A Randomized Double-Blind, Placebo-Controlled, Proof of Concept Study of Intravenous Sodium Nitroprusside in Adults with Symptomatic Schizophrenia

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B. Synopsis

Protocol Number:	2014P001204
Title:	A Randomized Double-Blind, Placebo-Controlled, Sequential Parallel Comparison Design (SPCD) Study of Intravenous Sodium Nitroprusside in Adults with Symptomatic Schizophrenia
Design:	This will be a 6-8 week, randomized, double-blind, placebo-controlled, Sequential Parallel Comparison Design (SPCD) study, where treatment will be intravenously administered twice (at Day 0 and Day 14) over 4 hours, with assessments during the infusion and weekly assessments post-infusion to assess the efficacy and safety of intravenous sodium nitroprusside (0.5 µg/kg/min for 4 hours).
Objectives:	The primary objective of this study is to investigate whether intravenous sodium nitroprusside (0.5 µg/kg/min for 4 hours) is superior to placebo (5% dextrose solution) in improving the positive and negative symptoms associated with schizophrenia as defined by the DSM-IV-TR. The secondary objective of this study is to evaluate the safety and tolerability of sodium nitroprusside (0.5 µg/kg/min for 4 hours) as compared to Placebo (5% dextrose solution for 4 hours).
Enrollment:	100 subjects total (randomize 60 subjects) 30 subjects at MGH Site (randomize 20 subjects)
Clinical Sites:	Approximately 4 US sites
Patient Population:	Men and women, 18-65 (inclusive) years of age with stable refractory schizophrenia, on an antipsychotic for 8 weeks with a stable dose for at least 4 weeks**,** and who meet all study inclusion and exclusion criteria.
Primary and Secondary Outcomes:	The Structured Clinical Interview for *DSM-IV-TR* (SCID) will be used to confirm the diagnosis of schizophrenia. An independent SAFER interview will confirm both diagnosis and severity of symptoms. PANSS total, positive- and-negative subscale scores will be used to measure the primary outcomes. The PANSS will be collected at Visits 1, 2, 3, 4 (Hour 3 to 4), 5, 6. 7,

	(Hour 3 to 4), 8 and 9. The SAFTEE and the Abnormal Involuntary Movement Scale (AIMS), together with blood pressure and heart rate, will be used to assess the secondary outcomes of this study (safety and tolerability). AIMS and SAFTEE will be collected at Visits 3, 4, 6, 7 and 9. The MATRICS Consensus Cognitive Battery (MCCB) is the standard tool for assessing cognitive change in trials of cognitive-enhancing agents in schizophrenia. The MCCB domains include: Speed of Processing; Attention/Vigilance; Working Memory; Verbal Learning; Visual Learning; Reasoning & Problem-Solving; and Social Cognition and the battery takes about 80 minutes to complete. The MCCB is accepted by the US FDA as a primary outcome measure for registry trials for cognition in schizophrenia. The MCCB composite score represents a global measure of cognition and is than outcome measure for inclusion in the efficacy analyses. MCCB will be collected at Visits 3, 6, and 9. The University of California San Diego (UCSD) Performance-based Skills Assessment Brief (UPSA-B) (Mausbach et al., 2007). The UPSA-B will be administered Visits 3, 6, and 9. The UPSA-B consists of two of the five original UPSA domains, finances and communication, and has been shown to be highly correlated with the long form of the UPSA and to have substantial test–retest reliability (Green et al., 2011). Each subscale contributes 50 points; total scores range from 0 to 100 points with higher scores reflecting better performance. The UPSA-Brief requires approximately 10–15 minutes to complete and may be administered by a suitably trained lay professional.
Inclusion Criteria:	Males and Females, 18-65 years of age, inclusive. Meets Diagnostic and Statistical Manual of Mental Disorders Forth Edition (DSM-IV-TR) criteria for a primary diagnosis of Schizophrenia established by a structured psychiatric evaluation (SCID) based on DSM-IV-TR criteria. Written informed consent in compliance with 21 CFR part 50 and in accordance with the International Conference

	on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines. Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1994) total score ≥ 70 with a score of ≥ 4 on two or more of the following PANSS items: delusions, conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content. A score of ≥ 4 on the Clinical Global Impression—Severity (CGI-S) (Guy, 1976). Confirmation of both diagnosis and severity of psychosis symptoms by an independent SAFER interview. Must have ongoing antipsychotic treatment for at least 8 weeks, with a stable dose for at least 4 weeks. Subjects who have failed to achieve clinically-recognized symptom reduction to at least 1 marketed antipsychotic agent, given at a Physician Desk Reference (PDR)-defined therapeutic dose for ≥ 8 weeks during the past 12 months, as assessed by the MGH FAST, will be eligible. Understands and is able, willing, and (in the opinion of the investigator) likely to fully comply with the study procedures and restrictions.
Exclusion Criteria:	Subjects with renal insufficiency, congestive heart failure, cardiac arrhythmias or history of myocardial infarction. Subjects with a history of symptomatic orthostatic hypotension defined as supine to standing systolic blood pressure ≤ 90mmHg or diastolic blood pressure ≤ 60mm Hg with any of the following symptoms: lightheaded or dizzy upon standing up, blurry vision, weakness, fainting (syncope),confusion, nausea. Subjects with any clinically significant abnormalities as determined by medical history, physical exam, clinical and lab evaluation suggestive of an underlying disease state that may, in the opinion of the investigator, may confound the results of study, increase risk to the subject or lead to difficulty complying with the protocol. Subjects on chlorpromazine, PDE-5 inhibitors, nitrites and any medication with CNS effects with the exception of antipsychotic drugs (other than chlorpromazine), anticholinergics, b-adrenergic antagonists, amantadine, biperiden, diphenhydramine, lorazepam, zolpidem, and temazepam (see Appendix 3).

	Medications which in the opinion of the PI, and in conjunction with the medical monitor, may be expected to significantly interfere with the metabolism or excretion of sodium nitroprusside, and/or may be associated with a significant drug interaction with sodium nitroprusside that may pose a significant risk to subjects' health and/or confound the study data. Pregnant or breast-feeding patients. Women of childbearing potential must have a negative pregnancy test performed at screening visit prior to randomization and prior to baseline at visits 3 and 6. Women enrolled in this trial must use adequate birth control. Subjects with a current (within the last 3 months) DSM-IV-TR diagnosis of alcohol or substance use disorder or dependence (excluding nicotine) as established by the clinical assessment (SCID) at the screening visit (Visit 1) will be excluded. Has tested positive for any of the following: cannabis, opioids, cocaine, amphetamines, barbiturates methadone, methamphetamine and phencyclidine at the screening or baseline visits. If positive, the urine drug toxicology screen may be repeated once based on investigator judgment, but due to safety concerns, the result must be negative for the subject to continue in the study. 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 Columbia Suicide Severity Rating Scale (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. In the opinion of the investigator, participation for any reason would compromise patient safety or integrity of the study
Statistical Methodology	To test the hypothesis that a statistically significant difference exists in the degree of improvement over 2 weeks between intravenous sodium nitroprusside (0.5 µg/kg/min for 4 hours) and placebo, treatment effect estimates and two-sided 95% confidence intervals (CIs) for sodium nitroprusside and placebo will be provided. A constrained longitudinal data analysis (cLDA) model (Liu et al., 2009) including terms for treatment group, time (as

	a categorical variable), and treatment by time will be used to evaluate the mean difference in slope between sodium nitroprusside and placebo for the primary efficacy endpoint over 2 weeks. All time point data starting at the baseline measurement will be included in the analysis model, and treatment difference estimates at specific time points (e.g., Day 0, 7, 14, 21 and 28) will also be provided to assess the time course of effects.

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C. INTRODUCTION AND BACKGROUND

Statement of Intent

The design, conduct and reporting of this trial shall be conducted in accordance with the protocol, the United States 45 Code of Federal Regulations (CFR) part 46 known as "The Common Rule", 45 CFR 164.502(d), and 164.514(a)-(c) known as "The Privacy Rule" of the Health Insurance Portability and Accountability Act (HIPAA), and the International Conference on Harmonization (ICH)/Good Clinical Practice (GCP). The data collection will also comply with 21 CFR part 11. All Investigators will have documented training in The Collaborative Institutional Training Initiative (CITI Program) in Biomedical Research and GCP. Independent monitoring of the trial will be conducted by a Contract Research Organization (CRO).

Background

The typical and atypical antipsychotic medications have been repeatedly demonstrated to be efficacious for the treatment of schizophrenia. However, these treatments have substantial limitations which have prompted aggressive, but thus far, unsuccessful efforts to find more effective and better tolerated alternatives. In particular, current pharmacologic treatments for schizophrenia are ineffective for negative symptoms and require weeks to produce antipsychotic effects; during this period of delay, patients continue to experience distress associated with delusions and hallucinations, are often incarcerated, and are at high risk for injury. Refractory symptoms are common and, if clozapine is not effective, no other treatment options have demonstrated efficacy. Antidepressant therapeutics faced a similar situation of delayed therapeutic response and limited treatment options for refractory symptoms until the recent finding that a single ketamine infusion produces rapid, persistent and marked symptom response in refractory cases. Ketamine infusion is now increasingly offered in cases of refractory depression and is being studied as an acute emergency room-based treatment for suicidal depression (Machado-Vieria et al., 2009).

A similar approach could fundamentally change treatment of psychosis. Several years ago it was hypothesized that a single dose of an agent acting on the N-Methyl-D-aspartate (NMDA)/ nitric oxide synthase (NOS) / cyclic guanosine monophosphate (cGMP) pathway might produce just such an effect. Nitric oxide diffuses across cell membranes to activate guanylate cyclase and produce cyclic guanosine monophosphate. In smooth muscles, cGMP causes vasodilation; in neurons cGMP promotes neuroplasticity. Activation of NMDA receptors similarly produces intracellular release of diffusible nitric oxide via activation of neuronal nitric oxide synthase (nNOS). The NMDA/nNOS/cGMP pathway is involved in long term potentiation (LTP) and neuroplasticity, including memory, which is mediated in part by cGMP's role in regulating the phosphorylation of CREB and AKT (Mohn et al. 1999, Allen et al. 2008).

Indeed, initial studies with the nitric oxide donor nitroprusside completely attenuated behavioral effects of PCP in rats, a standard albeit limited rodent model of psychosis and antipsychotic action, and blocked PCP-induced c-fos expression in all brain regions without producing behavioral change when administered alone (Wang et al 2011). Nitroprusside also inhibited cocaine-induced immediate early gene expression in the frontal cortex and striatum (Thirlet et al. 2002). While the precise means by which NO donors may exert therapeutic effects remains unclear, these animal studies suggest that effects on NMDA signaling and/or modulation of excess corticostriatal dopamine may be relevant.

The recent report by Hallak et al. (Hallak 2013) of a positive placebo-controlled trial of nitroprusside infusion provides the first human data supporting the use of nitric oxide donors to achieve rapid, robust antipsychotic effect. Hallak and colleagues randomly assigned 20 symptomatic subjects with schizophrenia to a single 4 hour infusion of nitroprusside 0.5 μg/kg/min or placebo and observed a large improvement of positive and negative symptoms (effect size of approximately 1.7) which achieved statistical significance at 2 hours and persisted for 28 days. Although high-dose nitroprusside may produce hypotension and cyanide toxicity, the investigators observed no side effects at the substantially lower doses used in their pilot study. This novel approach is supported by converging evidence from human genetics and animal models and represents a potential paradigm shift in the treatment of schizophrenia. If replicated, the potential impact on clinical care might include both emergency-room treatment of acute psychosis and office-based treatment of refractory symptoms. Once efficacy and safety are established, future studies can examine repeated administration for maintenance treatment while pursuing other agents targeting this pathway. Drugs acting on this pathway are currently in development for Alzheimer's disease (Lahti et al 1995).

Sodium Nitroprusside

Intravenous sodium nitroprusside was approved by the FDA in 1979 for management of hypertension and reduction of intra-operative blood loss; it releases nitric oxide by interacting with oxyhemoglobin, and thus represents a balanced arterial and venous vasodilator. It has been widely used clinically for related indications including exacerbation of congestive heart failure (Carlson 2013).

Summary of Prior Clinical Studies

In the only prior investigation of intravenous (i.v) sodium nitroprusside (SNP) in a psychiatric population, described above (Hallak et al), i.v. SNP or placebo was administered to 20 subjects at a dose of 0.5 μg/kg/min over 4 hours. No subjects experienced significant adverse effects and none were discontinued from the study because of safety or tolerability concerns.

As noted above, SNP has been used therapeutically for more than 30 years in a variety of acute cardiac settings. Systematic reviews conclude that i.v. SNP remains a generally safe and efficacious medication despite the availability of newer vasodilators. Another recent review of pediatric populations also concludes that i.v. SNP can be safely administered, with low risk of cyanide toxicity (Thomas 2009).

Because of its established safety profile, there are relatively few recent investigations of sodium nitroprusside. In one of the largest, SNP was studied in comparison with other anti-hypertensives in cardiac surgery. While efficacy was less than the calcium channel blocker clevidipine, safety profile among the agents studied was similar (Aronson 2008).One recent study examining control of hypertension in neurosurgical intensive care unit confirmed its safety. (Ritberg 2008). Likewise, a head-to-head study with IV adenosine for assessments of cardiac perfusion, also indicated safety. (Rudzinski 2013)

D. STUDY RATIONALE

We plan to conduct a Phase II or Proof of Concept (POC), randomized, double-blind, placebo-controlled, multi-center study using a Sequential Parallel Comparison Design (SPCD) to demonstrate the efficacy and safety of sodium nitroprusside (0.5 µg/kg/min for 4 hours) in treating the positive and negative symptoms of subjects with schizophrenia.

E. OBJECTIVES

Primary Objective

The primary objective of this study is to evaluate the effectiveness of sodium nitroprusside (0.5 µg/kg/min for 4 hours) as compared to Placebo (5% dextrose solution for 4 hours), in improving the positive symptoms and negative symptoms associated with schizophrenia.

Secondary Objectives

The secondary objective of the study is to evaluate the safety and tolerability of sodium nitroprusside (0.5 µg/kg/min for 4 hours) as compared to Placebo (5% dextrose solution for 4 hours).

