PROTOCOL

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Number: NICHD-2003-09-LT

Study Drug: Sodium Nitroprusside

IND: 71,979

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Sponsor: National Institute of Child Health and Human Development

Final Date:

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APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

And I have fully discussed the objectives of this trial and the contents of this protocol with representatives of PODS Center and BPCA–Coordinating Center.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations.

I understand that, should the decision be made by the PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

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PROTOCOL SYNOPSIS

Protocol Title:	A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects
Protocol Number:	NICHD-2003-09-LT
Sponsor:	National Institute of Child Health and Human Development
Product:	Sodium Nitroprusside
Objectives:	1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours 2. To assess the potential for rebound hypertension following administration of sodium nitroprusside for 12 hours or more
Study Design:	This is a phase II, randomized, double blind, withdrawal to placebo study examining the efficacy, safety and tolerability of sodium nitroprusside in pediatric subjects.
Study Population:	Children up to 17 years of age who require long term (at least 12 hour) blood pressure control will be eligible for study.
Number of Subjects:	A target of approximately 60 patients will be enrolled.
Number of Sites:	Up to 15
Duration of Subject Participation:	Enrollment is anticipated to begin in 2008 and to be complete in approximately 12 months. Patients will be followed for up to 30 days following receipt of study drug.
Treatment:	Subjects who require vasodilator therapy for relatively long time periods will receive open-label infusion of sodium nitroprusside for at least 12 hours but not greater than 24 hours.
Dose Schedule:	Patients will be randomized to receive either placebo or sodium nitroprusside for 30 minutes following at least 12- hours but not more than 24 hours of open-label infusion of sodium nitroprusside.
Estimated Start:	Q2 2008
Estimated Finish:	Q1 2009
Ethics	All regulations stated in 21 CFR Parts 50, 56, and 312 and recommendations outlined in the ICH Guidelines for Good Clinical Practice, as well as all other applicable local and national laws and regulations, will be adhered to throughout this trial.

Safety:	The safety of the drug will be assessed by multiple subject assessments of vital signs, physical exams, clinical tests and laboratory evaluations. Adverse events will be monitored and tracked. All SAEs will be closely monitored throughout the course of the study.
Statistical Consideration:	The trial will be sized to detect the loss of as little as 50% of the expected blood pressure lowering effect of the chosen dose of sodium nitroprusside during the 30 minutes of withdrawal to placebo.

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BP	Blood Pressure
BPCA-CC	Best Pharmaceuticals for Children Act Coordinating Center
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
cGMP	cyclic Guanosine Monophosphate
CN^-	Cyanide
CRA	Clinical Research Associate
CRF	Case Report Form
DCRI	Duke Clinical Research Institute
DBP	Diastolic Blood Pressure
DSMB	Data and Safety Monitoring Board
ECMO	Extracorporeal Membrane Oxygenation
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
hr	Hour
HR	Heart Rate

ACRONYMS AND ABBREVIATIONS

IB	Investigational Brochure
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
kg	Kilogram
MAP	Mean Arterial Pressure
mcg	Microgram
mEq/L	Milliequivalent per Liter
mcgs	Micrograms
min	Minute
mL	Milliliter
mm Hg	Millimeters of Mercury
mmol/L	Millimoles per Liter
NO	Nitric Oxide
NICHD	National Institute for Child Health and Human Development
NONMEM	Nonlinear Mixed Effect Model
NTG	Nitroglycerin
PD	Pharmacodynamic
PaO_2	Arterial Partial Pressure of Oxygen
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SCN	Thiocyanate
SNP	Sodium Nitroprusside
μM	Micromoles per liter, Micromolar

1.0 Background and Rationale

Blood pressure control in children is a significant concern in the intensive care unit (ICU), where management of arterial pressure is often necessary during periods of acute physiologic stress such as occurs after certain surgical and medical procedures. Examples of surgical procedures that require blood pressure control in the intensive care unit following surgery include aortic coarctation repair, Ross procedure (pulmonary valve autograft), and solid organ transplantation. Medical conditions requiring control of systemic arterial pressure include renal disease, drug therapy (corticosterioids and immunosuppression agents), and procedures such as extracorporeal membrane oxygenation (ECMO).

A wide variety of drugs of various therapeutic classes have been utilized for either controlled hypotension in the operating room or prevention of hypertension in the pediatric ICU. These drug classes include calcium channel blockers (Tobias et al, 1996), beta-adrenergic antagonists (Kay et al, 2001), ganglionic blockers (DuToit, 1970 and Gallagher and Milliken, 1979), inhalation anesthetics (Tobias, 1998) and direct acting vasodilators such as nitroglycerin and sodium nitroprusside (SNP) (Kaplan, 1980, and Tinker, 1976 Groshong, 1996, and Sinaiko, 1996). Although many vasodilator agents are available to lower blood pressure in the operating room and intensive care unit setting, few have been systematically studied in children.

SNP is a direct acting vasodilator commonly used for blood pressure control. It produces vascular smooth muscle relaxation when its metabolism in the red blood cell results in the liberation of nitric oxide (NO). NO then activates the enzyme guanylyl cyclase. This activation results in the formation of increased intracellular levels of cyclic guanosine monophosphate (cGMP). The result is vasodilation.

1.1 Metabolism

Five molecules of cyanide (CN^-) are released when SNP is metabolized in the red blood cell. The major metabolic pathway for CN^- is conversion to thiocyanate (SCN). This conversion occurs enzymatically via two sulfur transferase systems: 1) rhodenase (the primary pathway) and 2) beta-mercaptopyruvate-cyanide sulfurtransferase. Rhodenase is ubiquitous throughout the body, but it is highly concentrated in the liver. Rhodenase catalyzes the transfer of sulfur from a sulfur donor molecule such as thiosulfate (Na_2SO_3) to cyanide and thereby the formation of thiocyanate (SCN). SCN is subsequently eliminated in the urine and can therefore serve as a marker of cyanide exposure.

The ability of rhodenase to catalyze the conversion of cyanide to thiocyanate (SCN) is limited by the availability of sulfur donors in the body. Thus the provision of exogenous sulfur donors such as thiosulfate (Na_2SO_3) in the setting of acute cyanide intoxication is a potentially life-saving intervention (Pasch et al, 1983, Cole and Vesey, 1987).

One out of every five CN^- ions liberated by the metabolism of SNP binds to methemoglobin to form the non-toxic cyanomethemoglobin. The creation of additional quantities of methemoglobin by the intravenous infusion of sodium nitrite can thus provide additional CN^- buffering capacity. The resultant methemoglobinemia can then be treated with the administration of intravenous methylene blue.

Additional metabolic pathways for CN^- include the conversion of hydroxycobalamine (vitamin B12a) to cyanocobalamine, and conversion to 2-aminothiazoline 4-carboxylic acid.

If the above three pathways (rhodenase, methemoglobin, hydroxycobolamine) are overwhelmed, cyanide will bind to mitochondrial cytochrome oxidases and poison cellular oxidative phosphorylation. Cellular hypoxia is induced when cyanide inhibits the electron transport chain at cytochrome a_3. Oxygen cannot be utilized, mixed venous

oxygen tension rises and the generation of high-energy adenosine triphosphate (ATP) is blocked. The cell reverts from aerobic to anaerobic metabolism, with the subsequent generation of pyruvate and lactate. Acidosis ensues, and with it, deterioration in the organ systems most dependent on oxidative metabolism: the central nervous system and heart.

Clinical manifestations of cyanide toxicity to the central nervous system include headache, anxiety, agitation, confusion, lethargy, convulsions and coma. Cardiovascular manifestations include progressive heart failure with both loss of contractile force (negative inotropy) and slowing of rate (negative chronotropy). Bradycardia and hypotension are commonly observed pre-morbid events associated with cyanide toxicity.

In patients receiving SNP, the earliest, most sensitive signs of cyanide toxicity are acidosis, elevated mixed venous oxygen tension, and rising blood lactate levels. Venous blood that appears "bright" red due to the inability of the tissues to extract oxygen should suggest cyanide toxicity. Arterial and mixed venous blood gas analysis with co-oximetry can help confirm the diagnosis.

1.2 Previous Studies

SNP was first discovered in 1850. Its hypotensive effects were noticed in 1929, and its first therapeutic use was reported by Page et al. in 1955. Moraca et al. first described the clinical use of SNP for deliberate hypotension during surgical procedures in 1962. Since then, it has been widely used to control blood pressure in infants and children in the perioperative period.

Despite its widespread use, there is a paucity of information on its safety, efficacy, and pharmacokinetic/pharmacodynamic relationships in children. Davies et al (1975) and Bennett and Abbott (1977) described their retrospective experience with SNP used to induce deliberate hypotension in small cohorts of children. Both authors observed that younger patients required more SNP than older ones to achieve comparable degrees of

blood pressure control. In their small retrospective cohort, Bennett and Abbott recommended that doses of 10 micrograms/kilogram/minute were necessary to achieve satisfactory blood pressure response. Davies et al described three possible responses to SNP administration in children: 1) a constant response to "conventional" doses < 3 mg/kg; 2) a tachyphylactic response characterized by continuously escalating dose requirement (> 3 mg/kg) to achieve a satisfactory blood pressure; and 3) resistance to the blood pressure lowering effects of the drug. They cautioned against using total doses that exceeded 3 mg/kg or continuing administration of SNP in the latter two scenarios. Firm conclusions cannot be drawn because these small case series were not randomized controlled trials with specific pharmacodynamic endpoints.

Yaster et al (1986) compared SNP to nitroglycerin (NTG) for inducing hypotension in a group of 14 adolescents. They found doses of SNP between 6-8 micrograms/kg/minute superior to NTG at any dose in the reliable induction of hypotension for children and adolescents undergoing scoliosis, craniofacial or hepatic surgery.

Hersey et al (1997) performed a randomized trial comparing SNP to the dihydropyridine calcium channel antagonist nicardipine in 20 healthy adolescents with idiopathic scoliosis undergoing spinal fusion. Target blood pressures were easily obtainable in both groups and operating conditions were comparable. The time to restoration of baseline blood pressure after termination of the infusion was significantly longer in the nicardipine group. Interestingly, blood loss was significantly greater in the SNP group. Details on SNP dose requirements were not provided.

Przybylo et al (1995) described CN^- and SCN blood levels in ten children who received SNP at doses up to 10 micrograms/kg/min (mean infusion rate 6 microgram/kg/min) while undergoing cardiopulmonary bypass for repair of complex congenital cardiac defects. CN^- levels rose as a function of time while SNP was infused, and rapidly fell when SNP was discontinued. Despite the fact that some children demonstrated serum CN^- levels above the generally accepted threshold of 0.5 micrograms/ml, no patient

developed clinically apparent toxicity. Kazim et al (1996) questioned the validity of the results of this study because of the CN^- assay methods utilized.

Linakis et al (1991) retrospectively examined physician-ordering practice as it pertained to blood cyanide levels in children receiving SNP. They sought to determine how the laboratory determinations were used to monitor patients and if there was clinically apparent toxicity in children found to have cyanide concentrations exceeding the "normal" limit of 500 micrograms/liter. They found poor correlation between blood cyanide concentration and dose or duration of therapy in patients whose cyanide levels were "toxic." Thiocyanate determinations were normal and no child manifested signs or symptoms of cyanide toxicity. They concluded that further pediatric studies were needed.

2.0 Study Objectives

We propose a multicenter trial that will provide guidance for the use of SNP to reduce blood pressure in pediatric patients. The trial is a randomized, blinded withdrawal to placebo trial. The aims of the trial are:

1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours
2. To assess the potential for rebound hypertension following administration of sodium nitroprusside for 12 hours or more when the infusion is temporarily discontinued.

To meet these study aims, the following study phases, defined in Section 3.1.1, will have the following objectives:

- Open-Label Study Drug Administration (Dose-Titration) Phase

The objective during this phase of the study is to determine the effectiveness and safety of SNP for controlling blood pressure during stable infusion lasting at least 12 hours.

- Blinded Study Drug Administration Phase

  The primary endpoint for the study will be determined during this phase of the study. The primary endpoint is Delta (MAP) recorded during the Blinded Study Drug Administration Phase in the absence of other stimuli. The primary objective is to determine the persistence of sodium nitroprusside versus placebo for reducing blood pressure in pediatric patients

  The secondary objectives during this phase of the study are as follows:

  1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience offset during the 30-minute blinded study drug period.
  2. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience rebound hypertension during the 30-minute blinded study drug period.
  3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the 30-minute blinded study drug period.
  4. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the 30-minute blinded study drug period.
  5. To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.

6. To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in individual laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo.

- Follow-up Phase

The following objectives to be evaluated during this phase of the study are as follows:

1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the Follow-up Period.
2. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the Follow-up Period.
3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience changes in individual physical examination parameters represented as either normal or abnormal from the Pre-Study Period to the end of the Follow-up Period.
4. To compare the changes (values recorded during the end of the Follow-up Period minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.

3.0 Investigational Plan

3.1 Overall Study Design and Plan: Description

This is a phase II, multicenter, randomized, double-blind placebo-controlled, parallel group study to determine the persistence of the effect of SNP on blood pressure and to assess the potential for rebound hypertension associated with prolonged infusion in pediatric subjects.

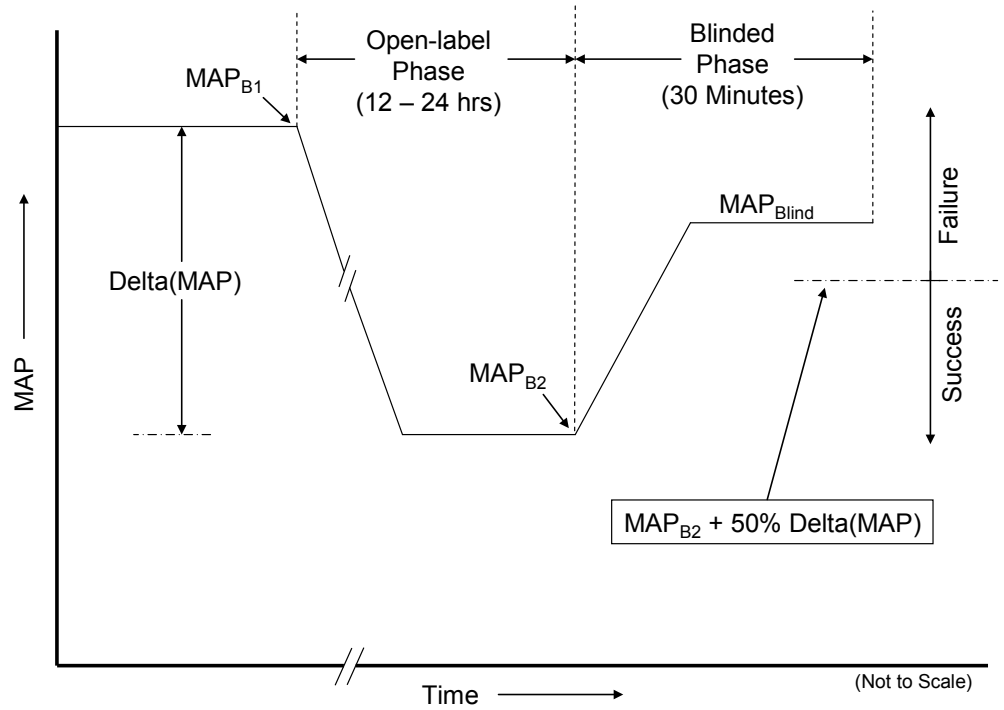
Target MAP is defined as the clinically appropriate MAP as determined by the investigator taking into account the clinical presentation and medical needs of the subject. The investigator may change the target MAP at his/her discretion based on clinical needs during the course of the study.

The initial baseline MAP (B1) is defined as the blood pressure measurement taken just prior to the initiation of open label study drug after at least a 5-minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). See Figure 1 below.

The subsequent baseline MAP (B2) is defined as the blood pressure measurement taken just prior to the initiation of blinded study drug after at least a 5 minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). Prior to establishing B2, there shall have been no changes in the SNP infusion rate for a period of at least 20 minutes.

Figure 1

Study Treatment Phases with MAPs and Delta(MAP)



- MAP$_{B1}$ = MAP immediately prior to start of Open-label Study Drug Administration Phase
- MAP$_{B2}$ = MAP immediately prior to start of Blinded Study Drug Administration Phase
- Delta(MAP) = MAP$_{B1}$ – MAP$_{B2}$
- MAP$_{Blind}$ = Highest MAP during Blinded phase that is sustained for at least 30 seconds
- Treatment Success: MAP$_{Blind}$ < MAP$_{B2}$ + 50% Delta(MAP)

Example: Subject's MAP immediately prior to start of the Open-label Study Drug Administration Phase is 100 mmHg (MAP$_{B1}$). At the end of the Open-label Study Drug Administration Phase, MAP is 60 mmHg (MAP$_{B2}$). Thus, Delta(MAP) = 40 mmHg. For *treatment success*, MAP during the Blinded Study Drug Administration Phase cannot exceed MAP$_{B2}$ + 50% Delta(MAP), or 80 mmHg, for ≥30 seconds per excursion.

Approximately 12 centers will participate in subject recruitment to complete the study.

Approximately sixty (60) patients who require long term (at least 12 hours) blood pressure control will be enrolled. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment.

Any patient who starts the blinded study drug administration period will be considered complete for analysis. Enrolled subjects will be randomized in equal proportions to receive either placebo or SNP for the duration of the blind-treatment period, which will immediately follow the open-label infusion of SNP.

3.1.1 Definition of Study Periods

Study periods are as follows:

- Pre-study drug administration: a period of up to 3 days preceding the start of study drug administration during which informed consent, randomization and other enrollment procedures takes place.
- Open-label study drug administration (Dose-Titration): The period of open label study drug administration will be at least 12 hours but not greater than 24 hours.
- Blinded study drug administration: The period beginning with the start of blinded study drug administration and ending with the discontinuation of blinded study drug. It immediately follows the open-label period and will be no longer in duration than 30 minutes.
- Follow up: The period immediately following blinded study drug administration and ending 30 days after completion of study drug administration. AEs will be followed for 24 hours after termination of study drug. SAEs will be followed for 30 days.

Safety will be assessed via the evaluation of adverse events, pre- and post-treatment laboratory results, and vital sign data. The efficacy endpoints will mainly be assessed by examining blood pressure parameters.

3.2 Selection of Study Population

Children up to 17 years of age who require pharmacologic blood pressure control for at least 12 hours will be eligible for study. Blood pressure control is defined as the maintenance of the subject's mean arterial pressure to within 90-110% of a target MAP specified by the physician.

Five pediatric age groups will be enrolled in this trial:

Group A: Neonates from birth to less than 30 days of age
Group B: Infants and toddlers from 30 days to < 2 years
Group C: Preschool children from 2 years - < 6 years
Group D: School age children from 6 yrs - < Tanner stage III
Group E: Adolescents from Tanner stage III - < 17 years.

Tanner III refers to the onset of puberty and occurs at different ages in different individuals. The mean age at onset of Tanner III ranges from 12.4 to 13.1 years in males, and 11.9 to 12.6 years in females. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment (see Appendix A).

3.2.1 Inclusion Criteria

Subjects must meet all of the following criteria:

1. Subject is less than 17 years of age.
2. An in-dwelling arterial line is clinically indicated.

3. Subject's parent or legal guardian is willing and able to give informed consent signing and dating an IRB-approved informed consent, and subject provides assent, signing an IRB-approved and –required informed assent.
4. Subject is anticipated to require a minimum of 20 mm Hg reduction in MAP for at least 12 hours using SNP.

3.2.2 Exclusion Criteria

Subjects will be excluded from study if any of the following criteria exist:

1. Subject weighs < 2.5 kg.
2. Subject has a known allergy to SNP.
3. Subject has a known mitochondrial cytopathy with a disorder of oxidative phosphorylation or of respiratory chain enzymes.
4. Subject has a contraindication to vasodilator therapy for control of blood pressure during surgery or in the intensive care unit.
5. Subject has raised intracranial pressure.
6. Subject is anticipated to need anti-hypertensive drugs other than study drug either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) during the period of study drug administration. However, patients receiving stable doses of an anti-hypertensive drug(s) prior to the initiation of study drug may be enrolled, provided they will not have received IV vasodilator therapy for greater than 8 hours prior to receiving study drug.
7. Subject has any serious medical condition which, in the opinion of the investigator, is likely to interfere with study procedures.
8. Subject is moribund (death likely to occur within 48 hours).
9. Subject has a positive result for the urine or serum HCG test administered at screening.

3.2.3 Prior and Concomitant Therapy

Initiation of new anti-hypertensive drugs either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) other than the study drug during study drug administration is prohibited. However, in patients receiving stable doses of non-study anti-hypertensive drugs, these agents may be continued during study-drug administration If open label study drug is initiated during anesthesia, the anesthetic medications will be recorded on the CRFs but are not considered as anti-hypertensive drugs. All concomitant medications and clinically meaningful, unexpected, and invasive procedures will be recorded for the period beginning 72 hours prior to study drug administration through 24 hours post study drug conclusion. The dates of administration, dosage and reason for use must be included. Concomitant medications will be collected for SAEs occurring within 30 days following study drug administration. Vaccines are considered a concomitant medication.

3.3 Efficacy and Safety Assessments

3.3.1 Efficacy and Safety Measurements

Table 1 is a schematic representation of study assessments and procedures.

