d. Use of Audio and Video Recordings

Audio and video recordings will be used during the Autism Diagnostic Observation Schedule, 2nd Edition, Childhood Autism Rating Scale, 2nd Edition, and the Autism Diagnostic Interview – Revised assessments. A senior clinical psychologist (Dr. Jennifer Phillips or Dr. Cristiana Vattuone) will supervise diagnostic testing. Video recording will be used in order to quantify the participant's social mimicry while they view videos of people yawning and laughing. Two independent raters will study the videos to assess for mimicry behavior.

The analyses of these recordings can be shared for scientific purposes, shown in classrooms to students for educational purposes, shown in public presentations to non-scientific groups, and submitted for public distribution (sold on a DVD). However, audio and video recordings will not be used for any other purpose or shown outside of the research group unless explicit written permission is obtained in the consent form.

e. Alternative Procedures or Courses of Treatment

An alternative is not participating in this study. No standard treatment will be withheld. Participants would remain on their regular medications.

f. Will it be possible to continue the more (most) appropriate therapy for the participant(s) after the conclusion of the study?

Yes.

g. Study Endpoint(s)

Primary Outcome Measure:

1. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Total scores during treatment. [Time Frame: 4-week; 8-week]

Secondary Outcome Measures:

2. Change from baseline in Clinical Global Impression (CGI) scores during treatment. [Time Frame: 4-week; 8-week]
3. Change from baseline on Reading the Mind in the Eyes Test (RMET) during treatment. [Time Frame: 4-week; 8-week]
4. Change from baseline on the Facial Emotion Recognition Test during treatment. [Time Frame: 4-week; 8-week]
5. Change from baseline in parent rated Repetitive Behavior Scale Revised (RBS-R) scores during treatment. [Time Frame: 4-week; 8-week]
6. Change from baseline in parent rated Spence Children's Anxiety Scale (SCAS) during treatment. [Time Frame: 4-week; 8-week]
7. Change from baseline on electrocardiogram (EKG) P Duration during treatment. [Time Frame: 4-week; 8-week]
8. Change from baseline on electrocardiogram (EKG) PR Interval during treatment. [Time Frame: 4-week; 8-week]
9. Change from baseline on electrocardiogram (EKG) QRS Interval during treatment. [Time Frame: 4-week; 8-week]
10. Change from baseline on electrocardiogram (EKG) QT Interval during treatment. [Time Frame: 4-week; 8-week]
11. Change from baseline on blood clinical labs (Sodium) during treatment. [Time Frame: 4-week; 8-week]
12. Change from baseline on blood clinical labs (Potassium) during treatment. [Time Frame: 4-week; 8-week]
13. Change from baseline on blood clinical labs (Chloride) during treatment. [Time Frame: 4-week; 8-week]
14. Change from baseline on blood clinical labs (CO2) during treatment. [Time Frame: 4-week; 8-week]
15. Change from baseline on blood clinical labs (Anion Gap) during treatment. [Time Frame: 4-week; 8-week]

16. Change from baseline on blood clinical labs (Glucose) during treatment.
[Time Frame: 4-week; 8-week]
17. Change from baseline on blood clinical labs (Creatinine) during treatment.
[Time Frame: 4-week; 8-week]
18. Change from baseline on blood clinical labs (Urea Nitrogen) during treatment.
[Time Frame: 4-week; 8-week]
19. Change from baseline on blood clinical labs (Calcium) during treatment.
[Time Frame: 4-week; 8-week]
20. Change from baseline on blood clinical labs (Osmolality) during treatment.
[Time Frame: 4-week; 8-week]
21. Change from baseline on urine clinical labs (Osmolality) during treatment.
[Time Frame: 4-week; 8-week]
22. Change from baseline on vital signs (systolic blood pressure) during treatment.
[Time Frame: 4-week; 8-week]
23. Change from baseline on vital signs (diastolic blood pressure) during treatment.
[Time Frame: 4-week; 8-week]
24. Change from baseline on vital signs (pulse) during treatment.
[Time Frame: 4-week; 8-week]
25. Change from baseline on height during treatment.
[Time Frame: 4-week; 8-week]
26. Change from baseline on weight during treatment.
[Time Frame: 4-week; 8-week]
27. Change from baseline on the Dosage Record Treatment Emergent Symptom Scale (DOTES) during treatment.
[Time Frame: 2-week, 4-week; 6-week, 8-week]
28. Change from baseline in Overt Aggression Scale (OAS) during treatment.
[Time Frame: 2-week, 4-week; 6-week, 8-week]
29. Baseline vasopressin concentration predicting primary and secondary behavioral outcome measures.
[Time Frame: 4-week; 8-week]

Other Pre-specified Outcome Measures:

30. Change from baseline in Vineland Adaptive Behavior Scales, 3rd edition (VABS-3) - Social Skills and Relationships Domain during treatment.
[Time Frame: 4-week; 8-week]
31. Change from baseline in parent rated Pediatric Quality of Life (PedsQL) inventory scores during treatment.
[Time Frame: 4-week; 8-week]
32. Change from baseline on Eye Gaze Assessment (eye tracking) during treatment.
[Time Frame: 4-week; 8-week]
33. Change from baseline on the Developmental Neuropsychological Assessment, 2nd edition (NEPSY-II) Theory of Mind Test during treatment.
[Time Frame: 4-week; 8-week]
34. Change from baseline the Diagnostic Analysis of Nonverbal Accuracy, 2nd edition (DANVA-2) Child Voices Prosody Test during treatment.
[Time Frame: 4-week; 8-week]

35. Change from baseline in parent rated Stanford Social Motivation Scale (also known as the Stanford Social Dimensional Scale) Total scores during treatment.
[Time Frame: 4-week; 8-week]
36. Change from baseline in Parent Rated Anxiety Scale - Autism Spectrum Disorder (PRAS-ASD) score during treatment.
[Time Frame: 4-week; 8-week]
37. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Social Avoidance Factor Score during treatment.
[Time Frame: 4-week; 8-week]
38. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Emotion Recognition Factor Score during treatment.
[Time Frame: 4-week; 8-week]
39. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Interpersonal Relatedness Factor Score during treatment.
[Time Frame: 4-week; 8-week]
40. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Insistence On Sameness Factor Score during treatment.
[Time Frame: 4-week; 8-week]
41. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Repetitive Mannerisms Factor Score during treatment.
[Time Frame: 4-week; 8-week]
42. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Attachment and Affiliation Factor Score during treatment.
[Time Frame: 4-week; 8-week]
43. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Non-facial Communication Production Factor Score during treatment.
[Time Frame: 4-week; 8-week]
44. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Facial Communication Production Factor Score during treatment.
[Time Frame: 4-week; 8-week]
45. Change from baseline in parent rated Social Responsiveness Scale, 2nd edition (SRS-2) Mental States Understanding Factor Score during treatment.
[Time Frame: 4-week; 8-week]
46. Change from baseline in parent rated Child's Sleep Habits Questionnaire (CSHQ) Score during treatment.
[Time Frame: 4-week; 8-week]
47. Change from baseline on spectral power in the alpha, theta, and gamma frequencies as measured by electroencephalogram (EEG) during treatment.
[Time Frame: 4-week; 8-week]

3. BACKGROUND

Impact and relevance. ASD is a pervasive developmental disorder characterized by core deficits in social behavior and communication, and the presence of repetitive or stereotyped behaviors. ASD affects an estimated 1 in 68 children in the US [1]. Individuals with ASD demonstrate diminished eye gaze, abnormal facial and emotion processing, and impaired social judgment.

These deficits jeopardize the development of appropriate social skills and maintenance of social relationships [2-4]. Although several medications are approved for the treatment of ASD (i.e., the atypical antipsychotics risperidone and aripiprazole), none are effective in ameliorating the characteristic social deficits. Moreover, these medications have unfavorable side effects (e.g., lethargy, weight gain). Identifying the underlying pathophysiology of the core social deficits associated with ASD and developing new medications that target these impairments is clearly an important challenge [5]. The proposed project leverages promising preclinical findings to test whether intranasal administration of the neuropeptide vasopressin enhances social functioning in children, particularly males, with ASD. Vasopressin represents enormous potential for enhancing an individual's quality of life through improved social cognition and more meaningful social relationships. The discovery of effective novel therapeutics provides an outstanding opportunity to reduce the emotional and financial burden of ASD on patients, family members, and society.