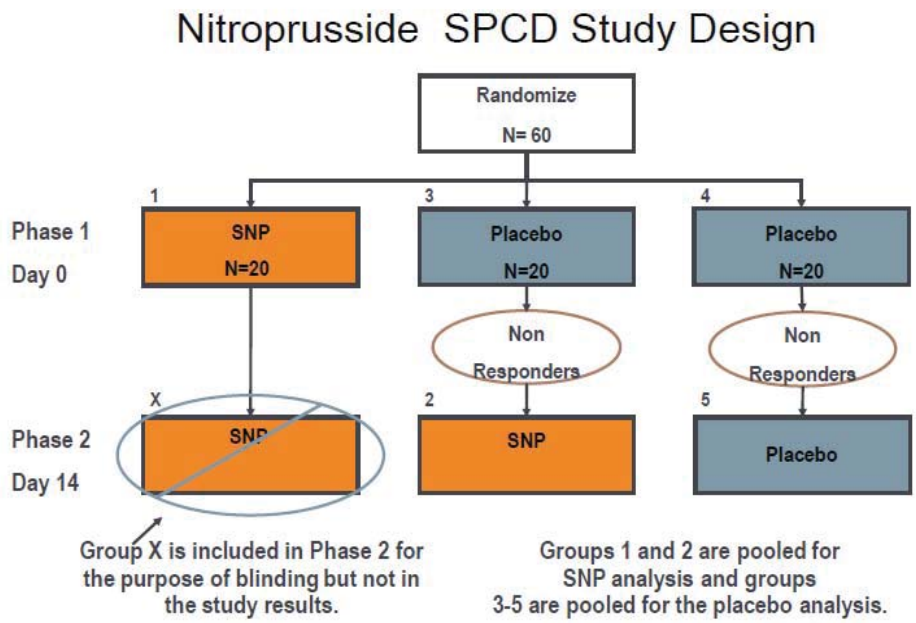
F. STUDY DESIGN

This is a phase II proof of concept (POC), multi-center, prospective, randomized, placebo-controlled, Sequential Parallel Comparison Design (SPCD) study, in which a total of 60 subjects with schizophrenia will be randomized studywide.

The study will be conducted in two phases. The study treatment will be administered in a double-blind fashion for all subjects throughout both phases of the study. A total of 60 subjects with schizophrenia will be randomized in a 1:1:1 ratio to one of three treatment sequences: drug-drug, placebo-drug, or placebo-placebo. Subjects will be randomized directly following confirmation of eligibility (at completion of Visit 2). Subjects randomized to the drug-drug sequence (n=20) will receive i.v. sodium nitroprusside (0.5 µg/kg/min for 4 hours) at Day 0 and Day 14. Subjects randomized to the placebo-drug sequence (n=20) will receive i.v. placebo at Day 0 and i.v. sodium nitroprusside (0.5 µg/kg/min for 4 hours) at Day 14. Subjects randomized to the placebo-placebo sequence (n=20) will receive i.v. placebo at Day 0 and again at Day 14. The double-blind treatment will be divided into two phases: Phase 1 from Day 0 to Day 14, and Phase 2 from Day 14 through Day 28.

At the end of Phase 1 the randomized subjects will be assessed and categorized into responders and non-responders, based on 20% or more reduction in their PANSS total score between Visit 4 (Baseline, Day 0) and Visit 7(Day 14). The data from the subjects deemed placebo non-responders in Phase 1 who go on to either stay on placebo or to receive treatment with sodium nitroprusside will be pooled with the data from Phase 1 from all subjects, according to SPCD.

Figure 1 –


Nitroprusside SPCD Study Design
Randomize
N= 60
1
3
4
Phase 1
Day 0
SNP
N=20
Placebo
N=20
Placebo
N=20
Non
Responders
Non
Responders
X
2
5
Phase 2
Day 14
SNP
SNP
Placebo
Group X is included in Phase 2 for the purpose of blinding but not in the study results.
Groups 1 and 2 are pooled for SNP analysis and groups 3-5 are pooled for the placebo analysis.

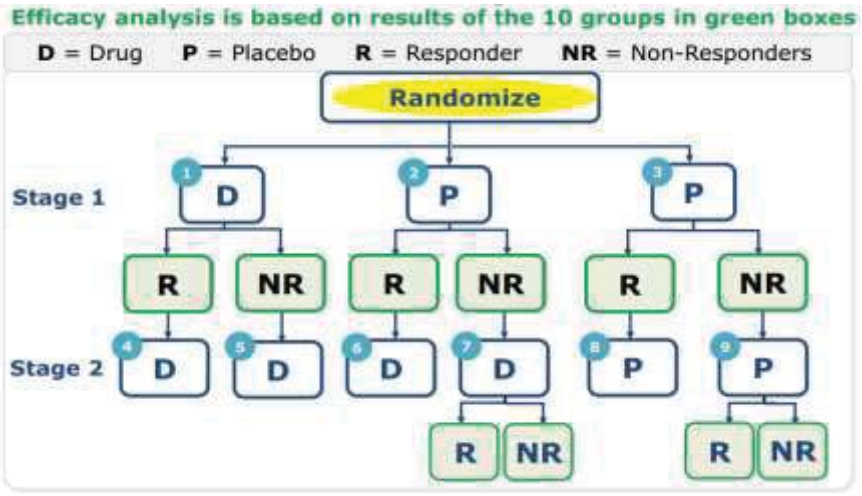
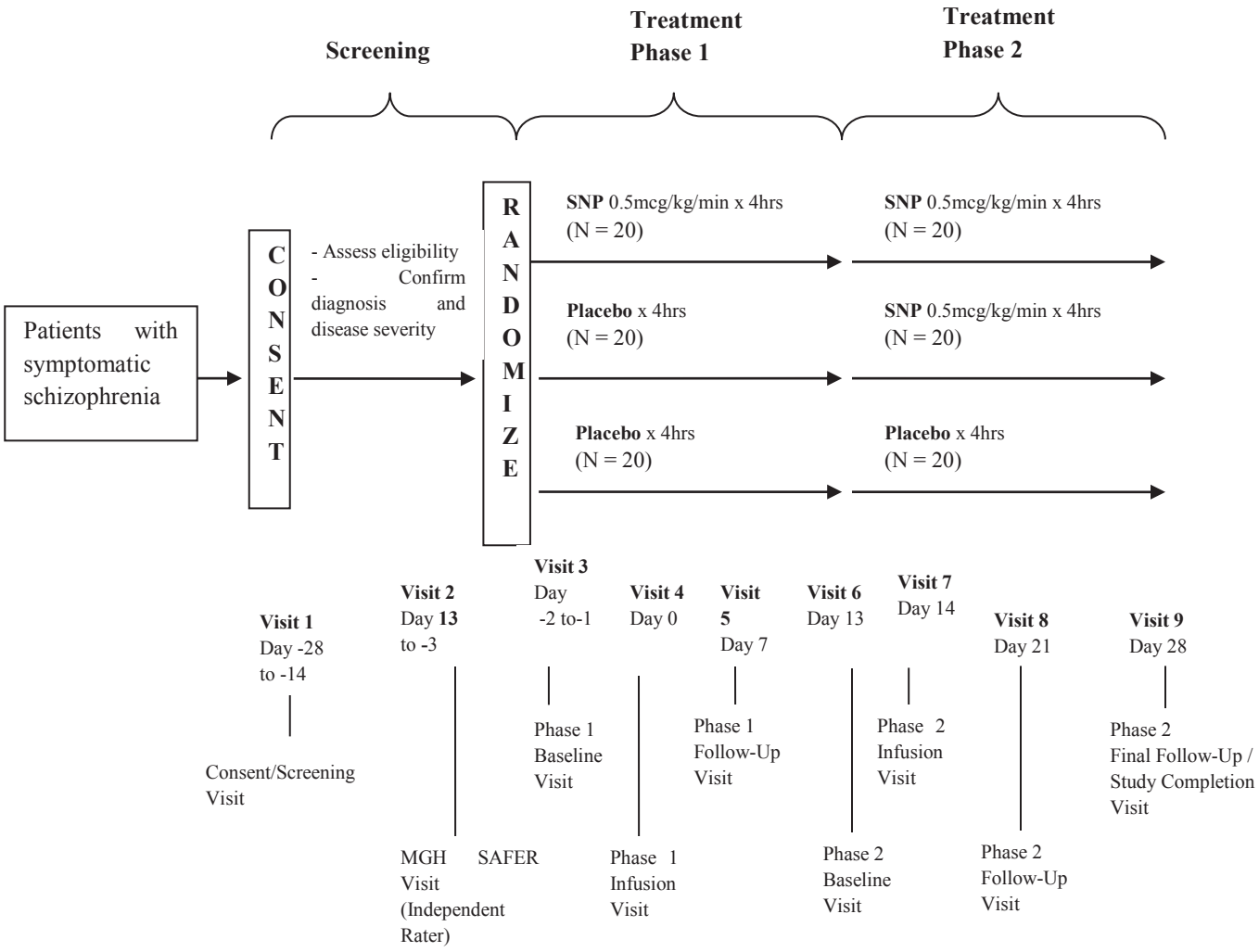

Efficacy analysis is based on results of the 10 groups in green boxes
D = Drug
P = Placebo
R = Responder
NR = Non-Responders
Randomize
Stage 1
Stage 2
D
P
P
R
NR
R
NR
R
NR
D
D
D
D
P
P
R
NR
R
NR

Figure 2 – Study Schemata



Study Visit Overview

Screening

- Visit 1 (Screening Visit, Day -28 to -14): Consists of signing a HIPAA compliant informed consent form and study qualification based on evaluation of inclusion/exclusion criteria. Medical records will be requested from the participant's primary care physician for confirmation of relevant eligibility criteria.
- Visit 2 (Remote Independent Interview, Day -13 to -3): Consists of independent assessment to confirm the diagnosis and the appropriateness of the subject for the study. Eligibility will also be assessed at this visit by conducting a PANSS,

and reviewing the SCID, MGH FAST and CGI-S. Pending confirmation of eligibility, the subject will be randomized.

Phase 1:

- Visit 3 (Phase 1 Baseline, Day -2 to -1): Subjects will undergo full battery of study efficacy and safety evaluations. Subjects will also have a urine drug screen, vital signs, 12-lead ECG, and concomitant medications. Visit 4 must occur the day after Visit 3.

- Visit 4 (Phase 1 Study Treatment, Day **0**): Anthropometrics, vital signs, concomitant medications, CBC, electrolytes**,** urinalysis, and urine pregnancy test will be assessed between Hours -1 and 0, before the infusion begins. Randomized i.v. study treatment will be administered from Hours 0 to 4. During these hours there will be safety monitoring (See: Infusion Monitoring and Discharge Procedures). During Hours 5 to 7 following the infusion, there will be a post-infusion debrief, neurological assessment, and assessment of adverse events. Should an infusion room be unavailable on Day 0, the infusion may be scheduled one day before or one day after with prior notification provided to CTNI.

- Visit 5 (Phase 1 Follow-up, Day 7): Subjects will undergo full battery of study efficacy and safety evaluations. Subjects will also have vital signs, 12-lead ECG, and concomitant medications.

Phase 2:

- Visit 6 (Phase 2, Day 13): Subjects will undergo full battery of study efficacy and safety evaluations. Subjects will also have urine drug screen, vital signs, 12-lead ECG, and concomitant medications. Visit 7 must occur the day after Visit 6.

- Visit 7 (Phase 2 Study Treatment Day 14): Anthropometrics, vital signs, concomitant medications, CBC, electrolytes, urinalysis, and urine pregnancy will be assessed between Hours -1 and 0, before the infusion begins. Randomized i.v. study treatment will be administered from Hours 0 to 4. During these hours there will be safety monitoring (See: Infusion Monitoring and Discharge Procedures). During Hours 5 to 7 following the infusion, there will be a post-infusion debrief, neurological assessment, and assessment of adverse events. Should an infusion room be unavailable on Day 7, the infusion may be scheduled one day before or one day after with prior notification provided to CTNI.

- Visit 8 (Phase 2 Follow-up, Day 21): Subjects will undergo full battery of study efficacy and safety evaluations. Subjects will also have vital signs, 12-lead ECG, and concomitant medications.

- Visit 9 (Final Follow-up/Study Completion, Day 28): Subjects will undergo full battery of study efficacy and safety evaluations. Subjects will also have laboratory assessments, urinalysis, vital signs, 12-lead ECG, and concomitant medications.

Screening will ensure the subject meets inclusion/exclusion criteria and is appropriate for a clinical trial. An independent Massachusetts General Hospital (MGH) SAFER interview will confirm both diagnosis and severity of symptoms. Studies have revealed that external, site-independent assessment of patient eligibility and symptom severity prior to randomization can improve the patient selection process (Fava et al., 2003). The MGH-SAFER assessment will be administered by CTNI trained staff psychiatrists and psychologists from MGH.

Subjects will be recruited from out-patient clinics and those who meet the DSM-IV-TR diagnostic criteria for Schizophrenia and have a total PANSS score of 70 or greater upon screening, with a score of 4 or greater on at least two of the following five items of the PANSS positive subscale: hallucinatory behavior, delusions, conceptual disorganization, suspiciousness, and unusual thought content will be eligible for the trial. If the diagnosis

and appropriateness of the patient for the study is confirmed by an independent SAFER rater, and the subject meets all of the inclusion criteria and none of the exclusion criteria, the subject will be eligible for Phase 1 and will be randomized to one of three treatment sequences: intravenous SNP in Phase 1 and intravenous SNP in Phase 2; placebo in Phase 1 and placebo in Phase 2; or placebo in Phase 1 and intravenous SNP in Phase 2 (1:1:1 ratio) to be administered over 4 hours in a controlled and medically monitored intensive care-type setting (as per institutional guidelines). After a post-treatment debriefing and period of observation (2-3 hours total), the subject will be permitted to return home. Follow-up visits will be conducted in the outpatient setting at Day 7 (Phase 1) and Day 21 (for Phase 2). A final follow-up outpatient visit will be conducted at Day 28. Efficacy, safety and cognitive outcome measures will be collected at each of the study visits.

G. STUDY POPULATION

Sample Size

The study will be conducted at approximately 4 centers within the USA, including hospitals and outpatient psychiatric clinics. A 20% discontinuation rate is anticipated for this SPCD study, thus, a total of 60 subjects will be initially randomized to have at least 48 subjects complete the study. To account for potential screen failures, up to 100 subjects may be consented in order to randomize 60 subjects total.

Subject Eligibility

Inclusion Criteria

Each subject must meet all of the following criteria to be eligible for this study:

1. Males or Females aged 18-65 years inclusive.
2. Primary diagnosis of Schizophrenia established by a structured psychiatric evaluation (SCID) based on Diagnostic and Statistical Manual of Mental Disorders Forth Edition (DSM-IV-TR) criteria.
3. Written informed consent in compliance with 21 CFR part 50 and in accordance with the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines.
4. A Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1994) total score ≥ 70 with a score of ≥ 4 on two or more of the following PANSS items: delusions, conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content.
5. A score of ≥4 on the Clinical Global Impression—Severity (CGI-S) (Guy, 1976).
6. Confirmation of both diagnosis and severity of psychosis symptoms by an independent MGH SAFER interview.

7. Must have ongoing antipsychotic treatment for at least 8 weeks, with a stable dose for at least 4 weeks. Subjects who have failed to achieve clinically-recognized symptom reduction to at least 1 marketed antipsychotic agent, given at a Physician Desk Reference (PDR)-defined therapeutic dose for ≥ 8 weeks during the past 12 months, as assessed by the MGH FAST, will be eligible
8. Understands and is able, willing, and (in the opinion of the investigator) likely to fully comply with the study procedures and restrictions.

Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study.

