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| Official Title: | Sodium Nitroprusside in Early Course Schizophrenia |
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# PART B
# STUDY DESCRIPTION

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| **Title of Protocol** | Proof of Mechanism Study using a Retinal Biomarker to Predict Treatment Response with Intravenous Sodium Nitroprusside in Symptomatic Early Course Schizophrenia |
| Principal Investigator | Paulo Lizano |

## B1. PURPOSE OF PROTOCOL

1. The main purpose of this proposal is to determine the effectiveness of a single intravenous administration of sodium nitroprusside (SNP) (0.5 mcg/kg/min for 4 hours) on positive and negative symptoms in symptomatic Early Course Schizophrenia (ECS).
2. To examine the effect of SNP on cognition and retinal imaging phenotypes in ECS, and whether baseline retinal microvasculature or inflammation (CRP levels) correlate with a change in psychosis symptoms or cognition.

We hypothesize that SNP will improve psychosis symptoms and cognition in symptomatic ECS and that this change can be predicted by retinal vessel morphology or CRP levels. We predict that SNP will improve oxygen supply and reduce inflammation, which will be evidenced by reductions in retinal vessel phenotypes.

## B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

**Background:** Despite decades of research, little clarity exists regarding the pathogenic mechanisms of schizophrenia (SZ). Since the microvascular environment is the chief interface for systemic factors influencing the brain[1], it is a logical focus for understanding SZ neurobiology. Altered microvasculature is central to the pathophysiology of many CNS disorders[1], but it has received less attention in SZ[2]. However, our understanding of the immunological underpinnings of SZ, and enhanced methodologies for detecting microvascular impairment, have led to increased research in this area[2]. We showed that inflammatory subtypes exist in psychosis and that an increased pattern of peripheral inflammation (including C-Reactive Protein, CRP) displayed worse overall cognition and elevated subcortical and neocortical gray matter thickening compared to those with low inflammation-mediated microvascular injury[3]. Since retinal and cerebral microvessels share embryological origins and are homologous in structure/function, the former can be used to gauge the condition of cerebral microvessels[4], including the detrimental effects of increased peripheral inflammation on retinal microvascular changes in psychiatric disorders[5]. Retinal arteriolar and venular diameter are commonly studied in relation to cerebrovascular disease and advances in retinal imaging and image analysis now allow for the accurate quantitative assessment of the condition of retinal microvessels, including diameter, length, density, and capillary network[6].

In a population-based birth cohort study using fundus imaging to assess retinal arteriolar and venular diameter, Meier et al, (2013) found that individuals who later developed SZ had significantly wider retinal venules compared to other medical groups, and that wider venules were associated with adult and childhood symptoms of psychosis[7]. Meier's findings were replicated in a longitudinal twin eye study where individuals with >1 symptom of psychosis had wider retinal venules than controls, and unaffected co-twins had intermediate values[4]. These findings support the view that retinal venular diameter could be used as a prognostic and/or endophenotypic biomarker that can bridge the gap between underlying neurobiological mechanisms and phenotypic expression of psychosis[6,8,9]. In a patient with 22q11 deletion syndrome, a genetic disorder associated with a 30x

increase SZ risk, ocular examination identified bilateral retinal vascular tortuosity[10]. In a cross-sectional study, Appaji et al, (2019) demonstrated that patients with SZ had significantly increased retinal venules[11], tortuosity[12], capillary network[13], and vessel trajectory[14] compared to controls, with retinal vessel diameter being associated with cognitive deficits in SZ[15]. While these findings have laid the foundation for understanding retinal pathology in SZ, there are several limitations associated with fundus imaging, including reduced image resolution and absence of retinal depth determination. Advances in retinal imaging, such as swept-source optical coherence tomography angiography (SS-OCTA), provide greater microvascular clarity to visualize the retina non-invasively, in a more detailed, quicker, and cost-effective manner. In a pilot study using SS-OCTA, we identified greater vessel length (SD), density (VD), capillary network (FD), and diameter (VDI) in the superficial layer of patients with chronic psychosis compared to HC[5].

The pathophysiological mechanisms underlying these retinal microvascular changes are not entirely understood, but they have been associated with inflammation (including CRP), endothelial dysfunction, reactive oxygen species and hypoxia/ischemia, which have also been consistently observed in SZ. While it is currently unclear whether venular diameter plays a causal role in the development of SZ, it has been hypothesized that smaller retinal arterioles and wider venules may reflect cumulative structural damage to the microvasculature from inflammation/endothelial dysfunction and indicate problems with the oxygen supply to the brain.

Nitric oxide (NO) signaling is a potential mechanism for protecting the microvasculature against oxidative stress, inflammation and endothelial dysfunction[16]. NO-donors (NOD) can protect nerve tissue from injury and preserve the integrity of the blood brain barrier, suggesting that cerebral vascular endothelium is essential in the control of vascular inflammatory and oxidative responses, leukocyte migration, and the production of inflammatory mediators[1]. In addition to reducing oxidative stress and inflammation, NOD can increase cerebral and retinal blood flow[16]. In healthy volunteers, SNP dose dependently increases retinal arteriolar/venular diameter, and leukocyte flow[17]. However, in pathologic states such as ischemia, SNP can enhance neuroprotection by increasing arteriolar diameter and reducing venular leukocyte adhesion (i.e., reduced venular diameter), which together facilitate blood flow[16]. Preclinical and clinical evidence have shown that SNP may have an antipsychotic profile. In preclinical studies, SNP has been used to prevent SZ-related behaviors in a spontaneously hypertensive rat model[18]. Five clinical studies to date have examined the effects of SNP on psychosis symptom improvement with mixed results. In one study, a single infusion of SNP resulted in an immediate improvement in overall psychosis and negative symptoms in an antipsychotic medicated ECS group with <5 years illness duration, an effect that persisted for 4 weeks[19]. However, four studies performed in chronic SZ did not find any evidence of symptomatic improvements, with two of these studies providing repeated infusions of SNP[20–23]. There were several limitations with these negative studies: older patient population, longer durations of illness (8-17 years), and the absence of a treatment biomarkers. These studies neglected to consider an alternative mechanism of action for SNP, which would include the protection against inflammatory mediated microvascular dysfunction. Another important factor to consider is that while ~70 randomized control trials to date have examined adjunctive anti-inflammatory drugs on psychosis symptoms or cognition in SZ[24], six of these have performed post hoc analyses of participants with elevated levels of individual inflammatory markers (such as CRP), and their findings strongly suggest that ECS individuals with elevated peripheral inflammation have the greatest benefit with anti-inflammatory drugs[24]. Thus, it is conceivable that treatment effects could be stronger in participants with elevated markers of inflammation or disruptions in related pathways. As a result, in order to SNP to be effective it should be administered to patients with ECS. Thus, further work is needed to determine whether the effect of SNP treatment is dependent on a patient's illness duration and whether a retinal biomarker for microvascular dysfunction/inflammation can predict treatment response to SNP.