TABLE 1: Schedule of Assessments: Sodium Nitroprusside Long-Term Infusion Study

Procedure	Pre-study Drug Period	Open-label Period	30 minute Blinded Study Drug Period	Study Drug d/c	Follow-up (24 hours post blinded study drug)[1]
Assessments					
Review Entry Criteria	X				
Informed Consent/ HIPAA Consent	X				
Collect Demographic Data	X				
Medical History	X				
Physical Examination	X			X	X[7]
Vital Signs (SBP, DBP, MAP, HR) [2]	X	X	X	X	X
Growth Parameters[3]	X				
Urine Output[10]		X	X	X	
Serious Adverse Events/Adverse Events		X	X	X	X[5]
Concomitant Medication/Proceedure[8]	X[9]	X	X	X	X
Randomization of Blinded study drug	X				
Blinded Study Drug Administration			X		
Open-label Study Drug Administration		X			
Laboratory Assessments					
Pregnancy test (post-menarche females)	X				
Electrolytes, BUN, creatinine	X			X	
Hematology (CBC & platelet count)	X			X	
Liver Enzymes (AST, ALT)	X			X	
Arterial Plasma Lactate level	X	Q 8 hours		X	
Arterial Blood Gas with Co-oximetry (includes Methemoglobin)[4]	X	Q 8 hours		X	
Mixed Venous Blood Gas with Co-oximetry (includes Methemoglobin)[4]	X	Q 8 hours		X	
Plasma Thiocyanate and cyanide (central lab)	X	Q 8 hours		X & 12 hr post d/c	
Urine Thiocyanate (central lab)[6]	X	Q 8 hours		X	X

1. End of Study assessment will be done at 24 hours post blinded study drug administration.
2. Vital sign measurements as described in protocol, sections 3.4.2 – 3.4.7.1. Vital signs will then be collected every 12 ± ½ hours for 24 hours post blinded study drug administration.
3. Growth parameters will include weight, height/ length.
4. ABG sampling preferred, sample collected at drug d/c only if line is still in.
5. AEs will be followed for 24 hours & SAE will be followed for 30 days, after the completion of study drug administration.
6. Urine collection details are described in section 3.4.7.2 and the MOP.
7. To be performed 18-30 hours following the termination of study-drug administration
8. Clinically meaningful, unexpected, and invasive procedures only
9. Within 72 hours of study drug administration
10. Measurements to be performed at time of urine thiocyanate sample collection

3.3.2 Safety Assessments

Safety assessments will include monitoring the tolerability of the SNP infusion and assessing physical examinations, vital signs, clinical laboratory values, concomitant medications and procedures, and adverse events throughout the study. SAEs will be collected for 30 days following completion of study drug administration .

In cases of discharge from the hospital before 30 days, parent (or guardian) will be contacted to determine if any SAE's occurred following discharge but within 30 days of study drug discontinuation. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. See section 4.2 for specific adverse event parameters and actions to be taken.

3.3.3 Drug Concentration Measurements

Cyanide, thiocyanate, methemoglobin, lactic acid, and arterial blood gas analysis will be performed throughout the trial to indirectly query SNP levels and determine subject safety.

3.4 Study Visits and Procedures

3.4.1 Informed Consent

Prior to the start of any study-related procedure, a signed and dated informed consent and, if applicable, assent must be obtained and documented in the subject's medical record (See Appendix B).

3.4.2 Pre-study drug administration procedures

The following procedures will be completed prior to the administration of study drug:

1) Obtain signed and dated informed consent/HIPAA authorization/assent.
2) Collect demographic data and medical/surgical history.
3) Record diagnosis.
4) Perform a pertinent physical examination.
5) Obtain vital sign measurements.
6) Determine subject height in centimeters and subject weight in kilograms (for calculation of appropriate study drug dose).
7) Collect blood samples for laboratory evaluations as per Table 1. Pregnancy test if required must be done within 48 hours of study drug administration. (If the screening pregnancy test will have been more than 48 hours prior to the start of the study drug administration, then the test will be repeated.)
8) Document concomitant medications (including over-the-counter preparations).
9) Randomize subject.

3.4.3 Open-Label Study Drug Administration (Dose-Titration) Procedures:

The following procedures should be performed sequentially unless otherwise indicated.

1) Stabilize sedation/analgesia.
2) Insert arterial line if not already in place.
3) Obtain vital sign measurements immediately prior to the start of the open-label study drug administration. This defines B1.
4) Determine and record target MAP.
5) If the difference between B1 and the target MAP is <20 mm, the patient will be withdrawn from the study and not given study drug.
6) Begin administration of open-label study drug at a dose not to exceed 1.0 mcg/kg/min; titrate to target MAP.

7) The dose of open-label SNP will be titrated according to the subject's BP response such that the target MAP, chosen by the study physician, is achieved ±10%. If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose. The duration of open-label study drug administration will be at least 12 hours but less than 24 hours.
8) Obtain vital sign measurements every one minute for the first 10 minutes then every 5 ± 1 minutes for an additional 30 minutes after initiation of open-label study drug infusion and after each dosage adjustment. After the initial 40 minutes, once a stable dose is achieved and BP control is satisfactory, vital sign measurements will be obtained at least every 15 minutes. Additionally, obtain vital sign measurements in a similar manner whenever it is necessary to change the open-label drug infusion rate.
9) Collect blood samples for laboratory evaluations as per Table 1.
10) Record concomitant medications, procedures, and adverse events.
11) Whenever an adverse event occurs, obtain vital sign measurements. If clinically appropriate, a blood sample for safety including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations may be drawn.

3.4.4 Blinded Study Drug Administration Procedures

1) Obtain vital sign measurements immediately prior to the start of blinded study drug administration. This defines MAP_{B2}. There must be 5 min of stable conditions and 20 minutes of no changes in Open-label study drug prior to starting the blinded study drug administration phase.
2) Begin 30-minute blinded study drug administration as described in Section 3.4.8.

3) Record blood pressure and heart rate every one minute for the duration of blinded study drug administration.
4) If blood pressure control is lost (defined as loss of 50% of delta MAP sustained for 30 sec in the absence of stimulation) the blinded study drug is discontinued when there is a safety concern or the MAP reaches 120% of MAP_{B1}.
5) Record concomitant medications and procedures and adverse events. Whenever an adverse event occurs, obtain vital sign measurements and, if appropriate, a blood sample for safety analysis including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations.

3.4.5 Study Drug Discontinuation

1) Within 2 hours after discontinuation of the study drug administration, collect blood samples for laboratory evaluations
2) Conduct a pertinent physical examination and perform all other assessments listed in Table 1 for this study phase.

3.4.6 Follow up Procedures

The following will be performed after completion of study drug administration through 24 hours post study drug end:

1) If applicable, record the estimated blood loss and fluid intake, including blood and blood products and output during the trial period.
2) Record concomitant medications and clinically meaningful, unexpected, and invasive procedures.
3) Record vital signs at 12 and 24 ± ½ hours after the end of study drug administration.
4) Perform a pertinent physical examination 18 – 24 hour following discontinuation of study drug administration.

5) Collect adverse events for 24 hours following discontinuation of study drug administration.
6) Collect serious adverse events plus associated concomitant medications and clinically meaningful, and invasive procedures for 30 days following discontinuation of study drug administration.

SAEs and associated concomitant medications and procedures will be collected for 30 days following the discontinuation of study drug administration, either through telephone contacts and/or study visits or spontaneously reported by the subjects.

3.4.7 Methods of Assessment

3.4.7.1 Vital Sign Measurements

Vital signs: systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate will be measured.

The principal method of obtaining blood pressure measurements will be from an intra-arterial catheter inserted in an upper or lower extremity artery. Manual blood pressures from a non-invasive blood pressure cuff will only be used prior to insertion of and during a malfunction of the arterial catheter. The blood pressure transducer is internally calibrated by the instrument upon performing the zeroing procedure. All blood pressure and heart rate data will be acquired electronically when possible.

3.4.7.2 Blood Draws and Urine Samples

Blood drawn for study related purposes will not exceed the maximum amounts specified by the American Association of Blood Banks for healthy infants, children, and adolescents. Normally, this value is 7 ml/kg over an eight week period. This study is an inpatient trial of short duration, therefore, the amount of blood withdrawn for study related purposes will take into account the patient's pre-existing hemoglobin and

hematocrit, and local Institutional Review Board limitations on maximum allowable blood draws for study-related purposes. A reasonable and conservative value is 3 ml/kg.

Urine will be sampled for thiocyanate concentrations every 8 hours, or fraction thereof, commencing with initial study drug administration to the end of study drug completion and then Q 8 hours times 3 after the discontinuation of study-drug administration, or until the urinary catheter is removed, whichever occurs first. All urine samples will be from a pooled urine collection from the 8-hour time period or fraction of 8-hour time period. The sample will be stored cold until shipment to the central lab. Details of urine collection procedures are described in the MOP.

3.4.8 Dispensing of Study Drug

Study drug will be dispensed to the sites in 2 ml vials containing 25 mg/ml of SNP. The pharmacist will dispense two preparations of study drug, one for the open-label study drug period and one for the blinded study drug period.

The open-label study drug administration phase will utilize a fixed study drug concentration and variable infusion rate scheme. Syringes or bags will be prepared by the investigational drug pharmacy by adding 25 mg of SNP to 50 ml 5% dextrose. The syringe will have a label indicating the concentration of the solution, and the infusion rate necessary to provide 1.0 microgram per kilogram per minute (1.0 mcg/kg/min). Clinicians can then make the necessary dosage adjustment for adequate blood pressure control. All dosage adjustments will be captured on the case report forms (CRFs).

The pharmacist will supply blinded study drug for each subject according to a randomization assignment generated by the IVRS. The blinded study drug will be prepared by the pharmacist such that either the concentration of drug is the same as in the initial open label period or placebo. Subjects will receive blinded study drug at the same rate of infusion that was used at the conclusion of the initial open-label study drug administration period.

Syringes or bags will be wrapped in opaque or amber plastic to protect from light.

3.4.9 Delivery of Study Drug

Infusion pumps capable of reliable delivery at low infusion rates (to 0.1 ml/hr) will be used. All pumps will have free flow protection and will be internally calibrated for accuracy by the manufacturer. Accuracy will be verified at each site by the biomedical engineering department as part of their equipment management program. Quality assurance checks will be performed periodically according to manufacturer specifications.

Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. Microbore low compliance tubing, with volumes of approximately 1 mL will be used.

Study drug will be infused via a dedicated peripheral intravenous catheter or via a dedicated lumen of a multi-orifice central venous catheter. Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population.

3.5 Removal of Subjects from Therapy or Assessment

3.5.1 Early Discontinuation of Study Drug and Subject Withdrawal

If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose.

If the subject withdraws participation in the study for any reason, every effort will be made to collect safety data, vital sign measurements, samples for safety and laboratory analyses. The date, time and reason for discontinuation must be recorded on the case report form (CRF). Additionally, every attempt should be made to complete all other study related procedures on discontinued subjects who have received any amount of study drug as the data will be included in the safety and intention to treat analyses. Subjects who prematurely discontinue from the study will not be replaced.

Any subject who does not start the blinded study drug administration period will be considered prematurely withdrawn from the study. Any patient who starts the blinded study drug administration period will be considered complete for analysis.

Potential reasons for subject withdrawal from the study are as follows:

1) Subject's parent or legal guardian wishes to have the subject withdrawn for any reason;
2) Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the subject's safety;
3) The investigator feels that it is in the subject's best medical interest to be withdrawn.
4) Subject no longer needs blood pressure control.

3.5.2 Data Safety and Monitoring Board

To ensure that the welfare of trial patients receives appropriate consideration, an independent Data and Safety Monitoring Board (DSMB) has been organized by the BPCA-CC on behalf of the NICHD to review relevant safety and/or efficacy data during the course of the trial. The DSMB may recommend discontinuation of the study, or modifications to the study protocol for safety reasons.

The DSMB consists of four core members (Chair, ethicist, statistician, community representative) plus additional ad hoc members for the various medical subspecialties involved in the BPCA protocols.

Each DSMB will have a presenting statistician who will be responsible for presenting the interim data. This member will write the reports and will be one non-voting member of the DSMB. Except as their role in the DSMB, all DSMB members are not participating in the design or conduct of this study, as an investigator or otherwise, and lack any financial conflict that would introduce any bias.

3.5.2.1 DSMB Responsibilities

- Monitoring the safety of trial patients;
- Recommending discontinuation of the trial for safety reasons;
- Recommending changes to the study protocol for safety reasons;
- Providing written reports on an ongoing basis following scheduled and ad hoc meetings that will be archived and may be provided to regulatory agencies.

These responsibilities will be broadened to include decisions regarding efficacy if the trial is an efficacy trial. The DSMB will monitor the safety of trial patients by reviewing the occurrence of adverse events and deaths, on a real-time basis as SAE reports are transmitted. The DSMB may also monitor compliance with the protocol, and factors affecting patient safety or the integrity of the trial. The DSMB may request any additional data that are not included in the report if deemed necessary for effective monitoring.

If the DSMB finds any major concerns about safety, it may recommend discontinuing the trial or modifying the study protocol. Following each data review, the DSMB will send a written recommendation regarding the trial, (e.g., to continue according to the protocol, or recommendations for specific actions) to the sponsor.

3.6 Investigational Product

3.6.1 Identity of Investigational Product

Sodium nitroprusside (sodium nitropentacyanoferrate (III) dihydrate) is a reddish-brown crystalline powder that is freely soluble in water. Its molecular formula is $Na_2[Fe(CN)_5NO] \cdot 2H_20$. Study drug will be supplied by the BPCA Coordinating Center to the Investigational Drug Service at each clinical center in a standard concentration of 25 mg/ml. The Investigational Drug Service at each clinical center will then prepare the drug in syringes or bags of sterile 5% dextrose for administration to randomized patients according to the guidelines provided above. Sterile 5% dextrose will be utilized as placebo.

3.6.1.1 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature from 15°- 30°C and protected from light in its carton until used. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug must be maintained under adequate security.

3.6.2 Methods of Assigning Subjects to Treatment Groups

After meeting all inclusion and exclusion criteria, subjects will be considered to be enrolled and will be randomly assigned to receive either placebo or active drug treatment groups during the blinded study drug administration phase of the trial using a single centralized randomization schedule. Randomization into the blinded portion of the trial will be performed via a centralized interactive voice response system (IVRS).

The BPCA-CC will provide a system for the pharmacist to obtain each subject's randomized treatment assignment in a timely manner prior to the administration of study drug.

3.6.3 Assigning Subject Numbers

Study participants will be assigned a subject number upon successful enrollment into the study. The subject number will consist of five digits. The first two digits will be the site number of the enrolling institution followed by a three digit enrollment-sequence number. For example, Subject #10-023 would be the 23rd subject enrolled at Site #10.

3.6.4 Blinding

The subject, as well as all caregivers, will remain blinded to the treatment assignment throughout the course of the study. For subjects' safety, the pharmacist will be aware of the treatment group for each subject. The BPCA-CC will maintain the double-blinded randomization schedule.

The randomization for an individual subject may be revealed in an emergency; however, investigators are discouraged against requesting that the blind be broken for an individual subject. Notification of any unblinding must be sent via facsimile to the BPCA-CC within 24 hours.

3.6.5 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms.

3.6.6 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the Clinical Supply Shipment Request and Verification or similar document. An accurate inventory of study drug will be kept by the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and returned to the BPCA-CC if requested. The investigator agrees not to supply study medication to any persons not enrolled in the study or not named as a sub-investigator for the trial.

4.0 Adverse Events

4.1 Definition

An adverse event is defined as any unintended and unfavorable medical occurrence in a clinical investigation subject, administered a pharmaceutical product, regardless of the causal relationship with treatment. An adverse event can therefore be any untoward sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not the event is considered causally related to the use of the product. Pre-existing conditions that remain stable throughout the study period will not be considered adverse events. Any worsening of a pre-existing condition or illness is considered an adverse event.

Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional over-dosage, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events if they result in discontinuation from the study, necessitate therapeutic medical intervention, or the investigator considers them to be adverse events.

Adverse events will also include electronically-monitored vitals signs that meet the definitions of section 4.2; however, electronically-captured data excursions, which in the opinion of the investigator represent data artifacts, such as might be produced by the turning of the patient or brief interruption of the electronic circuit of the monitor device(s) or which do not likely reflect an actual untoward event will not be considered to adverse events.

New or worsening physical-exam findings that occur following the initiation of study-drug administration will be considered to be adverse events, without regard to causality.

4.1.1 Serious Adverse Events

A serious adverse event is one that meets the above criteria and also results in one of the following conditions:

1. Death
2. A threat to life
3. Requirement for inpatient hospitalization
4. Prolongation of hospitalization
5. Production of a congenital anomaly or birth defect
6. A persistent or significant disability or incapacity (excluding experiences of minor medical significance such as headache, nausea, vomiting, diarrhea, and accidental injury)
7. The requirement for a medical or surgical intervention in order to prevent a serious outcome.

If an adverse event meets any of the above criteria, it must be reported to the BPCA-CC as a serious adverse event (SAE) within 24 hours of the investigative site's awareness of its occurrence.

4.2 Adverse Event Severity

The criteria for rating adverse events are as follows:

Mild	The adverse event is transient and easily tolerated by the subject.
Moderate	The AE causes subject discomfort and interrupts usual activities.
Severe	The AE causes significant interference with normal activities and may be incapacitating or life threatening.

Safety data are a primary objective of this trial. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. Safety will be evaluated throughout the study and during the follow-up period by evaluating the tolerability of the study drug infusion and by monitoring clinical and laboratory signs of SNP toxicity such as hypotension, tachycardia, bradycardia, acid base status, serum lactate concentration, methemoglobin levels, cyanide levels, and when available, mixed venous oxygen tension.

- Co-oximetric arterial blood gas analysis with methemoglobin determination will be performed Q8. Frequent arterial blood gas monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity.
- Lower than expected methemoglobin concentrations may reflect indirect evidence of cyanide toxicity because cyanide has a high affinity for methemoglobin, combining with it to form the non-toxic molecule cyanomethemoglobin.
- Other laboratory findings suggestive of cyanide toxicity that will be monitored in this trial include serum lactate levels and mixed venous oxygen saturation.

Lactate levels will rise and mixed venous oxygen tension will increase when cyanide toxicity is occurring due to the reduced ability of the tissues to extract oxygen.

- Plasma and urine thiocyanate levels are indirect markers of cyanide exposure because the majority of the cyanide ions liberated by the metabolism of SNP in the red blood cell are converted to thiocyanate by the rhodenase enzyme system in the liver and excreted in the urine. Free cyanide levels will also be measured.

The adverse event of rebound hypertension shall be considered mild if the MAP rises >10% above the baseline (B1), moderate if the MAP rises >20% above baseline (MAP_{B1}), and severe if the MAP rises >30% above baseline (MAP_{B1}).

The adverse event of excessive hypotension shall be considered mild if the MAP falls >20% below target; fluid therapy may be administered. The adverse event of excessive hypotension shall be considered moderate if the MAP falls >25% below target, fluid therapy is required, and pharmacologic therapy is required. The adverse event of excessive hypotension shall be considered severe if the MAP falls >30% below target, fluid therapy is required, and repeated and/or continuous pharmacologic support is required.

The rating of the adverse event of tachycardia shall be defined for sustained rates exceeding the age adjusted maximums (Table 3) by using the mild, moderate, and severe ranges outlined in Table 4A.

TABLE 3: Age-Adjusted Maximums for Pediatric Heart Rate

Subject Age	**Maximum Heart Rate (bpm)**
1 month <6 months	180
6 months <3 years	160
3 <8 years	150
8 <17 years	130

TABLE 4A: Severity of Tachycardia (Heart Rate in Beats per Minute)

Age	Mild	Moderate	Severe
Age <6 months	180-199	200-219	220 and higher
6 months - <3 years	160-179	180-199	200 and higher
3 years to < 8 years	150-164	165-179	180 and higher
8 years to <17 years	130-149	150-169	170 and higher

The adverse event of lactic acidosis shall be considered mild if the serum lactate concentration is between 5 and 7.5 mM, moderate if the serum lactate concentration is between 7.6 and 10 mM, and severe if the serum lactate concentration exceeds 10 mM.

The adverse event of cyanide toxicity will be considered mild if the serum cyanide concentration is 0.5 mcg/L – 1.0 mcg/L, moderate if the serum cyanide concentration is >1.0 – 1.5 mcg/L, and severe if the serum cyanide concentration is > 1.5 mcg/L. Corresponding erythrocyte (vs. serum) cyanide values are < 10 μM/L (mild), 10-20 μM/L (moderate), and > 20 μM/L (severe).

Severity ratings for these selected adverse events are displayed in Table 4B.

TABLE 4B: Severity of Selected Adverse Events

Event	Mild	Moderate	Severe
Rebound Hypertension (during blinded phase)	MAP rises >10% above baseline (MAP_{B1})	MAP rises >20% above baseline (MAP_{B1})	MAP rises >30% above baseline (MAP_{B1})
Hypotension	MAP falls >20% below target	MAP falls >25% below target IV therapy required; Pharmacological therapy required	MAP falls >30% below target IV therapy required; Repeated or continuous pharmacological therapy required
Lactic acidosis	5 -7.5 mM	7.6-10 mM	>10 mM
Cyanide toxicity	serum cyanide 0.5 0.5 – 1.0 mcg/L	serum cyanide >1.0-1.5 mcg/L	serum cyanide >1.5 mcg/L
Cyanide toxicity (erythrocyte)	cyanide <10 μM	cyanide ≥10–20 μM	cyanide >20 μM

Frequent monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity. If the base deficit exceeds 8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the patient will be discontinued from study and the SNP infusion terminated. If the lactate level rises by more than 4 mmol/L in a 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output) SNP administration will be discontinued. If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes

percent between arterial and mixed venous blood in the absence of an explainable cause, SNP administration will be discontinued and treatment for suspected cyanide toxicity will be initiated.

Suspected cyanide toxicity will be further assessed and treated as follows:

1) Obtain blood for arterial and venous blood gases with co-oximetry, plasma lactate, and cyanide and thiocyanate levels.

2) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration (see below).

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10ml) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result).

TABLE 5: Dosage Chart for Children

Patient's Hemoglobin g/dL	Initial Dose of Sodium Nitrate (3%) mL/kg IV	Initial Dose of Sodium Thiosulfate mL/kg IV
8	0.22 mL/kg (6.6 mg/kg)	1.10 mL/kg
10	0.27 mL/kg (8.7 mg/kg)	1.35 mL/kg
12	0.33 mL/kg (10.0 mg/kg)	1.65 mL/kg
14	0.39 mL/kg (11.6 mg/kg)	1.95 mL/kg

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals. [Micromedex Poisondex www.micromedex.duhs.duke.edu , see also, Berlin, 1970]

4.3 Relationship to Study Drug

The criteria for determining the relationship of the AE to the study drug are as follows:

1) Probably related: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.
2) Possibly related: An AE that is likely to be related to the administration of the study drug and an alternative cause is equally or less likely when compared to the study drug.
3) Probably not related: An AE that has little or no relationship to the study drug and there exists a more likely, or equally likely, alternative cause.
4) Not related: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.