Preclinical findings: Vasopressin and oxytocin regulate social behavior and show translational promise as pharmacotherapies to treat social impairments. The neurobiological systems critical for normative social functioning are arguably the most promising candidates by which to identify "druggable" targets to treat ASD [5,6]. Two such candidates are vasopressin and oxytocin [8]. Vasopressin and oxytocin are primarily synthesized in the hypothalamus and released into the brain via distributed neural pathways and systemic circulation via the posterior pituitary [7]. Vasopressin and oxytocin are nearly structurally identical nonapeptides and likely evolved due to duplication of a common ancestral gene [6]. Extensive animal research during four decades has demonstrated the importance of both vasopressin and oxytocin in social functioning. Specifically, brain vasopressin and oxytocin systems promote social behavior (e.g., partner preference formation, parenting behavior) and social cognition (e.g., learning and memory for social information) in a variety of mammalian species [8]. In contrast, vasopressin and oxytocin peptide and receptor impairments induced by pharmacological or genetic manipulations produce numerous social deficits in animals [9-12].

Clinical findings: The behavioral effects of intranasal vasopressin and oxytocin administration in neurotypical and patient populations and gaps in our knowledge. On the basis of these promising preclinical findings, multiple investigators have begun to test the potential of these neuropeptides as therapeutic agents to enhance social functioning in people. These studies have mostly focused on the prosocial effects of intranasal oxytocin on social functioning in both neurotypical individuals and individuals with various psychiatric disorders (e.g., anxiety, schizophrenia, ASD) [13-25]. Several independent groups have recently demonstrated that single doses of oxytocin administered to teenage and adult males with ASD improve the processing and retention of social information [20], improve emotion recognition when viewing the eye region of faces [15], and increase trust and preference for social partners during a computer simulated ball-toss game [14]. Several groups, including our own, are now testing the effects of longer-term oxytocin administration to younger individuals, inclusive of boys and girls, with ASD.

In contrast to oxytocin, only a handful of studies have investigated the effects of intranasal vasopressin on social functioning in humans. These studies have shown that vasopressin enhances memory for happy and angry faces [26], increases recognition of positively and negatively valenced social words [27], and enhances neural activity in known vasopressin brain circuitry during a cooperation task [28]. Single intranasal doses of vasopressin or analogs have also been shown to enhance speech and word formation in patients with post-stroke aphasia and improve

short and long-term memory in patients with central diabetes insipidus suggesting potential cognitive enhancing properties of this hormone [29,30]. In humans, intranasally administered vasopressin results in elevated cerebrospinal fluid levels of measured vasopressin, strongly suggesting that it achieves access to the brain [31].

Intranasally administered vasopressin and its analogs (e.g., desmopressin) are well tolerated in humans with no significant side effects reported in any of the studies outlined above. Vasopressin and its analogs have been used for more than 30 years in both children and adults to treat central diabetes insipidus, nocturnal enuresis, and varying states of shock. It has caused very few side effects given that precautions are adhered to (e.g., water restriction to prevent hyponatremia/water intoxication) [32-36]. Despite a growing body of evidence documenting the prosocial effects of vasopressin, and the relevance of this system to ASD [37,38], no studies have tested whether vasopressin administration enhances social functioning in this disorder. Given the research effort now directed toward testing oxytocin as a treatment for the social deficits in ASD, there is an obvious need to investigate the treatment potential of this closely related neuropeptide in order to ameliorate the social deficits in individuals with ASD.

Why is a vasopressin treatment trial critically needed in people with ASD? In addition to its structural and functional similarity to oxytocin, vasopressin merits investigation as a candidate drug for use in ASD for several other reasons. First, preclinical findings from genetically modified and wild type animals suggest that important aspects of social cognition are mediated through vasopressin V1a receptors. In oxytocin receptor (OXTR) null (Oxtr-/-) mice, central administration of either oxytocin or vasopressin directly in to the brain improves sociability deficits, enhances exploration of social novelty, and reverses cognitive flexibility deficits [39]. Pre-treatment with a selective V1a receptor antagonist prior to central oxytocin administration almost completely attenuates the prosocial and cognitive enhancing effects of oxytocin, indicating that V1a receptors mediate this effect. Experiments using wild-type mice, rats, and voles likewise show that administration of selective V1a antagonists in the presence of normal brain oxytocin signaling impairs social functioning [40,41]. These findings are particularly evident in males, in which vasopressin has been shown to selectively regulate social functioning. An intriguing and important aspect of vasopressin and oxytocin physiology is that they exert largely sexually dimorphic behavioral effects as determined by a series of influential pharmacological and autoradiographical studies [42-44]. These studies have shown that, whereas oxytocin preferentially regulates pair bond formation, reproductive physiology (e.g., parturition and lactation), and parental care in females, vasopressin preferentially regulates mating behavior, pair bond formation, and parental care in males [45].

Second, recent genetic association studies indicate that several single nucleotide polymorphisms and their haplotypes in the OXTR gene increase risk for ASD [46,47]. Genome-wide scans likewise indicate that the 3p25 region, which contains the OXTR gene, may be a linkage site for ASD [48]. These findings suggest the intriguing supplementary hypothesis that in people with OXTR impairments, vasopressin treatment may be even more efficacious than oxytocin administration. Although both vasopressin and oxytocin ligands show high affinity for all four receptors (i.e., vasopressin: V1a, V1b, V2; and oxytocin: OXTR, respectively), they bind with greatest affinity to their own receptor(s) [49]. This hypothesis is further supported by evidence that Oxtr-/- mice have higher vasopressin V1a receptor expression in the brain than do wild-type mice, which suggests that these animals may be more sensitive to vasopressin administration than

oxytocin [39,50]. These findings clearly highlight the need for careful studies that clarify the underlying neurobiological actions of these neuropeptides as pharmacotherapeutic agents in individuals with ASD [50]. To address this larger research question, we must first test whether intranasal vasopressin administration improves social functioning in individuals with ASD, and whether this medication is more efficacious in males than females.

Factors that contribute to treatment response efficacy. In addition to gender differences in treatment response, most vasopressin and oxytocin pharmacology studies have found treatment responders and non-responders. For example, previous preclinical research conducted by our group, led by Dr. Karen Parker, has shown that baseline social phenotype significantly influences the efficacy of vasopressin treatment to induce or enhance prosocial behaviors in male rodents [51]. Similarly, a single-dose oxytocin treatment study in male adults with ASD provides preliminary evidence for enhanced social functioning post-treatment in a subgroup of individuals who show higher social motivation at baseline [14]. Another recent study using transcutaneous electrical acupoint stimulation therapy demonstrated enhanced social functioning post-treatment, which was associated with higher plasma vasopressin levels in children with ASD. Treatment benefits and higher vasopressin levels were most pronounced in children with “passive” and ”aloof,”, but not “active but odd” behavioral phenotypes as assessed by the Wing Subgroup Questionnaire at baseline [52]. These findings suggest that individual differences in pre-treatment social impairments may contribute to treatment response efficacy and that these changes are associated with increased plasma neuropeptide levels. This intriguing hypothesis will be explored in the present study.

Innovation. Vasopressin is one of the most promising candidate drugs to treat the presently intractable social deficits of ASD. This large-scale clinical trial will to test the efficacy of vasopressin treatment to enhance social abilities in individuals with ASD. Inclusion of female participants is a strength of this study. Female exclusion, which frequently occurs in ASD treatment studies, may jeopardize optimal testing of effective pharmacotherapies, and preclude identification of disease mechanisms. Our inclusion of females is particularly important because preclinical evidence suggests that males may respond more robustly to vasopressin treatment than females [53]. Findings from both preclinical and clinical studies have shown that differences in pre-treatment social functioning and/or neuropeptide biology are associated with vasopressin and oxytocin treatment response outcomes. Ours will be the first study to test concomitantly whether pre-treatment social measurements and baseline neuropeptide levels contribute to individual differences in vasopressin treatment response efficacy.

The use of both eye-tracking methodology to assess change in social abilities and an automated prosody assessment in this trial represent crucial steps toward objective assessment of core symptoms of ASD and generalization of treatment. No clear biologically-based marker, such as eye tracking, has yet been used extensively in published controlled trials of children with ASD in order to test treatment effects and moderators of response.