There is a growing literature on how increased peripheral inflammation and microvascular dysfunction are central to the pathophysiology of SZ. Advances in retinal imaging

now allow for the accurate quantitative assessment of the condition of retinal microvessels, and early studies implicate microvascular dysfunction in SZ, but the specific pathophysiological mechanisms underlying greater length, density, capillary network, and diameter are not yet entirely understood. A potential target is NO signaling, which is involved in vessel protection from oxidative stress, inflammation and endothelial dysfunction, and treatment with NOD have been shown to reduce oxidative stress/inflammation and to increase cerebral blood flow in cerebrovascular disorders. Anti-inflammatory drug trials in SZ suggest that ECS individuals with elevated peripheral inflammation show the greatest benefit to adjunctive anti-inflammatory treatments. Also, there is a growing interest in the use of SNP in SZ and studies to date have been mixed. Hallak et al (2013) demonstrated that a single infusion of SNP in patients with ECS was both safe and associated with immediate and longer-term clinical outcome. While three other studies demonstrated that SNP was well-tolerated in patient with multi-episode SZ, they were not able to replicate Hallak's finding, which was likely due to the disease heterogeneity, the inclusion of an older population with a longer duration or multi-episodes of illness, and the lack of treatment biomarkers. Thus, the principal goal in the field of SZ is to identify biomarker-based targets for early intervention, evidence of engaging this target by selective interventions and assessing therapeutic efficacy[25].

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## B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

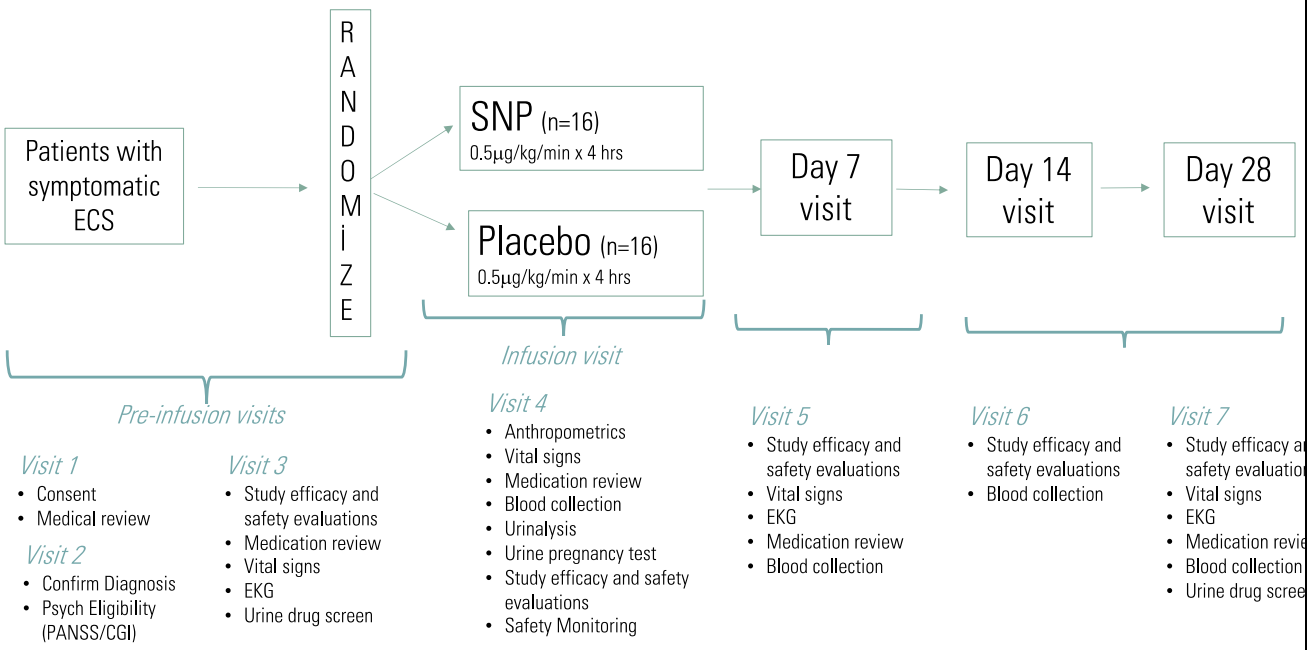
**Study Design**

This is a 4-week, single-center, double-blind, randomized, parallel-group, placebo-controlled pilot trial of SNP in symptomatic ECS patients.

Participants who meet eligibility criteria and are taking antipsychotics for 8 weeks with a stable dose of 4 weeks, will be randomized using a block design to either the SNP or placebo arm. A randomization identifier taking into account gender and race will be generated for each participant and this identifier will be accessible to the site pharmacy to prepare the corresponding infusion treatment.

**Follow up visits (1st week, 2nd week, 4th week)**

Patients will be observed for 28 days. All participants should complete their follow-up study visits, including efficacy assessments, safety assessments, vital sign assessments, and blood collection.




**Clinical evaluation:** A detailed medical history will be obtained by the PI (Dr. Lizano) or Co-I (Dr. Berg). This will include information regarding the subjects' full history of medical and psychiatric conditions, diagnoses, procedures, treatments, demographic information, and any other noteworthy medical information, including suicidality, with dates of start and finish. This information will be collected either in person or virtually. Any updates to medical history information that the PI or designee becomes aware of will be captured throughout the study. The PI's will perform the physical examinations (PEs) as indicated in the Schedule of Events (SOE). A complete PE, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, chest/lungs, heart, abdomen, lymph nodes, extremities, and general appearance. The physical examination will not include a pelvic, breast, or rectal examination. A neurological examination will also be performed, which includes mental status, cranial nerves, strength, sensation, deep tendon reflexes, and coordination.

**Vital Signs**: Evaluation of vital signs will be performed by qualified site personnel after the subject has been supine for 5 minutes and will include a measurement of systolic and diastolic blood pressure, pulse rate, oral temperature, and respiratory rate. Systolic and diastolic blood pressure should be then measured from supine to standing to assess orthostatic hypotension. Subjects will be asked about lightheaded or dizzy, blurry vision, weakness, syncope, confusion, or nausea why performing orthostatic vital signs. Vital sign measurements will be obtained at the time points indicated in the study flow chart and following the supine ECG assessments, if taken at the same time. Blood pressure should be taken on the same arm throughout the study.