If an AE is considered to be not related, probably not related or possibly related, an explanation of other probable causes must be included in the CRF.

4.4 Adverse Event Collection Period

SAEs will be monitored and reported from the time the subject receives study drug through 30 days following termination of study drug. Knowledge of adverse events will be gained from direct monitoring of the study subject as well as from clinician observation, and self reporting by the study subject or his/her guardians. Adverse events that have not resolved, or are ongoing, will be monitored to resolution if felt to be related to study drug, or until it is felt that the subject has stabilized.

5.0 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual subject, the investigator or other responsible physician must contact one of the Co-Principal Investigators immediately so that a timely decision can be made as to whether or not the subject should be enrolled or continue in the study. If a deviation is being requested by one of the Co-Principal Investigators, he must contact the other Co-Principal Investigator for a decision. The deviation from the protocol will be authorized only for that particular subject. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF.

6.0 Statistical Considerations

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a Statistical Analysis Plan, which will be finalized prior to unblinding. Any changes in the statistical methods described in this protocol that occur prior to unblinding will be documented in the Statistical Analysis Plan and will not result in a protocol amendment.

6.1 General Overview

The primary efficacy variable is the intra-patient change in Delta (MAP) during the blinded phase of the study. The primary null hypothesis to be tested is that there is no difference between the active study drug and placebo in the proportion of patients who experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP). Statistical analyses will be performed using two-sided tests. A 0.05 significance level will be used in all tests of treatment differences. Tests for interactions will utilize a 0.10 statistical significance level. Individual secondary endpoints will be evaluated using a hierarchical testing procedure. The Statistical Analysis Plan will include a detailed description of all statistical methods, testing procedures, and methods of data imputation.

The Data Monitoring Committee charter will contain the specific details regarding the re-estimation of the target sample size.

Data will be summarized by treatment group with respect to demographic and baseline characteristics, efficacy variables, and safety variables. For parameters measured at baseline, the outcome variables of interest are the changes from baseline (Pre-Study Drug Period). Summary statistics will include the mean, N, standard deviation, median, minimum, and maximum values for continuous variables, and frequencies and percentages for categorical variables. Prior to summarizing results by study center, or performing analyses that include center as a factor in the analysis, small centers will be pooled. All efficacy variables will be summarized by treatment and by visit. Analyses will be performed to explore whether there are treatment-by-center interactions. If a treatment-by-center interaction is detected, the interaction will be explored in an ad-hoc manner. Categorical variables will be analyzed using Cochran-Mantel-Haenszel (CMH) test blocking on centers. Details of the model and the analyses will be specified in the Statistical Analysis Plan and all statistical analyses will be performed using SAS, Version 8.2 or higher.

6.2 Study Objectives

The study objectives are as defined in Section 2.0 of this protocol.

6.3 Patient Population(s) for Analysis

6.3.1 Efficacy

The intent to treat (ITT) population will contain all patients who were exposed to the study drug during the Open-Label Study Drug Administration (Dose-Titration) Phase.

The Per-Protocol population will contain all patients randomized to the double-blind phase of the trial. The efficacy analysis will be based on the Per-Protocol population. A patient will be classified as a *treatment success* if they meet the following criteria:

• Complete the 30-minute double-blind phase without having an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP) and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

A patient will be classified as a *treatment failure* if they meet the following criteria:

• Fail to complete the entire 30-minute double-blind phase without receiving additional treatment to control their blood pressure in addition to the study drug.

• Fail to complete the entire 30-minute double-blind phase for any reason.

• Experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP) for ≥30 seconds at any time during the 30-minute double-blind phase.

6.3.2 Safety

All patients who receive any study medication (ITT population) will be included in the safety analyses and summaries, independent of the patient actually reaching the double-blind phase of the study. All non-serious adverse events recorded within 72 hours of either completion of the double-blind phase, or within 72 hours of premature discontinuation of the study, will be reported.

All serious adverse events recorded within 30 days of either completion of the double-blind phase, or premature discontinuation of the study, will be reported.

6.4 Background and Demographic Characteristics

All baseline information, including demographic factors, physical examination parameters, vital signs, growth parameters (if applicable), laboratory and blood gas information will be summarized by treatment group for all enrolled patients (ITT population). Additionally, nonrandomized patients versus randomized patients will be summarized and compared by age, gender, and race to determine if there are any differences among the 2 subsets. Analyses will be conducted to determine differences in the demographic and baseline characteristics of the treatment groups. For continuous variables (e.g., age, weight), the number of non-missing and missing values and the median, mean, standard deviation, minimum, and maximum will be displayed for each treatment group. For categorical variables (e.g., race, gender), the counts and proportions will be tabulated.

Baseline comparability will be evaluated based on the pooled data from all centers. To determine comparability of the treatment groups at baseline, continuous demographic and clinical variables will be analyzed using an analysis of variance test (with an appropriate transformation, if necessary). Baseline, demographic, and clinical variables that are ordinal will be analyzed using the Cochran Mantel Haenszel test; parameters that are dichotomous will be analyzed using a chi-square ($\chi 2$) test or Fisher's exact test,

depending on the individual cell counts. If there are treatment group differences at the 0.10 level of significance in demographic or baseline clinical variables, these variables may be added as stratification variables or covariates to the efficacy analyses.

6.5 Study Medication

The duration of exposure to study medication will be summarized for all enrolled patients, and separately for all randomized patients.

6.6 Concomitant Therapy

Concomitant medications (medications present while on study medication) will be recorded throughout the study and at early termination. These medications will be coded using the WHO drug dictionary. The number of randomized patients using prior or concomitant medications will be categorized by the WHO drug category and preferred term, and presented for each treatment group. In any given category [e.g., drug category] a patient will be counted only once.

6.7 Statistical Design and Models for Analysis

This is a biphasic (open-label dose-titration phase, followed by a randomized phase), randomized, double-blind placebo-controlled study. Patients who are enrolled into the initial phase of the study will have their dose of sodium nitroprusside titrated and must receive a minimum of 12-hours of treatment to be eligible for the randomized phase of the study. Patients who cannot be adequately titrated during the initial 12-hour period will not proceed to the randomization phase of the study. Patients who reach the randomization phase of the study will be assigned to receive placebo, or continue to receive sodium nitroprusside based on a stratified permuted block central randomization scheme.

Five age groups (A through E) will be enrolled in this trial:

Age Group A: Age Group A: Neonates from birth to less than 30 days of age

Age Group B: Infants and toddlers from 30 days to < 2 years

Age Group C: Preschool children from 2 years - < 6 years

Age Group D: School age children from 6 yrs - < Tanner stage III

Age Group E: Adolescents from Tanner stage III - < 17 years.

In order to efficiently account for the effect of SNP on the different age groups, neonates from birth to less than 30 days of age (Age Group A) and infants and toddlers from 30 days to < 2 years (Age Group B) will be pooled for analysis. Based on the planning estimates of the study, patients from these two pooled age groups should represent approximately 25% of the target enrollment (~60 patients).

Preschool children from 2 years - < 6 years (Age Group C) and school age children from 6 yrs - < Tanner stage III (Age Group D) will also be pooled for analysis; patients from these two pooled age groups should also represent approximately 25% of the target enrollment (~60 patients).

In order to accurately determine the target number of patients required for enrollment, a Data Monitoring Committee will examine the results from the Blinded Study Drug Administration Phase after the following number of patients have been enrolled and randomized:

- The initial 12 patients in Age Groups A & B
- The initial 12 patients in Age Groups C & D
- The initial 16 patients in Age Group E

The specific objective of the Data Monitoring Committee is to determine the target sample size based on the observed magnitude of the effect, expressed as a proportion (*treatment success* (sodium nitroprusside) / Randomized to receive sodium nitroprusside vs. *treatment success* (Placebo) / Randomized to receive Placebo. Re-estimation of the

sample size will be based on conditional power after 67% (40/60) of the target sample size has been enrolled.

6.7.1 Primary Efficacy Analysis

The primary efficacy analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} = \pi_{\text{ Patients randomized to receive placebo}}$
- H_A: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} \neq \pi_{\text{ Patients randomized to receive placebo}}$

where

- $\pi_{\text{Patients randomized to receive SNP}}$ = Proportion of *treatment successes* (sodium nitroprusside)
- $\pi_{\text{Patients randomized to receive placebo}}$ = Proportion of *treatment successes* (placebo)

6.7.2 Primary Safety Analysis

The primary safety analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} = \pi_{\text{ Patients randomized to receive placebo}}$
- H_A: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} \neq \pi_{\text{ Patients randomized to receive placebo}}$

where

- $\pi_{\text{Patients randomized to receive sodium nitroprusside}}$ = Proportion of patients randomized to receive SNP who experience a serious adverse event

- $\pi_{\text{Patients randomized to receive placebo}}$ = Proportion of patients randomized to receive placebo who experience a serious adverse event

6.7.3 Interim Monitoring Based on Conditional Power

The interim assessment for sample size adjustment will be predicated on the primary efficacy endpoint and conducted using a Conditional Power (CP) approach as described by Chen (2004). The Data Monitoring Committee will conduct the analysis to determine if a sample size adjustment is required. This assessment will be conducted after 67% of the patients from the original target sample size have either completed or withdrawn from the study. The instructions to the Data Monitoring Committee for the sample size adjustment will be described in detail in the Data Monitoring Committee Charter. An alpha level adjustment will not be necessary for the procedure described below, based on the procedure proposed by Chen, DeMets and Lan (2004).

In order for the Data Monitoring Committee to calculate conditional power using the observed data, the treatment assignment codes will need to be provided to the Data Monitoring Committee statistician. The intra-patient MAP_{B2} values will be required, including listings of the intra-patient post-baseline values during the blinded phase of the trial. If an increase in the sample size is required, and the re-estimation is within the defined sample size limits pre-specified for the study, the Data Monitoring Committee will communicate the revised target sample size to the IVRS vender for the trial. Enrollment will continue towards the new target sample size, and the data will remain blinded to all involved parties with the exception of the Data Monitoring Committee. Additionally, the Data Monitoring Committee will not have any direct contact with the study Sponsor, or the clinical investigators.

The following steps will be used to evaluate the sample size after the initial randomized patients from each pooled age group (either 12 or 16) have been enrolled and have completed the Blinded Study Drug Administration Phase, or withdrawn prematurely.

Compute conditional power for the primary hypothesis, using data from the initial patients randomized from each pooled age group (either 12 or 16).

If the conditional power is ≥ 0.5, compute the sample size necessary to increase conditional power to 0.8.

The Data Monitoring Committee will compare the re-estimated sample size calculated using the observed data, relative to the initial estimates for the study. Based on this evidence, the Data Monitoring Committee will proceed with the following action:

If the conditional power is ≥ 0.5, the sample size will be increased up to the maximum sample size pre-specified for the study.

If the conditional power is < 0.5, an analysis will be performed based on the predictive probability of achieving the endpoint within the maximum target sample size allocated for the study.

6.7.4 Sample Size Estimation

The overall sample size was calculated based on performing an un-stratified analysis of the proportion of patients classified as a *treatment success* between the 2 randomized treatment groups. With a balance randomization (1:1, SNP:Placebo), a difference in the proportion of *treatment successes* ranging from 34% to 40% would have 80% power to reject the null hypothesis in favor of the alternative (ref. Sample Size Table No. 1.0).

Sample Size Table No. 1.0

Two group χ^2 Test of Equal Proportions

Scenario	**1**	**2**	**3**	**4**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
SNP, π_1	0.080	0.160	0.240	0.320
Placebo, π_2	0.420	0.530	0.630	0.710
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	8.328	5.920	5.392	5.203
Power (%)	80	80	81	80
n per group	30	30	30	30

Based on the target sample size and the distribution of enrollment relative to the pooled analysis groups, approximately 30 patients from Age Group E (Adolescents from Tanner stage III - < 17 years) are scheduled to be randomized. However, based on the difference in the proportion of patients who are classified as a *treatment success* between the

randomized treatment groups, 30 patients may not be required to detect a significant difference at the alpha = 0.05 level. For this reason, the proportion of patients classified as *treatment successes* will be compared after the initial 12 or 16 patients are randomized, depending on the pooled age group. If the difference in proportions has an odds ratio >12, then less than the 30 patients would be required to be randomized from Age Group E. Under this scenario, the randomization of patients from this specific age group would be stopped. Drawing from Age Groups A & B or Age Groups C & D, if the difference in the proportion of *treatment successes* between the randomized treatment groups requires more than 12 patients, the study could still meet its intended goal within the pre-defined total sample size of 60 patients by enrolling the minimum sample size, based on the conditional power calculated at the interim evaluation. Power estimates at the interim evaluation for 16 patients are presented in Sample Size Table No. 2.0. The re-estimated sample size is presented in Sample Size Table No. 3.0, again for 16 patients.

Sample Size Table No. 2.0

Two group χ^2 Test of Equal Proportions

Scenario	**5**	**6**	**7**	**8**	**9**	**10**
Test significance level, α	0.050	0.050	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2	2	2
Group 1 proportion, π_1	0.125	0.125	0.125	0.125	0.125	0.125
Group 2 proportion, π_2	0.250	0.375	0.500	0.625	0.750	0.875
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	2.333	4.200	7.000	11.667	21.000	49.000
Power (%)	9	20	35	54	76	94
n per group	8	8	8	8	8	8

Sample Size Table No. 3.0

Two group χ^2 Test of Equal Proportions

Scenario	**11**	**12**	**13**	**14**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
Group 1 proportion, π_1	0.125	0.125	0.125	0.125
Group 2 proportion, π_2	0.500	0.625	0.750	0.875
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	7.000	11.667	21.000	49.000
Power (%)	80	80	80	80
N per group	23	14	9	6

6.7.5 Strategy for the Statistical Analysis

The primary method for analysis will be a comparison of the proportion of *treatment success* between patients randomized to receive placebo compared to patients randomized to remain on sodium nitroprusside. Additional analysis will be described in the Statistical Analysis Plan that will include a comparison of the event time distribution functions for the time until an increase in MAP_{B2} + 50% Delta (MAP) is initially observed. During the Open-Label Study Drug Administration (Dose-Titration) Phase, the sustainability of the blood pressure will be graphed over time to determine the effectiveness of SNP to maintain the target MAP.

6.7.6 Handling Missing Data in the Analyses

The following method of imputation will be used:

Last observation carried forward (LOCF): The goal of this imputation scheme is to create an observation for a completely missing observation at the end of the study for every patient in the ITT population. If a patient evaluation for a post-baseline observation is missing, then the immediately preceding non-missing evaluation will be used.

Specific algorithms for imputing missing or partially missing dates will be discussed in the SAP. Imputed or derived data will be identified in the individual patient data listings. Imputed data will not be incorporated into the case report form datasets. Imputed data will be used in the preparation of the derived datasets.

6.7.7 Pooling of Small Sites for Analysis

Small sites (i.e., sites that have less than 4 patients per treatment arm) will be identified and the following method will be used for combining the data. Data from all small sites (< 4 patients) will be combined to form a single site in order to obviate non-estimable situations (i.e., at least 2 intra-group observations are needed to estimate variance) in the evaluation of site and site interaction effects. Once combined, the pooled site will remain as such for all analyses for which a site interaction effect is determined. If the pooled smaller sites represent a single site that has more than twice as many patients as the largest single site, however less than 3 times as many patients, the small sites will be ranked by size and divided into 2 pooled groups using an alternating sequence (ABABAB). If the pooled smaller sites represent a site that has more than three times as many patients as the largest single site, however less than 4 times as many patients, the small sites will be ranked by size and divided into 3 pooled groups using an alternating sequence (ABCABCABC). This methodology will be applied, based on the initial pooling of the smaller sites.

6.7.8 Dropouts, Protocol Violations, and Exclusions

Randomized patients who fail to complete the study will not be replaced. All protocol violations will be documented and categorized in the final study report.

The rate of attrition will be evaluated by the Data Monitoring Committee during the interim evaluation to re-estimate sample size. The reasons for withdraw will be classified into 3 mutually-exclusive classes:

- Withdraw due to tolerability of the study drug
- Withdraw due to lack of treatment effect
- Withdraw not due to tolerability or lack of treatment effect

The proportion of patients who withdraw prematurely will be compared between treatment groups to determine if there is a disproportionate rate of attrition. If the rates differ by a pre-specified amount, the reasons for withdraw will be examined to determine causation. The specific monitoring rules, boundaries, and actions will be described in detail in the Data Monitoring Committee Charter.

6.8 Safety Evaluation

The primary assessment of safety will be based on the frequency of treatment-emergent adverse events and on the frequency of clinically notable abnormal vital signs and laboratory values. The primary safety analysis will be based on a comparison of the proportion of patients receiving sodium nitroprusside vs. placebo who experience a serious adverse event during the Blinded Study Drug Administration Phase.

6.8.1 Adverse Events and Medical Conditions

Adverse events will be recorded throughout the study and at early termination. Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Treatment-emergent adverse events will be defined as those events, which are newly occurring or worsening from baseline. In all cases only treatment emergent adverse events will be summarized. Treatment-emergent adverse events will be summarized by presenting, for each treatment group, the number and percentage of patients having any treatment-emergent AE, having an AE in each body system, and having each individual AE. (Note: In any given category [e.g. body system] a patient will only be counted once.) Similar displays will be provided for prior

(conditions ending prior to the first exposure to sodium nitroprusside) and current (conditions present while on study medication) medical conditions. Adverse events will further be categorized by severity, relationship to study medication, and action taken. Other information collected will be listed, as appropriate. Any event starting more than 3 days after the final dose of study medication will be excluded from the above tables and only presented in a listing, unless the adverse event was serious or caused discontinuation from the study.

6.8.2 Clinical Laboratory Results

6.8.2.1 Overview

The primary presentation of the results by individual laboratory parameter will focus on the intra-patient changes from baseline (Pre-study Period). The presentation and analysis of laboratory data will be based on the observed data. All patients who have a baseline and at least one follow-up laboratory assessment will be included in the presentation of the clinical laboratory data. For each clinical laboratory test, there will be three sets of descriptive statistics that summarize the results at baseline, post-baseline assessment, and the change from baseline to post-baseline assessment. Descriptive statistics include N, mean, standard deviation, median, and the minimum and maximum values. Within treatment group changes will be analyzed using a paired-difference t-test. Between treatment group differences will be compared using a one-factor analysis of variance test.

Shifts from baseline to each pre-specified post-baseline endpoint will also be summarized based on the laboratory categorization (*abnormally and clinically significant*, *abnormal but not clinically significant*, or *normal*) using the worst reported post-baseline observation that occurs within the pre-specified interval. The proportion of patients will be compared using a 2-tailed Fisher's exact test, pooling *abnormally and clinically significant* with *abnormally but not clinically significant.*

In the case that more than one laboratory is used, laboratory values will be transformed for mean change summaries to the same units and normal range as were provided by the central laboratory used in the study, using the formula:

$$y = (x - Li)\frac{Uc - Lc}{Ui - Li} + Lc$$

where x = original value, Li and Ui = lower and upper limits of normal for individual laboratory, Lc and Uc = lower and upper limit for central laboratory

In cases where the lower limit of central laboratory is 0, values that are below the lower limit of normal for a laboratory value prior to transformation will be assigned a value of 0.

6.8.3 Vital Signs

6.8.3.1 Overview

Vital signs of particular interested (blood pressure, MAP, heart rate) will be assessed during each phase of the study.

6.8.3.2 Presentation of Results

Descriptive statistics (n, mean, SD, median, minimum and maximum values) will be used to summarize systolic and diastolic blood pressure, MAP, and heart rate and compared between the randomized treatment groups using a one-factor analysis of variance test.

6.8.4 Physical Examination

6.8.4.1 Overview

The presentation of physical examination data is based on the dichotomous classification (normal or abnormal) of each of the 9 regions or body systems (General Appearance, HEENT, Cardiovascular, Respiratory, Abdomen, Extremities, Neurological, Hair and Skin, and Genitourinary). In addition to these 9 specific body systems, any other region recorded by the investigator under "other" will also be summarized and reported.

6.8.4.2 Presentation of Results

Results will be presented by treatment assignment using counts and percentages. Shift tables will be prepared containing the count and percentage of patients who transitioned from normal at baseline to abnormal at the end of the study. The number and percentage of patients that did not change (normal at baseline and normal at the end of the study, abnormal at baseline and abnormal at the end of the study) are also presented to frame the 2*2 contingency table. Shifts from baseline to each pre-specified post-baseline endpoint will be summarized using the worst reported post-baseline observation that occurred within the pre-specified interval. The count of the disagreements (normal to abnormal and abnormal to normal) by treatment assignment (active and placebo) will be compared for each parameter using McNemar's test.

7.0 Ethics

7.1 Independent Ethics Committee or Institutional Review Board

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator Brochure (IB), the informed consent and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an Independent Ethics Committee (IEC) or Institutional Review Board (IRB). IEC/IRB approval of the protocol, informed consent and subject information and/or advertising as relevant will be obtained prior to the authorization of drug shipment to a study site. Any amendments to the protocol will

require IEC/IRB approval prior to implementation of any changes made to the study design.

7.2 Ethical Conduct of Study

This study will be conducted in accordance with the protocol, GCP, FDA regulations governing clinical study conduct, ethical principles that have their origin in the Declaration of Helsinki, 2000 revision (see Appendix E) and all applicable local regulations. The investigator must assure that the study is conducted in accordance with the provisions as stated in the FDA regulations and complies with prevailing local laws and customs. Responsibilities of the Investigator are specified in Appendix D.