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4. RADIOISOTOPES OR RADIATION MACHINES

a. Standard of Care (SOC) Procedures

Identify Week/Month of Study	Name of Exam	Identify if SOC or Research
NA	NA	NA

b. Radioisotopes

i. Radionuclide(s) and chemical form(s)

NA

ii. Total number of times the radioisotope and activity will be administered (mCi) and the route of administration for a typical study participant

NA

iii. If not FDA approved: dosimetry information and source documents (package insert, Medical Internal Radiation Dose [MIRD] calculation, and peer reviewed literature)

NA

c. Radiation Machines – Diagnostic Procedures

i. Examination description (well-established procedures)

NA

ii. Total number of times each procedure will be performed (typical study participant)

NA

iii. Setup and techniques to support dose modeling

NA

iv. FDA status of the machine and information on dose modeling (if procedure is not well-established)

NA

d. Radiation Machines – Therapeutic Procedures

i. Area treated, dose per fraction/number of fractions, performed as part of normal clinical management or due to research participation (well-established procedures)

NA

ii. FDA status of the machine, basis for dosimetry, area treated, dose per fraction and number of fractions (if procedure is not well-established)

NA

5. DEVICES USED IN THE STUDY

a. Investigational Devices (Including Commercial Devices Used Off-Label)

Investigational Device 1	
Name:	NA

b. IDE-Exempt Devices

IND-Exempt Device 1	
Name:	NA

6. DRUGS, BIOLOGICS, REAGENTS, OR CHEMICALS USED IN THE STUDY

a. Investigational Drugs, Biologics, Reagents, or Chemicals

Investigational Product 1	
Name:	Vasopressin Injection, USP
Dosage:	16 IU BID
Administration Route:	Intranasal

b. Commercial Drugs, Biologics, Reagents, or Chemicals

Commercial Product 1	
Name:	NA

7. DISINFECTION PROCEDURES FOR MEDICAL EQUIPMENT USED ON BOTH HUMANS AND ANIMALS

NA

8. PARTICIPANT POPULATION

a. Planned Enrollment

All participants will be enrolled at Stanford. We plan to enroll and screen as many as 200 children to meet our target of a minimum of 100 randomized participants.

b. Age, Gender, and Ethnic Background

The participants will be between the ages of 6 and 17 years (maximum 17 years and 11 months). Males and females from all racial and ethnic backgrounds will be eligible to participate in this study.

c. Vulnerable Populations

We are recruiting a minimum of 100 potentially decisionally impaired children (ages 6 to 17 years) with ASD. This study is specifically directed at children with ASD in order to test the tolerability and efficacy of vasopressin in this population.

d. Rationale for Exclusion of Certain Populations

NA

e. Stanford Populations

NA

f. Healthy Volunteers

NA

g. Recruitment Details

Potential participants will be identified from the Autism and Developmental Disorders Clinic at Stanford and from the Autism and Developmental Disorders Research Registry participants (eProtocol #9345; PI: Antonio Hardan). An email letter and flyers will be distributed to the Autism and Developmental Disorders Research Registry participants.

Participants may also be recruited via the Simons Foundation Powering Autism Research for Knowledge (SPARK). SPARK will inform families about our study via email.

Flyers will be posted throughout the community in public places and also on craigslist, Facebook, Berkeley Parent Network, and various websites such as the Stanford Autism Research website (PI: Antonio Hardan), the Stanford Autism Center website, the Stanford Social Neuroscience Research Program (PI: Karen Parker), the Stanford Department of Psychiatry Recruitment website, the Bay Area Autism Consortium website, ClinicalTrials.gov, resourcesmatch.org, the Stanford Clinical Trials website, and centerwatch.com.

Additionally, participants may be recruited via the Interactive Autism Network (IAN). The Interactive Autism Network Project will inform families about our study in two distinct ways: via email (for Interactive Autism Network Research families meeting our study criteria) and via the Interactive Autism Network Community Research Opportunities Bulletin Board (for the public at large). If participants are interested in participating, they can fill out an online survey with basic contact information, or call the research staff who will explain the study using the advertisement phone script. If participants remain interested in participating, the research staff will obtain their informed consent as outlined above. The same process outlined above will be used for participants recruited from the flyers and email letters.

---

h. Eligibility Criteria

i. Inclusion Criteria

All participants will meet the following:
a) Medically healthy outpatients between 6 and 17 years of age;
b) Diagnostic and Statistical Manual 5th edition (DSM-5) criteria for Autism Spectrum Disorder on the basis of clinical evaluation, confirmed with the Autism Diagnostic Interview Revised and Autism Diagnostic Observation Schedule, 2nd edition or the Childhood Autism Rating Scale, 2nd Edition;
c) Males and females;
d) IQ of 40 and above;
e) Rating of 4 or higher on the Social Communication domain of the Clinical Global Impressions Severity;
f) Social Responsiveness Scale, 2nd edition Total score of 70 and above;
g) Care provider who can reliably bring participant to clinic visits, provide trustworthy ratings, and interact with participant on a regular basis;
h) Stable concomitant psychotropic medications or medications potentially affecting vasopressin for at least 4 weeks (with the exception of fluoxetine, 6 weeks);
i) No planned changes in psychosocial and biomedical interventions during the trial;
j) Willingness to provide blood samples and ability to participate in key study procedures (i.e., diagnostic assessments and laboratory safety measurements).

ii. Exclusion Criteria

Participants will be excluded if one or more of the following are met:
a) DSM-5 diagnosis of schizophrenia, schizoaffective disorder, or psychotic disorder;
b) Regular nasal obstruction or nosebleeds;

c) Unstable medical conditions such as migraine, asthma attacks, or seizures, and significant physical illness (e.g., serious liver disease, renal dysfunction, or cardiac pathology);
d) Clinically significant abnormal EKG reading;
e) History of hypersensitivity to vasopressin, its analogs, or compounding preservatives (e.g., chlorobutanol);
f) Evidence of a genetic mutation known to cause ASD or intellectual disability (e.g., Fragile X Syndrome); or metabolic or infectious etiology for ASD on the basis of medical history, neurologic history, and available tests for inborn errors of metabolism and chromosomal analysis;
g) Significant hearing or vision impairments;
h) Habitually drinks large volumes of water;
i) Pregnant or sexually active females not using a reliable method of contraception;
j) Current use of any medications known to interact with vasopressin including: 1) carbamazepine (i.e., Tegretol); chlorpropamide; clofibrate; urea; fludrocortisone; tricyclic antidepressants (all of which may potentiate the antidiuretic effect of vasopressin when used concurrently); 2) demeclocycline; norepinephrine; lithium; heparin; alcohol (all of which may decrease the antidiuretic effect of vasopressin when used concurrently); 3) ganglionic blocking agents including benzohexonium, chlorisondamine, pentamine (all of which may produce a marked increase in sensitivity to the pressor effects of vasopressin);
k) Previous participation in a vasopressin clinical trial or current use of vasopressin;
l) Current use of desmopressin (DDAVP) or oxytocin.

iii. Screening Procedures:

Consent will be obtained before any screening procedures can begin. A review of the medical and psychiatric history of participants will occur during the screening procedures in order to determine study eligibility. Clinically significant abnormal vital signs, EKG readings, and clinical chemistry labs will be determined according to Lucile Packard Children's Hospital standards and expert opinion from trained physicians.

i. Participation in Multiple Protocols

Participants will not be allowed to be enrolled in any other medication or behavioral trials during their participation in the current research study.

j. Payments to Participants

Participants will be paid $60 for the first visit and $30 for each additional completed visit.

k. Costs to Participants

There will be no costs charged to the participants.

l. Planned Duration of the Study

The entire study will last as long as six years to complete and will be followed by a period of 6 months of data analysis. A participant could be in the study for approximately 14 weeks including the initial screening period, the 8-week blinded treatment trial. The

optional open-label extension study (only for those participants randomized to the placebo-placebo group) will take an additional 4 weeks to complete.

9. RISKS

a. Potential Risks

i. Investigational devices

NA

ii. Investigational drugs

Vasopressin side effects: There is presently no readily available list of side effects based on a common-rare continuum for vasopressin. There are several routes of administration for vasopressin: intramuscular (IM), subcutaneous (SC), intravenous (IV), and intranasal (IN). In the present study, the intranasal administration route will be used. There are limited side effects associated with the intranasal route of administration for vasopressin. However, as discussed below, there are a number of potential side effects observed when vasopressin is administered by injection (i.e., intramuscular, subcutaneous, intravenous). It is crucial to differentiate between the side effects specifically associated with different routes of administration.

Intranasal administration: Information regarding the safety profile of intranasal vasopressin administration comes mostly from several clinical trials examining the efficacy of vasopressin for enhancing neurocognitive functioning. Additionally, although the typical administration route for treatment of central diabetes insipidus (CDI) is some form of vasopressin injection, some patients (including children) have primarily used a nasal route of administration to treat their disease, or use a vasopressin nasal spray as an adjunct to vasopressin injections [1-4]. Importantly, a large number of studies have provided convincing evidence for intranasal vasopressin being very well tolerated [1-13]. As mentioned, the majority of these studies have aimed to investigate the impact of intranasal vasopressin on neurocognitive processes.