**Anthropometric Measurements:** Weight (kg) (assessed in ordinary indoor clothing with shoes off) will be recorded at the time points indicated in the flow chart. Height (cm) will be recorded at Visit 1 (screening) only. Body Mass Index (BMI) will be calculated at all visits in which anthropometrics are collected. Waist circumference will be measured to the nearest 0.1 cm.

**Laboratory Procedures:** Screening blood work will be fasting, and blood work taken as part of the study treatment monitoring process will be non-fasting as a precautionary measure to reduce the risk of a hypotensive episode.

Routine laboratory panels will include (*see Section C below for exclusionary laboratory criteria*):

- Hematology (absolute and percentage): White blood cell (WBC) count with differential (absolute neutrophil count, lymphocytes, monocytes, basophils, and eosinophils), red blood cell (RBC) count, hemoglobin (Hgb), hematocrit (Hct), and platelet count.
- Comprehensive Metabolic Panel: Glucose, sodium, potassium, calcium, chloride, carbon dioxide, blood urea nitrogen (BUN), creatinine, calculated glomerular filtration rate, uric acid, phosphorus, magnesium, total protein, albumin, lipid panel (triglycerides, total cholesterol, HDL-cholesterol, calculated LDL-cholesterol) aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total bilirubin, creatine phosphokinase (CPK), creatine kinase (CK) thyroid stimulating hormone (TSH) and uric Acid, and C-Reactive Protein (CRP).
- Serum Pregnancy: Serum beta human chorionic gonadotropin (Beta- hCG).
- Urinalysis: color, appearance, specific gravity, pH, ketones, protein, glucose, nitrite, leukocyte esterase, and occult blood; microscopic examination of sediment will be performed only if the results of the urinalysis evaluation are positive (microscopic examination may include but is not limited to WBC count, red blood cell (RBC) count, casts, and crystals).
- Urine drug screen: cannabis, opioids, cocaine, amphetamines, methadone, cannabinoids, barbiturates, benzodiazepines, methamphetamine, and phencyclidine.
- Urine Pregnancy Test: urine pregnancy test will be performed in female subjects of childbearing potential using a dipstick urine test.

**12-Lead Electrocardiogram (ECG)**: A 12-lead ECG will be taken at following a supine rest for 5 minutes. ECG parameters including the QT interval, Bazett's corrected QT interval (QTcB), and QTcF Fridericia's corrected QT interval (QTcF), pulse rate (PR), and QRS intervals, and heart rate will be recorded. The ECGs will be reviewed by the Co-I to assess any immediate abnormalities.

**Efficacy Measures:** Symptoms will be assessed with the Positive and Negative Syndrome Scale and antipsychotic use with chlorpromazine (CPZ) equivalents. Global severity of illness will be assessed using the Clinician Global Impression severity scale (CGI-S). Clinical information will be collected using electronic forms when possible and they will be de-identified and maintained on Beth Israel Deaconess Medical Center (BIDMC) servers. IQ will be estimated with the Wechsler Abbreviated Scale of Intelligence. Cognition will be assessed with the Brief Assessment of Cognition in Schizophrenia (BACS). BACS is available electronically and can be performed on a tablet to assess cognition and a participant's information will remain de-identified on BIDMC servers. Social functioning will be assessed with the global and social functioning scales, and nicotine dependence with the Fagerstrom Test for Nicotine Dependence.

**Safety Measures:**
The Systematic Assessment for Treatment Emergent Effects–Systematic Inquiry (SAFTEE-SI) is a self-rated questionnaire assessing possible adverse events during the course of the trial. The time frame is the past 7 days. The abnormal Involuntary Movement Scale (AIMS) is a 12-item scoring tool that is commonly used to monitor the occurrence and severity of tardive dyskinesia for patients receiving neuroleptic medications. In this study, the highest severity observed for each item on the AIMS scoring sheet will be recorded. For movements that occur upon activation, one severity grade less than the spontaneously observed movement will be selected for the item. The Columbia Suicide Severity Rating Scale (C-SSRS) is a low-burden measure of the spectrum of suicidal ideation and behavior that was developed in the National Institute of Mental Health Treatment of Adolescent Suicide Attempters Study to assess severity and track suicidal events through any treatment. It is a clinical interview providing a summary of both

ideation and behavior that can be administered during any evaluation or risk assessment to identify the level and type of suicidality present. The C-SSRS can also be used during treatment to monitor for clinical worsening. The C-SSRS will be performed to assess suicidal ideation and behavior.

**SS-OCTA:** Images will be obtained using the Zeiss PLEX Elite 9000, which is a quick, inexpensive, and non-invasive method for acquiring retinal images with an axial resolution of ~5μm and a scan depth of 3.0mm. Automated layer segmentation provides four horizontal depth-resolved retinal slabs: superficial (SRL), deep (DRL), outer retinal layer (ORL) and the choriocapillaris. Participant images will be excluded for low signal strength (≤6), blink artifacts, doubling artifacts, media opacity obscuring visualization of the vasculature, and/or segmentation errors. Manual segmentation editing will be conducted to ensure proper layer assessment. Microvessels for the SRL, DRL, and choriocapillaris slab will be processed using a custom semiautomated algorithm we developed to rapidly quantify several vessel parameters including vessel density, length, diameter, and capillary network. All images will be independently analyzed and compared among study subjects by two graders blinded to the group assignment.

**Outcome:** Primary outcomes include the change in PANSS positive and negative scores (2hrs, 5hrs, 1wk, 2wk, and 4wks) in the SNP group compared to placebo. The secondary outcomes include the change in overall cognition (0hr, 1wk, 2wk, and 4wks) and retinal microvascular measures (0hr, 5hrs), as well as examining the relationship between baseline or change in retinal vessel measures or CRP and the change in psychosis symptoms and cognition after SNP treatment compared to placebo.

**SNP Treatment Protocol**

The BIDMC ***IV DRUG ADMINISTRATION GUIDELINE*** for clinical use of **Nitroprusside Sodium (Nipride®)** recommends use of this drug in the ICU setting**.** We are proposing the use of intravenous (IV) SNP for a research study in stable (not hospitalized) patients with early course schizophrenia (Clinical Trials.gov registry: NCT04986072; IND Exemption approved on 8/31/2021). Per our discussions with Critical Care leadership (Drs. Molly Hayes and Michael Cocchi), there are limited intensive care unit (ICU) beds available for research subjects who aren't already admitted to the ICU. It is important to note that five prior clinical trials of SNP in similar populations have also been conducted in non-ICU settings with no adverse effects reported[1–5]. For these reasons, we are proposing that the SNP study in early course schizophrenia take place in the Clinical Research Center (CRC) at BIDMC. We have received approval from the Critical Care Executive and Critical Care Practice Committees for execution of the protocol in the CRC at BIDMC (**please see attached approval letters**).