1. Subjects with a history of renal insufficiency, congestive heart failure, cardiac arrhythmias or history of myocardial infarction.
2. Subjects with a history of symptomatic orthostatic hypotension defined as sitting to standing systolic blood pressure ≤ 90mmHg or diastolic blood pressure ≤ 60mm Hg with any of the following symptoms: lightheaded or dizzy upon standing, blurry vision, weakness, fainting (syncope), confusion, or nausea.
3. Subjects with any clinically significant abnormalities as determined by medical history, physical exam, clinical and lab evaluation suggestive of an underlying disease state that may, in the opinion of the investigator, confound the results of study, increase risk to the subject, or lead to difficulty complying with the protocol.
4. Subjects on chlorpromazine, PDE-5 inhibitors, nitrites and any medication with CNS effects with the exception of antipsychotic drugs (other than chlorpromazine) anticholinergics, b-adrenergic antagonists, amantadine, biperiden, diphenhydramine, lorazepam, zolpidem, and temazepam.
5. Medications which in the opinion of the PI, and in conjunction with the medical monitor, may be expected to significantly interfere with the metabolism or excretion of sodium nitroprusside, and/or may be associated with a significant drug interaction with sodium nitroprusside that may pose a significant risk to subjects' health and/or confound the study data.
6. Pregnant or breast-feeding patients. Women of childbearing potential must have a negative pregnancy test performed at screening visit prior to randomization and prior to baseline at visits 3 and 6. Women enrolled in this trial must use adequate birth control.
7. Subjects with a current (within the last 3 months) DSM-IV-TR diagnosis of alcohol or substance use disorder or dependence (excluding nicotine) as established by the clinical assessment (SCID) at the screening visit will be excluded.
8. Has tested positive for any of the following: cannabis, opioids, cocaine, amphetamines, barbiturates methadone, methamphetamine and phencyclidine at the screening or baseline visits. If positive, the urine drug toxicology screen may be repeated once based on investigator judgment, but due to safety concerns, the result must be negative for the subject to continue in the study.

9. Subjects at imminent risk of suicide or injury to self or others, as per the opinion of the investigator, or history of significant suicide attempt within the last 6 months as per C-SSRS.
10. Subjects that have taken an investigational drug or taken part in a clinical trial within 30 days prior to screening.
11. Any other reason that, in the opinion of the investigator, would compromise patient safety or integrity of the study

Table 1- Exclusionary Safety Values of Potential Clinical Concern

Hematology	
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
Carbon Dioxide (Bicarbonate)	<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 the upper limit of the reference range
Creatine kinase (CK)	>3 times the upper limit of the reference range
Thyroid Stimulating Hormone (TSH)	>1.5 times upper limit of the reference range
Uric acid	>1.5 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

Pregnancy

Serum beta human chorionic gonadotropin (Beta- hCG)	< 6 IU/L negative

Urine Screen for Drugs of Abuse

Per Exclusionary Criteria #6, a subject is excluded from the study if they meet DSM-IV-TR criteria for diagnosis of alcohol or substance use disorder or dependence (excluding nicotine) within the last 3 months as established by the SCID at the Visit 1 (screening). Per exclusionary criterion 8, for all subsequent urine drug screens (performed at Visits 3 and 6) any individual testing positive for a potential drug of abuse without a documented prescription for that medication will be evaluated by the investigator who will determine if the subject should be terminated from the study due to concerns with respect to subject safety and/or confounding of study data.

Rationale for the Inclusion/Exclusion Criteria

Eligibility criteria for this study have been carefully considered to ensure the safety of the study subjects and to ensure that the results of the study are meaningful in support of the research objectives.

Nitroprusside infusion has been widely used for decades to control profound hypertension and most recent reviews have concluded that it is quite safe at doses less than 2 µg/kg/min. The most important adverse reactions to sodium nitroprusside are the avoidable ones of excessive hypotension and cyanide toxicity. (Package Insert http://medlibrary.org/lib/rx/meds/nitropress/ - last revised January 16, 2014).

No adverse effects were observed in 10 schizophrenia subjects who received nitroprusside 0.5 µg/kg/min in the study by Hallak et al., including no cases of hypotension despite administering nitroprusside to two subjects treated with chlorpromazine (500 mg/d and 300 mg/d); chlorpromazine has the greatest propensity for orthostatic hypotension of any antipsychotic and subjects taking this drug will be excluded from the study. Nitroprusside was also added to high-dose ziprasidone (240 mg/d) and high dose risperidone (8 mg/d) without measurable effects on blood pressure or EKG.

The potential for cyanide toxicity is greatest when more than 500 µg /kg of sodium nitroprusside is administered faster than 2 µg /kg/min, as cyanide is generated faster than the unaided patient can eliminate it. Cyanide toxicity will be avoided in this study as the dose administered is very low, and individuals with renal disease will be excluded. Also, the study treatment will be infused within 3 hours of preparation as sodium nitroprusside is stable in solution for 24 hours.

Subjects will be continuously monitored for adverse effects during the infusion and blood pressure and heart rate will be taken and recorded 2 hours post infusion.

Given that subjects with renal disease, a history of orthostatic hypotension or medications that destabilize blood pressure will be excluded from the study, and that the infusion rate of SNP for subjects randomized to active drug in this study will be very low (0.5 µg/kg/min), the risk of excessive hypotension and cyanide toxicity is very low.

H. STUDY ASSESSMENTS – PLAN AND METHODS

Study Conduct

Subjects will sign a HIPAA-compliant informed consent form (ICF) before any study related screening procedures are performed. An overview of the study objectives and a summary of study procedure will be explained to the subject prior to signing the ICF. At any point during the study, subject will be encouraged to seek answers to any questions they may have.

Study Procedures

Medical History

A detailed medical history will be obtained by the PI or designee during Visit 1 (screening visit). 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. Any updates to medical history information that the PI or designee becomes aware of will be captured throughout the study. Medical records will also be requested from the participant's primary care physician for confirmation of relevant eligibility criteria.

Physical Examination

The PI, or medically-qualified designee, will perform the physical examinations (PEs) at Visit 1 (screening) 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 at Visit 1 (screening), which includes mental status, cranial nerves, strength, sensation, deep tendon reflexes, and coordination.

If any clinically significant change is noted from screening, it will be reported as an AE and will be followed up to resolution or upon reaching a stable end point.

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. Vital sign measurements will be obtained at the time points indicated in the SOE and following the supine ECG assessments, if taken at the same time. Blood pressure should be taken on the same arm throughout the study.

If clinically significant findings, as determined by the PI or medically qualified Sub-Investigator, occur in any vital sign measurement, that measurement should be captured

as an adverse event and will be repeated at medically appropriate intervals until the value returns to an acceptable range, a specific diagnosis is established, or the condition is otherwise explained

Anthropometric Measurements

Weight (kg) (assessed in ordinary indoor clothing with shoes off) will be recorded at the time points indicated in the SOE. 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 and is defined as the subject's weight in kilograms divided by the square of the subject's height in meters (kg/m^2). Waist circumference will be measured to the nearest 0.1 cm.

Laboratory Procedures

Laboratory tests will be performed at the site, and each site will provide their laboratory reference ranges to the coordinating center. 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:

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, caluculated 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.

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 at visits 4 and 7.

Laboratory Panels assessed at Visit 1 only:

Serum Pregnancy Test: serum beta human chorionic gonadotropin test for pregnancy in female subjects of childbearing potential and FSH for post-menopausal female subjects will be conducted.

Other: glycosylated hemoglobin (Hb)A1c

Study physicians should mark either “CS” for Clinically Significant or “NCS” for Not Clinically Significant in the margin of the laboratory result source document for items outside the normal range.

12-Lead Electrocardiogram (EGG)

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 PI or medically qualified Sub-Investigator to assess any immediate abnormalities. The findings of the ECGs will be marked by the PI or medically qualified Sub-Investigator as normal, abnormal-not clinically significant, or abnormal-clinically significant.

The QTcB and QTcF will be manually calculated and recorded on a worksheet that will serve as the source document. All ECGs that are considered abnormal and clinically significant should be evaluated for a change from baseline and captured as an AE.

If any clinically significant change is noted from screening or an ECG is considered abnormal and clinically significant, it will be reported as an AE and will be appropriately recorded in both the source documents and the eCRF.

Study Instruments

The following instruments will be administered according to the schedule in the Schedule of Events in Appendix 1:

Diagnostic Instruments

Structured Clinical Interview for DSM-IV-TR™ (SCID I/P): The SCID, administered by the clinician, includes modules aimed at diagnosing possible Axis I disorders (First et al, 1996). Questions here are asked exactly as written, and each is based on the individual criteria from DSM-IV-TR™.

Massachusetts General Hospital- Fast Additive Summary of Treatment (FAST) - This questionnaire (Appendix 2) provides an efficient, structured means of capturing

historical treatments across mood and psychotic disorders. It allows extraction of other historical measures for antidepressants: ATRQ (Fava and Davidson, 1996; Fava, 2003); for lithium: the Alda Scale (Grof JCP 2002): The questionnaire captures treatment duration, response, and reason for discontinuation. In the present study, FAST is a means of recording number and type of prior antipsychotic trials, to define treatment resistance for purposes of stratification.

SAFER Rater Assessment

In order to confirm diagnosis and to ensure patients are appropriate for the study, remote diagnostic assessments (SCID-I/P Psychosis module), and disease severity assessments (PANSS, MGH FAST, and CGI-S) will be performed remotely by an independent MGH CTNI rater. The subject will be provide contact information and a SAFER interview will be scheduled within 27 days of Visit 1 (screening). Sites will be notified of the results within 24 hours of the interview.

Efficacy Measures

The Positive and Negative Syndrome Scale (PANSS) (Kay, 1987) will be used to measure symptom severity in this trial. PANSS is a 30-item questionnaire used to evaluate schizophrenia symptoms, based on the clinical interview as well as reports of family members or primary care hospital workers. PANSS consists of Positive scale (7 items), Negative scale (7 items), and General Psychopathological scale (16 items) sections. Each item (symptom) will be scored on a 7-point scale with higher scores representing increasing levels of psychopathology: 1) Absent, 2) Minimal, 3) Mild, 4) Moderate, 5) Moderate severe, 6) Severe, and 7) Extreme.

Each of the 30 items is accompanied by a specific definition as well as detailed anchoring criteria for all seven rating points. Ratings will be performed by the Investigator or qualified sub-Investigator. The highest applicable rating point is always assigned, even if the subject meets criteria for lower points as well. In judging the level of severity, the rater must utilize a holistic perspective in deciding which anchoring point best characterizes the subject's functioning and rate accordingly, whether or not all elements of the description are observed. The determined results must be documented on the source documents and in the CRF.

For the purpose of this study, the total PANSS score along with sub-scores for Positive scale, Negative scale, General Psychopathology scale, and Composite scale will be derived as follows:

- **Positive Subscale Score:** the total of scores for items P1 through P7. This score could range from a minimum of seven to a maximum of 49, with higher scores representing increasing levels of psychopathology.

- **Negative Subscale Score:** the total of scores for items N1 through N7. This score could range from a minimum of seven to a maximum of 49, with higher scores representing increasing levels of psychopathology.
- **General Psychopathology Score:** the total of scores for items G1 through G16. This score could range from a minimum of 16 to a maximum of 112, with higher scores representing increasing levels of psychopathology.
- **Total PANSS Score:** the total of scores for items P1 through P7, N1 through N7, and G1 through G16. This score could range from a minimum of 30 to a maximum of 210, with higher scores representing increasing levels of psychopathology.

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Clinical Global Impressions–Severity (CGI-S) and Clinical Global Impressions–Improvement (CGI-I) Scales (Guy, 1976): The CGI-S is an observer-rated scale that measures illness severity. The severity is measured using a 7 point Likert scale: 1) Normal, not at all ill, 2) Borderline mentally ill, 3) Mildly ill, 4) Moderately ill, 5) Markedly ill, 6) Severely ill, and 7) Among the most extremely ill patient. The CGI-I is an observer-rated scale that measures illness improvement. Improvement is measured using a 7 point Likert scale: 1) Very much improved, 2) Much improved, 3) Minimally improved, 4) No change 5) Minimally worse, 6) Much worse, 7) Very much worse.

Safety Measures

The Systematic Assessment for Treatment Emergent Effects–Systematic Inquiry (SAFTEE-SI) (Levine and Schooler, 1986): This is a self-rated questionnaire assessing possible adverse events during the course of the trial. The time frame is the past 7 days.

Site personnel should also review the SAFTEE-SI before the subject has left the clinic to check for any symptoms which are endorsed as “moderate” or “severe.” This process is meant to ensure that the study physician is given the opportunity to evaluate these symptoms adequately for adverse event reporting. Study physicians should mark either “CS” for Clinically Significant or “NCS” for Not Clinically Significant in the margin of the source document for items endorsed as moderate or severe.

It is up to the study physician to determine if symptoms represent either a worsening of pre-existing conditions or an unfavorable/ unintended sign, symptom or disease which is temporally related with study participation; and to document AEs in the AE log accordingly.

Abnormal Involuntary Movement Scale (AIMS) (Riezen 1988)
The 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 12-item AIMS to be used in this study consists of five categories, as outlined below:

I. **Facial and Oral Movements** (items 1 through 4) includes an item for each of the following: muscles of facial expression, lips and perioral area, jaw, and tongue. Each item will be assessed using a 5-point scale (0 = none, 1 = minimal (may be extreme normal), 2 = mild, 3 = moderate, and 4 = severe).

II. **Extremity Movements** (items 5 and 6) includes an item for each of the following: upper extremities (including arms, wrists, hands, and fingers) and lower extremities (including legs, knees, ankles, and toes). Each of these items will be assessed using the same 5-point scale as items 1 through 4.

III. **Trunk Movements** (item 7) includes one item for assessing the movements in the neck, shoulders, and hips. This item will be assessed using the same 5-point scale as items 1 through 4.

IV. **Global Judgment** (items 8 through 10) includes three items. Item 8 assesses severity of abnormal movements overall. Item 9 assesses the severity of incapacitation due to the abnormal movements. These two items (i.e., items 8 and 9) will be assessed using the same 5-point scale as items 1 through 4. Item 10 assesses the subject's awareness of his/her abnormal movements, as judged by the subject. For Item 10, the following 5-point scale will be used: 0 = no awareness; 1 = aware, no distress; 2 = aware, mild distress; 3 = aware, moderate distress; 4 = aware, severe distress.

The Columbia Suicide Severity Rating Scale (C-SSRS) (Posner 2007):

The 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. The C-SSRS tool was first developed for a prospective national study of treatment for adolescent suicide attempts. C-SSRS was developed by reliance on evidence stemming from two decades of research. It contains a 1-to-5 rating scale for suicidal ideation of increasing severity (from a "wish list to die" to an "active thought of killing oneself with plan and intent"). The time frame is the past six months for the Baseline/Screening scale and since the last visit for the Since Last Visit scale.

Neurocognitive Measures

MATRICS: The Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB) (Nuechterlein 2008): The MATRICS Consensus Cognitive Battery is the standard tool for assessing cognitive change in trials of cognitive-enhancing agents in schizophrenia. The MCCB domains include: Speed of Processing; Attention/Vigilance; Working Memory; Verbal Learning; Visual Learning; Reasoning & Problem-Solving; and Social Cognition and the battery takes about 80 minutes to complete. The MCCB is accepted by the US FDA as a primary outcome measure for registry trials for cognition in schizophrenia. The MCCB composite score represents a global measure of cognition and is than outcome measure for inclusion in the efficacy analyses. MCCB will be administered at Visits 3 (baseline), 6, and 9.