Given the relatively large size of vasopressin (>1 kD), injections of this polypeptide are not considered to reach the brain in significant quantities due to the restrictions of the blood-brain barrier (BBB) [14]. Nasal routes of administration are purported to overcome the restrictions of the blood-brain barrier for large molecule peptides, including vasopressin, making it a preferred administration route for targeted central nervous system treatment. Born et al. [15] provided convincing evidence of this by showing that intranasal vasopressin (40 and 80 IU) administered to healthy adult males and females significantly increased cerebrospinal fluid concentrations of the peptide compared to placebo administration; vasopressin levels had not returned to baseline at the end of the 80-minute sampling period. Blood concentrations of vasopressin also increased but were only slightly (non-significantly) correlated with cerebrospinal fluid levels. The nasal route of administration is generally considered much safer than injections of vasopressin for multiple reasons. Not only is the risk of

local infection or transfer of contagious blood borne disease substantially reduced, far fewer peripheral effects are noted with intranasal administration of vasopressin [5-13]. For example, several recent studies have outlined the ability of intranasal vasopressin (20 and 40 IU) to enhance complex psychosocial functioning and associated brain activity when given in single-doses to healthy adults [5,6,11-13]. Only minor and transient side effects (relaxation/tiredness, headache, dizziness, bitter taste, nasal irritation, increased diastolic blood pressure) were reported by a small number of participants and were experienced following both administration of vasopressin and placebo.

Chronic dosing with intranasal vasopressin, as proposed in the current study, has also been shown in several studies to be very well tolerated [8-10]. In a study of 26 elderly individuals utilizing a blinded, randomized, placebo-controlled, 10-week trial of intranasal vasopressin administration (20 IU, bid) for treatment of sleep disturbances, no major side effects were reported across multiple safety measures [10]. A self-rating scale of complaints did not reveal any side effects of the intranasal vasopressin treatment. Cardiovascular monitoring revealed a systolic blood pressure that was slightly elevated 60 minutes after vasopressin administration, but not 8-12 hours later. Measurements of diastolic blood pressure and heart rate did not indicate any treatment effects. Examination of blood pressure and heart rate on a weekly basis during the 10-week study period revealed values in the normal range and no treatment related differences. At the end of the trial, measurement of sodium, osmolality, and hematocrit did not reveal any treatment effects, and values were entirely within the normal range.

In our pilot intranasal vasopressin trial, minimal side effects were reported, including fever, cough, body aches, excitement/agitation, increased motor activity, insomnia, headache, decreased motor activity, akathisia, depressive affect, drowsiness, head banging, nasal congestion, blurred vision, dry mouth, decreased appetite/anorexia, nausea/vomiting, constipation, increased urination, bed wetting, skin rash, bug bite, lethargy/tiredness, feeling emotional, bloody nose, stinging sensation in the nose, and burning sensation in the throat.

Taken together, the studies outlined above suggest that intranasal vasopressin administration at the doses proposed here is very well tolerated. Below is a list of side effects reported from a small number of participants following intranasal vasopressin (and placebo) treatment.

In our prior pilot vasopressin trial at Stanford, sneezing, gagging, and sore throat, have also been reported.

Reported side effects following intranasal vasopressin (and placebo) administration (NB: These side effects were all minor and transient):

- Relaxation or tiredness
- Headache
- Dizziness
- Increased systolic blood pressure

- Increased diastolic blood pressure
- Bitter taste
- Nasal irritation

Vasopressin administered by injection: Ten IU of vasopressin (0.5 mL) given intramuscularly will usually elicit full physiologic response in adult patients. The manufacturer recommends that in the average postoperative adult, an initial 5 IU dose, increased to 10 IU, if necessary, should be given intramuscularly every 3 to 4 hours for treating abdominal distention. In preparation for roentgenography, the average patient will require two intramuscular injections of 10 IU given two hours and one half-hour, respectively, before films are exposed. For treating central diabetes insipidus, vasopressin may be administered subcutaneously or intranasally. The dose of injection is 5 to 10 IU repeated two or three times daily as needed. When vasopressin is administered intranasally for treating central diabetes insipidus, the dosage and interval should be adjusted for each patient [16].

Many of the side effects listed by the manufacturer are associated with injections of vasopressin and result from its vasoconstrictive properties mediated by vasopressin V1a receptors. These side effects include cardiac arrest and/or shock, circumoral pallor, arrhythmias, decreased cardiac output, angina, myocardial ischemia, and peripheral vasoconstriction. Gastrointestinal side effects may include abdominal cramps, diarrhea, intestinal hyperactivity, nausea and vomiting. Bronchial constriction, tremor, vertigo and "pounding" in the head have also been reported. Skin changes may also occur following vasopressin injection and include sweating, urticarial (rash), and cutaneous gangrene. Additionally, some individuals may experience a severe hypersensitivity to vasopressin or its excipients (e.g., chlorobutanol as preservative) (see Kim et al. [17] for a review of vasopressin side effects). In recent years, an increase has been noted in the use of intravenous vasopressin treatment for hemodynamically unstable infants and children following cardiothoracic surgery [18].

This treatment has been associated with reports of water intoxication/hyponatremia associated with prolonged intravenous vasopressin. Careful monitoring of cardiovascular parameters and serum sodium values is important when vasopressin is given to intensive care patients or to those with underlying cardiovascular pathology or a known sensitivity to the drug or its excipients. For outpatients, drinking a glass of water when taking vasopressin is reported to reduce some of the skin and gastrointestinal effects. However, close monitoring of fluid intake and output should also be considered during vasopressin treatment. Less free water should be given in cases where hyponatremia may be a concern. Several studies have shown that regulation of fluids and close monitoring of electrolyte balance substantially reduce the risk of hyponatremia in children treated with intravenous vasopressin [19,20].

Reported side effects following injections (intramuscular, subcutaneous, intravenous) of vasopressin:
- Abdominal cramps
- Diarrhea

- Intestinal hyperactivity
- Nausea
- Vomiting
- Passage of gas
- Blanching of skin
- Sweating
- Tremor
- Vertigo
- "Pounding" in head
- Bronchial constriction
- Urticarial
- Circumoral pallor
- Cutaneous gangrene
- Anaphylaxis (cardiac arrest and/or shock)
- Arrhythmias
- Decreased cardiac output
- Angina
- Myocardial ischemia
- Peripheral vasoconstriction
- Gangrene
- Hyponatremia/water intoxication (possibly resulting in seizure or coma)

FDA indications for vasopressin administration: The formulation of vasopressin to be used in the proposed research is VasostrictTM (Vasopressin Injection, USP; manufactured by PAR Pharmaceutical, an Endo International Company, Chestnut Ridge, NY). The drug has FDA marketing approval for increasing blood pressure in adults with vasodilatory shock. This study is a Phase II clinical trial that aims to investigate both the efficacy and tolerability of intranasal vasopressin treatment for social, cognitive, and behavioral problems in children with ASD. Several other US research groups have received FDA approval to investigate the effects of intranasal vasopressin administration for enhancing neurocognitive functioning in both healthy adults and people with ASD (e.g., see NCT01327027, NCT01566539, NCT01680718, NCT01093768).

Contraindications and precautions for vasopressin administration: The safety concerns listed in the section above for vasopressin injections are considered rare if precautions are adhered to. The package insert for Vasopressin Injection, USP lists several precautions and contraindications associated with this drug. Contraindications for the use of vasopressin include anaphylaxis or hypersensitivity to the drug or its components (e.g., chlorobutanol as a preservative). This drug is not recommended for use in patients with vascular disease, especially disease of the coronary arteries, except with extreme caution. In such patients, even small doses may precipitate angina pain; with larger doses, the possibility of myocardial infarction should be considered. Vasopressin may also produce hyponatremia/water intoxication and is contraindicated in patients who habitually drink large volumes of water (e.g., habitual or psychogenic polydipsia). For this reason, the early signs of drowsiness, listlessness, and headaches should be recognized to prevent terminal coma and

convulsions. Vasopressin should also be used with caution in the presence of epilepsy, migraine, asthma, heart failure, or any state in which a rapid addition to extracellular water may produce hazards for an already overburdened system. These contraindications are reflected in the extensive exclusion criteria and safety monitoring used in this study. Research utilizing vasopressin treatment in children provides convincing evidence for vasopressin being well tolerated if fluid intake is monitored closely during the treatment period [e.g., 20].