**Paulo Lizano, MD, PhD** (Neuropsychiatrist, clinical investigator, and Assistant Professor of Psychiatry at BIDMC/HMS) is the PI for this study. Dr. Lizano will conduct efficacy and safety assessments, perform medical reviews, recommend medical clearance for study inclusion, and conduct assessments prior to discharge. Dr. Lizano will be available at the CRC through the study visit.

**Katherine Berg MD,** (Pulmonary and Critical Care Physician, clinical investigator, and Assistant Professor of Medicine at BIDMC/HMS) is the co-investigator, has prior experience with administering SNP, and will be the physician of record for the study. Dr. Berg will provide oversight of medical clearance, ordering the study drug, and responding to clinical issues during the study visit. Dr. Berg will be available before, during, and after the SNP infusion. Specifically, Dr. Berg will be present in the CRC 30 minutes prior to SNP infusion and 30 minutes after the initiation of SNP. Dr. Berg will be available on the East Campus of BIDMC after stable titration of SNP, in close to proximity to the CRC.

The **BIDMC CRC** is prepared to support this study and has the appropriate resources to conduct the study, including 1:1 ACLS/BLS certified nursing. CRC nursing staff involved with the study will

undergo training by critical care nursing to be able to safely administer nitroprusside in the CRC. The CRC is equipped with an Omnicell and can be stocked with rescue medications needed for the study. The CRC has the capability to conduct continuous monitoring of heart rate, blood pressure, pulse oximetry and telemetry (**see attached support letter from the CRC**).

The CRC and the PI and co-investigators are prepared to ensure that this study protocol is conducted safely outside of an ICU setting, and an outline of the protocol, including procedures for study drug administration and monitoring, is as follows:

**Patient Selection**

Medically Relevant Exclusion Criteria:

- Presence of renal, hepatic, cardiac, hematologic, neurologic (seizures or elevated intracranial pressure) impairments.
- Pregnant or breastfeeding. Women of childbearing potential must have a negative pregnancy test performed at the screening visit prior to the baseline assessment and randomization.
- Physical exam suggestive of an underlying disease state that may, in the opinion of the investigators, confound the results of study, increase risk to the subject or lead to difficulty complying with the protocol.
- Baseline systolic BP <100mmHg or symptomatic orthostatic hypotension (systolic BP <90 mmHg or diastolic BP <60 mmHg with any of the following: lightheaded or dizzy, blurry vision, weakness, syncope, confusion, nausea).
- Abnormal laboratory testing (see appendix)
- Medications that interfere with the metabolism or excretion of nitroprusside or are associated with drug-drug interactions (see appendix below)
- Medications that could pose a significant risk to the participants' health
- Hypersensitivity to SNP
- Currently taking clozapine or chlorpromazine
- Subjects with a current (within the last 3 months) DSM-V diagnosis of alcohol or substance use disorder or dependence (excluding nicotine) at the screening visit
- Has tested positive for any of the following: cannabis, opioids, cocaine, amphetamines, barbiturates methadone, methamphetamine and phencyclidine at the screening or baseline visits.
- Subjects at imminent risk of suicide or injury to self or others or history of significant suicide attempt within the last 12 months.

**Screening Pre-infusion Visit**

Visits will consist of informed consent signing, inclusion/exclusion criteria review, medical record review, and psychiatric diagnostic assessments. Some of these assessments will be performed virtually. Medical clearance will also be performed and includes a review of concomitant medications, urine pregnancy and drug testing, blood collection for laboratory testing, electrocardiogram, and vital sign monitoring. Safety measures will also be performed, and they include the SAFTEE-SI, AIMS, and C-SSR.

**Protocol for Nitroprusside Infusion**

*Protocol created with reference to BIDMCs Nitroprusside IV Drug Administration Guideline (Approved by Critical Care Practice, Critical Care Exec, Pharmacy and Therapeutics Committee on December 2005 and revised in February 2019) with amendments from the CRC and Dr. Berg, as well as from the Critical Care Practice and Critical Care Executive Committees.*

**Scheduling:**

- Participants will be scheduled for the single SNP infusion when CRC staff and PIs (Dr. Berg and Dr. Lizano) are all available on site

- We will follow BIDMC guidelines, as well as our study staff and CRC recommendations for decision on pausing the study due to COVID19 restrictions

**Infusion Monitoring:**

- Pre-infusion orders
  - Semi-recumbent position, IV-line placement, Volumetric pump with a carrier line
  - nicotine patch [if appropriate, see cigarettes per day (cpd) break down below]
    - if 15 cpd: 21 mg patch
    - if 8-15 cpd: 14 mg patch
    - if 5-8 cpd: 7 mg patch
    - if <5 cpd: PRN nicotine gum or lozenges 2 mg (1 piece every 3-4 hours)
- 12-lead EKG
  - Pre-infusion (60 mins before)
  - During (30 mins after start of infusion)
  - Post-infusion (60 after end of infusion)
- VS (BP, HR, O2 sat)
  - Pre-infusion (baseline)
  - During
    - Phase I (q5 Xs 10 mins)
    - Phase II (q5min Xs 10 mins, then q10min Xs 40 mins, then q20min Xs 3 hours)
  - Post-infusion (q20mins Xs 60 mins)
- Study Investigators
  - Co-I: Dr. Berg will be present 30 minutes prior to SNP infusion
  - PI: Dr. Lizano will be present during the entire study visit

**Phase I Infusion Protocol**

- Co-I (Dr. Berg) will be present during Phase I (10 mins)
- A 10-minute run-in period at 0.25 mcg/kg/min using a carrier line and infusion pump
- If SBP ≤ 89 and/or if SBP decreased by >20% PAUSE infusion. Lie flat, feet up, call PIs
- If HR > 120 bpm and/or >20 bpm over baseline HR, PAUSE infusion. Lie flat, feet up, call PIs Continue to check BP.
- If SpO2 <92%, PAUSE infusion. Lie flat, feet up, call PIs
- If patient reports headache, ask: “do you feel this headache is tolerable?”
  - If patient says “yes,” continue dose
  - If patient says “no,” PAUSE infusion. Call PIs
- If patient experiences symptomatic hypotension (nausea, dizziness) PAUSE infusion. Call PIs
- If Phase I is tolerated per the above guidelines after the 10-minute run-in period, then start Phase II