The University of California San Diego (UCSD) Performance-based Skills Assessment Brief (UPSA-B) (Mausbach et al., 2007) consists of two of the five original UPSA domains, finances and communication, and has been shown to be highly correlated with the long form of the UPSA and to have substantial test–retest reliability (Green et al., 2011). Each subscale contributes 50 points; total scores range from 0 to 100 points with higher scores reflecting better performance. The UPSA-Brief requires approximately 10–15 minutes to complete and may be administered by a suitably trained lay professional. The UPSA-B will be administered at Visits 3 (baseline), 6, and 9.

Study Visit Assessment Schedule

Screening

Screening will take place from Visit 1 (Day -28 to -14) through Visit 2 (Day -13 to -3). All screening assessments must be completed prior to randomization.

Visit 1 (Consent, Day -28 to -14)

The following procedures will be conducted at Visit 1 (screening) for all subjects:

- The signed informed consent will be obtained before any screening or other study related procedures are initiated.
- Assignment of a subject ID. When a subject signs the ICF, she/he will be assigned a unique subject ID in numerical sequence. The subject ID will be recorded in the source document and eCRF.
- The DSM-IV-TR diagnosis of Schizophrenia will be made using the Structured Clinical Interview (SCID) and documented by an adequately trained clinician.
- The PANSS will be performed to assess symptoms associated with Schizophrenia. Subjects are required to have a total PANSS score of 70 or greater, and have a score of $\geq$4 on at least two of the following five items of the PANSS positive subscale: hallucinatory behavior, delusions, conceptual disorganization,

suspiciousness, or unusual thought content at screening in order to be included in the trial.

- The MGH FAST, a structured questionnaire for recording psychotropic treatment, will be used to assess prior antipsychotic and other psychotropic trials.
- The CGI-S will be performed.
- Recording of the subject's comprehensive medical history, including medical and psychiatric conditions;
- Subject's demographic information and tobacco use will be obtained
- Recording a complete history of use of central nervous system (CNS)-active compounds, including details (eg, drug name, dose, and frequency), as well all other medications, including prescription and non-prescription medications, taken within 30 days of screening.
- After a minimum 5 minute rest in the seated position, vital sign measurements (systolic and diastolic blood pressure, pulse, respiration rate, and oral temperature).
- Anthropometrics (height, weight, waist circumference and body mass index (BMI))
- Fasting blood samples will be collected at screening for clinical laboratory tests including hematology and serum chemistry (using a venous blood sample)
- Urine will be collected for urinalysis
- For female subjects of childbearing potential, a serum pregnancy test.
- For female subjects that are post-menopausal, an FSH test, using a venous blood sample.
- Urine drug screen (cannabis, opioids, cocaine, amphetamines, methadone, cannabinoids, barbiturates, benzodiazepines, methamphetamine, and phencyclidine).
- HbA1c testing using a venous blood sample.
- Physical examination (including a neurological examination).
- 12-lead ECG will be performed at rest after the subject has been lying down for approximately 5 minutes. ECG will be evaluated at the investigational site to determine the subject's eligibility and to monitor safety.
- The Columbia Suicide Severity Rating Scale (C-SSRS) will be conducted and together with the clinical opinion of the investigator will be used to assess risk for suicide.
- AEs will be monitored starting at the time the informed consent is signed.
- Review of the inclusion/exclusion criteria.

A blood sample will be collected at Visit 1 (screening) to obtain DNA to enable pharmacogenetic studies. Samples will be sent to The Stanley Center for Psychiatric Research at the Broad Institute at Massachusetts Institute of Technology in Cambridge, Massachusetts for banking and future extraction/genotyping. The MGH CTNI will ensure that appropriate informed consent process and privacy and de-identification procedures are in place for the collection of biomaterials.

Visit 2 (Day -13 to -3)

The following procedures will be conducted at Visit 2 for all subjects:

- MGH SAFER Interview
- MGH-FAST
- PANSS
- CGI-S
- Randomization

Randomization: When all of the screening assessments, including the SAFER assessment, have been completed and entered into the CTMS, the subject's status will be moved to eligible and the CTMS will generate a randomization ID for the subject. This ID will be accessible to the site pharmacy and will be used to prepare the corresponding study treatment. Once a subject has been assigned a Randomization ID, they will be identified in the CRF by this Randomization ID for the duration of their study participation. The subject ID should no longer be used.

Phase 1

Visit 3 (Baseline, Day -2 to -1)

The following procedures will be conducted at Visit 3 for all subjects:

- Concomitant Medications
- Vital Signs
- Anthropometrics (height, weight, waist circumference, BMI)
- 12-Lead ECG
- Urine Drug Screen
- PANSS
- CGI-S
- CGI-I
- MATRICS
- UPSA-B
- AE/SAE Assessment
- AIMS
- SAFTEE-SI
- C-SSRS

Visit 4 (Phase 1 Study Treatment, Day 0)

Anthropometrics, vital signs, urinalysis, and concomitant medications will be collected for all subjects at the start of Visit 4 (Hours -1 to 0) prior to initiating the infusion. Subjects

will also have blood sample drawn for chemistry and electrolytes. Female subjects of childbearing potential will have a pregnancy test prior to the infusion. If pregnancy test is positive, subject will not continue with the infusion. Subjects who use tobacco will be provided transdermal nicotine in order to prevent withdrawal symptoms. The nicotine replacement will be based on the number of cigarettes per day (cpd) or tobacco equivalent collected at screening.

- ≥15 cpd: 21 mg patch
- 8-15 cpd: 14 mg patch
- 5-8 cpd: 7 mg patch
- <5 cpd: as needed nicotine gum or 2mg lozenges (1 piece every 3-4 hours)

Intravenous sodium nitroprusside or Placebo infusion will take place during Hours 0-4. Cardio-pulmonary monitoring will take place throughout intravenous infusion (see Infusion Monitoring and Discharge Procedures).

Following completion of infusion, the subject will have a post-infusion debrief. The following measurements will also be collected for all subjects between hours 4 and 7:

- Vital Signs
- 12-Lead ECG
- AE/SAE Assessment
- AIMS
- SAFTEE-SI
- PANSS
- CGI-S
- CGI-I

Following the completion of all study assessments, a study psychiatrist or nurse practitioner will perform discharge procedures in order release the subject at the end of the visit.

Visit 5 (Phase 1 Follow Up, Day 7)

The following procedures will be conducted at Visit 5 for all subjects:

- Concomitant Medications
- Vital Signs
- Anthropometrics (weight, height, waist circumference, BMI)
- 12-Lead ECG
- PANSS
- CGI-S
- CGI-I

- AE/SAE Assessment
- C-SSRS

Phase 2

Visit 6 (Phase 2 Baseline, Day 13)

The following procedures will be conducted at Visit 6 for all subjects:

- Concomitant Medications
- Vital Signs
- Anthropometrics (weight, height, waist circumference, BMI)
- 12-Lead ECG
- Urine Drug Screen
- PANSS
- CGI-S
- CGI-I
- MATRICS
- UPSA-B
- AE/SAE Assessment
- AIMS
- SAFTEE-SI
- C-SSRS

Visit 7 (Phase 2 Infusion, Day 14)

Anthropometrics, vital signs urinalysis, and concomitant medications will be collected for all subjects at the start of Visit 7 (Hours -1 to 0) prior to initiating the infusion. Subjects will also have blood sample drawn for chemistry and electrolytes. Female subjects of childbearing potential will have a pregnancy test prior to the infusion. If pregnancy test is positive, subject will not continue with the infusion. Subjects who use tobacco will be provided transdermal nicotine in order to prevent withdrawal symptoms. The nicotine replacement will be based on the number of cigarettes per day (cpd) or tobacco equivalent collected at screening.

- ≥15 cpd: 21 mg patch
- 8-15 cpd: 14 mg patch
- 5-8 cpd: 7 mg patch
- <5 cpd: PRN nicotine gum or lozenges 2 mg (1 piece every 3-4 hours)

Intravenous sodium nitroprusside or Placebo infusion will take place during hours 0-4. Cardio-pulmonary monitoring will take place throughout intravenous infusion (see Infusion Monitoring and Discharge Procedures).

Following completion of infusion, the subject will have a post-infusion debrief. The following measurements will be collected for all subjects between hours 4 and 7:

- Vital Signs
- 12-Lead ECG
- AE/SAE Assessment
- AIMS
- SAFTEE-SI
- PANSS
- CGI-S
- CGI-I

Following the completion of all study assessments, a study psychiatrist or nurse practitioner will perform discharge procedures in order release the subject at the end of the visit.

Visit 8 (Phase 2 Follow Up, Day 21)

The following procedures will be conducted at Visit 8 for all subjects:

- Concomitant Medications
- Vital Signs
- Anthropometrics (weight, height, waist circumference, BMI)
- 12-Lead ECG
- PANSS
- CGI-S
- CGI-I
- AE/SAE Assessment
- C-SSRS

Visit 9 (Phase 2 Final Follow Up/Study Completion, Day 28)

The following procedures will be conducted at Visit 9 for all subjects:

- Concomitant Medications
- Vital Signs
- Anthropometrics (weight, height, waist circumference, BMI)
- 12-Lead ECG
- CBC with differential and electrolytes
- PANSS

- CGI-S
- CGI-I
- MATRICS
- UPSA-B
- AE/SAE Assessment
- AIMS
- SAFTEE
- C-SSRS

Safety Profile of Sodium Nitroprusside

We recognize that acceptability of risk may differ across various treatment populations. However, we believe the risk-benefit ratio to be similar for sodium nitroprusside's current indication and the proposed use in this study. In fact, as the dose being tested in this study is very low, we anticipate the risk to be modest at most.

Recent reviews have concluded that sodium nitroprusside has an excellent safety profile at doses less than 2 µg/kg/min. The most important adverse reactions are the avoidable ones of excessive hypotension and cyanide toxicity. (Package Insert http://medlibrary.org/lib/rx/meds/nitropress/ - last revised January 16, 2014). No adverse effects were observed in 10 schizophrenia subjects who received nitroprusside 0.5 µg/kg/min in the study by Hallak et al., including no cases of hypotension despite administering nitroprusside to two subjects treated with chlorpromazine (500 mg/d and 300 mg/d); chlorpromazine has the greatest propensity for orthostatic hypotension of any antipsychotic and subjects taking this drug will be excluded from the study. Nitroprusside was also added to high-dose ziprasidone (240 mg/d) and high dose risperidone (8 mg/d) without measurable effects on blood pressure or EKG.

The potential for cyanide toxicity is greatest when more than 500 µg /kg of sodium nitroprusside is administered faster than 2 µg /kg/min, as cyanide is generated faster than the unaided patient can eliminate it. 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. Subjects will be continuously monitored for adverse effects during the infusion and blood pressure and heart rate will be taken and recorded hourly for 2 hours post infusion.

Given that subjects with renal disease, cardiac disease, hypotension or medications that destabilize blood pressure will be excluded from the study, and that the infusion rate of SNP for subjects randomized to active drug in this study will be low (0.5 µg/kg/min), the risk of excessive hypotension and cyanide toxicity is low.

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 course of the study.

Infusion Monitoring and Discharge Procedures

The study treatment will be infused with a volumetric infusion pump and the blood pressure of a subject will be continuously monitored by a cardiologist, anesthesiologist, or medically qualified designate as per institutional guidelines. A study physician will be available for consultation on any participant safety issues that arise during the infusion.
Each participant will be allowed to rest in a semi-recumbent position. Each subject will receive an intravenous line. The study treatment will be infused with a volumetric infusion pump and the blood pressure of a subject will be continuously monitored by a cardiologist, anesthesiologist, or medically qualified designate as per institutional guidelines. To maintain the double blind, each subject will receive an infusion for 4 hours. Nitroprusside or placebo will be administered at the dose specified in the randomization scheme. Subjects will have one-to-one nursing care during the infusion of the study treatment.
The assigned nurse or medically qualified designate will perform the following monitoring procedures, as developed by the consulting cardiologist:

- Pre-Infusion: 12-Lead ECG (Time -1 to Time 0, baseline)
- During Infusion:
    - 12-Lead ECG @ 30 min
- Post-Infusion:
    - 12-Lead ECG @ 60 min post-infusion
- Vital Signs: BP, HR and O2 sat (same arm, non-IV arm)
  Pre-Infusion (baseline)
  During Infusion:
    - Q5 min Xs 30 minutes then Q10 min Xs 3.5 Hrs (210 min)
  Post-Infusion:
    - Q5 min Xs 30 minutes then Q10 min Xs 1.5 Hrs (90 min)

A 10-minute run-in period (“Phase I”) has been added to ensure that the patient tolerates the drug. As determined by consulting cardiologist, this will include the administration of the drug at half of the rate (0.25 mcg/kg/min). The following Safety parameters will be followed for Phase I:

Safety Parameters
Safety Parameters Phase I dose:
-If SBP $\leq$ 89 STOP infusion. Lie flat, feet up, call N.P. & P.I.
-If HR > 120 bpm or >20 bpm over baseline HR, abort study
-If SpO2 <92%, stop infusion
-If patient reports headache, ask: “do you feel this headache is tolerable?”
    -If patient says “yes,” continue dose
    -If patient says “no,” STOP dose

-If patient experiences symptomatic hypotension (nausea, dizziness) STOP infusion.

If Phase I is tolerated per the above guidelines after the 10 minute run-in period, the drug will be administered at the rate of 0.5 mcg/kg/min ("Phase II") with the following parameters:

Safety Parameters for Phase II dose:
- If SBP between 81 and 89, lower dose to Phase I dose for 15 min. Monitor with CRC NP.
- If SBP rises to > 89mm Hg continue Phase I dose
- If SBP remains between 81 and 89mm Hg abort and call P.I.
- If SBP < 80, STOP infusion, lie flat, feet up & call P.I.
- If HR > 120 bpm or > 20 bpm over baseline HR, lower dose to Phase I dose for 15 min
- If SpO2 < 92% Stop infusion
- 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, STOP dose
- If patient experiences symptomatic hypotension (nausea, dizziness) *lower dose to Phase I dose for 15 min*
- If better, continue Phase I dose
- *If not, Stop infusion, call P.I.*

Should the patient require PRN rescue medications, the following procedures will take place:

PRN Rescue medications:
In consultation with CRC N.P.
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 IV line will be left in place for at least 1 hour following the infusion to allow for additional medications to control emergent side effects. If additional IV medication is necessary, an anesthesiologist, cardiologist, or medically qualified designate will start the infusion and a medical team will be present during the entire infusion period.

A study psychiatrist or medically qualified designate will release the subject at the conclusion of the testing period at the end of the visit. A trained research assistant along with a research nurse 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. 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 psychiatrist or medically qualified designate 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.

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.

Reliability Training

All physicians/raters involved in the assessment of study subjects will use standardized clinician-rated measures such as the SCID I/P, the PANSS, and CGI-S. Training in the use of these instruments occurs through videotaped and live interviews of mock patients. Both intra-individual and inter-individual reliability measures will be reported.