An additional concern with using vasopressin is the potential for increased aggression. This concern is based on specific preclinical animal studies; however, to the investigators' knowledge no evidence has demonstrated that vasopressin increases aggressive behavior when administered to humans. Research on vasopressin and aggression in rodents has generally shown that vasopressin increases aggression in: 1) non-monogamous adult male residents when an adult male intruder is introduced into his "territory" (i.e., home cage), and 2) monogamous adult male residents when an adult male intruder is introduced into his territory, which sometimes includes the mate and pups [21,22]. One can argue that increased aggression in these behavioral assays is adaptive. In the context of the second example, increased aggression in the presence of mate/pups in the home territory is a protective response that is prosocial in nature, at least regarding the resident male's family. Nevertheless, we acknowledged that monitoring aggression in children in our pilot treatment trial was an important precaution. We found no evidence that blinded vasopressin treatment increased aggression in our pilot vasopressin trial. We will continue to regularly inquire about aggressive behavior throughout this larger phase II study.

No reliable data are available on the developmental risks of vasopressin administration in children. The main purpose of our study is to determine the safety and efficacy of intranasal vasopressin administration in children with ASD. We will report any serious adverse events to the IRB and FDA according to law and will detail this information in published articles and presentations.

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iii. Commercially available drugs, biologics, reagents or chemicals

NA

iv. Procedures

Screening measures

Medical and psychiatric screening will be conducted under the supervision of Dr. Antonio Hardan, a board-certified child and adolescent psychiatrist, to determine participants' eligibility to take part in the trial. A physical examination will be completed on all participants. Parents and children will be interviewed to determine relevant information pertaining to the child's lifetime medical and psychiatric health.

The Clinical Global Impression Scale (CGI) [1] consists of 3 subscales: severity of illness (CGI-S), global improvement (CGI-I), and therapeutic index. The severity of the illness is scored from 1= normal to 7= extremely ill. The patient's improvement is scored on a 7-point scale which ranges from markedly improved (1), to no change (4), and to very much worse (7). The Clinical Global Impressions Scale – Severity has been widely used in psychopharmacological studies and has been shown as highly sensitive to medication effects [2]. Consistent with the Research Unit on Pediatric Psychopharmacology (RUPP) Autism Network, a score of 3 (mildly ill) on the severity of illness scale will be assigned to participants based upon the core features of ASD. Higher scores will be assigned based on the presence of other, secondary behaviors. The Clinical Global Impressions Scale – Improvement is a subscale scored from 1 (very much improved) to 7 (very much worse). A score of 4 reflects no change. Positive response is defined by a score of 2 (much improved) or 1 (very much improved) at week 4. The scores on the severity of illness and improvement scale will be based on parent interview, parent-completed behavior problem checklists, as well as direct observation of the child. The CGI-S will be used as a screening tool and as an outcome measure. CGI-I will be used as an outcome measure to assess improvement observed during the trial that is due to vasopressin treatment.

The Autism Diagnostic Interview-Revised (ADI-R) [3] is a reliable and well validated research diagnostic instrument administered to participants' parents and consists of 88 items that are informed by the ICD-10 and DSM-5 diagnostic criteria for ASD. To meet these criteria, the participant must have a score higher than a pre-specified cut-point in the three symptom areas of the ICD-10 diagnostic system, and must also have an age of onset prior to 3 years.

The Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2) [4,5] is a reliable and well validated semi-structured clinical and research diagnostic instrument that allows assessment through behavioral observations during specific play, social, and language tasks. The ADOS-2 provides empirically-based thresholds for diagnoses of autism and autism spectrum. The ADI-R and ADOS-2 will be used in conjunction with expert clinical opinion guided by DSM-5 criteria to assess for the presence of a diagnosis of ASD.

The Childhood Autism Rating Scale, 2nd Edition (CARS-2) is a clinician-rated questionnaire that allows for ASD diagnosis assessment through behavioral observations and additional information such as parent and/or teacher reports [6]. CARS-2 consists of 15 items that cover social, emotional, adaptive, communicative, and cognitive functions. CARS-2 (standard form) is "for use with individuals

younger than 6 years of age and those with communication difficulties or below-average estimated IQs" and CARS-2-HF (high functioning) is used for "assessing verbally fluent individuals, 6 years of age and older, with IQ scores above 80". The Childhood Autism Rating Scale, 2nd edition will be used as an alternative to the ADOS-2 for participants who enroll in the trial after the COVID-19 protocol changes; CARS-2 can be administered via Zoom.

The Stanford Binet, 5th edition (SB-5) [7] will be administered to determine intellectual functioning. Participants must have an IQ of 40 or higher to meet inclusion criteria. The SB-5 provides an overall (Full Scale) IQ score, as well as Verbal and Nonverbal IQ scores. Tasks are broken into five domains of cognitive functioning: Fluid Reasoning, Knowledge, Quantitative Reasoning, Visual-Spatial Processing, and Working Memory. Age-referenced standard scores, as well as raw scores for cognitive domains, are available. If participants have already undergone previous IQ testing with a different instrument, such as one of the Weschler scales, the research team will review the assessment report to determine its suitability in place of the SB-5.

The Wechsler Intelligence Scale for Children, 5th Edition (WISC-V) is an individually administered, comprehensive clinical instrument that will be administered to assess intelligence [8]. The primary index scores represent intellectual functioning in five cognitive areas: Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and the Processing Speed Index. The assessment also includes a Full-Scale IQ composite score representing general intellectual ability. The WISC-V will be used as an alternative to the SB5 for participants who enroll in the trial after the COVID-19 protocol changes; the WISC-V can be administered in a modified manner via Zoom, following publisher guidelines.

Phenotyping measures:

The Wing Subgroups Questionnaire (WSQ) [9] will be administered to classify participants into distinct social subtypes to test whether social phenotype affects treatment efficacy. The three subtypes are: active-but-odd (actively seeks social interaction with others but does so in an awkward manner); passive (does not engage in social approach but responds when approached and will remain socially engaged); or aloof (fails to approach others and withdraws if approached). The Wing Subgroup Questionnaire has criterion-referenced validity and internal consistency [10].

Safety measures

Clinical chemistry labs, including blood urea nitrogen sodium, potassium, chloride, CO2, anion gap, glucose, creatinine, calcium, and blood and urine osmolality, will be conducted at screening prior to participants being allocated to treatment groups to determine eligibility status and Visits 4 and 6 to assess for drug safety. Blood samples will be collected at the Lucile Packard Children's Hospital phlebotomy lab or locally through the participant's primary care physician's office. As much as 30mL of blood will be collected (including the blood collected for research biomarker measures).

An EKG will be performed at screening prior to participants being allocated to treatment groups to determine eligibility status and at Visits 4 and 6 to assess for drug safety. A trained investigator will place the leads for measurement of basal cardiac function.

Vital signs including heart rate, blood pressure, and body temperature will be assessed at each visit. Participants enrolled in the trial after the COVID-19 protocol changes will have the above assessed at Visits 1, 4, 6, and 8 (if applicable).

Height and weight will be measured at each visit. Participants enrolled in the trial after the COVID-19 protocol changes will have height and weight measured at screening, Visit 4, Visit 6, and Visit 8 (if applicable). Fluctuations in weight can provide important information on the diuretic effects of vasopressin and will be used to determine alterations in weight gain or loss potentially related to changes in appetite/metabolism resulting from the treatment.

The Dosage Record and Treatment Emergent Symptom Scale (DOTES) will be used to assess general physical and psychiatric side effects. The Dosage Record and Treatment Emergency Symptom Scale is a general rating scale published by the Early Clinical Drug Evaluation Unit of the National Institute of Mental Health [11]. The scale has been widely used clinically for children and adults to assess many central nervous system side effects as well as some behavioral side effects [12]. We have modified the Dosage Record and Treatment Emergency Symptom Scale to include relevant questions regarding hydration status.

The Overt Aggression Scale (OAS) will be administered to determine changes in aggressive behavior throughout the study. The Overt Aggression Scale is designed to assess observable aggressive or violent behavior and has been used in children with different neuropsychiatric disorders [13,14].

All safety assessments outlined above will be repeated during the optional open-label vasopressin treatment extension period. We will also perform pharmacokinetics analysis during the open-label treatment period as outlined previously.

Urine pregnancy tests will be administered to all female participants of childbearing age. Females, who are at least 12.5 years old or have a history of menstruating, must have a negative pregnancy test before participating in this study.

Primary outcome measure

The Social Responsiveness Scale, 2nd edition (SRS-2) [15-17] is a norm-referenced questionnaire developed to measure social behavior in both clinical and non-clinical populations. The Social Responsiveness Scale, 2nd edition provides age- and gender-

referenced scores, as well as raw scores for the following domains: Total score, Receptive, Cognitive, Expressive, and Motivational aspects of social behavior, and Autistic preoccupations. The psychometric properties were tested in younger and older participants [15,18]. The Social Responsiveness Scale, 2nd edition Total score was continuously distributed within each group and minimally correlated with IQ. Post-treatment parent Social Responsiveness Scale, 2nd edition ratings will be used as the primary outcome measure in each treatment arm, and pre-treatment parent Social Responsiveness Scale, 2nd edition ratings will be used to assess whether social deficit severity affects treatment response efficacy.