**Phase II Infusion Protocol**

- Co-I (Dr. Berg) will be present for the first 20 mins of Phase II to ensure safe up titration of SNP
- Increase to max dose of 0.5 mcg/kg/min with the following parameters
- If SBP between 81 and 89 and/or if SBP decreased by >20%, lower dose to Phase I dose for 15 min. Monitor with CRC Nurse.
  - If SBP rises to >89mm Hg continue Phase I dose
  - If SBP remains between 81 and 89mm Hg PAUSE and call PIs
  - If SBP <80, PAUSE infusion, lie flat, feet up & call PIs

- If HR >120 bpm and/or >20 bpm over baseline HR, lower dose to Phase I dose for 15 min. Continue to check BP
- If SpO2 < 92% PAUSE infusion and call PIs
- If patient reports headache, ask, "Do you feel this headache is tolerable?"
    - If patient says "yes", continue dose
    - If patient says "no", *lower dose to Phase I dose for 15 min*
        - If better, continue Phase I dose
        - If still intolerable, PAUSE infusion. Lie flat, feet up, call PIs
- If patient experiences symptomatic hypotension (nausea, dizziness) lower dose to Phase I dose for 15 min
    - If better, continue Phase I dose
    - If not, PAUSE infusion and call PIs

**PRN Rescue Medications**

- In consultation with CRC Nurse and study PIs
- Omnicell automated medication dispensing cabinet system available for dispensing rescue medication
- For SBP <80 mmHg, may give 250 ml 0.9% NS IV bolus via Sigma pump @999 ml/hr over 15 min X 2
- For rash may give Diphenhydramine 25 mg by mouth X 1
- The need for additional potential rescue medications to be available in the Omnicell will be discussed between study staff and pharmacy

**Infusion Considerations**

- IV line will be left in place for at least 1 hour.
- If additional IV medication is necessary, Dr. Berg and the CRC nurse will start the infusion and a medical team will be present during the entire infusion period.
- A study PI or Co-I will release the subject at the conclusion of the visit.
- A trained RA, research nurse, and study PI (Dr. Lizano) will be present during the entire infusion and monitoring period.
- The psychiatry research staff member will administer rating scales.
- All side effects and vital parameters will be recorded during the infusion and 2 hours following the infusion by CRC nursing.
- Any abnormal, clinically significant vital signs will be recorded as adverse events.

**Discharge Procedure**

- Gross motor and cognitive function, ataxia, reflexes/clonus will be evaluated and documented by a study PI (Dr. Lizano) prior to discharge.
- Before being discharged patients should be awake and alert, feeling well, with no nausea, vertigo, or dizziness.
- There should be no significant change in vital signs from prior to infusion.
- They will be given a small meal before discharge from the CRC.
- Patients will be instructed not to drive. If necessary, the study nurse will release them to the care of an adult family member or caregiver, who will accompany the patient home. If needed, a trained Research Assistant will contact the participants support system to ensure transportation is coordinated.

**Study Drug Handling**

- Nitroprusside will be compounded by research pharmacy in 5% dextrose
- 5% dextrose will be used as placebo
- Study drug will be stored at room temperature and the required amount will be released from the pharmacy to the CRC at the time a patient is scheduled for the protocol.
- Study drug will be provided in light-blocked bags with labeling to ensure blinding of study staff.
  Nitroprusside standard concentration is 50mg/250mL of D5W and the concentrated version is 100mg/250mL.

**Institutional Approvals**

Critical Care Executive – Todd Sarge and Jane Foley – Approval granted on 9/8/21
Critical Care Practice – Joanna Anderson and John Whitlock – Approval granted on 9/8/21

### B. Statistical Considerations

**Power calculation:** With 16 ECS individuals in the SNP group and 16 in the placebo group we will have >80% power to detect a 10-point PANSS difference between the placebo and treatment groups, with a SD of 6, alpha of 0.05, 2-sided alternative hypothesis, 2-sample t-test, based on the sample size suggested by the retinal vessel differences in our pilot data. The above-mentioned sample size was determined based on our pilot SS-OCTA data, which suggests that we will have >70% power to detect group differences of at least 0.007 (estimated standard deviations of skeletal density =0.008, significance level=0.05, two-sided t-tests).

Baseline demographic details will be compared between the SNP and placebo groups using independent-samples t tests or Mann-Whitney U tests where appropriate using R software. Categorical data will be compared using chi-square tests. Effect sizes will be provided using Cohen's d. The primary outcome (PANSS) will be analyzed using a 2 (treatment group) × 5 (time point) mixed analysis of variance (ANOVA). Analyses of secondary outcome measures will be compared using a 2-group x 4 time point mixed ANOVA for cognition and a 2 x 2 for retinal measures. The relationship between baseline or change in retinal vessel measures or CRP and change in PANSS scores or cognition will be performed using Pearson's (parametric) or Spearman's (non-parametric) correlation.

### C. Subject Selection

**Population**: Men and women, 18-60 years of age, from diverse background, symptomatic, with early course schizophrenia, and meet all study inclusion and exclusion criteria.

**Inclusion criteria** for ECS patients are: a DSM-V diagnosis of SZ or schizoaffective disorder with ≤5 years from the onset of psychosis and up to 2 years of lifetime exposure to antipsychotics, total score of ≥55 on the PANSS with a score of ≥3 on 1 or more PANSS Positive Scale items (delusions, conceptual disorganization, hallucinatory behavior, suspiciousness, or unusual thought content) and/or score of ≥3 on the CGI-S scale, taking antipsychotic for 8 weeks with a stable dose of 4 weeks (no medication change will be allowed during the trial), English proficiency, understands and is able, competent and willing to give informed consent in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines.

**Exclusion criteria are**:

- Presence of renal, hepatic, cardiac, hematologic, neurologic impairments or abnormal physical exam.
- Presence of retinal disease (myopia >4.0 diopters)

- Baseline systolic BP <100mmHg or symptomatic orthostatic hypotension (systolic BP <90 mmHg or diastolic BP <60 mmHg) with any of the following: lightheaded or dizzy, blurry vision, weakness, syncope, confusion, nausea.
- Pregnant or breastfeeding (pregnancy test at screening visit and prior to randomization).
- Abnormal laboratory testing (see appendix)
- Treatment with medications that may interfere with the metabolism or excretion of SNP or are associated with drug-drug interactions (**see list of medications below**).
- Prior history of intolerance to SNP
- Currently taking clozapine or chlorpromazine
- Treatment with anti-inflammatory drugs, hormones or immunosuppressant agents in the 6 months before study entry
- Subjects with a current DSM-V diagnosis of alcohol or substance use disorder or dependence (excluding nicotine)
- Urine drug screen tested positive for any of the following: cannabis, opioids, cocaine, amphetamines, barbiturates methadone, methamphetamine and phencyclidine at the screening or baseline visits
- Subjects at imminent risk of suicide or injury to self or others or history of significant suicide attempt within the last 12 months as measured by the C-SSRS together with the opinion of the trial investigator.
- Subjects that have taken an investigational drug or taken part in a clinical trial within 30 days prior to screening.
- Participation for any reason would compromise patient safety or integrity of the study in the opinion of the investigator.