Patient Adherence to Protocol

Every effort will be made to encourage patients to comply with the procedures and the assessments involved in study. Patients will be compensated for their time at each visit, at a rate determined appropriate by each individual IRB (estimated compensation: $20.00/hour). This level of subject compensation, while non-coercive, facilitates adherence to assessments and procedures that require weekly visits.

Subjects and clinicians will be asked to guess which arm of the study the subjects were in at the end of the study to assess if blinding was successful.

Evaluation of Adverse Events (AEs)

Definition

In accordance with International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) guidance, any study related event incurred by a subject that occurs after the first study-related procedure to the completion of the protocol-defined safety surveillance period, that represents a change (positive or negative) in frequency or severity from a baseline (pre-study) event (if any), regardless of the presence of causal relationship or medical significance, is a reportable AE.

Abnormal results of diagnostic procedures, including laboratory test abnormalities, are considered AEs if they:

- Result in discontinuation from the study,
- Require treatment or any other therapeutic intervention,

- Require further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality), or
- Are associated with clinical signs or symptoms that would have a significant clinical impact, as determined by the PI.

Performing Adverse Events Assessments

The PI is ultimately responsible for assessing and reporting all adverse events as outlined in the protocol. The assessment of AEs may be delegated to a medically qualified Sub-Investigator, trained on this study protocol, who is listed on the FDA Form 1572 or equivalent document, and on the delegation of authority form.

AEs should be volunteered by the subject, or solicited from the subjects using a standard statement, from examination of the subject at a clinic visit, or from observations of clinically significant lab values or special exam abnormal values. AEs will not be solicited by the use of a specific list of anticipated events.

All AEs are to be assessed and recorded in a timely manner and followed to resolution or until the Investigator determines that there is not an anticipated resolution. Each AE is to be documented with reference to severity, date of occurrence, duration, treatment, and outcome. Furthermore, each AE is to be classified as being serious or non-serious (as per definitions). In addition, the PI or delegated medically qualified Sub-Investigator must assess whether the AE is drug-related or not. Changes in severity of AEs and resolution dates should be documented as separate events.

Timing

AEs will be captured from the first study-related procedure through to the completion of the protocol defined safety surveillance period. For the purposes of this study, the period of observation for collection of AEs extends from the time the subject gives informed consent until the final visit.

Surgical procedures, planned before enrollment of the subject in the study, are not considered AEs if the condition was known before study inclusion. In this case the medical condition should be reported in the subject's medical history. Intermittent adverse events will not be reported as multiple adverse events. The definition of an intermittent adverse event is "a recurring event of consistent severity, frequency, and causality."

Severity

Each AE is to be documented with reference to severity. Changes in severity of AEs and resolution dates should be documented as separate events. Any event that ameliorates over more than one assessment time point may be considered for listing as a single event at the highest severity.

Table 2. Definition of Intensity of Adverse Events

Intensity	Definition
Mild	Causes transient or mild discomfort; no limitation of usual activities; no medical intervention required.
Moderate	Causes mild to moderate limitation in activity; some limitation of usual activities: no or minimal medical intervention or therapy is required.
Severe	Causes marked limitation in activity; some assistance is usually required; medical intervention or therapy is required; hospitalization is probable.

The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is not the same as “serious,” which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Relationship

The PI or a medically qualified Sub-Investigator, trained on this study protocol, listed on the 1572 or equivalent document and on the delegation of authority form, is responsible for determining the adverse event relationship to the investigational product.

The following categories will be used to define the relationship of an AE to the administration of the IMP:

- Not Related: Data are available to identify a clear alternative cause for the AE other than the investigational product.
- Related: The cause of the AE is related to the investigational product and cannot be reasonably explained by other factors (e.g., the subject’s clinical state, concomitant therapy, and/or other interventions).

Expectedness

An unexpected AE is any AE, the nature and severity of which is not consistent with the applicable product information (e.g., Package Insert of product characteristics for an approved product).

Clinical Significance

The PI or a medically qualified Sub-Investigator, trained on this study protocol who is listed on the 1572 or equivalent document and on the delegation of authority form, is responsible for determining the clinical significance of abnormal results (e.g., labs, ECG results) for the subject.

Clinical Laboratory Adverse Events

Changes in laboratory values or vital signs, or other safety parameters (e.g., ECG, study assessments) as noted in the protocol, are a subset of AEs and are reportable only if considered to be clinically significant by the PI or medically qualified Sub-Investigator.

Screening assessment exams are differentiated from adverse events/symptoms that are incurred post informed consent. Pre-dose abnormal results without clinical symptoms will not be reported as adverse events.

Pregnancy

If a subject (or subject's partner) becomes pregnant during the study, it must be reported in within 24 hours of the time the investigator becomes aware of the event and in accordance with the procedures described on the Pregnancy Report Form. Pregnancy in itself is not regarded as an AE unless there is a suspicion that a study drug may have interfered with the effectiveness of a contraceptive medication. Any pregnancy that occurs from Day 1 to 30 days following the last dose given will be followed for Serious Adverse Events (SAEs).

Evaluation of Serious Adverse Events (SAEs)

Definition

An SAE is defined by federal regulation as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening event
- Hospitalization or prolongation of hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

Reporting Serious Adverse Events

All SAEs will be reported to MGH-CTNI by the PI through telephone or email within 24 hours of discovery, using the form provided by the CTNI.

In the event of a SAE, the PI or designate will notify the MGH-CTNI Medical Monitor by phone or email within 24 hours of the event and will email supporting documentation

within 48 hours of the event and then as relevant data is available within the following week.

The PI must inform the IRB immediately regarding any AE (does not have to be causally related) that is both serious and unexpected; or that represents a series of AEs that, on analysis, is unanticipated, or occurs at an unanticipated frequency, or otherwise represents an unanticipated safety risk to the study subject. The IRB may subsequently choose to modify the informed consent or request changes to the protocol.

Treatment-Emergent Adverse Events

A treatment emergent adverse event (TEAE) is an AE that either began following initiation of study treatment or was present prior to the initiation of the treatment, but increased in frequency or severity following initiation treatment, regardless of causality.

All TEAEs (post infusion) will be captured on the subject source documents for all required assessments and data transferred to the eCRF. The reporting PI or medically qualified Sub-Investigator will sign and date the source document and eCRF.

Procedures and follow-up of Subjects Experiencing AEs after Completion of or Withdrawal from the Study

If a subject experiences the onset of a SAE within a period of 30 days following study completion and, in the opinion of the PI or medically qualified Sub-Investigator, it is associated with the study, it will be followed up and reported as described above.

Protocol Waivers and Violations

Protocol Waivers

Protocol waivers will be assessed on a case by case basis by the medical monitor and the principle investigator for this study.

Procedure for Non-Compliant Subjects

Subjects who miss a study visit will be allowed (at the discretion of the PI) to make up that visit the next day. Subjects who miss more than one visit will be discontinued from the study. Subjects who withdraw consent will no longer have data collected for the study. Every effort will be made to maintain contact with patients who are discontinued, but who do not withdraw consent, according to the study schedule for 30 days post-treatment in order to collect safety data.

Protocol Deviation and Violation Definitions

Protocol violations as defined in the table below will be reported as described in the Manual of Operations.

Table 3. Major and Minor Protocol Violations

	Major Protocol Violation	**Minor Protocol Violation**
The list of examples is intended as a guide and is not all-inclusive.		
Definition	A violation that **<u>may</u>:** Impact subject safety, Affect the integrity of study data, and/ or Affect the subject's willingness to participate in the study.	A violation that **<u>does not</u>:** Impact subject safety, Compromise the integrity of study data, and/ or Affect the subject's willingness to participate in the study.
Examples ***(not all-inclusive)***	Failure to obtain informed consent Informed consent obtained by an unauthorized individual Enrollment of a subject who did not meet eligibility criteria for whom a protocol exception was not obtained Performing a study procedure that is not approved by the IRB and/or is not in the protocol Failure to perform a required lab test that, in the opinion of the Site Investigator, may affect subject safety or data integrity Failure to perform or follow a study procedure that, in the opinion of the Site Investigator, may affect subject safety or data integrity Failure to follow safety (AE) management plan Failure to report a SAE to the IRB and/or Coordination Center	Implementation of unapproved recruitment procedures Only a photocopy of the signed/ dated consent form is available (the original is missing) Pages are missing from the signed/ dated informed consent form Use of invalid consent form (i.e. without IRB approval or outdated/expired form) Failure to perform or follow an approved study procedure that, in the opinion of the Site Investigator, **does not** affect subject safety or data integrity Study procedure conducted out of sequence Failure to perform a required lab test Missing lab results Study Visit out of approved window Over-enrollment Enrollment of subjects after IRB approval of the study has expired

		Failure to submit a continuing review application to the IRB before study expiration
Reporting Requirements	Record the date discovered, date occurred, description of event in the Protocol Deviation Log. Notify the Coordinating Center within 24 hours.	Record the date discovered, date occurred, description of event in the Protocol Deviation Log. Notify the Coordinating Center

I. TREATMENT

Dosing

Subjects will receive either sodium nitroprusside diluted with 5% dextrose and infused at a rate 0.5 µg/kg/min for 4 hours, or a placebo solution of 5% dextrose infused over 4 hours. The study treatment will not be exposed to light prior to and during the infusion as exposure of SNP to light has the potential to form cyanide ions. Subjects will be recumbent and blood pressure, heart rate, blood oxygen saturation, and ECG will be continuously monitored during infusion of the study treatment.

Study Product Supplies and Administration

The Institutional Pharmacy will issue either undiluted sodium nitroprusside in sterile 5% dextrose or sterile 5% dextrose only. For subjects randomized to sodium nitroprusside, an unblinded pharmacist will dilute the SNP with 5% dextrose to achieve a concentration allowing infusion of 0.5 µg/kg/min over the course of 4 hours.

Packaging and Labeling

Sodium nitroprusside or placebo will be prepared within 12 hours from the scheduled delivery time and once prepared in solution will be protected from UV light by covering the iv bag with an aluminum foil bag. The intravenous drip chamber will also be covered for blinding purposes but the tubing does not need to be covered.

A study label that includes the subject ID and randomization ID will be pasted to the foil bag so that it is clearly visible. The dextrose solution will also be covered with an identical foil bag and study label so that medical personal administering the i.v. will be blinded to the study treatment.

Dose Modification during Treatment

As previously stated, the subject's blood pressure will be monitored continuously during the study treatment. If the subject's systolic blood pressure falls below 90 mm Hg the

infusion rate will be reduced by 50% and a drop below 80 mm Hg will terminate the infusion. Nitroprusside has a circulating half-life of 2 minutes and blood pressure is usually restored within 1-5 minutes of stopping the infusion. Trendelenburg position and IV fluids will be used if subjects experience symptomatic hypotension. The medical monitor will receive all safety data within 24 hours after each infusion and will inform the PI who will inform the IRB of any safety concerns.

Possible 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.

Concomitant Therapy

The subject may not have adjustment of psychotropic medications except for benzodiazepines or anticholinergic agents if deemed absolutely necessary by the Investigator for relief of transient symptoms. (See Appendix 3: Medications Allowed and Not Allowed as Concomitant Medications). Subjects must be questioned at each study visit concerning any new medications or changes in current medications including over-the-counter medication and topical medication. All medications and therapies administered or taken by the subject beginning 30 days prior to signing the ICF and throughout the study will be recorded. The subjects' antipsychotic medication type should have remained unchanged for at least eight (8) weeks prior to screening into the study, at constant dose for at least 4 weeks, and should be expected to remain unchanged during the study.

For each medication and non-study treatment, the following will be documented:

- Medication/treatment name (generic name may be used if trade name is unknown)
- If an anti-psychotic medication, whether typical or atypical.
- Dose, unit, and frequency of dosing (individual dosages, not total daily dose). Note: Each new dose of medication should be recorded as a separate entry, with the exception of medications that are given on a sliding scale. For these, it is acceptable to enter the range of the dosage, including the start and stop dates for which the specified dosage range was used.
- Route of dosing
- Indication for use
- The start date
- The stop date (if medication/therapy is not ongoing)

Discontinuation from Study Treatment

Every effort will be made to keep the patient in the study for the full study period consisting of two phases of treatment with follow-up assessments. Acceptable reasons for early discontinuation include the following: 1) request of patient, 2) decision of physician, 3) serious adverse event, and 4) protocol violation.

Medical Reasons for Discontinuation:

Treatment (infusion) discontinuation will be determined by the criteria outlined in the Infusion Monitoring and Discharge Procedures section of this Protocol. A study physician will be available for consultation on any participant safety issues that arise during the infusion.

A patient who decides to discontinue participation in the study will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an investigator. AEs will be followed up until resolution.

Procedures for Discontinuation from Study:

If a patient discontinues from the study before randomization, then no further follow-up will be expected. However, if the patient discontinues after randomization, but before receiving any study treatment, the patient will be asked to return for a final study visit, during which the procedures outlined in the Visit 9 Final Visit/Early Termination Visit procedures will be completed, including AEs and concomitant medication assessments.

If a patient discontinues from the study before completion and has received the dose of study drug, the patient will be asked to return for a final study visit, at which the procedures outlined in the Early Termination Visit will be completed. Every effort should be made to follow up with patients who discontinue from the study prior to Visit 9 (Day 28). If patients refuse to return to the clinic for the study-related assessments, a modified follow-up through, for example, regular telephone contact or a contact at study closure should be arranged, if agreed to by the patient and in compliance with local data privacy laws/practices. If the patient refuses follow-up, the reason for the refusal and last contact date should be documented in the eCRF and source documents.

Patients who discontinue from the study will not be replaced.

If an Early Termination Visit is warranted, the following procedures will be performed:

- Concomitant medications
- Vital Signs
- 12-lead ECG
- Anthropometrics (weight, height, waist circumference, BMI)
- CBC with differential and Comprehensive Metabolic Panel (CMP)
- Urinalysis
- Serum Pregnancy test
- PANSS
- CGI-S

- CGI-I
- AE/SAE assessment
- AIMS
- SAFTEE
- C-SSRS
- MATRICS
- UPSA-B

Unblinding Procedure

Methods for Ensuring Blinding

The investigator, subject, and study staff will be blinded. The preparation and labeling of the study drugs will be performed by the site pharmacy in a way to ensure blinding throughout the study.

No members of the study team at study sites, will have access to the randomization scheme during the conduct of the study, with the exception of the Site's unblinded pharmacist or nurse as designated by the PI.

The randomization schedule for blinding of randomized treatment will be maintained by MGH CTNI or representative and will not be disclosed until after database lock.

During the infusion visits, the study staff conducting the clinical efficacy assessments must not be able to view the bag containing the infusion solution to ensure that they remain blinded. The bag must be protected from view with an opaque covering.

Methods for Unblinding the Study

Individual treatment codes, indicating the treatment randomization for each randomized patient, will be available to the investigator(s) or pharmacists from MGH CTNI or its designee.

The treatment code must not be broken except in medical emergencies when the appropriate management of the patient necessitates knowledge of the treatment randomization. The investigator documents and reports the action to MGH CTNI or representative, without revealing the treatment given to the patient to MGH CTNI or representative's staff.