7.3 Subject Information and Consent

The investigator or designated representative will explain the nature of the study to the subject, to the extent compatible with the subject's understanding, or the subject's parents or legal guardian, and answer all questions regarding this study. Prior to any study related screening procedures being performed on the subject, the informed consent statement will be reviewed, signed and dated by the subject, if capable, or the subject's parent or legal guardian, and by the person who administered the informed consent. A copy of the informed consent form will be given to the subject and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that informed consent was obtained prior to any study-related procedures and that the subject received a signed copy.

8.0 Source Documents and CRF Completion

8.1 Source Documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information,

subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

8.2 Case Report Forms

Data for individual subjects will be recorded on CRFs provided to the BPCA-CC. All entries must be complete. A case report form must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the CRF by the investigator. The principal investigators must review and approve each CRF.

Case report forms must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested during an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

Because of the potential for errors and inaccuracies in entering data into CRFs, laboratory and other test results must be kept on file with the subject's study dossier. Case report forms and copies of test results must be available at all times for inspection by the CRA for the site and the FDA.

9.0 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA-CC, the investigators and their study coordinators and the CRAs for the study. This

meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures by a CRA at a study initiation visit and will be given an CRF completion workbook for reference.

The CRAs will monitor each site throughout the study. At each visit, 100% source document review will be made against entries on the CRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and all applicable regulations.

After completion of the entry process, computer logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary correction will be made to the database and documented via addenda or audit trail. A manual review of selected line listings will also be performed at the end of the study.

10.0 Use of Information and Publication

10.1 Use of Information

This trial is sponsored by the NICHD. The NICHD endorses the sharing of final research data to expedite the translation of research results into new scientific knowledge in order to improve human health.

This contract is part of a collaborative program involving multiple sites. A data sharing dissemination plan will be developed jointly with the BPCA-CC, the NICHD, and the collaborating institutions following announcement of the award.

10.2 Publication

The BPCA-CC and steering committee for this study shall have the right to review and approve any paper for publication, including oral presentation and abstracts, which utilize data generated from this study. At least 90 days before any such paper or abstract is presented or submitted for publication, a complete copy shall be given to the BPA-CC for review by the steering committee. Any individual investigator agrees to defer publication of any such paper or abstract until the BPCA-CC and Steering Committee have reviewed and approved it.

11.0 Completion of Study

The investigator will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon in writing by the investigator, the NICHD, the BPCA-CC and the PODS. The investigator will provide a summary of the study's outcome to the IRB/IEC following the conclusion of the study.

The PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. The investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the NICHD and BPCA-CC within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

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12.0 Investigator Agreement

I have received and reviewed the investigator brochure for sodium nitroprusside (SNP).

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulation.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Signature of Principal Investigator Date

Name of Principal Investigator (printed or typed)

Appendices

Appendix A: Tanner Stages of Sexual Maturity

	Pubic Hair		Breasts	Penis	Testes
SMR Stage[1,2]	Boys	Girls	Girls	Boys	Boys
1	None	Preadolescent	Preadolescent	Preadolescent	Preadolescent
2	Scanty, long, slightly Pigmented	Sparse, lightly pigmented, straight, medial border of labia	Breast and papilla elevated as small mound; areolar diameter increased	Slight Enlargement	Enlarged scrotum, pink texture altered
3	Darker, starts to curl, small Amount	Darker, beginning to curl, increased amount	Breast and areola enlarged, no contour separation ,	Longer	Larger
4	Resembles adult type, but less in quantity; coarse, cu rly	Coarse, curly, abundant but amount less than in adult	Areola and papilla form secondary mound	Larger; glans and breadth increase in size	Larger, scrotum dark
5	Adult distribution, spread to medial surfaces of thighs	Adult feminine triangle, spread to medial surface of thighs	Mature; nipple projects, areola part of general breast contour	Adult size	Adult size

1. Adapted from Tanner, JM: Growth at Adolescence, 2 ed. Oxford, Blackwell Scientific Publications, 1962.
2. MR = Sexual Maturity

Appendix B: Research Consent Form with HIPAA

Protocol Title: A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects

Protocol Director: [PROGRAM PI]
IRB Approval Date: _____ IRB Expiration Date: _____

Is your child participating in any other research studies? _____Yes _____No

INTRODUCTION

You are being asked to agree to let your child be a part of a drug research study. He or she is scheduled for surgery, or needs to stay in an intensive care unit (ICU). During this operation or stay in the ICU, it will be necessary for the doctor to lower your child's blood pressure for a long period of time, up to 24 hours. We will tell you more about how he/she will do this and the drug that will be used later in this paper. Before you decide whether to let your child be involved in this study, *[Site PI]* wants you to read the following information. He wants you to ask him any questions you may think of. He wants to be sure that you understand what your child's participation will mean. You need to fully understand the type of treatment and its risks. Your doctor is responsible for providing you with the necessary information so that you understand the possible risks. Your child's participation in this study is entirely your choice.

Your child cannot participate in another research study at the same time as this research study. Your child cannot be taking another experimental drug while enrolled in this research study. Your child will be a part of this study for approximately 30 days. There may be risks that we cannot predict. We will tell you about any new information that may affect your child's condition or affect your willingness to stay in this study.

NATURE AND PURPOSE OF THE RESEARCH STUDY

In certain kinds of surgeries, doctors often need to control the blood pressure of the patient. The doctor may also need to lower the patient's blood pressure below normal. This can reduce blood loss and avoid blood transfusions during stressful periods. Sodium nitroprusside is a drug that is approved by the Food and Drug Administration (FDA) for use in adults. Scientific studies show that this drug works well when doctors need to control blood pressure during surgeries in adult patients. Doctors also often use sodium nitroprusside in children. However, not many scientific studies tell us how best to use sodium nitroprusside in children.

In this study, we hope to learn the best dose of sodium nitroprusside to use in children who need it in the ICU for more than 12 hours. We also want to learn the same thing for children who need certain kinds of surgeries. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children. This research study is looking for 50-100 children at several hospitals in the United States.

Your child's participation in this study is entirely voluntary.

Your decision whether or not to allow your child to participate will not prejudice your child or his/her medical care. If you decide to allow your child to participate, you are free to withdraw your consent, and to discontinue participation at any time without prejudice to your child or effect on your child's medical care. If you decide to terminate your child's participation in this study, you should notify *(name)* at *(telephone number)*.

PROCEDURES

If you agree to your child's participation in this research study, he or she will undergo the following types of procedures:

- [Site PI] and his research staff will talk to you (and your child) about his or her health. They will ask you about your child's medical history. They will ask you about any medications your child is currently taking. They will give your child three physical examinations, including

measurement of blood pressure, pulse, and weight. The first one will be within 24 hours before study drug is given. The other two physical exams will be after your child receives study drug. A small amount of blood, much less than one teaspoon, will be drawn at these visits. We need this blood for our study, but the same blood work may also be needed as part of your child's regular medical care. If this is the case, we will use these results instead of having to take another blood sample.

- Your child will receive study treatment in stages:
  1. First, after your child is stable, sodium nitroprusside will be given into a vein. This will be done through tubing your child will already have in place. This drug may be the treatment of choice to control your child's blood pressure even if your child is not enrolled in this study. This first stage will last up to 24 hours. He or she will receive sodium nitroprusside at a pre-set initial rate. That rate will be changed until your child's blood pressure is in the range that his or her doctor has decided is the best.

  2. The second stage of study treatment will begin between 12 and 24 hours after the medication was first started. This stage will last up to 30 minutes. During this stage your child will receive one of two treatments. He or she might receive sodium nitroprusside at the rate that was being used before to keep his or her blood pressure at a stable level. Or, he or she might receive a placebo; this is a solution like salt water that is known to have no effect on your child's health. Your child's vital signs (blood pressure and heart rate) will be watched very closely. If your child's blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end immediately. Then your child will again be given sodium nitroprusside at the rate that successfully kept his or her blood pressure at a safe level. This phase of the research study is blinded. That means that the study doctor will not know which treatment group your child will be placed into. The choice of which treatment group your child will be in (placebo or sodium nitroprusside) will be random. The choice will be made in a way that is like flipping a coin.

  3. If your child stills needs sodium nitroprusside treatment to control his/her blood pressure, the treatment may continue after the research study ends.

During the research study, vital signs (blood pressure and heart rate) will be checked often at specific time points.

There will be a follow up evaluation. About 30 days after the research study is over, we will call you at home to ask questions about your child's health in the last month, since he or she participated in the research study. If your child is readmitted to the hospital before our call, please let us know.

Blood samples will be taken between 3 and 6 times (to total between 1 to 2.5 teaspoons) during the research study. This is to see how much of the drug is circulating in the blood at varying time points. If indicated, additional blood samples may be taken to help determine the amount of drug in your child's blood. We are very careful to minimize the amount of blood drawn from your child, and anticipate that 3 1/2 teaspoons is the most we will draw for these tests.

Whenever possible, blood will be taken from tubing already in place. The nurse will often take the study blood samples at the same time that routine blood samples are taken to check on your child's health. This is done to avoid any extra needle-sticks (drawing blood from a vein in the arm with a needle). It is very unlikely that there would not be a catheter (IV line) in place; however, if that were to happen, blood drawing would require a needle stick that might cause minor bruising. Blood drawing is done to assess safety and to measure the activity of the study drug.

The total amount of blood drawn during the entire research study for children less than 2 years of age is approximately 1 to 2 ½ teaspoons. For children more than 2 years of age, the total amount of blood is about 1 to 3 ½ teaspoons.

The information gained during this research study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help patients in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

YOUNG WOMEN OF CHILD-BEARING POTENTIAL

If your child is a young woman who is able to become pregnant, it is expected that she will use an effective method of birth control to prevent exposing a fetus to a potentially dangerous agent with unknown risk. If your child is pregnant or currently breast feeding, she may not participate in this study.

To confirm to the extent medically possible that your child is not pregnant, you agree that she will have a pregnancy test done before beginning this research study. This test will be carried out right before your child undergoes surgery.

PARTICIPANT RESPONSIBILITIES

You should:

- Follow the instructions of the Protocol Director and study staff.
- Tell the Protocol Director or research study staff about any side effects that your child may have.
- Tell the Protocol Director or research study staff if you believe your child might be pregnant.
- Ask questions as you think of them.
- Tell the Protocol Director or research staff if you change your mind about staying in the study.

While participating in this research study, your child should not take part in any other research project without approval from all of the Protocol Directors. This is to protect your child from possible injury arising from such things as extra blood drawing, the possible interaction(s) of research drugs, or other similar hazards.

WITHDRAWAL FROM STUDY

If you first agree to participate and then you change your mind, you are **free to withdraw** your consent and discontinue your child's participation at any time. Your decision will not affect your child's ability to receive medical care for his or her disease and your child will not lose any benefits to which he or she would otherwise be entitled.

If you decide to terminate your child's participation in this study, you should notify (*name)* at (*phone number)*.

The Protocol Director may also withdraw your child from the study and the study medication may be stopped without your consent for one or more of the following reasons:

- Failure to follow the instructions of the Protocol Director and/or study staff.
- The Protocol Director decides that continuing your child's participation could be harmful to him or her.
- Pregnancy (if applicable).
- Your child needs treatment not allowed in the study.

- The study is cancelled.
- Other administrative reasons.
- Unanticipated circumstances.

POSSIBLE RISKS, DISCOMFORTS, AND INCONVENIENCES

There are risks, discomforts, and inconveniences associated with any research study. These deserve careful thought. This is true whether it is a normal kind of treatment or an experimental type. You should talk with the Protocol Director if you have any questions.

In spite of all safety measures, your child might develop medical problems while taking part in this research study. These risks include elevated blood pressure. Treatment of these potential medical problems will not be limited or delayed by your child's participation in the study.

Sodium nitroprusside is a drug that lowers blood pressure. Because of this, there is a chance that your child could develop hypotension (low blood pressure). The doctor will monitor your child very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable blood pressure.

Another side effect that might happen is that your child's heart rate may increase in response to sodium nitroprusside. Again, the doctor and research nurse will monitor your child's heart rate very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable heart rate.

Minor side effects due to sodium nitroprusside may also occur but will not cause the study to be ended. Since sodium nitroprusside makes blood vessels bigger, the following side-effects may occur: nausea, headache, restlessness, abdominal pain, redness or flushing of the skin, nervousness, and perspiring.

Another side effect that happens when using sodium nitroprusside is that as it is used in the body, another chemical, cyanide, is released in small amounts. This can affect the amount of oxygen in the

blood and have a number of other effects. The doctors will carefully watch for any of these signs or symptoms and treat your child if needed.

POTENTIAL BENEFITS

The information gained during this study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help children in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOUR CHILD WILL RECEIVE ANY BENEFITS FROM THIS STUDY.

ALTERNATIVES

If you choose not to enroll your child in this study, your child may receive sodium nitroprusside anyway. This could happen if your child's doctor feels it is the best medicine to use to control blood pressure. Or, your child's doctor could choose to use other types of blood pressure medications, such as esmolol or fenoldopam, instead. Whether or not your child is enrolled, the medical team will, of course, do everything possible to ensure the safety and comfort of your child.

If you do not wish your child to take part in this study, other treatments can be used for your child's condition. If you withdraw your child's participation, the study doctor will recommend an alternative treatment for blood pressure control for your child, such as esmolol or fenoldopam. If this study is discontinued, your child will receive one of these alternative treatments.

PARTICIPANT'S RIGHTS

You should not feel obligated to agree that your child participate in this study. Your questions should be answered clearly and to your satisfaction.

If you decide not to participate, tell the Protocol Director. Your child will still receive care for his/her disease and will not lose any benefits to which he/she would otherwise be entitled.

You will be told of any important new information that is learned during the course of this research study, which might affect your child's condition or your willingness to continue participation in this study.

CONFIDENTIALITY

Your child's identity will be kept as confidential as possible as required by law. Except as required by law, your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your child's research records may be disclosed outside of Stanford, but in this case, your child will be identified only by a unique code number. Information about the code will be kept in a secure location and access limited to research study personnel.

The results of this research study may be presented at scientific or medical meetings or published in scientific journals. However, your child's identity will not be disclosed**.**

Patient information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your child's identity if this study falls within its jurisdiction.

- The purpose of this research study is to obtain data or information on the safety and effectiveness of sodium nitroprusside in children; the results will be provided to the sponsor, the Food and Drug Administration and other federal and regulatory agencies as required.

Authorization to Use Your Health Information for Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed consent form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?

In this study, we hope to learn the best dose of sodium nitroprusside to use in children of different ages who need it in the ICU for more than 12 hours. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children as determined by the NIH and FDA.

Do I have to sign this authorization form?

You do not have to sign this authorization form. But if you do not, your child will not be able to participate in this research study, including receiving any research-related treatment.

Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?

If you decide to allow your child to participate, you are free to withdraw your authorization regarding the use and disclosure of your child's health information (and to discontinue any other participation in the study) at any time. After any revocation, your child's health information will no longer be used or disclosed in the study, except to the extent that the law allows us to continue using your child's information (e.g., necessary to maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must contact: *(researcher's name and contact information, including telephone number).*

What Personal Information Will Be Used or Disclosed?

Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to, vital sign measurements, laboratory results of blood collections, physical exams, related medical records, and other data.

Who May Use or Disclose the Information?

The following parties are authorized to use and/or disclose your child's health information in connection with this research study:

- The Protocol Director *(Insert Name of PD)*
- The *(Insert name of Institution)* Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary
- Research Staff

(List every other class of persons or organization affiliated with the hospital/university who might need to use and/or disclose the participant's information in connection with this study.)

Who May Receive or Use the Information?

The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- The National Institutes of Health
- The Food and Drug Administration
- Collaborating Institutions
- The Coordinating Center, Premier Research

Your child's information may be re-disclosed by the recipients described above, if they are not required by law to protect the privacy of the information.

When will my authorization expire?

Your authorization for the use and/or disclosure of your child's health information will expire December 31, 2055.

Will access to my child's medical record be limited during the study?

To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or

billing decision about your child (e.g., if included in your child's official medical record).

_____

Signature of Participant

_____

Signature of Legally Authorized Representative

_____

Date

_____

Description of Representative's Authority to Act for Subject

FINANCIAL CONSIDERATIONS

PAYMENT

You and your child will not be paid to participate in this research study.

COSTS

The sponsor will pay for the cost of sodium nitroprusside and for the extra blood tests that will be used to monitor the amount of drug in your child's blood and for safety tests. You or your insurance company will be responsible for the medical procedures, surgery, anesthesia, and other normal costs associated with standard medical care for treatment of your child's condition.

SPONSOR

The National Institute of Child Health and Development of the National Institutes of Health (NIH) is providing financial support and/or material for this study.

COMPENSATION FOR RESEARCH-RELATED INJURY

All forms of medical diagnosis and treatment – whether routine or experimental – involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. **You will be responsible for any associated co-payments or deductibles as required by your insurance.**

If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

Additionally, *{Institution name}* is not responsible for research and medical care by other institutions or personnel participating in this study. You do not waive any liability rights for personal injury by signing this form.

CONTACT INFORMATION

- Questions, Concerns, or Complaints: If you have any questions, concerns or complaints about this **research study**, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director, *{name}*. You may contact *{him/her}* now or later at *{phone number}*.
- Emergency Contact: If you feel your child has been **hurt by being a part of this study**, or need immediate assistance please contact *{Protocol Director's name and phone number}*.
- Alternate Contact: If you cannot reach the Protocol Director, please page the research team at *{phone number}*.
- Independent of the Research Team Contact: If you are not satisfied with the manner in which this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a research study subject, please contact the *{Institution's name}* Institutional Review Board (IRB) to speak to an informed individual who is independent of the research team at *{phone number}*. Or write the *{Institution's name}* IRB, *{IRB full address}*. In addition, please call the *{Institution's name}* IRB at *{phone number}* if you wish to speak to someone other than the research team or if you cannot reach the research team.

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

As a human subject your child has the following rights. These rights include but are not limited to the subject's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
- be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise;
- be given an opportunity to ask questions concerning the experiment or the procedures involved;

- be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated consent form;
- and be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING CONSENT, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____

Signature of Parent or Legal Guardian

_____

Signature of Second Parent or Legal Guardian

_____

Date

_____

Name of Patient

PERSON OBTAINING CONSENT

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied - that the Parent or Legal Guardian has been provided with the Experimental Subject’s Bill of Rights, if appropriate, that I have discussed the research project with

the Parent or Legal Guardian and explained to him or her in nontechnical terms all of the information contained in this consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the Parent or Legal Guardian to ask questions and that all questions asked were answered.

_____ _____

Signature of Person Obtaining Consent Date

Appendix C: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles
for
Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when

providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The

responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's

freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case, the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in

the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix D: Responsibilities of the Investigator

Investigator Responsibility/Performance

Prior to starting enrollment at a site, all investigators must read and understand the Investigational Plan and must sign and complete the Investigator Agreement Form. This documents that they accept all conditions of the Investigational Plan and will conduct the study accordingly. The investigator must provide a current copy of his or her curriculum vitae that is not more than 2 years old.

Informed Consent and IRB Approval

The investigator must have written approval from the IRB prior to enrolling patients in the study. A copy of the written approval that includes the following must be provided to the DCRI:

- A statement of IRB approval for the proposed study and ICF at the institution
- The date the study was approved and the duration of approval
- A listing of any conditions attached to the approval
- Identification of the approved primary investigator
- The signature of the IRB chairperson

Any amendments to the protocol, as well as associated consent form changes, will be submitted to the IRB, and written approval must be obtained prior to implementation. Serious adverse event reports will be submitted as requested by the BPCA.

The study will be explained to the patients in lay language. Patients will sign and receive a copy of the IRB-approved informed consent form prior to study participation. Patients will be assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Source Documentation

Regulations require that investigators maintain information in the study patient's medical records that corroborate data collected on the CRFs. In order to comply with these regulatory requirements, at the minimum, the following information should be maintained:

1. Medical history/physical condition of the study patient before involvement in the study sufficient to verify protocol entry criteria (if not already present)
2. Dated and signed notes on the day of entry into the study including clinical site, patient number assigned and a statement that consent was obtained
3. Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams)
4. Notations on abnormal laboratory results
5. Adverse events reported through 28 days from study drug administration
6. Notes regarding concomitant medications taken during the study (including start and stop dates)
7. Study patient's condition upon completion of or withdrawal from the study

Data Transmittal

Required data will be recorded on the CRFs as soon as possible after the patient is discharged, day 10, or death, whichever comes first. CRFs and any supporting documents must be sent to the BPCA and/or retrieved from the investigational site according to the outlined time windows. The 28-day follow-up CRF needs to be forwarded within 10 days of the follow-up visit.

Non-Protocol Research

BPCA has a legal responsibility to report fully to regulatory authorities all the results of clinical studies. No investigative procedures other than those in this protocol shall be undertaken on the enrolled patients without the agreement of the IRB and BPCA.

Publication Policies

At the conclusion of the study, a multicenter abstract reporting the primary results may be prepared and presented in an appropriate international forum. A multicenter, peer-reviewed manuscript will also be prepared for publication in a reputable scientific journal.

Appendix E: Treatment of Suspected Nitroprusside Toxicity

Signs of Toxicity:

- If the base deficit exceeds -8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the subject will be also be discontinued from study.
- If the lactate level rises by more than 4 mmol/L in a two to four hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output).
- If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes percent between arterial and mixed venous blood.
-

SUSPECTED CYANIDE TOXICITY SHOULD BE TREATED AS FOLLOWS:

1) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration.

2) Obtain blood for arterial and venous blood gases with co-oximetry, serum lactate, and cyanide and thiocyanate levels.

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10mL) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result)

Subject Hemoglobin g/dL	Initial dose Sodium NITRITE (3%) mL/kg IV	Initial dose Sodium Thiosulfate mL/kg IV
8 g/dL	0.22 mL/kg	1.10 mL/kg
(6.6 mg)/kg		
10 g/dL	0.27 mL/kg	1.35 mL/kg
(8.7 mg)/kg		
12 g/dL	0.33 mL/kg	1.65 mL/kg
(10 mg)/kg		
14 g/dL	0.39 mL/kg	1.95 mL/kg
X	(11.6 mg)/kg	

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals.