**Exclusionary Safety Values of Potential Clinical Concern**

| Complete Blood Counts | |
|---|---|
| Leukocytes | <2 or >17.5 x $10^3$/$mm^3$ |
| Platelets | <75 or >700 x $10^3$/$mm^3$ |

| Comprehensive Metabolic Panel | |
|---|---|
| Sodium | <1.1 times the lower limit or >1.1 times upper limit of the reference range |
| Potassium | <1.1 times the lower limit or >1.1 times upper limit of the reference range |
| Chloride | <1.1 times the lower limit or >1.1 times upper limit of the reference range |
| Glucose | >2 times the limits of the reference range |
| Blood Urea Nitrogen (BUN) | >1.3 times upper limit of the reference range |
| Creatinine | >1.3 times upper limit of the reference range |
| Calculated Glomerular Filtration | <60 |
| Total bilirubin | >2 times the upper limit of the reference range |
| Aspartate amino transferase (AST) | >3 times upper limit of the reference range |
| Alanine amino transferase (ALT) | >3 times upper limit of the reference range |

| | |
|---|---|
| Alkaline phosphatase (ALP) | >3 times upper limit of the reference range |
| Creatine phosphokinase (CPK) | >3 times upper limit of the reference range |
| Creatine kinase (CK) | >3 times upper limit of the reference range |
| Thyroid Stimulating Hormone (TSH) | >3 times upper limit of the reference range |
| Uric acid | >3 times upper limit of the reference range |
| Lipid panel (triglycerides, total cholesterol (C), HDL-C, calculated LDL-C) | >3 times the upper limit of the reference range |
| Serum beta human chorionic gonadotropin (Beta- hCG) | < 6 IU/L negative |

**Drug-Drug Interactions:**

-Risk X: Avoid combination
Bromperidol
Phosphodiesterase 5 Inhibitors
Riociguat

-Risk D: Consider therapy modification
Amifostine
Obinutuzumab

-Risk C: Monitor therapy

Alfuzosin
Amphetamines
Clozapine and Chlorpromazine
Barbiturates
Benperidol
Blood Pressure Lowering Agents
Brigatinib
Brimonidine (Topical)
Dapsone (Topical)
Dexmethylphenidate
Diazoxide
DULoxetine
Herbs (Hypertensive Properties)
Herbs (Hypotensive Properties)
Local Anesthetics
Lormetazepam
Methylphenidate
Molsidomine
Naftopidil
Nicergoline
Nicorandil
Nitric Oxide
Pentoxifylline
Pholcodine
Prilocaine
Prostacyclin Analogues
Quinagolide
Sodium Nitrite
Sildenafil or Tadalafil
Yohimbine

**References**

1. Brown, H. E. *et al.* Efficacy and Tolerability of Adjunctive Intravenous Sodium Nitroprusside Treatment for Outpatients With Schizophrenia: A Randomized Clinical Trial. *JAMA Psychiatry* **76**, 691 (2019).
2. Hallak, J. E. C. et al. Rapid Improvement of Acute Schizophrenia Symptoms After Intravenous Sodium Nitroprusside: A Randomized, Double-blind, Placebo Controlled Trial. JAMA Psychiatry 70, 668 (2013).
3. Stone J.M. et al. The effect of sodium nitroprusside on psychotic symptoms and spatial working memory in patients with schizophrenia: a randomized, double-blind, placebo-controlled trial. *Psychological Medicine* 46, 3443-3450 (2016).
4. Wang X. et al. Sodium nitroprusside treatment for psychotic symptoms and cognitive T deficits of schizophrenia: A randomized, double-blind, placebo-controlled trial. *Psychiatry Research* 269, 271-277 (2018).
5. Adelino et al. Treatment-resistant schizophrenia - A RCT on the effectiveness of repeated-dose sodium nitroprusside. Schizophr Res. 2021 May;231:70-72.

## B4. POSSIBLE BENEFITS

There are no direct benefits to subjects. However, as shown by previous clinical studies, an immediate improvement in overall psychosis and negative symptoms in participants with ESZ may be achieved with SNP treatment.

Potential benefits to society include increased understanding of the pathophysiology of schizophrenia and the personalization of treatment early in the course of schizophrenia illness. We are expecting to have an impact on the growing consensus in immunopsychiatry and neuro-ophthalmology that the retina provides a window into the brain that informs our understanding of brain pathophysiology and identifies biomarkers of treatment response with this study.

## B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

Psychological Interview: There is a possibility that a patient may become distressed when being asked about symptoms or personal information during the diagnostic evaluation or assessment session. The primary risks to the patients are discomfort or anxiety. Subjects may also experience some discomfort or anxiety from discussing personal information. Yet this anxiety is not expected to be greater than that subjects already experience and subjects will be assured that they will be able to withdraw from the study at any time. There is a possibility of suicidal ideation as a result of any anxiety or distress associated with discussing personal information. This risk is largely reduced by screening out patients with significant suicidal ideation or those who have enacted suicidal behaviors within 6 months prior to intake.

Cognitive Tests: The cognitive tasks pose no risks to participants. The tasks will require sustained attention and mental effort for a steady period of time. This could lead to minor temporary fatigue or boredom, but otherwise there are no known risks. We will ensure that data remain as confidential as possible. Also, these data generally are not considered to be of sensitive nature.

Confidentiality: Although every effort will be effort made to assure confidentiality, it is possible someone could without permission gain access to study related data during the time they are being used or stored for examination results.

Blood draw: Temporary irritation from the needle stick, the likelihood of pain or bruising at the site of the blood draw, occasional symptoms of lightheadedness, and, in rare cases, infection at the site of the blood draw are all risks and discomforts associated with the blood draw procedure.

SS-OCTA: OCTA is painless and safe techniques that will be used to investigate the structure and function of the retina. Performing these imaging techniques in individuals with psychotic disorders does not pose specific, additional unique challenges or risks to the participants. Participants will have their pupils dilated for OCT/OCTA retinal imaging, as is done with all ophthalmology imaging facilities, which may result in light sensitivity or acute angle-closure glaucoma. When possible, we will forgo pupil dilation, especially when high quality retinal images can be obtained without the need for pupillary dilation.