MGH CTNI retains the right to break the code for serious adverse events (SAEs) that are unexpected and are suspected to be causally related to a study drug and that potentially require expedited reporting to regulatory authorities. Treatment codes will not be broken

for the planned analyses of data until all decisions on the evaluability of the data from each individual patient have been made and documented.

J. ETHICAL CONSIDERATIONS

Risk/Benefit Assessment

The clinical use of nitroprusside has been restricted due to concerns about cyanide toxicity at high doses. In particular, the FDA established a black box warning for doses greater than 2 µg/kg/min. Upon review of the literature the threshold for safety with nitroprusside is probably substantially higher than a dose of 5 µg/kg/min (Kristiansen 2010). Recent work has suggested that neurotoxicity associated with high-dose nitroprusside is mediated by iron-related free radicals and can be prevented by the anti-oxidant, glutathione (Nong 2003) or by hydroxocobalamine (vitamin B12) (Burgdorf 2011). However, Hallak et al reported no adverse events (including hypotension) in association with an infusion rate of 0.5 µg/kg/min for 4 hours of nitroprusside, and the efficacy of the treatment was evident within 4 hours of treatment and persisted for 4 weeks post infusion. Thus, this study will potentially help provide a novel treatment for individuals with Schizophrenia. If this intervention is effective, these results will lead to a full clinical development program of nitroprusside as a treatment for Schizophrenia.

The treatment has been selected with consideration of safety in mind. Additionally, exposure to the intervention will be brief and carefully monitored. The risks of participation in the study are therefore judged to be small, and adequate protections are in place to monitor the medical wellbeing of participants.

The aforementioned data suggest that risks to subjects are minimal. The benefit to society from the development of efficacious interventions for schizophrenia would be a substantial public health benefit.

Informed Consent

The Investigator must ensure that patients are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB or Ethics Committee.

All subjects will receive the consent form for the study. Any questions, concerns, or ambiguities will be clarified by the site's PI or another study clinician prior to the patient signing consent. Subjects will sign informed consent and only then will begin participation in the study.

IRB or Ethics Committee Review

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the IRB for the protocol, consent form, patient recruitment materials and process (e.g., advertisements), and any other written information to be provided to subjects.

The Investigator should provide the IRB with reports, updates, and other information (e.g., Safety Updates, Amendments, and Administrative Letters) according to regulatory requirements and Institution procedures.

A detailed list of required regulatory documents also to be submitted to MGH CTNI will be sent upon final approval of the protocol.

K. DATA HANDLING AND RECORD KEEPING

A Clinical Trials Management Software (CTMS) package with a Microsoft SQL server database and internet browser interface will be implemented for data management. The system will be HIPAA compliant with transmission security, 21 CFR Part 11 compliance, audit trails, electronic signatures, user authentication, study site and role–base security and datasets can be extracted as de-identified data sets. The system uses electronic data capture (EDC) allowing data assessments to be entered directly into the browser interface. It supports multi-site projects with separate security for each site including Serious Adverse Event (SAE) Reporting.

Study specific data that has been outlined in the protocol will be entered into the clinical database via the EDC system by designated site staff in accordance with the eCRF Completion Guidelines. Data is verified electronically using a series of on-line programmed edit checks that have been created by the clinical data manager and programmed by the clinical data programmer or designee. Data discrepancies will be brought to the attention of the site staff and investigated by the clinical monitor and site study coordinator. The clinical monitor(s) will review and verify all eCRF data against acceptable source documentation during scheduled monitoring visits. The clinical monitor will work closely with the site study coordinator to address any discrepancies which have been found so that resolutions can be made and documented into the clinical database. An audit trail within the system will track all changes made to the data.

DATABASE Quality Assurance

The clinical database will be reviewed and checked for omissions, apparent errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification will be generated and addressed by the investigational site.

Only authorized personnel will make corrections to the clinical database, and all corrections will be documented in an audit trail.

This study will be organized, performed, and reported in compliance with the protocol, SOPs, working practice documents, and applicable regulations and guidelines. Site visit audits will be made periodically by a contract research organization's qualified compliance auditing team, which is an independent function from the study conduct team.

Record Retention

All documents pertaining to the study, including all versions of the approved study protocol, copy of the informed consent document and Health Insurance Portability and Accountability Act (HIPAA) documents, eCRFs, source documents (i.e., subject records, hospital records, laboratory records, medication records, etc.), and other study-related documents will be retained at the study site for 5 years after the study ends.

The PI must notify and obtain approval in writing from the CTNI prior to destruction of any study records or provide an opportunity for the CTNI to collect such records. If the Investigator withdraws from the study (e.g., relocation, retirement) all study-related records should be transferred, in a written agreement with the CTNI, to a mutually agreed upon designee and specified timeframe.

Data Confidentiality

Potential risks to data confidentiality will be mitigated by requirements for the de-identification of all study data and by security protocols for all data capture systems. All users of the EDC system will be tracked and provided access in a secure fashion following established SOPs for this process.

As with all research data, information gathered by the study will be used only for aggregate analysis; it will not be released with any information that identifies research participants. Pharmacogenetic information, in particular, will be coded and unlinked to individual respondent identifiers. The data manager, statistician, Investigator, and sponsor do not have access to the identities of patients. That information is retained only at the clinical centers. Uses and risks related to data collection will be outlined in the informed consent and reviewed with the subjects.

L. MONITORING AND OVERSIGHT

Evaluation of Study Sites

Study sites selected by MGH CTNI will provide facility details and site capabilities, past performance in similar studies, investigator, and staff experience, ongoing studies at the site, projected enrollment in this study, and FDA or other agency audit findings. Study sites may be asked to complete a study-specific Site Selection Questionnaire and other

documents for consideration for participation and MGH CTNI or clinical study monitors will complete a remote Site Qualification Visit prior to completing the evaluation.

Initiation of Study Sites

Prior to subject enrollment, a study initiation visit or evaluation will be completed at each investigational site to ensure the following: IRB approval has been obtained and documented prior to subject screening, the Investigators and study personnel are appropriately trained and clearly understand the protocol, the Investigators and study personnel accept the obligations incurred in undertaking this clinical investigation.

Periodic Monitoring Visits

Qualified clinical monitors representing MGH CTNI will conduct investigational site monitoring visits to ensure that all Investigators conduct the study in compliance with the protocol and applicable regulations. The site will receive notification prior to each monitoring visit during the course of the study. It is expected that the Investigator and/or sub-Investigator and other appropriately trained study staff are available on the day of the visit in the event any questions arise.

Periodic monitoring visits will be made in accordance with the approved monitoring plan at all active study sites throughout the clinical study to assure that the Investigator obligations are fulfilled and all applicable regulations and guidelines are being followed. These visits will assure that the facilities are still acceptable, the protocol and investigational plan are being followed, the IRB has been notified of approved protocol changes as required, complete records are being maintained, appropriate and timely reports have been made to the Sponsor and the IRB, and the Investigator is executing all agreed activities.

MGH CTNI retains the right to remove either the investigator or the investigational site from the study for issues of non-compliance with the protocol or regulatory requirements.

On one or more occasions, the study site may be inspected or audited by a MGH CTNI or a representative. The Investigator will be informed in advance of this visit.

Study Closeout Visit

Upon completion of the clinical study (when all subjects at the site have completed follow up visits, all data has been entered in the EDC system and cleaned, all queries resolved, and final electronic signatures have been obtained), a study closeout procedure will be performed. Any unused study materials and equipment will be collected and returned to MGH CTNI. The Monitor will ensure that the Investigator's regulatory files are up to date and complete and that any outstanding issues from previous visits have been resolved. Other issues which will be reviewed at this visit include: discussing retention of study files, possibility of site audits, publication policy, and ensuring that the Investigator will notify the local IRB regarding study closeout.

Medical Monitoring

The medical monitor for this trial will be a board certified psychiatrist designated by MGH CTNI. The MGH CTNI Medical Monitor will review all adverse events, review issues related to subject eligibility, assess the benefits and risks of protocols on an ongoing basis, and work in collaboration with the IRB to identify safety signals and trends.

M. DATA ANALYSIS

This section presents general information about statistical considerations and concepts such as randomization, stratification, statistical power, sample size, and a brief discussion on analysis methodology.

Treatment Groups

The following treatment groups will be assessed:

Table 4. Study Treatments

Group	Description
Test Treatment	Sodium Nitroprusside (0.5 µg/kg/min for 4 hours)
Control Treatment	Placebo (5% dextrose for 4 hours)

Description of Study Endpoints

Primary Efficacy Endpoint

- Mean change from baseline in Positive and Negative Syndrome Scale (PANSS) total score after 2 weeks of randomized treatment using SPCD.

Secondary Efficacy Endpoints

- Percent change from baseline in the PANSS total score after 2 weeks of treatment using SPCD
- Percentage of subjects with 20% or more reduction from baseline in PANSS total score after 2 weeks of treatment using SPCD
- Percent change in PANSS subscales using SPCD

Additional Efficacy Endpoints

- Percent change in Clinical Global Impression-Severity (CGI-S) using SPCD
- Percent change in MATRICS Consensus Cognitive Battery (MCCB) using SPCD

Safety Assessments

- Incidence of Treatment-Emergent Adverse Events (TEAE)
- Incidence of withdrawals from the study due to TEAEs
- Percent change in Abnormal Involuntary Movement Scale (AIMS)
- Percent change in Systematic Assessment for Treatment Emergent Effects–Systematic Inquiry (SAFTEE-SI)
- Assessment of suicidality per the Columbia-Suicide Severity Rating Scale (C-SSRS)
- Changes in blood pressure and heart rate
- Changes in electrocardiogram (ECG) parameters

Sample Size Determination and Rationale

Sixty (60) subjects will be randomized in an effort to assure that 48 subjects complete the study.

The RCT Logic Calculator (by Schoenfeld, D and Ivanova,A; http://www.rctlogic.com/calculator/calculator-continuous.aspx) was used for this sample size calculation. This sample size will provide “sufficient” statistical power for the selected primary endpoint. The sample size calculation is based on the assumption that there is a clinically meaningful weighted (50%) average difference of 10 points (9 points in Stage 1 and 11 points in Stage 2) between sodium nitroprusside and placebo group accordingly, (with standard deviation of 14) in PANSS total scores after 2 weeks of treatment between the two treatment groups. This power calculation is based on a 20% attrition by Week 4 and 30% placebo response at the end of Stage 1. Under the above assumptions, 60 subjects will be required to meet the Type I error rate of 0.05 and an 81% power.

Randomization

This is a double-blind, placebo-controlled, multi-center, randomized clinical trial. Subjects who have provided written informed consent and have met all the inclusion criteria and have met none of the exclusion criteria will be randomized to one of the three treatment sequences.

The study design contains two double-blind treatment phases (i.e. Phase 1 and Phase 2) of the same duration of two weeks. Subjects will be randomly assigned using stratified block randomization. The randomization scheme will be programmed in to the CTMS software and will generate a randomization code for each subject upon enrollment into the study. Subjects will be stratified by primary treatment status (clozapine, versus antipsychotic other than clozapine) and by site. The number of subjects taking clozapine will be restricted to 20 study wide.

Blinding and prevention of Bias

All subjects, Investigators and their staff involved in the management of the study will be blinded to treatment assignments.

Treatment unblinding for the study will occur after all clinical data have been received, data inconsistencies have been resolved, and the database is locked, except for safety reasons on a case by case basis (i.e., emergency unblinding).

Interim Analysis

There will be no interim analysis for this trial.

General Statistical Considerations

All collected study data will be presented in subject data listings. Statistical analyses will be performed using SAS® for Windows, version 9.3 or later. Descriptive statistics (n, mean, standard deviation, median, minimum and maximum) will be calculated by treatment group for continuous variables. Frequencies and percentages will be presented by treatment group for categorical variables.

Analysis Populations

The details of the analysis population to be used for the study are described in the below sections. All the subjects who were randomized in the study will be considered for analysis. For the primary and secondary endpoint analyses the following set of subject will be accounted:

- All subjects randomized to the sodium nitroprusside group in Phase 1 of the study (~ 20 subjects)
- All subjects randomized to the Placebo group in Phase 1 of the study (~ 40 subjects)

- All subjects who did not respond to placebo in Phase 1 and were pre-randomized to the sodium nitroprusside group in Phase 2 of the study (~11 subjects)
- All subjects who did not respond to placebo in Phase 1 and were pre-randomized to the placebo group in Phase 2 of the study (~11 subjects)

Intent-to-Treat (ITT) population

The Intent-to-Treat (ITT) population is defined as all randomized subjects. The ITT population will be the primary population for the analysis of the primary, secondary, and additional efficacy endpoints.

Per Protocol (PP) Population

The Per Protocol (PP) population is defined as all randomized subjects who were not associated with a major protocol violation. This population will be identified before the database lock. The PP analysis of primary, secondary and additional endpoints will be considered supportive.

Safety Population

The Safety population is defined as any subject receiving the treatment after randomization. This population will be used for the analysis of safety parameters.

Covariates

For efficacy analyses, baseline values will be used as covariates in the analysis models.

Missing data

For efficacy evaluation data points missing data will be imputed per the method that will be detailed in the SAP for the study.

Multiple Comparisons and Multiplicity

This is a two phase Sequential Parallel Comparison Design (SPCD) study.

A two-stage test (Weighted Z-test approach with fixed weight of 0.5 for each part of the study) will be used to combine the data on treatment effects from the two stages of the study. This method will address the Type I error rate adjustment to protect the trial wise Type I error at the final analysis.

For the primary endpoint there will be only one hypothesis testing and there will be no Type I error adjustment for testing this endpoint.

There will also be no Type I error adjustment on the hypotheses testing of the secondary endpoints, other than using the Closed Test procedure.

Statistical Methods

A Statistical Analysis Plan (SAP) will be developed and approved before the database is locked. The SAP will present the detailed statistical methodology to be used in analyzing the efficacy and safety data from this trial. Inferential statistics for all inferential statistical analysis will be based on a 2 sided test with Type I Error rate of 0.05.

All the efficacy endpoints presented here will be conducted using both the evaluable and ITT populations. All safety analysis will be conducted using the safety population.

All data collected will be summarized according to the variable type:

- Continuous data summaries will include:
    - Number of observations, mean, standard deviation, median, and minimum and maximum values.
    - Analysis of Covariance using the stratification factor for inferential statistics.
- Categorical data summaries will include:
    - Frequency counts and percentages.
    - Logit model will be used for inferential statistics using the stratification factors referenced.

Subject Disposition

The disposition of all subjects who sign an ICF will be provided. The numbers of subjects screened, randomized, re-randomized, completed, and discontinued during the study, as well as the reasons for all post-randomization discontinuations will be summarized by treatment group. Disposition and reason for study discontinuation will also be provided as a by-subject listing.

Demographics and baseline characteristics analysis

Demographics and baseline characteristics will be summarized by treatment group using appropriate descriptive statistics.

Concomitant Medications

Concomitant medications will be summarized separately for the Safety population. All prior and concomitant medications recorded in the case report form will be coded to all matching Anatomic Therapeutic Classification codes using the most recent version of WHO Drug version. Descriptive summaries, by treatment group and stage of the study, will be prepared using the coded term. All concomitant medications recorded in the case report form will be listed.