[Micromedex Poisondex www.micromedex.duhs.duke.edu , see also Berlin, 1970]

Appendix F: Assay of Nitroprusside Metabolites and Handling of Blood Samples for Assay of Nitroprusside Metabolites

A classical UV bioanalytical method is utilized for detection and quantitation of cyanide in whole blood. Cyanide concentrations are determined by measuring the absorbance of the chromophore formed by the interaction of the cyanide ion with 4-nitrobenzaldehyde and o-dinitrobenzene in 2-methoxyethanol (Rieders, 1971 and Guilbault, 1966). In summary, to a 1.0 ml aliquot of whole blood, 4-Nitrobenzaldehyde solution and o-Dinitrobenzene solution is added then made basic with sodium hydroxide. Following a specific incubation time a UV scan spectrum is obtained from 520 to 580 nm with a maximum reading at 555 nm. The exact details of the method are proprietary to NMS Labs. The method is sensitive to a LLOQ of 0.05 μg/mL which correlates to normal circulating levels. A toxic threshold is normally assessed as approximately 0.5 μg/mL and acute toxicity is observed at greater than 1 μg/mL. There are no known interferences with this assay method.

An ion chromatography method is used for detection and quantitation of thiocyanate in serum that is specific, accurate, precise and rugged. In summary, a 0.10 mL aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography (Vogel, 1981 and Vesey, 1976). The same procedure is employed to detect thiocyanate in urine. The normal range for non-smokers is 1-4 μg/mL in serum/plasma. For smokers, it is 3 – 12 μg/mL and the therapeutic range for sodium nitroprusside is generally between 6 and 29 μg/mL (Schulz, 1984).

Thiosulfate is detected in serum/plasma or urine also via a validated ion chromatography method with the analytes separated and detected via conductivity detection. For this method, a 0.5 ml aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography.

Sample Handling Procedures

At each specified blood collection for cyanide and thiocyanate, 2 mL of arterial blood is to be collected in a 2 mL gray top (BD reference number 367921). Samples should be inverted at least eight times to mix, then one half the whole blood in the gray top (1 mL) is removed and stored in a polypropylene screw capped container and stored on ice until it can be refrigerated. The remaining 1 mL of whole blood is centrifuged within 20 minutes of collection at approximately 1200 g for 10 - 12 minutes at ambient temperature to obtain 0.5 mL of plasma for thiocyanate analysis. Plasma and whole blood should be stored in a refrigerator before sending to National Medical Services as soon as possible. Samples should be shipped cold using "frozen cold packs" for overnight delivery. Whole blood samples should NEVER be frozen. NMS will accept deliveries on a Saturday so Friday shipments are possible.

For neonates or to spare the total amount of blood drawn for the analysis, the minimum blood draw is 1 mL of arterial blood. This translates to 0.5 mL of whole blood for cyanide analysis and 0.5 mLs to be centrifuged to obtain approximately 0.25 mLs of plasma for thiocyanate analysis.

Appendix G: Sedation Suggested Regimen

Intensive Care Unit:

For those patients who receive study drug in the intensive care unit for a surgical or medical procedure, the following guidelines may be utilized.

Sedation may be administered intravenously and initiated with a benzodiazepine and opiate agonist as follows:

Midazolam bolus 0.1-0.2 mg/kg followed by a continuous midazolam infusion of 0.06-0.3 mg/kg/hr or intermittent bolus of 0.1 mg/kg every 1-2 hours

Or

Lorazepam bolus 0.05-0.1 mg/kg followed by a continuous infusion of 0.025-0.05 mg/kg/hr or intermittent bolus of 0.05-0.10 mg/kg every 4-6 hours

And/or

Fentanyl bolus 1- 5 micrograms/kg (intubated, mechanically ventilated patients) followed by an infusion of fentanyl of 0.5 – 5 micrograms/kg/hour

Or

Morphine bolus 50-100 microgram/kg followed by a continuous infusion of 20-80 micrograms/kg/hour.

Sedation and analgesic medication may be titrated to patient response. Higher doses may be used in patients who exhibit benzodiazepine and/or narcotic habituation due to long term (> 4-7 day) usage. Where applicable, sedation assessment may be by clinical judgment of the responsible physician, and/or a quantitative scoring system such as the COMFORT score. Patients receiving neuromuscular blocking drugs such as vecuronium or rocuronium as part of their ICU management are eligible for study.

Appendix H: Measurement of Blood Pressure in Children

Excerpt from: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents

Correct measurement of BP in children requires use of a cuff that is appropriate to the size of the child's upper right arm. The equipment necessary to measure BP in children, ages 3 through adolescence, includes child cuffs of different sizes and must also include a standard adult cuff, a large adult cuff, and a thigh cuff. The latter 2 cuffs may be needed for use in adolescents.

By convention, an appropriate cuff size is a cuff with an inflatable bladder width that is at least 40% of the arm circumference at a point midway between the olecranon and the acromion. For such a cuff to be optimal for an arm, the cuff bladder length should cover 80% to 100% of the circumference of the arm. Such a requirement demands that the bladder width-to-length ratio be at least 1:2. Not all commercially available cuffs are manufactured with this ratio. Additionally, cuffs labeled for certain age populations (eg, infant or child cuffs) are constructed with widely disparate dimensions. Accordingly, the working group recommends that standard cuff dimensions for children be adopted (see Table 2).

TABLE 2. Recommended Dimensions for BP Cuff Bladders

Age Range	Width, cm	Length, cm	Maximum Arm Circumference, cm*
Newborn	4	8	10
Infant	6	12	15
Child	9	18	22
Small adult	10	24	26
Adult	13	30	34
Large adult	16	38	44
Thigh	20	42	52

* Calculated so that the largest arm would still allow the bladder to encircle arm by at least 80%.

BP measurements are overestimated to a greater degree with a cuff that is too small than they are underestimated by a cuff that is too large. If a cuff is too small, the next largest cuff should be used, even if it appears large. If the appropriate cuffs are used, the cuff-size effect is obviated

SBP is determined by the onset of the "tapping" Korotkoff sounds (K1). Population data in children and risk-associated epidemiologic data in adults have established the fifth Korotkoff sound (K5), or the disappearance of Korotkoff sounds, as the definition of DBP. In some children, Korotkoff sounds can be heard to 0 mm Hg. Under these circumstances, the BP measurement should be repeated with less pressure on the head of the stethoscope. Only if the very low K5 persists should K4 (muffling of the sounds) be recorded as the DBP.

Appendix I: Research Assent Form Template

Protocol Title: A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Director:

IRB Approval Date: _____IRB Expiration Date: _____

Are you taking part in any other research studies right now? ____ yes ____ no

Why Are We Doing this Research Study?

We are doing this study to learn more about a medicine called sodium nitroprusside. This study will help us find out more about how good this drug is in keeping blood pressure and heart rate under control in children who are in the intensive care unit (ICU). Altogether, there will be 60 kids under the age of 17 in this study.

What Will Happen During the Study?

You will be seen by a doctor, who will give you a physical exam and ask you and your parents some questions about your health history and medicines you take. At certain times during the study, blood will be drawn to check that you are okay and safe, and to see how the study drug is working in your body. Blood will be drawn either from a tube that is already connected to you to get blood, or from a needle. The amount of blood to be taken during the study is about 2½ teaspoons to 3½ teaspoons.

In the first part of the study, you will receive a study drug called sodium nitroprusside through a tube placed into a vein in your arm. Your doctor will make changes in the amount of drug you get to keep you safe and your vital signs (like your heart rate and blood pressure) stable. Your doctor will

change how much of the drug you get so you have the blood pressure the doctor thinks is right for you. This part of the study will last for at least 12 hours up to 24 hours during the time that your doctor needs to control your blood pressure. During the time you get study drug, your blood pressure and heart rate will be measured very often.

The second part of the study will last up to 30 minutes. During this part, you will get one of two treatments. First, you might receive the study drug at the same rate that was being used before to keep your blood pressure stable. Or, you might receive a placebo, which is a solution like salt water that doesn't have any effect on your blood pressure. Your blood pressure and heart rate will be watched very close. If your blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end right away. Then you will again be given sodium nitroprusside at the rate that keeps your blood pressure at a safe level. This part of the research study is blinded. That means that the study doctor will not know which treatment group you are in. The choice of which treatment you receive will be made in a way that is like flipping a coin.

What if you don't want to be in the Study?

You can say "no" to being in the study if you want. You can also stop the study anytime you want by telling anyone that is caring for you. Your doctor can explain to you and your parents other treatments that could be used instead.

What You Should Know about the Medicine?

The medicine is used to keep your blood pressure where your doctor thinks is safe or needed for your operation or some other treatment or test. It has been okayed for use in adults but there is not a lot of information about how the drug works in kids. Like any medicine, it can cause unwanted things to happen. These unwanted things are called risks. Some of the risks from using this medicine are: getting sick to your stomach, headache, getting hot, having your blood pressure go down too much or your heart beat go up too high.

Things Girls Need to Know….

Some medicine can cause bad things to happen to an unborn baby. If you are able to get pregnant (if you have started having a period), you need to take a pregnancy test which your doctor will give you. If you are pregnant, you should not take part in this study.

What Else Do You Need to Know?

Sometimes doctors write about the research studies when they are done. If a paper is written about this research study, your name won't be used in it, but the medical information they find out about you may be used. We will keep your medical information private. People who work for *[site name]*, the people who are running the study, and some parts of the government (the part that takes care of medicines) will be able to look at your medical information.

There is no cost to you or your parents to be in this study.

If you have questions about the study you can call Dr. _____ at

_____.

I have read this form. I have had a chance to ask questions about things I don't understand. I want to be in this research study and understand what will happen to me.

_____ _____

Signature of Patient Date

_____

Name of Patient

_____ _____

Signature of the Person Obtaining Assent Date

1) Study Contact Information, Project Administrators

    a. Contact information has been updated

2) Protocol Synopsis

    a. Start and stop dates have been modified

        i.

Estimated Start:	Q3 ~~Q2~~ 2008
Estimated Finish:	Q3 ~~Q1~~ 2009

3) Section 2.0

    a. Blinded Phase

        i. The description of the primary endpoint has been slightly modified.

            1. "The primary endpoint is the change in MAP ~~Delta(MAP)~~ recorded during the Blinded Study Drug Administration Phase in the absence of other stimuli."

        ii. Moved secondary objective #5 from Blinded phase to Follow-up because there will be few if any labs performed during Blinded phase. Thus, the last objective in Blinded phase was deleted

            ~~1. "To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in individual laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo."~~

    b. Follow-up Phase, Added new objective (#5), which was really Blinded-phase objective #5, just relocated to Follow-up phase.

        i. "To compare the changes (values obtained during the two-hour period immediately following the stop of blinded study drug minus values obtained during the Pre-Study Drug Period) in individual

laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo."

4) Section 3.1

   a. To allow SNP to be a prior med, the definition of B1 was revised

      i. The initial baseline MAP (B1) is defined as the blood pressure measurement taken just prior to the initiation of SNP administration, either institutionally-supplied or open-label study drug, after at least a 5-minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.).

   b. Repeated definition of Delta(MAP) in Section 3.1. Previously it was only in Figure 1.

      i. Delta(MAP) is defined as the difference between MAP_{B1} and MAP_{B2}.

   c. Added definition of "Offset"

      i. Offset is defined as MAP_{B2} plus 50% Delta(MAP)

   d. Revised Figure 1

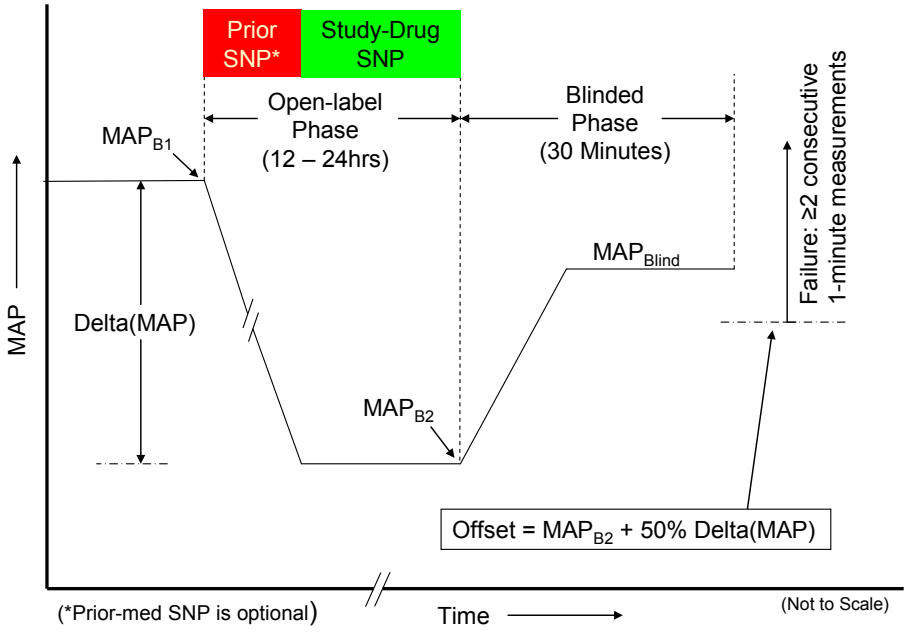
      i. Added "Prior SNP*" to Open-label phase (*Prior SNP is optional)

      ii. Modified failure from "~~>30 seconds above 50% delta(MAP)~~" to "≥2 consecutive 1-minute MAP measurements greater than Offset"

         1. We are only recording MAP once per minute, so this revision was needed

Figure 1

Study Treatment Phases with MAPs and Delta(MAP)



- MAP_{B1} = MAP immediately prior to start of Open-label Study Drug Administration Phase
- MAP_{B2} = MAP immediately prior to start of Blinded Study Drug Administration Phase
- Delta(MAP) = $MAP_{B1} - MAP_{B2}$
- Offset = MAP_{B2} + 50% Delta(MAP)
- Treatment Failure: During the blinded phase, ≥2 consecutive 1-minute MAP measurements greater than Offset [MAP_{B2} + 50% Delta(MAP)]

Example: Subject's MAP immediately prior to start of the Open-label Study Drug Administration Phase is 100 mmHg (MAP_{B1}). At the end of the Open-label Study Drug Administration Phase, MAP is 60 mmHg (MAP_{B2}). Thus, Delta(MAP) = 40 mmHg. For *treatment success*, MAP during the Blinded Study Drug Administration Phase cannot exceed MAP_{B2} + 50% Delta(MAP), or

80 mmHg, for any 2 consecutive MAP_{Blind} measurements, obtained at 1-minute intervals.

d. Increased the approximate number of sites from ~~12~~ to 15.

5) Section 3.1.1

   a. Increased the start of the Pre-study drug administration from up to ~~3~~ days to 7 days preceding the start of study drug administration.

   b. Moved subject randomization from the Pre-study drug period to the Open-label period.

      i. "Randomization will normally occur during this period"

   c. Revised start time of Open-label study drug administration period to include prior-med SNP

      i. "This period will begin at the start of SNP administration, either study drug or institutionally-supplied."

6) Section 3.2.1, #3, 4

   a. Wordsmithing of Inclusion Criterion #3

      i. Subject's parent or legal guardian is willing and able to give informed consent signing and dating an IRB-approved informed consent, and subject provides assent, signing an IRB-approved and –required informed assent, if applicable.

   b. Revised Delta(MAP) requirements for subjecta <2 years old

      i. Subject is anticipated to require a minimum of 20 mm Hg (15 mm Hg for subjects < 2 years old) reduction in MAP for at least 12 hours using SNP.

7) 3.2.2, #1, 10

   a. Exclusion criterion #1: Increased minimum subject weight from ~~2.5~~ to 3.0 kg because infusion rate is difficult to measure precisely at weight <3.0 kg.

b. Added new Exclusion criterion (#10)

i. "Subject has participated in other clinical trials for investigational drugs and/or devices within 30 days prior to enrollment"

8) Section 3.2.3 (Prior and Concomitant Therapy)

a. Added new paragraph to allow for SNP as prior med.

i. "Subjects may receive institutionally-supplied SNP prior to the initiation of study drug administration; however, administration of institutionally-supplied SNP will be discontinued immediately prior to the initiation of study drug administration, and the initial infusion rate of study-drug SNP will be the same as the discontinued institutionally-supplied SNP (see Section 3.4.3 #6)."

9) Table 1

a. Added Urine Output at Pre-study drug period and Follow-up

b. Moved Randomization from Pre-study drug period to Open-label period

c. To the top row of Table 1, added time descriptions of the study periods

i. Pre-study Drug Period

1. (Up to 7 days prior to Study Drug administration)

ii. Open-label Period

1. (12 -24 hrs duration)

iii. Study Drug d/c

1. (Within 2 hours)

iv. Follow-up

1. (Up to 24 hours post blinded study drug)

d. Added 30-minute window periods to the Q8h assessments

e. Increased to three the number of Urine thiocyanate tests at Follow-up

i. "X 3"

f. Footnote revisions

i. #1-- End of Study assessment will be done at 24 hours post blinded study drug administration, except where noted

ii. #3--Growth parameters will include weight, height/ length, and Tanner stage, if ≥6 years old.

iii. #10 -- Measurements to be performed at time of urine thiocyanate sample collection, if feasible

iv. New (#11) -- Blood for cyanide & thiocyanate at 12 hrs ± 30 min, post study drug d/c, only if arterial line in place

10) Section 3.4.2

a. #7 of this section has been modified to included the following

i. The first sentence now reads, "Collect urine and blood samples for laboratory evaluations as per Table 1."

ii. The following statement has be added to the end of this activity, "To minimize the blood volume obtained under this protocol, laboratory evaluations performed prior to the consenting of the patient as part of the standard of care of the patient and within 7 days of the administration of study drug may be substituted for these procedures."

11) Section 3.4.3, activities of the Open-Label Phase

a. #3 of this section was revised to allow for the use of SNP as prior med during the open-label period.

i. "Obtain vital sign measurements immediately prior to the start of SNP administration, either institutionally-supplied or open-label study drug ~~administration~~. This defines B1."

b. #5 of this section was revised to allow the minimum Delta(MAP) for subjects <2 years to be 15 mmHg.

i. "If the difference between B1 and the target MAP is <20 mmHg (15 mmHg for subjects <2 years old), the patient will be withdrawn from the study and not given study drug."

c. #6 of this section was revised to allow the initial infusion rate of study drug to be at the infusion rate of prior-med SNP

i. "Begin administration of open-label study drug at a dose not to exceed 0.3 mcg/kg/min, or, if applicable, at the infusion rate of the institutionally-supplied SNP."

d. #8 of this section is new.

i. "Revise target MAP as clinically indicated; titrate SNP to achieve new target MAP (±10%)."

e. #9 of this section has revised times for obtaining samples.

i. "Obtain vital sign measurements every one minute for the first 10 minutes then every 5 ± 1 minutes for an additional 20 ~~30~~ minutes after initiation of open-label study drug infusion and after each dosage adjustment. After the initial 30 ~~40~~ minutes, once a stable dose is achieved and BP control is satisfactory, vital sign measurements will be obtained every ≤ 20 minutes ~~at least every 15 minutes~~. Additionally, obtain vital sign measurements in a similar manner whenever it is necessary to change the open-label drug infusion rate."

f. #10 of this section moves subject randomization from the blinded-treatment period to open label.

i. "Randomize patient"

12) Section 3.4.5, activities occurring with study-drug discontinuation

a. Activity #1 was revised slightly

i. Collect urine and blood samples for laboratory evaluations listed in Table 1

b. A statement that activities during this period should be performed within 2 hours of discontinuation of study drug administration was moved to the header for this section.

13) Section 3.4.6, Follow-up procedures

a. #4 is a new statement. This statement could be is section 3.4.5, but it was place here as a reminder.

i. "Collect blood sample at 12 hours (± 30 min) after study drug discontinuation for cyanide and thiocyanate analysis."

b. #5 was revised to agree with Table 1

i. "Perform a pertinent physical examination 18 – 30 ~~24~~ hours following discontinuation of study drug administration."

c. #8 is a new statement.

i. "Collect blood samples for laboratory evaluations as per Table 1.

Blood samples for cyanide and thiocyanate analysis at the discontinuation of the non-study drug (concomitant) SNP to be done only if feasible (ICF must specify this blood sample will be drawn & only if an indwelling catheter is present). Note: This blood draw may be several days following the discontinuation of study-drug SNP administration."

14) Section 3.4.8,

a. 2nd paragraph, 3rd sentence

i. "The syringe will have a label indicating the concentration of the solution (0.5 mg/ml SNP), and the infusion rate necessary to provide 1 microgram per kilogram per minute (1 mcg/kg/min) — for example, for a 25-kg subject, an infusion rate of 3.0 ml/hr will deliver 1.0 mcg/kg/min SNP. The infusion rate of 0.5 mg/ml SNP can be calculated using the conversion factor:
Wt (kg) X 0.12 ml/kg/hr = 1.0 mcg/kg/min.

b. 3rd paragraph, 2nd sentence

i. "The blinded study drug will be prepared by the pharmacist such that either the concentration of drug is the same as in the ~~initial~~ open label period or placebo, see Section 3.6.1."

15) Section 3.4.9

a. 2nd paragraph, 2nd sentence

i. "Microbore low compliance tubing, with volumes of approximately 1 mL will be used, where possible."

b. 3rd paragraph, last sentence is new

i. "The carrier flow rate will be 5.0 mL/hr."

16) Section 3.6.3, last two sentences

a. The first two digits will be the site number of the enrolling institution followed by the number "2"—for the second trial under this IND—followed by a two ~~three~~ digit enrollment-sequence number. For example, Subject #10-2-23 would be the 23rd subject enrolled at Site #10.

17) Section 4.3

a. The last sentence of this section has been deleted

~~i. "If an AE is considered to be not related, probably not related or possibly related, an explanation of other probable causes must be included in the CRF."~~

18) Section 6.3.1

a. For clarity, the first bullet of this section has been revised

i. Complete the 30-minute double-blind phase without having an intra-patient increase greater than or equal to ~~MAP +~~ 50% Delta (MAP) [i.e., MAP $\geq$ (MAP_{B1} + MAP_{B2})/2] and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

b. Because the vital signs during the blinded treatment phase will be collected once per minute, the last bullet of this section has been revised

    i. "Experience an intra-patient increase greater than or equal to ~~MAP +~~ 50% Delta (MAP) for ≥2 consecutive MAP measurements, obtained at one-minute intervals, ~~≥30 seconds at any time~~ during the 30-minute double-blind phase."