IV infusion of SNP:
IV treatment is relatively safe, there can be complications if not administered properly. The most common complications include phlebitis, extravasation, air embolism, infection.
The single administration of IV Sodium nitroprusside has been reported to be safe and well tolerated. The most important adverse reactions are the avoidable ones of hypotension and cyanide toxicity and other side effects reported as; bradycardia, tachycardia, palpitations, headache, increased intracranial pressure, skin rash and nausea. The potential for cyanide toxicity

is greatest when more than 500 g/kg of sodium nitroprusside is administered faster than 2 g/kg/min, as cyanide is generated faster than the unaided patient can eliminate. Cyanide toxicity will be avoided in this study as the dose administered is very low, and individuals with renal impairment will be excluded. Also, the study treatment will be infused within 3-12 hours of preparation as sodium nitroprusside is only stable in solution for 24 hours. The infusion protocol will be 0.5mcg/kg/min which is the minimum therapeutic dose and patients will be continuously monitored during the infusion. The infusion procedure will take place at the Clinical Research Center at BIDMC, which is a fully staffed center with nurses and physicians, as well as all the necessary monitoring and personal protective equipment. In 4 other similar studies with the same protocol, there were no serious side effects.

Sodium nitroprusside does have some potential for drug-drug interactions. The hypotensive effect of sodium nitroprusside is augmented by that of most other hypotensive drugs, including ganglionic blocking agents, negative inotropic agents, and inhaled anesthetics. Subjects taking any of these drugs will be excluded from the study. Patients will also be advised not to take these medications during the study[1-4].

**Protections Against Risks**

Protection against potential clinical risks: All research procedures and assessments will be completed by trained and experienced personnel. We also strive to make the assessments as free from burden as possible. Assessments will be scheduled at the convenience of subjects, and we also arrange for transportation and parking. The research staff will be regularly in touch with the participants during participation and will encourage them to contact a 24-7 number in the event of an emergent need. If the participant develops worsening of symptoms, they will be referred for treatment as appropriate.

Protection for risks associated with retinal imaging: Oversight of this study will come from local IRBs. Subjects will be screened for exclusionary criteria, which include retinal diseases and past adverse effects from ophthalmic medication used for pupil dilation. Participants will also be provided with disposable sunglasses and asked to avoid driving and operating other potentially dangerous equipment for 2 hours after pupil dilation. In the event of an adverse effect from the medication for pupil dilation, there will be clinical staff (including my collaborators, Drs Miller) available to the participant at the Retinal Imaging site (Mass Eye and Ear) to promptly assess and treat the participant.

Protection against potential risks with SNP administration: All participants will undergo a physical examination (PE), routine laboratory tests (CMP, CBC, CRP, TSH, Lipid panel), urine toxicology tests and 12-lead-electrocardiogram to ensure medical stability. To monitor safety and tolerability, participants will also be administered the Abnormal Involuntary Movement Scale, the Systematic Assessment for Treatment Emergent Effects (SAFTEE), and the Columbia-Suicide Severity Rating Scale prior to each infusion, after each infusion, and at follow-up. Hypotension is unavoidable with this medication, but participants will be continuously monitored to avoid any potential complications and there will be nursing available to help address any potential complications by stopping the infusion and/or administrating fluids to help increase a patient's blood pressure. Cyanide toxicity will be avoided by providing low dosages of SNP to participants, which avoids this risk.

**References:**

1. Hallak, J. E. C. et al. Rapid Improvement of Acute Schizophrenia Symptoms After Intravenous Sodium Nitroprusside: A Randomized, Double-blind, Placebo Controlled Trial. JAMA Psychiatry 70, 668 (2013).

2. Stone, J. M. et al. The effect of sodium nitroprusside on psychotic symptoms and spatial working memory in patients with schizophrenia: a randomized, double blind, placebo-controlled trial. Psychol. Med. 46, 3443–3450 (2016).
3. Wang, X. et al. Sodium nitroprusside treatment for psychotic symptoms and cognitive deficits of schizophrenia: A randomized, double-blind, placebo-controlled trial. Psychiatry Res. 269, 271–277 (2018).
4. Brown, H. E. et al. Efficacy and Tolerability of Adjunctive Intravenous Sodium Nitroprusside Treatment for Outpatients With Schizophrenia: A Randomized Clinical Trial. JAMA Psychiatry 76, 691 (2019).
5. Holme MR, Sharman T. Sodium Nitroprusside. 2020 Oct 1. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID: 32491419.

## B6. RECRUITMENT AND CONSENT PROCEDURES

**Recruitment**

This randomized placebo control trial will include 32 early course schizophrenia subjects from a diverse background. Department of mental health clients will not be recruited for the study and thus IRB approval from Massachusetts Mental Health Center is not necessary. No healthy controls will be included in the study.

Patients will be recruited through BIDMC and BILH clinics and if necessary, through affiliated MAPNET clinics. Patients will be recruited via flyers/advertisements placed in clinic waiting rooms, public information sessions at the clinic about the project, and asking clinicians to hand out study flyers to subjects who they think may be interested. Patients will also be recruited by approaching the clinicians, and if they agree, then approaching the subject for consent into the study. Interested patients will be able to contact and call study staff for more information. ECS subjects will also be recruited from our IRB approved retinal imaging study where patients have provided consent to be recontacted about future studies via email (IRB 2019P000815). Subjects will be contacted by email only if they previously consented to this. If interested, they will then receive a phone screening questionnaire. Upon first contact, the study research assistant/coordinator will explain the study to patients. If they are interested in continuing, a brief initial telephone screen will be administered, assessing inclusion criteria. Those who agree to continue will be scheduled for an individual interview with the study research assistant at BIDMC or through Microsoft Teams to assure privacy.

In some cases, the consent procedure will occur remotely to allow only the screening and baseline portions of the study to occur via video conferencing. A written informed consent form that outlines what study procedures will occur remotely has been uploaded to RedCap. This consent form will be emailed to the participant ahead of a scheduled phone or Microsoft Teams video-conferencing call. During the phone/video call, a trained clinician or study research assistant will review the form with the participant (and, when applicable, with their legal guardian) in a private room in order to ensure HIPAA compliance. The research clinician or assistant will follow the same procedure as the in-person consent. All subjects must understand the requirements, risks, and benefits of the research protocol, which is approved by the Institutional Review Board BIDMC, before they are allowed to sign. Subjects must understand that they have the right to refuse participation at any time during the study. To more formally assess and document each subject's ability to give informed consent, the person obtaining consent will follow up the study description with an interactive questionnaire addressing the main points of the informed consent procedure via RedCap. Then, the participant may e-sign and date the RedCap consent form, after which the research clinician or assistant will do the same. A copy of the signed consent form will be emailed to the participant. When the participant is able to come into the laboratory for their first in-person visit, the PI, co-investigator, or their representative will review the full (non-verbal) consent form with the participant and obtain a physical signature on the consent form.