Efficacy Analyses

Primary Efficacy Analyses

Primary Analysis of the primary, secondary and additional efficacy endpoints will be conducted on the ITT population using SPCD.

- Primary Endpoint: Mixed Model Repeated Measures analysis (MMRM)/non-parametric methods will be used to compare using SPCD the difference between the two treatment groups on the primary endpoint depending on the distribution of the data. A weight of 50% will be used in pooling the data from Stages 1 and 2.

- Secondary Endpoints: To maintain the trial-wise Type I error rate at 0.05, a closed test procedure will be used for the secondary endpoints. The order of the endpoints will be pre-specified in the statistical analysis plan prior to database lock. A weight of 50% will be used in pooling the data from Stages 1 and 2, using SPCD.

    - Continuous data comparisons will be based on Mixed Model Repeated Measures (MMRM), if the Normality assumption is met, or a rank – ANCOVA analysis i.e., an ANCOVA analysis on rank-transformed data will be used if the data is not normally distributed.

    - Categorical data comparisons will be based on Logit model.

- Additional Efficacy Endpoints: These endpoints are considered exploratory endpoints. These additional Efficacy Endpoints will be analyzed using the similar methods outlined for the primary and secondary endpoints or additional

appropriate methods, if needed. The statistical methods will be detailed in the SAP.

Supportive Analysis

To assess the consistency of the Primary Analysis results, supportive analysis will be conducted using the PP population. Statistical methodology for the supportive analyses will be the same as that of the primary analysis, with the exception of the analysis population used. The PP population will be used for the supportive analysis while the ITT population will be used for the primary analysis.

Safety Analyses

Adverse Events

Adverse events will be coded using MedDRA. Treatment Emergent AE's (TEAE) are defined as events with an onset on or after the first randomized treatment. TEAEs will be summarized by treatment group and by stage of the study, System Organ Class, and preferred term. The following TEAE summaries will be provided:

- TEAEs by severity grade
- TEAEs by relationship to study treatment.

In addition, separate summaries of serious adverse events, and adverse events resulting in discontinuation of study treatment will be presented.

Abnormal Involuntary Movement Scale (AIMS)

All the data from the AIMS will be listed and the percent change in the total score will also be presented.

Systematic Assessment for Treatment Emergent Effects–Systematic Inquiry (SAFTEE-SI)

All the data from the SAFTEE-SI will be listed and the percent change in the total score will also be presented.

Columbia-Suicide Severity Rating Scale (C-SSRS)

All the data from C-SSRS will be listed and descriptive summaries will be presented for each of the subscales (i.e. Suicidal Ideation and Suicidal Behavior).

Clinical Laboratory Data

All laboratory values will be listed. Laboratory measurements will also be summarized as continuous variables and presented by treatment group and time point.

In addition, changes in vital signs, weight and ECG will be summarized over time.

N. PUBLICATION POLICY

A publication committee will be established by the Principal Investigator, with the assistance of the Stanley Center Scientific Advisory Board. The Publication Committee will develop a list of publication topics which will be assigned to site investigators for writing and analysis. In addition, investigators may submit topics for review by the Publication Committee. All publications will be reviewed by the Publication Committee for accuracy before submission to peer reviewed journals or scientific meetings. All manuscripts should be submitted to the SAB at least 30 days before submission to peer reviewed journals. Abstracts should be submitted for review at least 10.

O. PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the conduct of the study according to 21 CFR parts 50, 54, 56 and 812, 45 CFR 46, to GCP as described in ICH guideline E6 and to hospital Institutional Review Boards/Ethics Committees.

_____

Clinical Site

_____ _____

Principal Investigator Signature Date

_____

Principal Investigator

Printed Name

P. APPENDICES

APPENDIX 1: SCHEDULE OF EVENTS

SNP Study Schedule of Events	OPD	CTNI Interview	OPD	Monitoring Unit Double-Blind Treatment Phase 1			OPD	OPD	Monitoring Unit Double-Blind Treatment Phase 2			OPD	
N= 60 SPCD Design	Screen	Remote	Baseline	iv SNP -0.5 µg/kg/min or iv 5% glucose					iv SNP -0.5 µg/kg/min or iv 5% glucose				Final FU or ET
Visit	1	2	3	4			5	6	7			8	9
Day	-28 to -13	-13 to -3	-2 to -1	Day 0**	Hrs 0-4	Hrs 5-7	D7	D12-13	Day 14**	Hrs 0-4	Hrs 5-7	D21	D28
Informed Consent	X												
Subject ID Assignment	X												
SCID-I/P	X												
MGH-FAST	X												
PANSS	X		X			X	X	X			X	X	X
CGI-S / CGI-I	X		X			X	X	X			X	X	X
C-SSRS	X		X				X	X				X	X
I/E Criteria	X												
CTNI Rater (PANSS CGI-S MGH FAST)		X											
Demographics	X												
Physical Exam and Medical History	X												
Vital Signs and 12-Lead ECG	X		X	X		X	X	X	X		X	X	X
Anthropometrics (ht wt waist circumferen	X		X	X			X	X	X			X	X
Psychiatric History	X												
HbA1c	X												
CBC and CMP	X			X					X				X
Urinalysis	X			X					X				
Urine Drug Screen	X		X					X					
Serum Pregnancy Testing	X												
Blood sample for DNA	X												
AE/SAE Assessment	X		X		X	X	X	X		X	X	X	X
Conmed Assessment	X		X	X			X	X	X			X	X
AIMS			X			X	X*	X			X	X*	X
SAFTEE			X			X	X*	X			X	X*	X
MATRICS			X				X*	X				X*	X
UPSA-B			X				X*	X				X*	X
Randomization ID (allocation ratio 1:1:1)		X											
Pre-infusion neurological exam				X					X				
Cardio-pulmonary monitoring					X					X			
iv Na nitroprusside or Placebo					X					X			
Post-infusion neurological exam						X					X		
Post-infusion De Brief						X					X		

X* - discontinued as of 10/2015

** +/- 1 day with permission from CTNI

APPENDIX 2: MGH- FAST ADDITIVE SUMMARY OF TREATMENT

FAST: Fast Additive Summary of Treatment

Please complete the row for every treatment you tried. If you're not sure, use your best guess.

		a) Longest duration?				b) Benefit?			c) Why stopped?				d) Missed doses?		
		less than 4 weeks	4-8 weeks	8 weeks - 6 months	> 6 months	Back to normal	Better but not well	A bit/None	(Still treated)	Side effects	Lack of benefit	Cost/other	once a week or less	2+ times a week	Highest dose?
Mood stabilizers															
Lithobid, eskalith	Lithium carb/citrate														
Depakote, depakene	Valproate														
Equetro, Tegretol	Carbamazepine														
Lamictal	Lamotrigine														
At the same time : any two of the above															
Antipsychotics (including injected/shots)															
Zyprexa	Olanzapine														
Clozaril	Clozapine														
Risperdal	Risperidone														
Seroquel	Quetiapine														
Abilify	Aripiprazole														
Geodon	Ziprasidone														
Saphris	Asenapine														
Latuda	Lurasidone														
Invega	Paliperidone														
Prolixin	Fluphenazine														
Haldol	Haloperidol														
Orap	Pimozide														
Stelazine	Trifluoperazine														
Loxitane	Loxapine														
Molindone	Moban														
Trilafon	Perphenazine														
Navane	Tiotixene														
Thorazine	Chlorpromazine														
Serentil	Mesoridazine														
Mellaril	Thioridazine														
Other:															
Any weekly or monthly injection															
At the same time : Clozapine and another															
At the same time : Two antipsychotics															

OF ANTIPSYCHOTIC MEDICATIONS

FAST: Fast Additive Summary of Treatment

Please complete the row for every treatment you tried. If you're not sure, use your best guess.

		a) Longest duration?				b) Benefit?			c) Why stopped?				d) Missed doses?		
		less than 4 weeks	4-8 weeks	8 weeks - 6 months	> 6 months	Back to normal	Better but not well	A bit/None	(Still treated)	Side effects	Lack of benefit	Cost/other	once a week or less	2+ times a week	Highest dose?
Antidepressants (rate the longest trial)															
Prozac	Fluoxetine														
Paxil	Paroxetine														
Zoloft	Sertraline														
Celexa	Citalopram														
Lexapro	Escitalopram														
Luvox	Fluvoxamine														
Effexor	Venlafaxine														
Cymbalta	Duloxetine														
Wellbutrin	Bupropion, budeprion														
Remeron	Mirtazapine														
Viibryd	Vilazodone														
Brintellix	Vortioxetine														
Tofranil	Imipramine														
Pamelor	Nortriptyline														
Anafranil	Clomipramine														
Elavil	Amitriptyline														
Vivactil	Protriptyline														
Surmontil	Trimipramine														
Parnate	Tranylcypramine														
Nardil	Phenelzine														
Emsam	Selegiline														
At the same time : Two of the above															
Electroconvulsive therapy (ECT)															
Cognitive-behavioral therapy (CBT)															
Other depression medicines															
SAM-e	S-adenylmethionine														
Provigil/nuvigil	Modafinil/armodafinil														
Mirapex	Pramipexole														
Requip	Ropinirole														
Deplin	Methylfolate														
Dexedrine	Dextroamphetamine														
Ritalin, concerta	Methylphenidate														
Adderall	Amphetamine salts														
Buspar	Buspirone														
Synthroid	Thyroid hormone														

Atypical Antipsychotic Drugs

Aripiprazole (marketed as Abilify)

Asenapine Maleate (marketed as Saphris)

Clozapine (marketed as Clozaril)

Iloperidone (marketed as Fanapt)

Lurasidone (marketed as Latuda)

Olanzapine (marketed as Zyprexa)

Olanzapine/Fluoxetine (marketed as Symbyax)

Paliperidone (marketed as Invega)

Quetiapine (marketed as Seroquel)

Risperidone (marketed as Risperdal)

Ziprasidone (marketed as Geodon)

Source: http://www.fda.gov/drugs/drugsafety/ucm243903.htm#list

APPENDIX 3: CONCOMITANT MEDICATIONS ALLOWED

Drug Name or Class	Episodic Use (prn)	Chronic Use	Restrictions/Comment
Analgesics	Y	Y	Non-narcotic analgesics are allowed. Medically appropriate episodic use of narcotic analgesics is allowed for acute medical indications but is limited to no more than 3 days for each episode. Chronic NSAID use is allowed; tramadol is not allowed.
Anesthetics, general	Y	—	If procedures requiring general anesthesia are to occur/have occurred, please contact MGH CTNI to report the medical condition(s).
Anesthetics, local	Y	N	—
Anorexics	N	N	—
Antacids	Y	Y	—
Antiacne	Y	Y	Topical agents only, including topical antibiotics. Isotretinoin (Accutane) is not allowed.
Antianginal agents	N	N	—
Antiarrhythmics	N	N	—
Antiasthma agents	Y	Y	—
Antibiotics	Y	Y	Chronic use of topical or oral antibiotics for acne is allowed, with the exception of the MAOI linezolid (Zyvox) and isoniazid, which are not allowed. Erythromycin, clarithromycin, rifampin are excluded.
Anticoagulants	N	N	Warfarin (Coumadin) is not allowed. Antiplatelet agents are allowed (see “Antiplatelets”).
Anticholinergics	Y	Y	Except for scopolamine.
Anticonvulsants	N	YY	Gabapentin and pregabalin are allowed. Other anticonvulsants are not allowed, including lamotrigine and carbamazepine.
Antidepressants	N	Y	Stable (for at least 4 weeks prior to screening), ongoing antidepressant therapy is required during the course of the study. No dose changes are allowed during the study. Monoamine oxidase inhibitors (which may have unknown drug-drug interactions) are excluded. Concomitant use of trazodone (up to 200mg daily) is allowed. Nefazodone is excluded.

Antidiarrheal preparations	Y	N	Only loperamide HCl (Imodium), bismuth subsalicylate (Pepto-Bismol), and kaolin preparations are allowed.
Antifungals, systemic	N	Y	—
Antifungals, topical	Y	Y	Ketoconazole and itraconzole are excluded
Antihistamines	Y	Y	The use of combinations containing pseudoephedrine or phenylephrine is not allowed. Combination products containing the word nighttime or some synonym routinely include a sedating antihistamine and are not allowed. Combination products ending in "-D" routinely contain a stimulant such as phenylephrine, and the appropriate limits above apply to them.
Anti-hypertensives	N	Y	Diltiazem, verapamil are excluded
Anti-impotence medications	N	N	—
Anti-inflammatory drugs	Y	Y[a]	Indomethacin (Indocin) and systemic corticosteroids are not allowed.
Antifungal	Y	Y	Itraconazole is excluded
Antimigraine	Y	Y	Triptans are not allowed
Antinauseants/Antiemetics	Y	N	Phosphoric acid preparations (Emetrol, Emecheck), bismuth subsalicylate (Pepto-Bismol), cola syrup, 5-HT_3 receptor antagonists (e.g., ondansetron), and prokinetic agents (metoclopramide) are allowed. Scopolamine is not allowed (see section on antihistamines).
Antineoplastics/ Immunosuppressant agents	N	Y[c]	Interferons, methotrexate, and other immunosuppressant agents are not allowed. Call MGH CTNI for approval for certain cases in cancer remission maintaining therapy.
Antiobesity/Appetite suppressants	N	N	OTC Alli (Xenical), sibutramine (Meridia), and phentermine (Adipex-P and others) are not allowed.
Antiplatelet agents	N	Y[b]	Aspirin (maximum 325 mg/day) and clopidogrel (Plavix) are allowed.
Antipsoriatic treatments	Y	Y	Only topical agents are allowed. Acitretin (Soriatane) is not allowed.
Antipsychotics	N	Y	Stable in dosing at least 8 weeks prior to randomization is allowed.

Antismoking medications	N	Y[c]	Varenicline (Chantix) is not allowed. Chronic nicotine replacement may be allowed in certain cases after review with MGH CTNI.
Antiviral agents	Y	Y	Only oral or topical agents are allowed. Only acyclovir, famciclovir, valacyclovir, penciclovir, docosanal, trifluridine, and vidarabine are allowed. Amantadine,rimantadine, indinavir, nelfinavir, ritonavir, saquinavir are not allowed. Tamiflu (oseltamivir phosphate), and Relenza (zanamivir) inhalants are permitted for influenza prophylaxis but use is limited to a 7- to 14-day course in accordance with prescribing information. Interferons are not allowed.
Anxiolytics	Y	Y	Chronic, stable treatment with benzodiazepines for reasons other than sleep is allowed. Stable dosing at least four weeks prior to randomization is required for sleep agents. Up to 1mg of benzodiazepine in a 24h period can be utilized as needed during the study period.
Benign prostatic hyperplasia treatments	N	Y[b]	Male patients who have symptoms of obstructed voiding should not be included in the study. Surgically or medically treated patients must be asymptomatic and receiving a stable dosage of allowed medications (α-1 blockers, finasteride, or dutasteride) for 1 month before screening.
Buspirone	N	Y	Stable in dosing at least four weeks prior to randomization is allowed.
Cough/cold preparations	Y	N	Use of cough and cold preparations containing pseudoephedrine or dextromethorphan is not allowed, as are those containing phenylephrine. Combination products ending in “-D” routinely contain a stimulant such as phenylephrine, and the appropriate limits apply to them. (See “Antihistamines”.)
Diuretics	Y	Y[b]	Episodic use of diuretics is restricted to treatment of premenstrual symptoms. For chronic use, medication and dosage should be stable for 1 month before screening.
Dopaminergics	N	Y	Dopamine agonists for restless leg syndrome are allowed. Stable in dosing at least four weeks prior to randomization is allowed.