19) Section 6.3.2,

a. The 2nd sentence of the 1st paragraph has been revised reducing the time period for the collection of non-serious AE's.

    i. "All non-serious adverse events recorded within 24 ~~72~~ hours of either completion of the double-blind phase, or within 24 ~~72~~ hours of premature discontinuation of the study, will be reported."

20) Appendix B, "Template for the informed consent"

a. In the Introduction, the second sentence of the second paragraph of the Introduction

    i. Your child cannot be taking another experimental drug while enrolled in this research study, or within the previous 30 days.

b. In the section on Procedures, second bullet point, second paragraph

    i. The last sentence has been revised to allow for the possibility that the investigator decides to use an alternative drug to SNP to reduce the subject's MAP, "Then your child will again be given sodium nitroprusside, or similar drug, ~~at the rate that successfully kept~~ to keep his or her blood pressure at a safe level."

    ii. The last four sentences of the paragraph have been moved to follow the third sentence.

    iii. Similar changes were made to Appendix I, Template for the informed assent (Third paragraph of "What will happen during the study?")

21) Appendix D, “Responsibilities of the Investigator”

a. In Source Documentation, item #5, “Serious adverse events reported through ~~28~~ 30 days from the end of study drug administration.”

PROTOCOL

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Number: NICHD-2003-09-LT

Study Drug: Sodium Nitroprusside

IND: 71,979

Medical Monitor: Bernard Brownstein, M.D.

Principal Investigators:

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Sponsor: The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

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Confidential

APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

And I have fully discussed the objectives of this trial and the contents of this protocol with representatives of PODS Center and BPCA–Coordinating Center.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations.

I understand that, should the decision be made by the PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

PODS Center #1 Duke University Medical Center Box 3094 DUMC Durham, NC 27705	SUBCONTRACTOR: Institution: Address: Telephone: Fax: Email:
CO-PRINCIPAL INVESTIGATOR Scott Schulman, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

APPROVAL SIGNATURES

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I understand that, should the decision be made by the PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

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PROTOCOL SYNOPSIS

Protocol Title:	A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects
Protocol Number:	NICHD-2003-09-LT
Sponsor:	National Institute of Child Health and Human Development
Product:	Sodium Nitroprusside
Objectives:	1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours 2. To assess the potential for rebound hypertension following administration of sodium nitroprusside for 12 hours or more
Study Design:	This is a phase II, randomized, double blind, withdrawal to placebo study examining the efficacy, safety and tolerability of sodium nitroprusside in pediatric subjects.
Study Population:	Children up to 17 years of age who require long term (at least 12 hour) blood pressure control will be eligible for study.
Number of Subjects:	A target of approximately 60 patients will be enrolled.
Number of Sites:	Up to 15
Duration of Subject Participation:	Enrollment is anticipated to begin in 2008 and to be complete in approximately 12 months. Patients will be followed for up to 30 days following receipt of study drug.
Treatment:	Subjects who require vasodilator therapy for relatively long time periods will receive open-label infusion of sodium nitroprusside for at least 12 hours but not greater than 24 hours.
Dose Schedule:	Patients will be randomized to receive either placebo or sodium nitroprusside for 30 minutes following at least 12- hours but not more than 24 hours of open-label infusion of sodium nitroprusside.
Estimated Start:	Q3 2008
Estimated Finish:	Q3 2009
Ethics	All regulations stated in 21 CFR Parts 50, 56, and 312 and recommendations outlined in the ICH Guidelines for Good Clinical Practice, as well as all other applicable local and national laws and regulations, will be adhered to throughout this trial.

Safety:	The safety of the drug will be assessed by multiple subject assessments of vital signs, physical exams, clinical tests and laboratory evaluations. Adverse events will be monitored and tracked. All SAEs will be closely monitored throughout the course of the study.
Statistical Consideration:	The trial will be sized to detect the loss of as little as 50% of the expected blood pressure lowering effect of the chosen dose of sodium nitroprusside during the 30 minutes of withdrawal to placebo.

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BP	Blood Pressure
BPCA-CC	Best Pharmaceuticals for Children Act Coordinating Center
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
cGMP	cyclic Guanosine Monophosphate
CN^-	Cyanide
CRA	Clinical Research Associate
CRF	Case Report Form
d/c	Discontinuation
DCRI	Duke Clinical Research Institute
DBP	Diastolic Blood Pressure
DSMB	Data and Safety Monitoring Board
ECMO	Extracorporeal Membrane Oxygenation
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
hr	Hour

ACRONYMS AND ABBREVIATIONS

HR	Heart Rate
IB	Investigational Brochure
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
kg	Kilogram
MAP	Mean Arterial Pressure
mcg	Microgram
mEq/L	Milliequivalent per Liter
mcgs	Micrograms
min	Minute
mL	Milliliter
mm Hg	Millimeters of Mercury
mmol/L	Millimoles per Liter
NO	Nitric Oxide
NICHD	National Institute for Child Health and Human Development
NONMEM	Nonlinear Mixed Effect Model
NTG	Nitroglycerin
PD	Pharmacodynamic
PaO_2	Arterial Partial Pressure of Oxygen
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SCN	Thiocyanate
SNP	Sodium Nitroprusside
μM	Micromoles per liter, Micromolar

1.0 Background and Rationale

Blood pressure control in children is a significant concern in the intensive care unit (ICU), where management of arterial pressure is often necessary during periods of acute physiologic stress such as occurs after certain surgical and medical procedures. Examples of surgical procedures that require blood pressure control in the intensive care unit following surgery include aortic coarctation repair, Ross procedure (pulmonary valve autograft), and solid organ transplantation. Medical conditions requiring control of systemic arterial pressure include renal disease, drug therapy (corticosterioids and immunosuppression agents), and procedures such as extracorporeal membrane oxygenation (ECMO).

A wide variety of drugs of various therapeutic classes have been utilized for either controlled hypotension in the operating room or prevention of hypertension in the pediatric ICU. These drug classes include calcium channel blockers (Tobias et al, 1996), beta-adrenergic antagonists (Kay et al, 2001), ganglionic blockers (DuToit, 1970 and Gallagher and Milliken, 1979), inhalation anesthetics (Tobias, 1998) and direct acting vasodilators such as nitroglycerin and sodium nitroprusside (SNP) (Kaplan, 1980, and Tinker, 1976 Groshong, 1996, and Sinaiko, 1996). Although many vasodilator agents are available to lower blood pressure in the operating room and intensive care unit setting, few have been systematically studied in children.

SNP is a direct acting vasodilator commonly used for blood pressure control. It produces vascular smooth muscle relaxation when its metabolism in the red blood cell results in the liberation of nitric oxide (NO). NO then activates the enzyme guanylyl cyclase. This activation results in the formation of increased intracellular levels of cyclic guanosine monophosphate (cGMP). The result is vasodilation.

1.1 Metabolism

Five molecules of cyanide (CN^-) are released when SNP is metabolized in the red blood cell. The major metabolic pathway for CN^- is conversion to thiocyanate (SCN). This conversion occurs enzymatically via two sulfur transferase systems: 1) rhodenase (the primary pathway) and 2) beta-mercaptopyruvate-cyanide sulfurtransferase. Rhodenase is ubiquitous throughout the body, but it is highly concentrated in the liver. Rhodenase catalyzes the transfer of sulfur from a sulfur donor molecule such as thiosulfate (Na_2SO_3) to cyanide and thereby the formation of thiocyanate (SCN). SCN is subsequently eliminated in the urine and can therefore serve as a marker of cyanide exposure.

The ability of rhodenase to catalyze the conversion of cyanide to thiocyanate (SCN) is limited by the availability of sulfur donors in the body. Thus the provision of exogenous sulfur donors such as thiosulfate (Na_2SO_3) in the setting of acute cyanide intoxication is a potentially life-saving intervention (Pasch et al, 1983, Cole and Vesey, 1987).

One out of every five CN^- ions liberated by the metabolism of SNP binds to methemoglobin to form the non-toxic cyanomethemoglobin. The creation of additional quantities of methemoglobin by the intravenous infusion of sodium nitrite can thus provide additional CN^- buffering capacity. The resultant methemoglobinemia can then be treated with the administration of intravenous methylene blue.

Additional metabolic pathways for CN^- include the conversion of hydroxycobalamine (vitamin B12a) to cyanocobalamine, and conversion to 2-aminothiazoline 4-carboxylic acid.

If the above three pathways (rhodenase, methemoglobin, hydroxycobolamine) are overwhelmed, cyanide will bind to mitochondrial cytochrome oxidases and poison cellular oxidative phosphorylation. Cellular hypoxia is induced when cyanide inhibits the electron transport chain at cytochrome a_3. Oxygen cannot be utilized, mixed venous

oxygen tension rises and the generation of high-energy adenosine triphosphate (ATP) is blocked. The cell reverts from aerobic to anaerobic metabolism, with the subsequent generation of pyruvate and lactate. Acidosis ensues, and with it, deterioration in the organ systems most dependent on oxidative metabolism: the central nervous system and heart.

Clinical manifestations of cyanide toxicity to the central nervous system include headache, anxiety, agitation, confusion, lethargy, convulsions and coma. Cardiovascular manifestations include progressive heart failure with both loss of contractile force (negative inotropy) and slowing of rate (negative chronotropy). Bradycardia and hypotension are commonly observed pre-morbid events associated with cyanide toxicity.

In patients receiving SNP, the earliest, most sensitive signs of cyanide toxicity are acidosis, elevated mixed venous oxygen tension, and rising blood lactate levels. Venous blood that appears "bright" red due to the inability of the tissues to extract oxygen should suggest cyanide toxicity. Arterial and mixed venous blood gas analysis with co-oximetry can help confirm the diagnosis.

1.2 Previous Studies

SNP was first discovered in 1850. Its hypotensive effects were noticed in 1929, and its first therapeutic use was reported by Page et al. in 1955. Moraca et al. first described the clinical use of SNP for deliberate hypotension during surgical procedures in 1962. Since then, it has been widely used to control blood pressure in infants and children in the perioperative period.

Despite its widespread use, there is a paucity of information on its safety, efficacy, and pharmacokinetic/pharmacodynamic relationships in children. Davies et al (1975) and Bennett and Abbott (1977) described their retrospective experience with SNP used to induce deliberate hypotension in small cohorts of children. Both authors observed that younger patients required more SNP than older ones to achieve comparable degrees of

blood pressure control. In their small retrospective cohort, Bennett and Abbott recommended that doses of 10 micrograms/kilogram/minute were necessary to achieve satisfactory blood pressure response. Davies et al described three possible responses to SNP administration in children: 1) a constant response to "conventional" doses < 3 mg/kg; 2) a tachyphylactic response characterized by continuously escalating dose requirement (> 3 mg/kg) to achieve a satisfactory blood pressure; and 3) resistance to the blood pressure lowering effects of the drug. They cautioned against using total doses that exceeded 3 mg/kg or continuing administration of SNP in the latter two scenarios. Firm conclusions cannot be drawn because these small case series were not randomized controlled trials with specific pharmacodynamic endpoints.

Yaster et al (1986) compared SNP to nitroglycerin (NTG) for inducing hypotension in a group of 14 adolescents. They found doses of SNP between 6-8 micrograms/kg/minute superior to NTG at any dose in the reliable induction of hypotension for children and adolescents undergoing scoliosis, craniofacial or hepatic surgery.

Hersey et al (1997) performed a randomized trial comparing SNP to the dihydropyridine calcium channel antagonist nicardipine in 20 healthy adolescents with idiopathic scoliosis undergoing spinal fusion. Target blood pressures were easily obtainable in both groups and operating conditions were comparable. The time to restoration of baseline blood pressure after termination of the infusion was significantly longer in the nicardipine group. Interestingly, blood loss was significantly greater in the SNP group. Details on SNP dose requirements were not provided.

Przybylo et al (1995) described CN^- and SCN blood levels in ten children who received SNP at doses up to 10 micrograms/kg/min (mean infusion rate 6 microgram/kg/min) while undergoing cardiopulmonary bypass for repair of complex congenital cardiac defects. CN^- levels rose as a function of time while SNP was infused, and rapidly fell when SNP was discontinued. Despite the fact that some children demonstrated serum CN^- levels above the generally accepted threshold of 0.5 micrograms/ml, no patient

developed clinically apparent toxicity. Kazim et al (1996) questioned the validity of the results of this study because of the CN^- assay methods utilized.

Linakis et al (1991) retrospectively examined physician-ordering practice as it pertained to blood cyanide levels in children receiving SNP. They sought to determine how the laboratory determinations were used to monitor patients and if there was clinically apparent toxicity in children found to have cyanide concentrations exceeding the "normal" limit of 500 micrograms/liter. They found poor correlation between blood cyanide concentration and dose or duration of therapy in patients whose cyanide levels were "toxic." Thiocyanate determinations were normal and no child manifested signs or symptoms of cyanide toxicity. They concluded that further pediatric studies were needed.

2.0 Study Objectives

We propose a multicenter trial that will provide guidance for the use of SNP to reduce blood pressure in pediatric patients. The trial is a randomized, double-blinded withdrawal to placebo trial. The aims of the trial are:

1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours
2. To assess the potential for rebound hypertension during the 30-minute Blinded Phase following administration of sodium nitroprusside for 12 hours or more.

To meet these study aims, the following study phases, defined in Section 3.1.1, will have the following objectives:

- Open-Label Study Drug Administration (Dose-Titration) Phase
  The objective during this phase of the study is to determine the effectiveness and safety of SNP for controlling blood pressure during stable infusion lasting at least 12 hours.

- Blinded Study Drug Administration Phase

  The primary endpoint for the study will be determined during this phase of the study. The primary endpoint is the change in MAP recorded during the Blinded Study Drug Administration Phase in the absence of other stimuli. The primary objective is to determine the persistence of sodium nitroprusside versus placebo for reducing blood pressure in pediatric patients

  The secondary objectives during this phase of the study are as follows:

  1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience offset during the 30-minute blinded study drug period.
  2. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience rebound hypertension during the 30-minute blinded study drug period.
  3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the 30-minute blinded study drug period.
  4. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the 30-minute blinded study drug period.
  5. To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.

- Follow-up Phase

The following objectives to be evaluated during this phase of the study are as follows:

1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the Follow-up Period.
2. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the Follow-up Period.
3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience changes in individual physical examination parameters represented as either normal or abnormal from the Pre-Study Period to the end of the Follow-up Period.
4. To compare the changes (values recorded during the end of the Follow-up Period minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.
5. To compare the changes (values obtained during the two-hour period immediately following the stop of blinded study drug minus values obtained during the Pre-Study Drug Period) in individual laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo.

3.0 Investigational Plan

3.1 Overall Study Design and Plan: Description

This is a phase II, multicenter, randomized, double-blind placebo-controlled, parallel group study to determine the persistence of the effect of SNP on blood pressure and to assess the potential for rebound hypertension associated with prolonged infusion in pediatric subjects.

Target MAP is defined as the clinically appropriate MAP as determined by the investigator taking into account the clinical presentation and medical needs of the subject. The investigator may change the target MAP at his/her discretion based on clinical needs during the course of the study.

The initial baseline MAP (B1) is defined as the blood pressure measurement taken just prior to the initiation of SNP administration, either institutionally-supplied or open-label study drug after at least a 5-minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). See Figure 1 below.

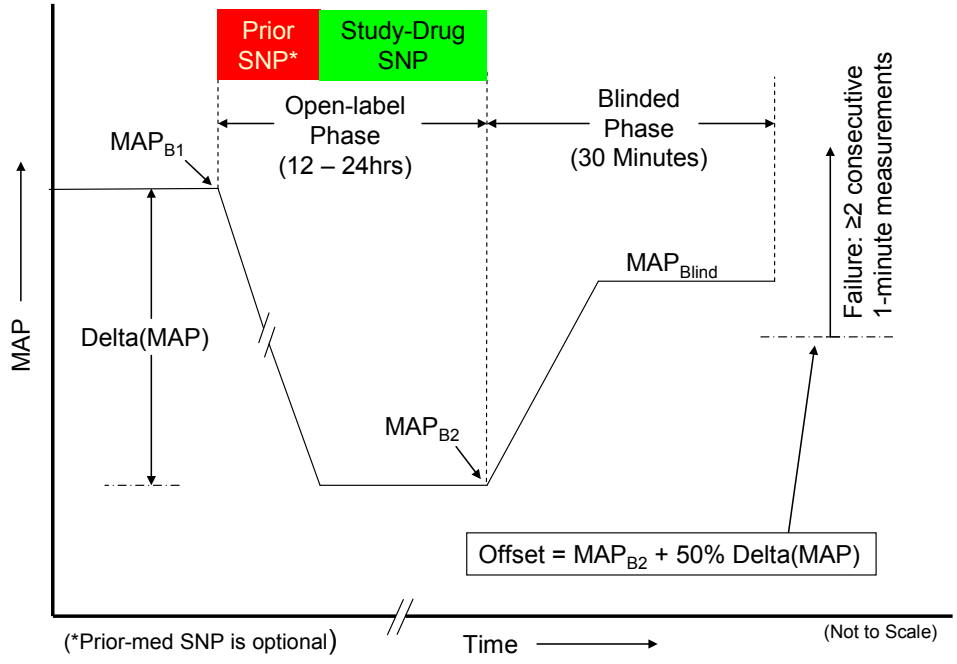
The subsequent baseline MAP (B2) is defined as the blood pressure measurement taken just prior to the initiation of blinded study drug after at least a 5 minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). Prior to establishing B2, there shall have been no changes in the SNP infusion rate for a period of at least 20 minutes.

Delta(MAP) is defined as the difference between MAP_{B1} and MAP_{B2}.

Offset is defined as MAP_{B2} plus 50% Delta(MAP)

Figure 1

Study Treatment Phases with MAPs and Delta(MAP)



- MAP_{B1} = MAP immediately prior to start of Open-label Study Drug Administration Phase
- MAP_{B2} = MAP immediately prior to start of Blinded Study Drug Administration Phase
- Delta(MAP) = $MAP_{B1} - MAP_{B2}$
- Offset = MAP_{B2} + 50% Delta(MAP)
- Treatment Failure: During the blinded phase, ≥2 consecutive 1-minute MAP measurements greater than Offset [MAP_{B2} + 50% Delta(MAP)]

Example: Subject's MAP immediately prior to start of the Open-label Study Drug Administration Phase is 100 mmHg (MAP_{B1}). At the end of the Open-label Study Drug Administration Phase, MAP is 60 mmHg (MAP_{B2}). Thus, Delta(MAP) = 40 mmHg. For *treatment success*, MAP during the Blinded Study Drug Administration Phase cannot exceed MAP_{B2} + 50% Delta(MAP), or 80 mmHg, for any 2 consecutive MAP_{Blind} measurements, obtained at 1-minute intervals.

Approximately 15 centers will participate in subject recruitment to complete the study.

Approximately sixty (60) patients who require long term (at least 12 hours) blood pressure control will be enrolled. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment.

Any patient who starts the blinded study drug administration period will be considered complete for analysis. Enrolled subjects will be randomized in equal proportions to receive either placebo or SNP for the duration of the blind-treatment period, which will immediately follow the open-label infusion of SNP.

3.1.1 Definition of Study Periods

Study periods are as follows:

- Pre-study drug administration: a period of up to 7 days preceding the start of study drug administration during which informed consent, and other enrollment procedures takes place.
- Open-label study drug administration (Dose-Titration): The period of open label study drug administration will be at least 12 hours but not greater than 24 hours. This period will begin at the start of SNP administration, either study drug or institutionally-supplied. Randomization will normally occur during this period.
- Blinded study drug administration: The period beginning with the start of blinded study drug administration and ending with the discontinuation of blinded study drug. It immediately follows the open-label period and will be no longer in duration than 30 minutes.
- Follow up: The period immediately following blinded study drug administration and ending 30 days after completion of study drug administration. AEs will be followed for 24 hours after termination of study drug. SAEs will be followed for 30 days.

Safety will be assessed via the evaluation of adverse events, pre- and post-treatment laboratory results, and vital sign data. The efficacy endpoints will mainly be assessed by examining blood pressure parameters.

3.2 Selection of Study Population

Children up to 17 years of age who require pharmacologic blood pressure control for at least 12 hours will be eligible for study. Blood pressure control is defined as the maintenance of the subject's mean arterial pressure to within 90-110% of a target MAP specified by the physician.

Five pediatric age groups will be enrolled in this trial:

Group A: Neonates from birth to less than 30 days of age
Group B: Infants and toddlers from 30 days to < 2 years
Group C: Preschool children from 2 years - < 6 years
Group D: School age children from 6 yrs - < Tanner stage III
Group E: Adolescents from Tanner stage III - < 17 years.

Tanner III refers to the onset of puberty and occurs at different ages in different individuals. The mean age at onset of Tanner III ranges from 12.4 to 13.1 years in males, and 11.9 to 12.6 years in females. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment (see Appendix A).

3.2.1 Inclusion Criteria

Subjects must meet all of the following criteria:

1. Subject is less than 17 years of age.
2. An in-dwelling arterial line is clinically indicated.
3. Subject's parent or legal guardian is willing and able to give informed consent signing and dating an IRB-approved informed consent, and subject provides assent, signing an IRB-approved and –required informed assent, if applicable.
4. Subject is anticipated to require a minimum of 20 mm Hg (15 mm Hg for subjects < 2 years old) reduction in MAP for at least 12 hours using SNP.

