

**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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Funding Provided by:

Stanley Medical Research Institute

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Clinical Trials.gov registry: NCT02164981

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.