Gastrointestinal: • H2-blockers/ • proton pump inhibitors/ • prokinetic agents	Y	Y	Cimetidine (Tagamet) is not allowed. Metoclopramide is not allowed.
Hormonal (noncontraceptive) therapies	N	Y	See below.
Hormone suppressants	N	Y[b]	Only finasteride (Proscar) and dutasteride (Avodart) are allowed.
Hormones: reproductive	N	Y	Systemic hormonal contraceptives (oral contraceptives of estrogen and progestin combinations, depot injections such as Depo-Provera, the contraceptive implant Implanon, or transdermally delivered contraceptives such as Ortho Evra) are allowed
Hormones: thyroid	N	Y	Thyroid hormone replacement is allowed (dosage of thyroid medication should be stable for 3 months before screening). Therapeutic use for psychiatric disorders (e.g., T3 augmentation) is not allowed
Hypoglycemic agents	N	Y	Oral hypoglycemic agents are allowed. Insulin is not allowed
Hypolipidemics	N	Y[b]	Ezetimibe (Zetia) is allowed
Hypolipidemics: bile acid sequestrants	N	N	—
Hypolipidemics: fibrates	N	Y[b]	Gemfibrozil and fenofibrate are allowed
Hypolipidemics: niacin	N	N	Niacin and niacinamide are not allowed
Hypolipidemics: statins	N	Y[b]	Lovastatin, simvastatin, pravastatin, atorvastatin, fluvastatin, and rosuvastatin are allowed
Laxatives	Y	Y[a]	Only fiber-based products and docusate sodium (Colace) are allowed
Lithium	N	Y	Stable dosing at least four weeks prior to randomization is allowed.

Medications that are primarily metabolized by CYP2C8 (e.g., cerivastatin, paclitaxel, repaglinide, sorafenib, and torsemide)	N	N	—
Muscle relaxants	N	N	—
NMDA receptor antagonist	N	N	Memantine is excluded.
Opioid agonists/analgesics (e.g., codeine, hydrocodone, methadone, morphine, maperidine, propoxyphene) and antagonists (e.g., naltrexone, naloxone, nalmefene)	N*	N*	See section on analgesics for exceptions
Proton pump inhibitors or H2 receptor blockers	Y	Y	—
Sedatives/hypnotics	N	Y	Ongoing, stable hypnotic therapy (e.g., zolpidem, zaleplon, benzodiazepine hypnotics, and low-dose trazodone 50-200mg) will be allowed during the course of the study. Eszopiclone is not allowed.
Steroids/systemic	Y	N	Systemic steroid treatment will be allowed only for medical emergencies, such as severe allergic reactions
Steroids/topical and inhalant	Y	Y	—
Steroids/intra-articular	Y	NA	—
Stimulants	N	N	Oral or transdermal methylphenidate, amphetamine products or prodrugs, pseudoephedrine, modafinil (Provigil), and other medications of same category are not allowed
Vaccines	Y	NA	—

a If being taken prior to enrolling in the study.

b If being taken for at least 3 months prior to enrolling in the study and the dose has been stable for at least 1 month.

c If approved by MGH CTNI Medical Monitor5-HT_3 = 5-hydroxytryptomine receptor type 3; 5-HTP = 5-hydroxytryptophan; ACE = angiotensin-converting enzyme; CR = controlled release; DHEA = dehydroepiandrosterone; N = no; NA = not applicable; OTC = over the counter; PRN = as needed (prorenataT3prorenataT3=triiodothyronine; Y = yes.

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APPENDIX 5: STUDY SCALES

The Positive and Negative Syndrome Scale (PANSS)

Link: http://www.emotionalwellbeing.southcentral.npanss-positive-and-negative-syndrome-scale-pdf-document.

Clinical Global Impressions–Severity (CGI-S) and Clinical Global Impressions–Improvement (CGI-I) Scales

Link: http://miksa.ils.unc.edu/unc-hit/media/CGI.pdf

Abnormal Involuntary Movement Scale (AIMS)

Link: http://www.atlantapsychiatry.com/forms/AIMS.pdf

Systematic Assessment for Treatment Emergent Effects–Systematic Inquiry (SAFTEE-SI)

Link: https://www.cognitiongroup.com/pdf/aims.pdf

Columbia-Suicide Severity Rating Scale (C-SSRS)

Link: http://cssrs.columbia.edu/docs/C-SSRS_1_14_09_Baseline.pdf

The Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB)

Link: MATRICS Consensus Cognitive Battery (MCCB)

The University of California San Diego (UCSD) Performance-based Skills Assessment Brief (UPSA-B)

http://www.researchgate.net/UPSA_B

A Randomized Double-Blind, Placebo-Controlled, Proof of Concept Study of Intravenous Sodium Nitroprusside in Adults with Symptomatic Schizophrenia

STATISTICAL ANALYSIS PLAN

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DATA ANALYSIS

This section presents general information about statistical considerations and concepts such as randomization, stratification, statistical power, sample size, and a brief discussion on analysis methodology.

Treatment Groups

The following treatment groups will be assessed:

Table 4. Study Treatments

Group	Description
Test Treatment	Sodium Nitroprusside (0.5 µg/kg/min for 4 hours)
Control Treatment	Placebo (5% dextrose for 4 hours)

Description of Study Endpoints

Primary Efficacy Endpoint

- Mean change from baseline in Positive and Negative Syndrome Scale (PANSS) total score after 2 weeks of randomized treatment using SPCD.

Secondary Efficacy Endpoints

- Percent change from baseline in the PANSS total score after 2 weeks of treatment using SPCD
- Percentage of subjects with 20% or more reduction from baseline in PANSS total score after 2 weeks of treatment using SPCD
- Percent change in PANSS subscales using SPCD

Additional Efficacy Endpoints

- Percent change in Clinical Global Impression-Severity (CGI-S) using SPCD
- Percent change in MATRICS Consensus Cognitive Battery (MCCB) using SPCD

Safety Assessments

- Incidence of Treatment-Emergent Adverse Events (TEAE)

- Incidence of withdrawals from the study due to TEAEs
- Percent change in Abnormal Involuntary Movement Scale (AIMS)
- Percent change in Systematic Assessment for Treatment Emergent Effects–Systematic Inquiry (SAFTEE-SI)
- Assessment of suicidality per the Columbia-Suicide Severity Rating Scale (C-SSRS)
- Changes in blood pressure and heart rate
- Changes in electrocardiogram (ECG) parameters

Sample Size Determination and Rationale

Sixty (60) subjects will be randomized in an effort to assure that 48 subjects complete the study.

The RCT Logic Calculator (by Schoenfeld, D and Ivanova,A; http://www.rctlogic.com/calculator/calculator-continuous.aspx) was used for this sample size calculation. This sample size will provide “sufficient” statistical power for the selected primary endpoint. The sample size calculation is based on the assumption that there is a clinically meaningful weighted (50%) average difference of 10 points (9 points in Stage 1 and 11 points in Stage 2) between sodium nitroprusside and placebo group accordingly, (with standard deviation of 14) in PANSS total scores after 2 weeks of treatment between the two treatment groups. This power calculation is based on a 20% attrition by Week 4 and 30% placebo response at the end of Stage 1. Under the above assumptions, 60 subjects will be required to meet the Type I error rate of 0.05 and an 81% power.

Randomization

This is a double-blind, placebo-controlled, multi-center, randomized clinical trial. Subjects who have provided written informed consent and have met all the inclusion criteria and have met none of the exclusion criteria will be randomized to one of the three treatment sequences.

The study design contains two double-blind treatment phases (i.e. Phase 1 and Phase 2) of the same duration of two weeks. Subjects will be randomly assigned using stratified block randomization. The randomization scheme will be programmed in to the CTMS software and will generate a randomization code for each subject upon enrollment into the study. Subjects will be stratified by primary treatment status (clozapine, versus antipsychotic other than clozapine) and by site. The number of subjects taking clozapine will be restricted to 20 study wide.

Blinding and prevention of Bias

All subjects, Investigators and their staff involved in the management of the study will be blinded to treatment assignments.

Treatment unblinding for the study will occur after all clinical data have been received, data inconsistencies have been resolved, and the database is locked, except for safety reasons on a case by case basis (i.e., emergency unblinding).

Interim Analysis

There will be no interim analysis for this trial.

General Statistical Considerations

All collected study data will be presented in subject data listings. Statistical analyses will be performed using SAS® for Windows, version 9.3 or later. Descriptive statistics (n, mean, standard deviation, median, minimum and maximum) will be calculated by treatment group for continuous variables. Frequencies and percentages will be presented by treatment group for categorical variables.

Analysis Populations

The details of the analysis population to be used for the study are described in the below sections. All the subjects who were randomized in the study will be considered for analysis. For the primary and secondary endpoint analyses the following set of subject will be accounted:

- All subjects randomized to the sodium nitroprusside group in Phase 1 of the study (~ 20 subjects)
- All subjects randomized to the Placebo group in Phase 1 of the study (~ 40 subjects)
- All subjects who did not respond to placebo in Phase 1 and were pre-randomized to the sodium nitroprusside group in Phase 2 of the study (~11 subjects)
- All subjects who did not respond to placebo in Phase 1 and were pre-randomized to the placebo group in Phase 2 of the study (~11 subjects)

Intent-to-Treat (ITT) population

The Intent-to-Treat (ITT) population is defined as all randomized subjects. The ITT population will be the primary population for the analysis of the primary, secondary, and additional efficacy endpoints.

Per Protocol (PP) Population

The Per Protocol (PP) population is defined as all randomized subjects who were not associated with a major protocol violation. This population will be identified before the database lock. The PP analysis of primary, secondary and additional endpoints will be considered supportive.

Safety Population

The Safety population is defined as any subject receiving the treatment after randomization. This population will be used for the analysis of safety parameters.

Covariates

For efficacy analyses, baseline values will be used as covariates in the analysis models.

Missing data

For efficacy evaluation data points missing data will be imputed per the method that will be detailed in the SAP for the study.

Multiple Comparisons and Multiplicity

This is a two phase Sequential Parallel Comparison Design (SPCD) study.

A two-stage test (Weighted Z-test approach with fixed weight of 0.5 for each part of the study) will be used to combine the data on treatment effects from the two stages of the study. This method will address the Type I error rate adjustment to protect the trial wise Type I error at the final analysis.

For the primary endpoint there will be only one hypothesis testing and there will be no Type I error adjustment for testing this endpoint.

There will also be no Type I error adjustment on the hypotheses testing of the secondary endpoints, other than using the Closed Test procedure.

Statistical Methods

A Statistical Analysis Plan (SAP) will be developed and approved before the database is locked. The SAP will present the detailed statistical methodology to be used in analyzing the efficacy and safety data from this trial. Inferential statistics for all inferential statistical analysis will be based on a 2 sided test with Type I Error rate of 0.05.

All the efficacy endpoints presented here will be conducted using both the evaluable and ITT populations. All safety analysis will be conducted using the safety population.

All data collected will be summarized according to the variable type:

- Continuous data summaries will include:
    - Number of observations, mean, standard deviation, median, and minimum and maximum values.
    - Analysis of Covariance using the stratification factor for inferential statistics.
- Categorical data summaries will include:
    - Frequency counts and percentages.
    - Logit model will be used for inferential statistics using the stratification factors referenced.

Subject Disposition

The disposition of all subjects who sign an ICF will be provided. The numbers of subjects screened, randomized, re-randomized, completed, and discontinued during the study, as well as the reasons for all post-randomization discontinuations will be summarized by treatment group. Disposition and reason for study discontinuation will also be provided as a by-subject listing.

Demographics and baseline characteristics analysis

Demographics and baseline characteristics will be summarized by treatment group using appropriate descriptive statistics.

Concomitant Medications

Concomitant medications will be summarized separately for the Safety population. All prior and concomitant medications recorded in the case report form will be coded to all matching Anatomic Therapeutic Classification codes using the most recent version of WHO Drug version. Descriptive summaries, by treatment group and stage of the study, will be prepared using the coded term. All concomitant medications recorded in the case report form will be listed.

Efficacy Analyses

Primary Efficacy Analyses

Primary Analysis of the primary, secondary and additional efficacy endpoints will be conducted on the ITT population using SPCD.

- Primary Endpoint: Mixed Model Repeated Measures analysis (MMRM)/non-parametric methods will be used to compare using SPCD the difference between the two treatment groups on the primary endpoint depending on the distribution of the data. A weight of 50% will be used in pooling the data from Stages 1 and 2.

- Secondary Endpoints: To maintain the trial-wise Type I error rate at 0.05, a closed test procedure will be used for the secondary endpoints. The order of the endpoints will be pre-specified in the statistical analysis plan prior to database lock. A weight of 50% will be used in pooling the data from Stages 1 and 2, using SPCD.

    - Continuous data comparisons will be based on Mixed Model Repeated Measures (MMRM), if the Normality assumption is met, or a rank –ANCOVA analysis i.e., an ANCOVA analysis on rank-transformed data will be used if the data is not normally distributed.

    - Categorical data comparisons will be based on Logit model.

- Additional Efficacy Endpoints: These endpoints are considered exploratory endpoints. These additional Efficacy Endpoints will be analyzed using the similar methods outlined for the primary and secondary endpoints or additional appropriate methods, if needed. The statistical methods will be detailed in the SAP.

Supportive Analysis

To assess the consistency of the Primary Analysis results, supportive analysis will be conducted using the PP population. Statistical methodology for the supportive analyses will be the same as that of the primary analysis, with the exception of the analysis population used. The PP population will be used for the supportive analysis while the ITT population will be used for the primary analysis.

Safety Analyses

Adverse Events

Adverse events will be coded using MedDRA. Treatment Emergent AE's (TEAE) are defined as events with an onset on or after the first randomized treatment. TEAEs will be summarized by treatment group and by stage of the study, System Organ Class, and preferred term. The following TEAE summaries will be provided:

- TEAEs by severity grade
- TEAEs by relationship to study treatment.

In addition, separate summaries of serious adverse events, and adverse events resulting in discontinuation of study treatment will be presented.

Abnormal Involuntary Movement Scale (AIMS)

All the data from the AIMS will be listed and the percent change in the total score will also be presented.

Systematic Assessment for Treatment Emergent Effects–Systematic Inquiry (SAFTEE-SI)

All the data from the SAFTEE-SI will be listed and the percent change in the total score will also be presented.

Columbia-Suicide Severity Rating Scale (C-SSRS)

All the data from C-SSRS will be listed and descriptive summaries will be presented for each of the subscales (i.e. Suicidal Ideation and Suicidal Behavior).

Clinical Laboratory Data

All laboratory values will be listed. Laboratory measurements will also be summarized as continuous variables and presented by treatment group and time point.

In addition, changes in vital signs, weight and ECG will be summarized over time.