Secondary outcome measures

The Clinical Global Impressions Scale – Severity (CGI-S) and Clinical Global Impressions Scale – Improvement (CGI-I) are included as secondary outcome measures. Please see above for more details about this instrument.

The Repetitive Behavior Scale – Revised (RBS-R) [19] is a rating tool used to capture the breadth of repetitive behavior in ASD. The Repetitive Behaviors Scale – Revised contains 43 items that are drawn from other instruments and grouped theoretically based on the authors' clinical experience. The Repetitive Behaviors Scale – Revised consists of five subscales: 1) Rituals/Sameness; 2) Self-injurious behavior; 3) Stereotypical behavior; 4) Compulsive behavior; and 5) Restricted interests. Items are rated on a four-point Likert scale ranging from (0) ''behavior does not occur'' to (3) ''behavior occurs and is a severe problem." Raters are asked to refer to the previous month when completing the scale. The Repetitive Behaviors Scale – Revised has been validated for use in young children with ASD.

The Spence Children's Anxiety Scale (SCAS) [20] assesses the frequency with which children experience symptoms relating to obsessive-compulsive disorder, separation anxiety, social phobia, panic/agoraphobia, generalized anxiety/overanxious disorder, and fears of physical injury. The Spence Children's Anxiety Scale has high internal consistency for the total score and factor scores, acceptable for 6-month test-retest reliability. Results of both confirmatory and exploratory factor analyses were supportive of the DSM-IV constructs of anxiety disorders.

The Reading the Mind in the Eyes Test (RMET) [21] will be used to test participants' ability to determine the emotions of others. The Reading the Mind in the Eyes Test is a widely used test of theory of mind that assesses individuals' ability to infer complex emotions from the eye region of faces. We will use the child version of the test, which consists of 28 images and has modified answer options for ease of use in pediatric populations.

The Facial Emotion Recognition Test will be used to assess participants' ability to identify facial emotions of others. The Facial Emotion Recognition Test was derived from the NimStim Set of Facial Expressions that required participants to identify the correct facial emotion displayed by each face stimulus [22]. Participants are required to select an emotion from a list of seven possible emotions (angry, calm, disgusted,

happy, sad, scared, and surprised) and are administered a total of 42 face stimuli per test session.

Optional high-density EEG: Outcome measures will include spectral power in the alpha, theta, and gamma frequencies. The exploratory outcome measure includes EEG coherence. Resting-state EEG will be recorded from participants comfortably seated with their eyes open during each visit at which EEG is collected. Participants will be familiarized with all experimental materials prior to recording and provided with an explicit visual schedule to diminish any undue stress associated with novel environments, equipment (e.g., electrode caps), or support staff. A practice cap will also be made available for participants should further familiarization be requested. Data will be collected using a 64-channel Ag/AgCl electrode cap (ANT North America, Madison, WI) and continuously digitized at 500Hz with an asalab Turnkey System (Madison, WI). Placement of electrodes will conform to the International 10–20 System. MNE will be used to pre-process data and monitor impedances to be less than 10kOhms. Approximately 5 minutes of continuous EEG will be recorded as participants sit with eyes open. Offline processing will be performed using the MNE neuroimaging suite and includes (but is not limited to) re-referencing to linked-ear mastoids, correction of DC drift, and the removal of eye-blink artifacts. For participants who enroll in the trial after the COVID-19 protocol changes, the EEG will not be available.

Other outcome measures

The Vineland Adaptive Behavior Scales, 3rd edition: Social Skills and Relationships Domain (VABS-3) will be administered to measure behavioral functioning [23]. The participants' level of expressive and receptive speech will be used as a covariable when examining performance on tasks that require adequate language and communication skills. The Vineland Adaptive Behavior Scales 3: Social Skills and Relationships Domain is a norm-referenced, standardized, parent report/interview that measures developmental and behavioral abilities in the areas of socialization, communication, daily living skills, and motor skills. This instrument was developed for use in people with developmental disabilities, has been used frequently in individuals with ASD, and maintains strong psychometric properties [24,25].

The Pediatric Quality of Life (PedsQL) [26] inventory is a 23-item brief parent proxy-report designed not only to measure core dimensions of health as delineated by the World Health Organization, but also to capture information about an individual's functioning in educational settings. The inventory has 4 subscales including physical, emotional, social, and school functioning. The Pediatric Quality of Life distinguishes between healthy children and children with acute and chronic health conditions and also distinguishes disease severity within a chronic health condition.
The NEPSY-II [27] is a widely used and highly validated norm-referenced measure of child (3-16 years old) neurocognitive processes. The Theory of Mind subtest will be used to assess social perception abilities. This test consists of participants being read various scenarios and then asked questions that require knowledge of another person's point of view to answer correctly.

Eye Gaze to social cues will be measured while participants view the emotional faces presented in the Facial Emotion Recognition assessment using a corneal reflection eye tracker. Eye tracking is routinely used with infants and children; it is no different from being videotaped with a camera that produces no sensation. For coding of eye gaze, four standard a priori regions of interest (the whole face, the eyes, the nose and mouth, and the forehead and cheeks) will be created on each face [28]. Data will be extracted for these regions of interest and analyzed for levels of gaze duration (total amount of time fixating on a region of interest) and fixation counts (number of gaze fixations toward a region of interest). The NimStim Set of Facial Expressions has been standardized for use with eye tracking and has been used in previous eye gaze studies in pediatric participants [29]. For participants who enroll in the trial after the COVID-19 protocol changes, the Eye Gaze Assessment will not be performed.

The Child's Sleep Habits Questionnaire (CSHQ) is the most frequently employed measure assessing the quality and type of sleep experienced by children as reported by parents/caregiver [30,31]. Thirty-three items are grouped into 8 subscales: bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night awakenings, parasomnias, sleep disordered breathing, and daytime sleepiness. To calculate subscale scores, each item is rated on a scale from 1 to 3; a higher score reflects more sleep disturbance. Owen et al., (2000) [30] demonstrated internal consistency and validity of the Child's Sleep Habits Questionnaire comparing a community sample and a clinical sample.

The Diagnostic Analysis of Nonverbal Accuracy-2 (DANVA-2) is a standardized measure that includes child vocal emotion recognition subtests. Diagnostic Analysis of Nonverbal Accuracy-2 scores are associated with subclinical characteristics of ASD in non-autistic individuals [32], and are widely utilized to evaluate emotional recognition in individuals with ASD [33-35]. The scores reflect the number of errors made in identifying the displayed emotion, with a lower score indicating stronger performance.

The Stanford Social Motivation Scale, also known as the Stanford Social Dimensional Scale (SSDS) [36] was developed by researchers from the Stanford Autism Center to enable detailed assessment of key social processes and skills that, if impaired, can impede one's ability to navigate the complexities of the social world. The Stanford Social Motivation Scale is a 71-item questionnaire measure. The first 58 items are appropriate for any child of any language level and have been factor analyzed. An additional 13 are language-based items suitable for verbal individuals. The Stanford Social Motivation Scale is a dimensional, quantitative measure designed to capture individual differences in social motivation, affiliation, social communication, and recognition, as well as unusual social approach in both normative and clinical populations.

The Parent Rated Anxiety Scale-ASD (PRAS-ASD) [37] is a 25-item, parent-reported measure of anxiety symptoms in youth with ASD (age 5-17 years). Parents rate the frequency and impairment of anxiety symptoms on a 4-point scale (0 = not

present; 1 = present sometimes, not a real problem; 2 = often present and a problem; 3 = very frequent and a major problem) during the past two weeks.

The Social Responsiveness Scale, 2nd edition (SRS-2) subscales: Five subscales have been proposed by the SRS manual and include Social Awareness, Social Cognition, Social Communication, Social Motivation, and Restricted Repetitive behaviors. Subsequent factor analysis indicated the presence of additional domains including Social Avoidance, Emotion Recognition, Interpersonal Relatedness, Insistence On Sameness, and Repetitive Mannerisms [38]. A factor analytic analysis that was based on the Research Domain Criteria (RDoC) revealed the following social domains: Attachment and Affiliation, Non-facial Communication Production, Facial Communication Production, and Understanding Mental States [39].