3.2.2 Exclusion Criteria

Subjects will be excluded from study if any of the following criteria exist:

1. Subject weighs < 3.0 kg.
2. Subject has a known allergy to SNP.
3. Subject has a known mitochondrial cytopathy with a disorder of oxidative phosphorylation or of respiratory chain enzymes.
4. Subject has a contraindication to vasodilator therapy for control of blood pressure during surgery or in the intensive care unit.
5. Subject has raised intracranial pressure.
6. Subject is anticipated to need anti-hypertensive drugs other than study drug either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) during the period of study drug administration. However, patients receiving stable doses of an anti-hypertensive drug(s) prior to the initiation of study drug may be enrolled, provided they will not have received IV vasodilator therapy for greater than 8 hours prior to receiving study drug.
7. Subject has any serious medical condition which, in the opinion of the investigator, is likely to interfere with study procedures.

8. Subject is moribund (death likely to occur within 48 hours).
9. Subject has a positive result for the urine or serum HCG test administered at screening.
10. Subject has participated in other clinical trials for investigational drugs and/or devices within 30 days prior to enrollment

3.2.3 Prior and Concomitant Therapy

Initiation of new anti-hypertensive drugs either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) other than the study drug during study drug administration is prohibited. However, in patients receiving stable doses of non-study anti-hypertensive drugs, these agents may be continued during study-drug administration. If open label study drug is initiated during anesthesia, the anesthetic medications will be recorded on the CRFs but are not considered as anti-hypertensive drugs. All concomitant medications and clinically meaningful, unexpected, and invasive procedures will be recorded for the period beginning 72 hours prior to study drug administration through 24 hours post study drug conclusion. The dates of administration, dosage and reason for use must be included. Concomitant medications will be collected for SAEs occurring within 30 days following study drug administration. Vaccines are considered a concomitant medication.

Subjects may receive institutionally-supplied SNP prior to the initiation of study drug administration; however, administration of institutionally-supplied SNP will be discontinued immediately prior to the initiation of study drug administration, and the initial infusion rate of study-drug SNP will be the same as the discontinued institutionally-supplied SNP (see Section 3.4.3 #6).

3.3 Efficacy and Safety Assessments

3.3.1 Efficacy and Safety Measurements

Table 1 is a schematic representation of study assessments and procedures.

3.3.2 Safety Assessments

Safety assessments will include monitoring the tolerability of the SNP infusion and assessing physical examinations, vital signs, clinical laboratory values, concomitant medications and procedures, and adverse events throughout the study. SAEs will be collected for 30 days following completion of study drug administration .

In cases of discharge from the hospital before 30 days, parent (or guardian) will be contacted to determine if any SAE's occurred following discharge but within 30 days of study drug discontinuation. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. See section 4.2 for specific adverse event parameters and actions to be taken.

3.3.3 Drug Concentration Measurements

Cyanide, thiocyanate, methemoglobin, lactic acid, and arterial blood gas analysis will be performed throughout the trial to indirectly query SNP levels and determine subject safety.

TABLE 1: Schedule of Assessments: Sodium Nitroprusside Long-Term Infusion Study

Procedure	Pre-study Drug Period (Up to 7 days prior to Study Drug administration)	Open-label Period (12 -24 hrs duration)	30 minute Blinded Study Drug Period	Study Drug d/c (Within 2 hours)	Follow-up (Up to 24 hours post blinded study drug)[1]
Assessments					
Review Entry Criteria	X				
Informed Consent/ HIPAA Consent	X				
Collect Demographic Data	X				
Medical History	X				
Physical Examination	X			X	X[7]
Vital Signs (SBP, DBP, MAP, HR) [2]	X	X	X	X	X
Growth Parameters[3]	X				
Urine Output[10]	X	X	X	X	X
Serious Adverse Events/Adverse Events		X	X	X	X[5]
Concomitant Medication/Proceedure[8]	X[9]	X	X	X	X
Randomization of Blinded study drug		X			
Blinded Study Drug Administration			X		
Open-label Study Drug Administration		X			
Laboratory Assessments					
Pregnancy test (post-menarche females)	X[14]				
Electrolytes, BUN, creatinine	X			X	
Hematology (CBC & platelet count)	X			X	
Liver Enzymes (AST, ALT)	X			X	
Arterial Plasma Lactate level	X	Q 8 hours (± 30 min)		X	X[13]
Arterial Blood Gas with Co-oximetry (includes Methemoglobin)[4]	X	Q 8 hours (± 30 min)		X	X[13]
Mixed Venous Blood Gas with Co-oximetry (includes Methemoglobin)[4, 12]	X	Q 8 hours (± 30 min)		X	X[13]
Plasma Thiocyanate and cyanide (central lab)	X	Q 8 hours (± 30 min)		X & 12 hr post d/c [11]	X[13]
Urine Thiocyanate (central lab)[6]	X	Q 8 hours (± 30 min)		X	Q8 hrs (± 30 min) X 3

1. End of Study assessment will be done at 24 hours post blinded study drug administration, except where noted
2. Vital sign measurements as described in protocol, sections 3.4.2 – 3.4.7.1. Vital signs will then be collected every 12 ± ½ hours for 24 hours post blinded study drug administration.
3. Growth parameters will include weight, height/ length, and Tanner stage, if ≥6 years old.
4. ABG sampling preferred, sample collected at drug d/c only if line is still in.
5. AEs will be followed for 24 hours & SAE will be followed for 30 days, after the completion of study drug administration.
6. Urine collection details are described in section 3.4.7.2 and the MOP.
7. To be performed 18-30 hours following the termination of study-drug administration
8. Clinically meaningful, unexpected, and invasive procedures only
9. Within 72 hours of study drug administration
10. Measurements to be performed at time of urine thiocyanate sample collection, if feasible
11. Blood for cyanide & thiocyanate at 12 hrs ± 30 min, post study drug d/c, only if arterial line in place

12. Mixed Venous Blood Gas done only if CVC is indwelling
13. Following concomitant SNP d/c, if feasible
14. To be performed within 48 hours of study drug administration

3.4 Study Visits and Procedures

3.4.1 Informed Consent

Prior to the start of any study-related procedure, a signed and dated informed consent and, if applicable, assent must be obtained and documented in the subject's medical record (See Appendix B).

3.4.2 Pre-study drug administration procedures

The following procedures will be completed prior to the administration of study drug:

1) Obtain signed and dated informed consent/HIPAA authorization/assent.
2) Collect demographic data and medical/surgical history.
3) Record diagnosis.
4) Perform a pertinent physical examination.
5) Obtain vital sign measurements.
6) Determine subject height in centimeters and subject weight in kilograms (for calculation of appropriate study drug dose).
7) Collect urine and blood samples for laboratory evaluations as per Table 1. Pregnancy test if required must be done within 48 hours of study drug administration. (If the screening pregnancy test will have been more than 48 hours prior to the start of the study drug administration, then the test will be repeated.)

   To minimize the blood volume obtained under this protocol, laboratory evaluations performed prior to the consenting of the patient as part of the standard of care of the patient and within 7 days of the administration of study drug may be substituted for these procedures.
8) Document concomitant medications (including over-the-counter preparations).

3.4.3 Open-Label Study Drug Administration (Dose-Titration) Procedures:

The following procedures should be performed sequentially unless otherwise indicated.

1) Stabilize sedation/analgesia.
2) Insert arterial line if not already in place.
3) Obtain vital sign measurements immediately prior to the start of SNP administration, either institutionally-supplied or open-label study drug. This defines B1.
4) Determine and record target MAP.
5) If the difference between B1 and the target MAP is <20 mmHg (15 mmHg for subjects <2 years old), the patient will be withdrawn from the study and not given study drug.
6) Begin administration of open-label study drug at a dose not to exceed 0.3 mcg/kg/min, or, if applicable, at the infusion rate of the institutionally-supplied SNP.
7) The dose of open-label SNP will be titrated according to the subject's BP response such that the target MAP, chosen by the study physician, is achieved $\pm$10%. If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose. The duration of open-label study drug administration will be at least 12 hours but less than 24 hours.
8) Revise target MAP as clinically indicated; titrate SNP to achieve new target MAP ($\pm$10%).
9) Obtain vital sign measurements every one minute for the first 10 minutes then every 5 $\pm$ 1 minutes for an additional 20 minutes after initiation of open-label study drug infusion and after each dosage adjustment. After the initial 30 minutes, once a stable dose is achieved and BP control is satisfactory, vital sign measurements will be obtained every $\leq$ 20 minutes. Additionally, obtain vital

sign measurements in a similar manner whenever it is necessary to change the open-label drug infusion rate.

10) Randomize patient
11) Collect blood samples for laboratory evaluations as per Table 1.
12) Record concomitant medications, procedures, and adverse events.
13) Whenever an adverse event occurs, obtain vital sign measurements. If clinically appropriate, a blood sample for safety including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations may be drawn.

3.4.4 Blinded Study Drug Administration Procedures

1) Obtain vital sign measurements immediately prior to the start of blinded study drug administration. This defines MAP_{B2}. There must be 5 min of stable conditions and 20 minutes of no changes in Open-label study drug prior to starting the blinded study drug administration phase.
2) Begin 30-minute blinded study drug administration as described in Section 3.4.8.
3) Record blood pressure and heart rate every one minute for the duration of blinded study drug administration.
4) If blood pressure control is lost (defined as loss of 50% of delta MAP sustained for 30 sec in the absence of stimulation) the blinded study drug is discontinued when there is a safety concern or the MAP reaches 120% of MAP_{B1}.
5) Record concomitant medications and procedures and adverse events. Whenever an adverse event occurs, obtain vital sign measurements and, if appropriate, a blood sample for safety analysis including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations.

3.4.5 Study Drug Discontinuation (Within 2 hours of discontinuing study drug)

1) Collect urine and blood samples for laboratory evaluations listed in Table 1
2) Conduct a pertinent physical examination and perform all other assessments listed in Table 1 for this study phase.

3.4.6 Follow up Procedures

The following will be performed after completion of study drug administration through 24 hours post study drug end:

1) If applicable, record the estimated blood loss and fluid intake, including blood and blood products and output during the trial period.
2) Record concomitant medications and clinically meaningful, unexpected, and invasive procedures.
3) Record vital signs at 12 and 24 ± ½ hours after the end of study drug administration.
4) Collect blood sample at 12 hours (± 30 min) after study drug discontinuation for cyanide and thiocyanate analysis.
5) Perform a pertinent physical examination 18 –30 hours following discontinuation of study drug administration.
6) Collect adverse events for 24 hours following discontinuation of study drug administration.
7) Collect serious adverse events plus associated concomitant medications and clinically meaningful, and invasive procedures for 30 days following discontinuation of study drug administration.
8) Collect blood samples for laboratory evaluations as per Table 1.

   Blood samples for cyanide and thiocyanate analysis at the discontinuation of the non-study drug (concomitant) SNP to be done only if feasible (ICF must

specify this blood sample will be drawn & only if an indwelling catheter is present). Note: This blood draw may be several days following the discontinuation of study-drug SNP administration."

SAEs and associated concomitant medications and procedures will be collected for 30 days following the discontinuation of study drug administration, either through telephone contacts and/or study visits or spontaneously reported by the subjects.

3.4.7 Methods of Assessment

3.4.7.1 Vital Sign Measurements

Vital signs: systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate will be measured.

The principal method of obtaining blood pressure measurements will be from an intra-arterial catheter inserted in an upper or lower extremity artery. Manual blood pressures from a non-invasive blood pressure cuff will only be used prior to insertion of and during a malfunction of the arterial catheter. The blood pressure transducer is internally calibrated by the instrument upon performing the zeroing procedure. All blood pressure and heart rate data will be acquired electronically when possible.

3.4.7.2 Blood Draws and Urine Samples

Blood drawn for study related purposes will not exceed the maximum amounts specified by the American Association of Blood Banks for healthy infants, children, and adolescents. Normally, this value is 7 ml/kg over an eight week period. This study is an inpatient trial of short duration, therefore, the amount of blood withdrawn for study related purposes will take into account the patient's pre-existing hemoglobin and hematocrit, and local Institutional Review Board limitations on maximum allowable blood draws for study-related purposes. A reasonable and conservative value is 3 ml/kg.

Urine will be sampled for thiocyanate concentrations every 8 hours, or fraction thereof, commencing with initial study drug administration to the end of study drug completion and then Q 8 hours times 3 after the discontinuation of study-drug administration, or until the urinary catheter is removed, whichever occurs first. All urine samples will be from a pooled urine collection from the 8-hour time period or fraction of 8-hour time period. The sample will be stored cold until shipment to the central lab. Details of urine collection procedures are described in the MOP.

3.4.8 Dispensing of Study Drug

Study drug will be dispensed to the sites in 2 ml vials containing 25 mg/ml of SNP. The pharmacist will dispense two preparations of study drug, one for the open-label study drug period and one for the blinded study drug period.

The open-label study drug administration phase will utilize a fixed study drug concentration and variable infusion rate scheme. Syringes or bags will be prepared by the investigational drug pharmacy by adding 25 mg of SNP to 50 ml 5% dextrose. The syringe will have a label indicating the concentration of the solution (0.5 mg/ml SNP), and the infusion rate necessary to provide 1.0 microgram per kilogram per minute (1.0 mcg/kg/min) —for example, for a 25-kg subject, an infusion rate of 3.0 ml/hr will deliver 1.0 mcg/kg/min SNP. The infusion rate of 0.5 mg/ml SNP can be calculated using the conversion factor: Wt (kg) X 0.12 ml/kg/hr = 1.0 mcg/kg/min. Clinicians can then make the necessary dosage adjustment for adequate blood pressure control. All dosage adjustments will be captured on the case report forms (CRFs).

The pharmacist will supply blinded study drug for each subject according to a randomization assignment generated by the IVRS. The blinded study drug will be prepared by the pharmacist such that either the concentration of drug is the same as in the open label period or placebo, see Section 3.6.1. Subjects will receive blinded study drug

at the same rate of infusion that was used at the conclusion of the initial open-label study drug administration period.

Syringes or bags will be wrapped in opaque or amber plastic to protect from light.

3.4.9 Delivery of Study Drug

Infusion pumps capable of reliable delivery at low infusion rates (to 0.1 ml/hr) will be used. All pumps will have free flow protection and will be internally calibrated for accuracy by the manufacturer. Accuracy will be verified at each site by the biomedical engineering department as part of their equipment management program. Quality assurance checks will be performed periodically according to manufacturer specifications.

Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. Microbore low compliance tubing, with volumes of approximately 1 mL will be used, where possible.

Study drug will be infused via a dedicated peripheral intravenous catheter or via a dedicated lumen of a multi-orifice central venous catheter. Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. The carrier flow rate will be 5.0 mL/hr.

3.5 Removal of Subjects from Therapy or Assessment

3.5.1 Early Discontinuation of Study Drug and Subject Withdrawal

If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to

within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose.

If the subject withdraws participation in the study for any reason, every effort will be made to collect safety data, vital sign measurements, samples for safety and laboratory analyses. The date, time and reason for discontinuation must be recorded on the case report form (CRF). Additionally, every attempt should be made to complete all other study related procedures on discontinued subjects who have received any amount of study drug as the data will be included in the safety and intention to treat analyses. Subjects who prematurely discontinue from the study will not be replaced.

Any subject who does not start the blinded study drug administration period will be considered prematurely withdrawn from the study. Any patient who starts the blinded study drug administration period will be considered complete for analysis.

Potential reasons for subject withdrawal from the study are as follows:

1) Subject's parent or legal guardian wishes to have the subject withdrawn for any reason;
2) Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the subject's safety;
3) The investigator feels that it is in the subject's best medical interest to be withdrawn.
4) Subject no longer needs blood pressure control.

3.5.2 Data Safety and Monitoring Board

To ensure that the welfare of trial patients receives appropriate consideration, an independent Data and Safety Monitoring Board (DSMB) has been organized by the BPCA-CC on behalf of the NICHD to review relevant safety and/or efficacy data during

the course of the trial. The DSMB may recommend discontinuation of the study, or modifications to the study protocol for safety reasons.

The DSMB consists of four core members (Chair, ethicist, statistician, community representative) plus additional ad hoc members for the various medical subspecialties involved in the BPCA protocols.

Each DSMB will have a presenting statistician who will be responsible for presenting the interim data. This member will write the reports and will be one non-voting member of the DSMB. Except as their role in the DSMB, all DSMB members are not participating in the design or conduct of this study, as an investigator or otherwise, and lack any financial conflict that would introduce any bias.

3.5.2.1 DSMB Responsibilities

- Monitoring the safety of trial patients;
- Recommending discontinuation of the trial for safety reasons;
- Recommending changes to the study protocol for safety reasons;
- Providing written reports on an ongoing basis following scheduled and ad hoc meetings that will be archived and may be provided to regulatory agencies.

These responsibilities will be broadened to include decisions regarding efficacy if the trial is an efficacy trial. The DSMB will monitor the safety of trial patients by reviewing the occurrence of adverse events and deaths, on a real-time basis as SAE reports are transmitted. The DSMB may also monitor compliance with the protocol, and factors affecting patient safety or the integrity of the trial. The DSMB may request any additional data that are not included in the report if deemed necessary for effective monitoring.

If the DSMB finds any major concerns about safety, it may recommend discontinuing the trial or modifying the study protocol. Following each data review, the DSMB will send a

written recommendation regarding the trial, (e.g., to continue according to the protocol, or recommendations for specific actions) to the sponsor.

3.6 Investigational Product

3.6.1 Identity of Investigational Product

Sodium nitroprusside (sodium nitropentacyanoferrate (III) dihydrate) is a reddish-brown crystalline powder that is freely soluble in water. Its molecular formula is $Na_2[Fe(CN)_5NO] \cdot 2H_20$. Study drug will be supplied by the BPCA Coordinating Center to the Investigational Drug Service at each clinical center in a standard concentration of 25 mg/ml. The Investigational Drug Service at each clinical center will then prepare the drug in syringes or bags of sterile 5% dextrose for administration to randomized patients according to the guidelines provided above. Sterile 5% dextrose will be utilized as placebo.

3.6.1.1 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature from 15°- 30°C and protected from light in its carton until used. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug must be maintained under adequate security.

3.6.2 Methods of Assigning Subjects to Treatment Groups

After meeting all inclusion and exclusion criteria, subjects will be considered to be enrolled and will be randomly assigned to receive either placebo or active drug treatment groups during the blinded study drug administration phase of the trial using a single centralized randomization schedule. Randomization into the blinded portion of the trial will be performed via a centralized interactive voice response system (IVRS).

The BPCA-CC will provide a system for the pharmacist to obtain each subject's randomized treatment assignment in a timely manner prior to the administration of study drug.

3.6.3 Assigning Subject Numbers

Study participants will be assigned a subject number upon successful enrollment into the study. The subject number will consist of five digits. The first two digits will be the site number of the enrolling institution followed by the number "2"—for the second trial under this IND—followed by a two digit enrollment-sequence number. For example, Subject #10-2-23 would be the 23rd subject enrolled at Site #10.

3.6.4 Blinding

The subject, as well as all caregivers, will remain blinded to the treatment assignment throughout the course of the study. For subjects' safety, the pharmacist will be aware of the treatment group for each subject. The BPCA-CC will maintain the double-blinded randomization schedule.

The randomization for an individual subject may be revealed in an emergency; however, investigators are discouraged against requesting that the blind be broken for an individual subject. Notification of any unblinding must be sent via facsimile to the BPCA-CC within 24 hours.

3.6.5 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms.

3.6.6 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the Clinical Supply Shipment Request and Verification or similar document. An accurate inventory of study drug will be kept by the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and returned to the BPCA-CC if requested. The investigator agrees not to supply study medication to any persons not enrolled in the study or not named as a sub-investigator for the trial.

4.0 Adverse Events

4.1 Definition

An adverse event is defined as any unintended and unfavorable medical occurrence in a clinical investigation subject, administered a pharmaceutical product, regardless of the causal relationship with treatment. An adverse event can therefore be any untoward sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not the event is considered causally related to the use of the product. Pre-existing conditions that remain stable throughout the study period will not be considered adverse events. Any worsening of a pre-existing condition or illness is considered an adverse event.

Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional over-dosage, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events if

they result in discontinuation from the study, necessitate therapeutic medical intervention, or the investigator considers them to be adverse events.

Adverse events will also include electronically-monitored vitals signs that meet the definitions of section 4.2; however, electronically-captured data excursions, which in the opinion of the investigator represent data artifacts, such as might be produced by the turning of the patient or brief interruption of the electronic circuit of the monitor device(s) or which do not likely reflect an actual untoward event will not be considered to adverse events.

New or worsening physical-exam findings that occur following the initiation of study-drug administration will be considered to be adverse events, without regard to causality.

4.1.1 Serious Adverse Events

A serious adverse event is one that meets the above criteria and also results in one of the following conditions:

1. Death
2. A threat to life
3. Requirement for inpatient hospitalization
4. Prolongation of hospitalization
5. Production of a congenital anomaly or birth defect
6. A persistent or significant disability or incapacity (excluding experiences of minor medical significance such as headache, nausea, vomiting, diarrhea, and accidental injury)
7. The requirement for a medical or surgical intervention in order to prevent a serious outcome.

If an adverse event meets any of the above criteria, it must be reported to the BPCA-CC as a serious adverse event (SAE) within 24 hours of the investigative site's awareness of its occurrence.

4.2 Adverse Event Severity

The criteria for rating adverse events are as follows:

Mild	The adverse event is transient and easily tolerated by the subject.
Moderate	The AE causes subject discomfort and interrupts usual activities.
Severe	The AE causes significant interference with normal activities and may be incapacitating or life threatening.

Safety data are a primary objective of this trial. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. Safety will be evaluated throughout the study and during the follow-up period by evaluating the tolerability of the study drug infusion and by monitoring clinical and laboratory signs of SNP toxicity such as hypotension, tachycardia, bradycardia, acid base status, serum lactate concentration, methemoglobin levels, cyanide levels, and when available, mixed venous oxygen tension.

- Co-oximetric arterial blood gas analysis with methemoglobin determination will be performed Q8. Arterial blood gas monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity.
- Lower than expected methemoglobin concentrations may reflect indirect evidence of cyanide toxicity because cyanide has a high affinity for methemoglobin, combining with it to form the non-toxic molecule cyanomethemoglobin.
- Other laboratory findings suggestive of cyanide toxicity that will be monitored in this trial include serum lactate levels and mixed venous oxygen saturation.