All subjects must understand the requirements, risks, and benefits of our research protocol before they are allowed to sign. Subjects must understand what to do should they experience any discomfort during the course of the study. Subjects must understand that they have the right to refuse participation at any time during the study. To more formally assess and document each subject's ability to give informed consent, the person obtaining consent will follow up the description of the study with an interactive PowerPoint presentation addressing the main points of the informed consent procedure. The study investigator will ask the subject each question and document their response. Subjects who are unable to answer the questions, even after additional information is provided, will be excluded from the study. This process is designed to enroll only subjects who have the capacity to make the decision to participate and to prevent undue influence on the subject to enroll in the study by their physician. The PI, co-investigators, or their representative will obtain written consent after explaining the procedures. These individuals are certified by the PI that they have in depth understanding of the study and procedures. Only when the subject declares that they are comfortable with all aspects will the subject be allowed to offer consent. The informed consent form will be signed by the subject and a member of the study staff. All key personnel will have passed the web-based training course on the responsible conduct of research**.**

Because people who are recruited for this investigation display symptoms of psychosis, we will use the attached Assessment of Capacity form to assess ability to consent to participation. This form has been used in previous studies with schizophrenia at our center. The form prompts the investigator to assure that the subject is voluntarily choosing to participate in the study, has a factual understanding of the study, has an appreciation of the significance of the facts about research participation and is able to reason about his or her participation.

**Subject Protection**

Patients with schizophrenia, schizoaffective disorder may be considered a vulnerable population. The multi-step consent process involving both a phone screening and in-person visit, or secure remote process provide safeguards and opportunities for subjects to understand the study and choose whether or not to partake. Subjects will be questioned to confirm their understanding of the protocol and possible risks or adverse side effects of Sodium Nitroprusside, thus helping ensure that subjects with incomplete understanding do not unwittingly consent to the study. Finally, we will also complete a one-page Assessment of Capacity form to provide informed consent.

We will assure subjects both on the informed consent form and in person that refusal to partake in the study or drop-out from the study at any point has no impact on continued services from their current clinic or at BIDMC. Clinical interviews will be performed by a trained clinical staff who will be able to reassure subjects or if necessary, provide counseling if they are distressed. Subjects will be forewarned throughout the pre-screening and screening process that the protocol involves sitting in a chair for up to 5 hours, and that they may take breaks at any time. Breaks will be offered to subjects regularly, though they may decline to take them.

Again, if during the consent process or any study visit, study staff notice the subject expressing suicidality, homicidality appearing agitated or distressed, or if they score 4 or higher on the PANSS question G6 "Depression", they will be instructed to pause the protocol and immediately contact the investigator (Drs. Lizano or Brady, all trained psychiatrists), who will perform a safety assessment, contacting outpatient providers or emergency services as deemed necessary.

## B7. STUDY LOCATION

**Privacy**

Screening will be performed by phone or via Microsoft Teams and we will assure that the participant is located in a place where they can securely answer any sensitive questions. Initial screening for the study will take place over the phone/Microsoft Teams via a screening questionnaire. At the start

of the phone call, the potential subject will be forewarned of the possibility of sensitive questions in the screening questionnaire. They will be offered the choice to defer the phone screening questionnaire to a different time if they are uncomfortable answering questions over the phone at that time. Assessments and procedures for the study will take place in private suites and rooms at BIDMC, and OCTA assessments will take place at Massachusetts Eye and Ear's, which will afford protection against a participant's violation of privacy. Some of these assessments can be performed virtually. We will also limit the information that is being collected to only the minimum amount of data necessary to accomplish the research purposes.

**Physical Setting**
Urine toxicology screen, urine pregnancy test, consenting, screening and baseline assessments will take place in Dr. Lizano's lab space at BIDMC. Subsequent medical screening (blood tests, vital signs, EKG) and SNP infusion visits will occur in the BIDMC Clinical Research Center. The visits will be completed with a single subject at a time, ensuring privacy. Data will also be stored at BIDMC.

## B8. DATA SECURITY

To ensure confidentiality, the entry of self-reported data into electronic format will be identified only by subject number, visit number and date of visit. By recording the study data in this manner, the information can be considered de-identified and therefore compliant with the Standards for Privacy of Individually Identifiable Health Information ("Privacy Rule") of the Health Insurance Portability Act of 1996 (HIPAA). Any data that is transmitted electronically will be fully encrypted and password protected. Subjects' names will never be entered into a database; each will uniquely be identified by an ID number. Consent forms and any study measures that are completed on paper will be kept and filed in separate locked office cabinets accessible only to lab personnel (75 Fenwood Road 5$^{th}$ floor office, Boston MA 02215). Computer data files are labeled using arbitrary identification codes to preserve confidentiality as well.

A master data file containing the associations between each participant's demographic information (e.g., age and gender) and their unique identification code will be kept on a password protected computer. This information is used to report the gender and age of the subject sample in publications, required by many journals. Only lab research will have access to this computer and this file.

Data collected by a computer will be stored on computers within the laboratory and also stored in a backup format on the lab's RAID array and/or on external media such as recordable DVDs or encrypted external hard drive. No identifying information will be kept in relation to these data. Data collected by questionnaire or written record by the experimenter will be kept in a locked office in the laboratory along with the Informed Consent Forms. Only lab research personnel will have access to informed consent forms or other such personal information about the subject.

The data collected from these studies will be conducted in private locations where subjects can be assured their information is collected privately and in a strictly confidential manner. All consent forms, questionnaires and related hard copy forms with personal identifiers will be securely stored in a file in a locked office. The files in this office are accessible only to lab research personnel. Each participant's computerized data will be labeled with a unique identification code that bears no resemblance to the participant's name. All behavioral, SS-OCTA and routine laboratory test data will be stored on computer hard drives in the laboratory. Back-ups of all data files will be stored on encrypted external storage devices within the laboratory (e.g., external hard drives, CD-R). Individually identifiable data will not be released to anyone outside of the lab unless required by law and/or necessary for oversight purposes.

## B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☐ No

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☐ No

## B10 Dissemination of Research Results

We appreciate the time and effort that our research subjects provide for the advancement of medicine. We will acknowledge our subjects in manuscripts that arise from this work. We will provide our participants with our contact information if they are requesting study progress. At the completion of the study, we will send each participant a letter thanking them and giving them an update regarding our findings.