Biological measurements

Plasma vasopressin concentrations, as well as related biological measures, will be quantified using techniques validated for use in humans. Blood samples for measuring plasma-based biomarkers will be collected once pre-treatment and at Visits 4 and 6 (for participants in open-label period, Visit 8 as well). Blood collection will take place at the Lucile Packard Children's Hospital phlebotomy lab. The blood samples will be immediately placed on wet ice and centrifuged at 1600g for 15 mins at 4°C. The plasma layer will be aliquoted, and stored at -80°C in Dr. Parker's lab. As much as 30 mL of blood will be collected (including the blood collected for clinical chemistry measurements).

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v. Radioisotopes/radiation-producing machines

NA

vi. Physical well-being

Vasopressin and its analogs have been used for more than 30 years, in both pediatric and adult patient groups, to treat central diabetes insipidus, nocturnal enuresis, and varying states of shock with very few serious adverse effects, given that simple

precautions are adhered to (e.g., water restriction to prevent hyponatremia and water intoxication). See above for a comprehensive discussion of potential risks associated with vasopressin administration. Obtaining blood can cause bruising and hurt briefly, which applying a topical anesthetic can minimize.

vii. Psychological well-being

There are minimal risks to individuals from interviewing, testing measures, and blood draws other than loss of time at school and parental work. The risks associated with the testing measures are minimal and are limited to performance anxiety or fatigue. There will be an attempt to schedule appointments for assessments at times that minimize loss of time from the child's school schedule and caregiver's work. Parents may experience some discomfort in responding to questions asked during the initial interview or to items on the various measures. Blood collection can also cause stress and anxiety for some individuals. Trained pediatric phlebotomists, who have extensive experience working with children with ASD, will collect blood at the Lucile Packard Children's Hospital Phlebotomy Clinic.

As detailed above in 9a, there is preclinical data showing that vasopressin can induce aggression in several rodent species, under specific circumstances. Although we did not observe an increase in aggression in children enrolled in our pilot vasopressin trial, we nevertheless will monitor aggression in children in the present trial as a precaution. We will regularly inquire about aggressive behavior throughout the study.

viii. Economic well-being

Low to almost no risk

ix. Social well-being

Low to almost no risk

x. Overall evaluation of risk

Medium – therapy with chemotherapy, antibodies, or a non-FDA approved potentially toxic drug, invasive procedures such some organ biopsies or catheter procedures, and some studies using biological agents

b. International Research Risk Procedures

NA

c. Procedures to Minimize Risk

Every precaution will be taken to use the safest and most gentle procedures.

Cognitive and behavioral assessments and paper-and-pencil tests: The risk of the cognitive and behavioral tests are minimal and are limited to performance anxiety or fatigue. To minimize these problems, testing will not begin until the participant is comfortable with the testing environment and the tester. Staff experienced in working with children with disabilities will administer the tests, provide breaks as needed, and adjust the length of the test sessions to each individual. Participants will be provided with

frequent positive feedback and will receive certificates in recognition of their participation. Each examiner will be attentive to participant fatigue and provide breaks accordingly. Parents/legal guardians may experience discomfort in revealing personal information regarding psychiatric and medical status; therefore, we will provide support and assure that personal information is kept in a locked cabinet with no personal identifiers on the forms except for a participant ID number.

Scheduling during school/caregiver work: Attempts will be made to schedule appointments for research assessments at times that minimize absence of the child from school and the caregiver from work.

Blood draws: Obtaining blood may hurt briefly and cause bruising, but will be minimized by both applying a topical anesthetic and having experienced staff draw blood samples. All Lucile Packard Children's Hospital laboratory staff have considerable experience working with children. The phlebotomists are trained to work with youth of a wide age range and different neuropsychiatric disorders, including ASD.

Vasopressin side effects: As discussed above, the use of intranasal vasopressin in several research studies has been associated with limited side effects. However, in light of the young age of participants, the lack of experience with intranasal vasopressin in individuals with ASD, and the theoretical pharmacological effects, a series of protective measures will be applied to minimize risk and increase monitoring. Briefly (see above for full list):

1. Vital signs (heart rate/blood pressure, temperature) monitoring will be conducted at each visit. Participants enrolled in the trial after the COVID-19 protocol changes will have vital signs measured at screening, Visit 4, Visit 6, and Visit 8 (if applicable).
2. Height and weight will be measured at each visit. Participants enrolled in the trial after the COVID-19 protocol changes will have height and weight measured at screening, Visit 4, Visit 6, and Visit 8 (if applicable).
3. Dosage Record and Treatment Emergency Symptom Scale side effects report will be collected at each visit.
4. Overt Aggression Scale will be collected at screening and Visits 3, 4, 5, 6, 7, and 8 (if applicable).
5. EKG monitoring will be conducted at screening and Visits 4, 6, and 8 (if applicable).
6. Clinical chemistry labs will be conducted at screening and Visits 4, 6, and 8 (if applicable).
7. A study investigator will be available 24 hours a day to respond to adverse events.
8. Participants and their families will be briefed on procedures for responding to potential adverse events (see Procedures for Handling Adverse Events section below).

All safety procedures will be repeated during the optional open-label extension period.

Procedures for Handling Adverse Events:

Outpatient dosing phase: For any adverse events, families will be instructed to contact the research coordinator directly during business hours, and through the Stanford page operator after hours and during weekends/holidays. (A project coordinator will be available 24/7 to respond to safety concerns.) If the adverse event is life threatening, participants will be asked to call 911 or referred to the nearest emergency room. If the adverse event is not life threatening, the study coordinator will contact the study physician who will contact the family directly and inquire about the adverse event. If the study physician judges the side effects as minor or benign, a decision will be made whether the participant will continue in the study. If side effects are judged as severe and might be related to vasopressin, the participants will be told to stop the study drug immediately. They will be invited to the clinic within 24 hours for a direct evaluation and termination of their participation in the study. If the study physician believes that the adverse event requires immediate medical attention, parents will be instructed to contact 911 or go to the nearest emergency room. The study physician will also have the ability to break the blind if it is felt that doing so is in the best interest of the participants' health.

Any serious adverse events will be reported to the Data Safety and Monitoring Board and Stanford IRB within 24 hours and to the FDA within seven calendar days.

Data Safety and Monitoring Board: A Data and Safety Monitoring Board will be established to monitor the proposed clinical trial that will be registered with, and receive approval from, the FDA as an Investigational New Drug. (This study will also be listed on clinicaltrials.gov and on Stanford University's Clinical Trials Directory.) The Data and Safety Monitoring Board will include several physicians who are not involved in the research and will be chaired by Glen Elliott, M.D., PhD. The Data and Safety Monitoring Board will meet every three months to assess data quality and timeliness, participant recruitment, accrual and retention, participant risk-versus-benefit (including review of all adverse reactions and SAEs), and any breaches of confidentiality. Reports will be submitted to the Stanford University IRB every 3 months, as part of the annual IRB review process, as well as for the annual report. We will also submit annual reports to the FDA.

Confidentiality: All information from the evaluation and assessment of all participants are subject to standard confidentiality procedures followed at Stanford as mandated by the IRB. Information will not be released to any party, except with the written consent of participants and parents/legal guardians. Participants' identity will be concealed in data records and files by assigned ID codes. Research data will be maintained in locked storage cabinets in a locked file room. Only project staff will be allowed in this room. The screening, diagnostic, and neuropsychological tests are maintained in folders for each participant, stored in locked cabinets, in locked rooms, within a locked suite. When data are transferred to data summary sheets, only the participant number will appear on the sheet and entered alone into the database.

We will continuously monitor both our procedures and our data to ensure compliance with confidentiality guidelines. Research staff members are carefully trained concerning both the critical nature of participants' confidentiality and also the procedures for

respecting and maintaining it. They are instructed to file data immediately after collection, never to leave unlocked or opened data files unattended, never to discuss participants' behavior outside of the research study, and never to mention the name(s) of participants except to other research staff on this project. They are also instructed on the procedures for assigning ID numbers and for filing data separately from participant-identification information. All staff members complete training modules on human subjects' protection and take an examination to obtain certification.

Data and safety monitoring by PIs: Drs. Parker and Hardan will oversee all data and safety monitoring. They will hold weekly meetings with staff to discuss progress of the research study (data quality, timeliness, and participant recruitment); outcome and adverse event data and changes to the benefit-to-risk ratio that would change the design of the experiment; and issues related to confidentiality, ensuring that procedures for obtaining information in a private manner were performed and that documentation was stored in the appropriate locked file cabinets. The PIs will also hold separate monthly educational meetings to discuss any new information relevant to this study. They will be responsible for reporting any serious and unexpected adverse reactions to the IRB and Data and Safety Monitoring Board within 24 hours and also to the FDA within seven calendar days. Any identifying information collected from participants in this study will be kept strictly confidential. As mentioned above, the participants will be assigned an ID code for all data, and all information in research files will be coded using that number rather than participants' names. A name log will be kept in a locked desk in a locked office at all times. All data will be entered onto a secured database. Scientifically trained and properly authorized employees of the FDA and/or Stanford University may inspect the relevant records. We are committed to complying with FDA and IRB policies for reporting serious and unexpected adverse events.

For maximal benefit to the field, de-identified data will be made available to other investigators through the National Database for Autism Research (NDAR) and related information will be included in our consent form.

Columbia-Suicide Severity Rating Scale: Because recent evidence has linked psychotropic medications to suicidal behaviors, closely monitoring the mental well-being of patients taking these psychotropic medications is important. During Visit 1, suicidal ideation/behaviors will be assessed using the Columbia-Suicide Severity Rating Scale, and will continue to be administered throughout the trial. The Columbia-Suicide Severity Rating Scale is an interview that prospectively assesses suicidal ideation and behavior using a semi-structured interview to probe participant responses. Determining the presence of suicidal ideation and/or behavior depends on the judgment of the individual administering the scale. A trained investigator will administer the Columbia-Suicide Severity Rating Scale. Parents/caregivers will complete the interview for participants. The study physician will proactively assess participants for suicidality and mood disturbances through discussions with the participant and/or parent. Any positive responses will be further investigated, and appropriate medical treatment will be initiated. The Columbia-Suicide Severity Rating Scale will be completed at baseline, at the end of the blinded phase, and at the end of the open-label phase. For baseline, the scale will evaluate the last 12 months and for subsequent time points, it will assess any changes

from the previous visit. If the study physician determines that the participant should be monitored more closely, the scale may be added as needed throughout the study. If, at any assessment, the Suicidal Ideation (questions #1-5) score is a 4 or higher, or the patient (or parents) answers "yes" to any Suicidal Behavior item (actual attempt, interrupted attempt, aborted attempt, preparatory acts or behavior, or suicidal behavior), a mental health care professional will further assess and/or treat. Once the participant has started study medication, the decision about whether the participant should be withdrawn from study treatment will be determined by the protocol director and study physician. To note, any study investigator or research staff can refer a participant to the designated mental health professional for any concerns about suicidality, even if the participant does not meet the criteria detailed above. The participant should not be allowed to leave the study center until the results of the Columbia-Suicide Severity Rating Scale are reviewed and the participant is not considered to be at risk. If there is doubt about whether a participant is at risk, the investigator should obtain appropriate psychiatric consultation prior to releasing the participant.

d. Study Conclusion

Outpatient dosing phase: For any adverse events, families will be instructed to contact the research coordinator directly during business hours, and through the Stanford page operator after hours and during weekends/holidays. (A project coordinator will be available 24 hours a day/7 days a week to respond to safety concerns.) If the adverse event is life threatening, participants will be told to call 911 or referred to the nearest emergency room. If the adverse event is not life threatening, the study coordinator will contact the study physician who will contact the family directly and inquire about the adverse event. If the study physician judges the side effects as minor or benign, a decision will be made whether the participant will continue in the study. If side effects are judged as severe and might be related to vasopressin, the participants will be told to stop the study drug immediately. They will be asked to come to the clinic within 24 hours for a direct evaluation and termination of their participation in the study. If the study physician ascertains that the adverse event requires immediate medical attention, parents will be instructed to contact 911 or go to the nearest emergency room. The study physician will also have the ability to break the blind if it is felt that doing so is in the best interest of the participants' health.

Any serious adverse events will be reported to the Data and Safety Monitoring Board and Stanford IRB within 24 hours, and to the FDA within seven calendar days.

e. Data Safety Monitoring Plan

i. Data and/or events subject to review

1. Vital signs (Heart Rate/Blood Pressure, temperature) monitoring will be conducted at each visit.
2. Height and weight will be measured at each visit.

3. Dosage Record and Treatment Emergency Symptom Scale side effects report will be conducted at each visit.
4. Overt Aggression Scale will be measured at screening and Visits 3, 4, 5, and 6.
5. EKG monitoring will be conducted at screening and Visits 4 and 6
6. Clinical chemistry labs will be conducted at screening and Visits 4 and 6.

All safety assessments outlined above will be repeated during the optional open-label extension period.

ii. Person(s) responsible for Data and Safety Monitoring

A Data and Safety Monitoring Board will be established to monitor the proposed clinical trial that will be registered with, and will receive approval from, the FDA as an Investigational New Drug. (This study will also be listed on clinicaltrials.gov and on Stanford University's Clinical Trials Directory.) The Data and Safety Monitoring Board will include several physicians who are not involved in the research investigations and will be chaired by Glen Elliott, M.D., PhD. The Data and Safety Monitoring Board will meet every three months to assess data quality and timeliness, participant recruitment, accrual and retention, participant risk-versus-benefit (including review of all adverse reactions and Serious Adverse Events [Serious Adverse Events]), and any breaches of confidentiality. Reports will be submitted to the Stanford University IRB every six months, as part of the annual IRB review process, as well as for the annual report. We will also submit annual reports to the FDA.

iii. Frequency of Data and Safety Monitoring Board meetings

The Data and Safety Monitoring Board will meet every three months to assess data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit (including review of all adverse reactions and Serious Adverse Events), and any breaches of confidentiality. Reports will be submitted to the Stanford University IRB every six months, as part of the annual IRB review process, as well as for the annual report. We will also submit an annual report to the FDA.

iv. Specific triggers or stopping rules

The study will end if a serious adverse event is related to the study medication resulting in hospitalization or emergency room visit or death. A serious adverse reaction is an untoward medical occurrence that at any dose: results in death; is life-threatening; requires inpatient hospitalization; results in persistent or significant disability/incapacity; or is an otherwise important medical event. An important medical event may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient/participant or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes. Cancer/Overdose: All cases of cancer and overdose will be reported immediately.

v. DSMB Reporting

Any serious adverse events will be reported to the Data and Safety Monitoring Board and Stanford IRB within 24 hours and to the FDA within seven calendar days.

vi. Will the Protocol Director be the only monitoring entity? (Y/N)

N

vii. Will a board, committee, or safety monitor be responsible for study monitoring? (Y/N)

Y

f. Risks to Special Populations

Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects. The research presents more than minimal risk to children, but holds out the prospect of direct benefit for the individual subject or is likely to contribute to the subject's well-being. Please provide rationale that: (a) the risk is justified by the anticipated benefit to the subjects; (b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches; and (c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians.

Rationale for category selected above:
a) ASD is a pervasive developmental disorder characterized by core deficits in social behavior and communication, and the presence of repetitive/stereotyped behaviors. Recent prevalence rates estimate that 1 in 68 children in the US meet criteria for ASD. Good evidence now exists for the potential of vasopressin treatment to improve social functioning and relieve other symptoms in patients with ASD. Importantly, in the present study vasopressin treatment occurs during childhood when the brain is most plastic, and when pharmacotherapies may be maximally beneficial.

b) Although several medications are approved for ASD (i.e., the antipsychotics risperidone and aripiprazole), none are effective in ameliorating the characteristic social deficits and often result in unfavorable side effects (e.g., lethargy and weight gain). No effective pharmacological or behavioral treatments are currently available for the core social deficits exhibited in people with ASD. Vasopressin is overall a very well tolerated agent with minimal risks if simple precautions are adhered to (e.g., water restriction to prevent hyponatremia and water intoxication).

c) Parents of participants will be consented in a private room by a trained investigator. All participants who are older than 7 years of age, and also capable of understanding the consenting process, will be assented by a trained investigator, in a private room, in the presence of the parent. Both parents of participants and the participants themselves will have ample opportunity to ask questions and to discuss the risks and potential benefits with Dr. Hardan and the other investigators.

10. BENEFITS

As of now, no effective intervention strategy is aimed at targeting the core symptoms of ASD and curbing the long course and guarded prognosis of ASD. Outcome studies indicate that about 2/3 of autistic adults remain severely handicapped and live in complete dependence or semi-

dependence. Available agents do aim at controlling associated behavioral features such as aggression, agitation and compulsive behaviors. Agents that treat underlying chronic neurobiological abnormalities are lacking. The suggested innovative agent (vasopressin), if shown to be effective, will lead to the development of new strategies to treat individuals with ASD.

11. PRIVACY AND CONFIDENTIALITY

All participant information and specimens are handled in compliance with the Health Insurance Portability and Accountability Act (HIPAA) and privacy policies of Stanford University, Stanford Health Care, and Stanford Children's Health.