  Lactate levels will rise and mixed venous oxygen tension will increase when cyanide toxicity is occurring due to the reduced ability of the tissues to extract oxygen.

- Plasma and urine thiocyanate levels are indirect markers of cyanide exposure because the majority of the cyanide ions liberated by the metabolism of SNP in the red blood cell are converted to thiocyanate by the rhodenase enzyme system in the liver and excreted in the urine. Free cyanide levels will also be measured.

The adverse event of rebound hypertension shall be considered mild if the MAP rises >10% above the baseline (B1), moderate if the MAP rises >20% above baseline (MAP_{B1}), and severe if the MAP rises >30% above baseline (MAP_{B1}).

The adverse event of excessive hypotension shall be considered mild if the MAP falls >20% below target; fluid therapy may be administered. The adverse event of excessive hypotension shall be considered moderate if the MAP falls >25% below target, fluid therapy is required, and pharmacologic therapy is required. The adverse event of excessive hypotension shall be considered severe if the MAP falls >30% below target, fluid therapy is required, and repeated and/or continuous pharmacologic support is required.

The rating of the adverse event of tachycardia shall be defined for sustained rates exceeding the age adjusted maximums (Table 3) by using the mild, moderate, and severe ranges outlined in Table 4A.

TABLE 3: Age-Adjusted Maximums for Pediatric Heart Rate

Subject Age	**Maximum Heart Rate (bpm)**
1 month <6 months	180
6 months <3 years	160
3 <8 years	150
8 <17 years	130

TABLE 4A: Severity of Tachycardia (Heart Rate in Beats per Minute)

Age	Mild	Moderate	Severe
Age <6 months	180-199	200-219	220 and higher
6 months - <3 years	160-179	180-199	200 and higher
3 years to < 8 years	150-164	165-179	180 and higher
8 years to <17 years	130-149	150-169	170 and higher

The adverse event of lactic acidosis shall be considered mild if the serum lactate concentration is between 5 and 7.5 mM, moderate if the serum lactate concentration is between 7.6 and 10 mM, and severe if the serum lactate concentration exceeds 10 mM.

The adverse event of cyanide toxicity will be considered mild if the serum cyanide concentration is 0.5 mcg/L – 1.0 mcg/L, moderate if the serum cyanide concentration is >1.0 – 1.5 mcg/L, and severe if the serum cyanide concentration is > 1.5 mcg/L. Corresponding erythrocyte (vs. serum) cyanide values are < 10 μM/L (mild), 10-20 μM/L (moderate), and > 20 μM/L (severe).

Severity ratings for these selected adverse events are displayed in Table 4B.

TABLE 4B: Severity of Selected Adverse Events

Event	Mild	Moderate	Severe
Rebound Hypertension (during blinded phase)	MAP rises >10% above baseline (MAP_{B1})	MAP rises >20% above baseline (MAP_{B1})	MAP rises >30% above baseline (MAP_{B1})
Hypotension	MAP falls >20% below target	MAP falls >25% below target IV therapy required; Pharmacological therapy required	MAP falls >30% below target IV therapy required; Repeated or continuous pharmacological therapy required
Lactic acidosis	5 -7.5 mM	7.6-10 mM	>10 mM
Cyanide toxicity	serum cyanide 0.5 0.5 – 1.0 mcg/L	serum cyanide >1.0-1.5 mcg/L	serum cyanide >1.5 mcg/L
Cyanide toxicity (erythrocyte)	cyanide <10 μM	cyanide ≥10–20 μM	cyanide >20 μM

Frequent monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity. If the base deficit exceeds 8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the patient will be discontinued from study and the SNP infusion terminated. If the lactate level rises by more than 4 mmol/L in a 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output) SNP administration will be discontinued. If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes

percent between arterial and mixed venous blood in the absence of an explainable cause, SNP administration will be discontinued and treatment for suspected cyanide toxicity will be initiated.

Suspected cyanide toxicity will be further assessed and treated as follows:

1) Obtain blood for arterial and venous blood gases with co-oximetry, plasma lactate, and cyanide and thiocyanate levels.

2) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration (see below).

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10ml) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result).

TABLE 5: Dosage Chart for Children

Patient's Hemoglobin g/dL	Initial Dose of Sodium Nitrate (3%) mL/kg IV	Initial Dose of Sodium Thiosulfate mL/kg IV
8	0.22 mL/kg (6.6 mg/kg)	1.10 mL/kg
10	0.27 mL/kg (8.7 mg/kg)	1.35 mL/kg
12	0.33 mL/kg (10.0 mg/kg)	1.65 mL/kg
14	0.39 mL/kg (11.6 mg/kg)	1.95 mL/kg

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals. [Micromedex Poisondex www.micromedex.duhs.duke.edu , see also, Berlin, 1970]

4.3 Relationship to Study Drug

The criteria for determining the relationship of the AE to the study drug are as follows:

1) Probably related: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.
2) Possibly related: An AE that is likely to be related to the administration of the study drug and an alternative cause is equally or less likely when compared to the study drug.
3) Probably not related: An AE that has little or no relationship to the study drug and there exists a more likely, or equally likely, alternative cause.
4) Not related: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.

4.4 Adverse Event Collection Period

SAEs will be monitored and reported from the time the subject receives study drug through 30 days following termination of study drug. Knowledge of adverse events will be gained from direct monitoring of the study subject as well as from clinician observation, and self reporting by the study subject or his/her guardians. Adverse events that have not resolved, or are ongoing, will be monitored to resolution if felt to be related to study drug, or until it is felt that the subject has stabilized.

5.0 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual subject, the investigator or other responsible physician must contact one of the Co-Principal Investigators immediately so that a timely decision can be made as to whether or not the subject should be enrolled or continue in the study. If a deviation is being requested by one of the Co-Principal Investigators, he must contact the other Co-Principal Investigator

for a decision. The deviation from the protocol will be authorized only for that particular subject. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF.

6.0 Statistical Considerations

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a Statistical Analysis Plan, which will be finalized prior to unblinding. Any changes in the statistical methods described in this protocol that occur prior to unblinding will be documented in the Statistical Analysis Plan and will not result in a protocol amendment.

6.1 General Overview

The primary efficacy variable is the intra-patient change in Delta (MAP) during the blinded phase of the study. The primary null hypothesis to be tested is that there is no difference between the active study drug and placebo in the proportion of patients who experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP). Statistical analyses will be performed using two-sided tests. A 0.05 significance level will be used in all tests of treatment differences. Tests for interactions will utilize a 0.10 statistical significance level. Individual secondary endpoints will be evaluated using a hierarchical testing procedure. The Statistical Analysis Plan will include a detailed description of all statistical methods, testing procedures, and methods of data imputation. The Data Monitoring Committee charter will contain the specific details regarding the re-estimation of the target sample size.

Data will be summarized by treatment group with respect to demographic and baseline characteristics, efficacy variables, and safety variables. For parameters measured at baseline, the outcome variables of interest are the changes from baseline (Pre-Study Drug Period). Summary statistics will include the mean, N, standard deviation, median, minimum, and maximum values for continuous variables, and frequencies and

percentages for categorical variables. Prior to summarizing results by study center, or performing analyses that include center as a factor in the analysis, small centers will be pooled. All efficacy variables will be summarized by treatment and by visit. Analyses will be performed to explore whether there are treatment-by-center interactions. If a treatment-by-center interaction is detected, the interaction will be explored in an ad-hoc manner. Categorical variables will be analyzed using Cochran-Mantel-Haenszel (CMH) test blocking on centers. Details of the model and the analyses will be specified in the Statistical Analysis Plan and all statistical analyses will be performed using SAS, Version 8.2 or higher.

6.2 Study Objectives

The study objectives are as defined in Section 2.0 of this protocol.

6.3 Patient Population(s) for Analysis

6.3.1 Efficacy

The intent to treat (ITT) population will contain all patients who were exposed to the study drug during the Open-Label Study Drug Administration (Dose-Titration) Phase.

The Per-Protocol population will contain all patients randomized to the double-blind phase of the trial. The efficacy analysis will be based on the Per-Protocol population. A patient will be classified as a *treatment success* if they meet the following criteria:

• Complete the 30-minute double-blind phase without having an intra-patient increase greater than or equal to 50% Delta (MAP) [i.e., $MAP \geq (MAP_{B1} + MAP_{B2})/2$] and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

A patient will be classified as a *treatment failure* if they meet the following criteria:

• Fail to complete the entire 30-minute double-blind phase without receiving additional treatment to control their blood pressure in addition to the study drug.

• Fail to complete the entire 30-minute double-blind phase for any reason.

• Experience an intra-patient increase greater than or equal to 50% Delta (MAP) for ≥2 consecutive MAP measurements, obtained at one-minute intervals, during the 30-minute double-blind phase.

6.3.2 Safety

All patients who receive any study medication (ITT population) will be included in the safety analyses and summaries, independent of the patient actually reaching the double-blind phase of the study. All non-serious adverse events recorded within 24 hours of either completion of the double-blind phase, or within 24 hours of premature discontinuation of the study, will be reported.

All serious adverse events recorded within 30 days of either completion of the double-blind phase, or premature discontinuation of the study, will be reported.

6.4 Background and Demographic Characteristics

All baseline information, including demographic factors, physical examination parameters, vital signs, growth parameters (if applicable), laboratory and blood gas information will be summarized by treatment group for all enrolled patients (ITT population). Additionally, nonrandomized patients versus randomized patients will be summarized and compared by age, gender, and race to determine if there are any differences among the 2 subsets. Analyses will be conducted to determine differences in the demographic and baseline characteristics of the treatment groups. For continuous variables (e.g., age, weight), the number of non-missing and missing values and the

median, mean, standard deviation, minimum, and maximum will be displayed for each treatment group. For categorical variables (e.g., race, gender), the counts and proportions will be tabulated.

Baseline comparability will be evaluated based on the pooled data from all centers. To determine comparability of the treatment groups at baseline, continuous demographic and clinical variables will be analyzed using an analysis of variance test (with an appropriate transformation, if necessary). Baseline, demographic, and clinical variables that are ordinal will be analyzed using the Cochran Mantel Haenszel test; parameters that are dichotomous will be analyzed using a chi-square ($\chi 2$) test or Fisher's exact test, depending on the individual cell counts. If there are treatment group differences at the 0.10 level of significance in demographic or baseline clinical variables, these variables may be added as stratification variables or covariates to the efficacy analyses.

6.5 Study Medication

The duration of exposure to study medication will be summarized for all enrolled patients, and separately for all randomized patients.

6.6 Concomitant Therapy

Concomitant medications (medications present while on study medication) will be recorded throughout the study and at early termination. These medications will be coded using the WHO drug dictionary. The number of randomized patients using prior or concomitant medications will be categorized by the WHO drug category and preferred term, and presented for each treatment group. In any given category [e.g., drug category] a patient will be counted only once.

6.7 Statistical Design and Models for Analysis

This is a biphasic (open-label dose-titration phase, followed by a randomized phase), randomized, double-blind placebo-controlled study. Patients who are enrolled into the initial phase of the study will have their dose of sodium nitroprusside titrated and must receive a minimum of 12-hours of treatment to be eligible for the randomized phase of the study. Patients who cannot be adequately titrated during the initial 12-hour period will not proceed to the randomization phase of the study. Patients who reach the randomization phase of the study will be assigned to receive placebo, or continue to receive sodium nitroprusside based on a stratified permuted block central randomization scheme.

Five age groups (A through E) will be enrolled in this trial:

Age Group A: Age Group A: Neonates from birth to less than 30 days of age
Age Group B: Infants and toddlers from 30 days to < 2 years
Age Group C: Preschool children from 2 years - < 6 years
Age Group D: School age children from 6 yrs - < Tanner stage III
Age Group E: Adolescents from Tanner stage III - < 17 years.

In order to efficiently account for the effect of SNP on the different age groups, neonates from birth to less than 30 days of age (Age Group A) and infants and toddlers from 30 days to < 2 years (Age Group B) will be pooled for analysis. Based on the planning estimates of the study, patients from these two pooled age groups should represent approximately 25% of the target enrollment (~60 patients).

Preschool children from 2 years - < 6 years (Age Group C) and school age children from 6 yrs - < Tanner stage III (Age Group D) will also be pooled for analysis; patients from these two pooled age groups should also represent approximately 25% of the target enrollment (~60 patients).

In order to accurately determine the target number of patients required for enrollment, a Data Monitoring Committee will examine the results from the Blinded Study Drug Administration Phase after the following number of patients have been enrolled and randomized:

- The initial 12 patients in Age Groups A & B
- The initial 12 patients in Age Groups C & D
- The initial 16 patients in Age Group E

The specific objective of the Data Monitoring Committee is to determine the target sample size based on the observed magnitude of the effect, expressed as a proportion (*treatment success* (sodium nitroprusside) / Randomized to receive sodium nitroprusside vs. *treatment success* (Placebo) / Randomized to receive Placebo. Re-estimation of the sample size will be based on conditional power after 67% (40/60) of the target sample size has been enrolled.

6.7.1 Primary Efficacy Analysis

The primary efficacy analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} = \pi_{\text{ Patients randomized to receive placebo}}$
- H_A: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} \neq \pi_{\text{ Patients randomized to receive placebo}}$

where

- $\pi_{\text{Patients randomized to receive SNP}}$ = Proportion of *treatment successes* (sodium nitroprusside)
- $\pi_{\text{Patients randomized to receive placebo}}$ = Proportion of *treatment successes* (placebo)

6.7.2 Primary Safety Analysis

The primary safety analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} = \pi_{\text{ Patients randomized to receive placebo}}$
- H_A: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} \neq \pi_{\text{ Patients randomized to receive placebo}}$

where

- $\pi_{\text{Patients randomized to receive sodium nitroprusside}}$ = Proportion of patients randomized to receive SNP who experience a serious adverse event

- $\pi_{\text{Patients randomized to receive placebo}}$ = Proportion of patients randomized to receive placebo who experience a serious adverse event

6.7.3 Interim Monitoring Based on Conditional Power

The interim assessment for sample size adjustment will be predicated on the primary efficacy endpoint and conducted using a Conditional Power (CP) approach as described by Chen (2004). The Data Monitoring Committee will conduct the analysis to determine if a sample size adjustment is required. This assessment will be conducted after 67% of the patients from the original target sample size have either completed or withdrawn from the study. The instructions to the Data Monitoring Committee for the sample size adjustment will be described in detail in the Data Monitoring Committee Charter. An alpha level adjustment will not be necessary for the procedure described below, based on the procedure proposed by Chen, DeMets and Lan (2004).

In order for the Data Monitoring Committee to calculate conditional power using the observed data, the treatment assignment codes will need to be provided to the Data Monitoring Committee statistician. The intra-patient MAP_{B2} values will be required, including listings of the intra-patient post-baseline values during the blinded phase of the trial. If an increase in the sample size is required, and the re-estimation is within the defined sample size limits pre-specified for the study, the Data Monitoring Committee will communicate the revised target sample size to the IVRS vender for the trial. Enrollment will continue towards the new target sample size, and the data will remain blinded to all involved parties with the exception of the Data Monitoring Committee. Additionally, the Data Monitoring Committee will not have any direct contact with the study Sponsor, or the clinical investigators.

The following steps will be used to evaluate the sample size after the initial randomized patients from each pooled age group (either 12 or 16) have been enrolled and have completed the Blinded Study Drug Administration Phase, or withdrawn prematurely.

Compute conditional power for the primary hypothesis, using data from the initial patients randomized from each pooled age group (either 12 or 16).

If the conditional power is ≥ 0.5, compute the sample size necessary to increase conditional power to 0.8.

The Data Monitoring Committee will compare the re-estimated sample size calculated using the observed data, relative to the initial estimates for the study. Based on this evidence, the Data Monitoring Committee will proceed with the following action:

If the conditional power is ≥ 0.5, the sample size will be increased up to the maximum sample size pre-specified for the study.

If the conditional power is < 0.5, an analysis will be performed based on the predictive probability of achieving the endpoint within the maximum target sample size allocated for the study.

6.7.4 Sample Size Estimation

The overall sample size was calculated based on performing an un-stratified analysis of the proportion of patients classified as a *treatment success* between the 2 randomized treatment groups. With a balance randomization (1:1, SNP:Placebo), a difference in the proportion of *treatment successes* ranging from 34% to 40% would have 80% power to reject the null hypothesis in favor of the alternative (ref. Sample Size Table No. 1.0).

Sample Size Table No. 1.0

Two group χ^2 Test of Equal Proportions

Scenario	**1**	**2**	**3**	**4**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
SNP, π_1	0.080	0.160	0.240	0.320
Placebo, π_2	0.420	0.530	0.630	0.710
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	8.328	5.920	5.392	5.203
Power (%)	80	80	81	80
n per group	30	30	30	30

Based on the target sample size and the distribution of enrollment relative to the pooled analysis groups, approximately 30 patients from Age Group E (Adolescents from Tanner stage III - < 17 years) are scheduled to be randomized. However, based on the difference in the proportion of patients who are classified as a *treatment success* between the

randomized treatment groups, 30 patients may not be required to detect a significant difference at the alpha = 0.05 level. For this reason, the proportion of patients classified as *treatment successes* will be compared after the initial 12 or 16 patients are randomized, depending on the pooled age group. If the difference in proportions has an odds ratio >12, then less than the 30 patients would be required to be randomized from Age Group E. Under this scenario, the randomization of patients from this specific age group would be stopped. Drawing from Age Groups A & B or Age Groups C & D, if the difference in the proportion of *treatment successes* between the randomized treatment groups requires more than 12 patients, the study could still meet its intended goal within the pre-defined total sample size of 60 patients by enrolling the minimum sample size, based on the conditional power calculated at the interim evaluation. Power estimates at the interim evaluation for 16 patients are presented in Sample Size Table No. 2.0. The re-estimated sample size is presented in Sample Size Table No. 3.0, again for 16 patients.

Sample Size Table No. 2.0

Two group χ^2 Test of Equal Proportions

Scenario	**5**	**6**	**7**	**8**	**9**	**10**
Test significance level, α	0.050	0.050	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2	2	2
Group 1 proportion, π_1	0.125	0.125	0.125	0.125	0.125	0.125
Group 2 proportion, π_2	0.250	0.375	0.500	0.625	0.750	0.875
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	2.333	4.200	7.000	11.667	21.000	49.000
Power (%)	9	20	35	54	76	94
n per group	8	8	8	8	8	8

Sample Size Table No. 3.0

Two group χ^2 Test of Equal Proportions

Scenario	**11**	**12**	**13**	**14**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
Group 1 proportion, π_1	0.125	0.125	0.125	0.125
Group 2 proportion, π_2	0.500	0.625	0.750	0.875
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	7.000	11.667	21.000	49.000
Power (%)	80	80	80	80
N per group	23	14	9	6

6.7.5 Strategy for the Statistical Analysis

The primary method for analysis will be a comparison of the proportion of *treatment success* between patients randomized to receive placebo compared to patients randomized to remain on sodium nitroprusside. Additional analysis will be described in the Statistical Analysis Plan that will include a comparison of the event time distribution functions for the time until an increase in MAP_{B2} + 50% Delta (MAP) is initially observed. During the Open-Label Study Drug Administration (Dose-Titration) Phase, the sustainability of the blood pressure will be graphed over time to determine the effectiveness of SNP to maintain the target MAP.

6.7.6 Handling Missing Data in the Analyses

The following method of imputation will be used:

Last observation carried forward (LOCF): The goal of this imputation scheme is to create an observation for a completely missing observation at the end of the study for every patient in the ITT population. If a patient evaluation for a post-baseline observation is missing, then the immediately preceding non-missing evaluation will be used.

Specific algorithms for imputing missing or partially missing dates will be discussed in the SAP. Imputed or derived data will be identified in the individual patient data listings. Imputed data will not be incorporated into the case report form datasets. Imputed data will be used in the preparation of the derived datasets.

6.7.7 Pooling of Small Sites for Analysis

Small sites (i.e., sites that have less than 4 patients per treatment arm) will be identified and the following method will be used for combining the data. Data from all small sites (< 4 patients) will be combined to form a single site in order to obviate non-estimable situations (i.e., at least 2 intra-group observations are needed to estimate variance) in the evaluation of site and site interaction effects. Once combined, the pooled site will remain as such for all analyses for which a site interaction effect is determined. If the pooled smaller sites represent a single site that has more than twice as many patients as the largest single site, however less than 3 times as many patients, the small sites will be ranked by size and divided into 2 pooled groups using an alternating sequence (ABABAB). If the pooled smaller sites represent a site that has more than three times as many patients as the largest single site, however less than 4 times as many patients, the small sites will be ranked by size and divided into 3 pooled groups using an alternating sequence (ABCABCABC). This methodology will be applied, based on the initial pooling of the smaller sites.

6.7.8 Dropouts, Protocol Violations, and Exclusions

Randomized patients who fail to complete the study will not be replaced. All protocol violations will be documented and categorized in the final study report.

The rate of attrition will be evaluated by the Data Monitoring Committee during the interim evaluation to re-estimate sample size. The reasons for withdraw will be classified into 3 mutually-exclusive classes:

- Withdraw due to tolerability of the study drug
- Withdraw due to lack of treatment effect
- Withdraw not due to tolerability or lack of treatment effect

The proportion of patients who withdraw prematurely will be compared between treatment groups to determine if there is a disproportionate rate of attrition. If the rates differ by a pre-specified amount, the reasons for withdraw will be examined to determine causation. The specific monitoring rules, boundaries, and actions will be described in detail in the Data Monitoring Committee Charter.

6.8 Safety Evaluation

The primary assessment of safety will be based on the frequency of treatment-emergent adverse events and on the frequency of clinically notable abnormal vital signs and laboratory values. The primary safety analysis will be based on a comparison of the proportion of patients receiving sodium nitroprusside vs. placebo who experience a serious adverse event during the Blinded Study Drug Administration Phase.

6.8.1 Adverse Events and Medical Conditions

Adverse events will be recorded throughout the study and at early termination. Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Treatment-emergent adverse events will be defined as those events, which are newly occurring or worsening from baseline. In all cases only treatment emergent adverse events will be summarized. Treatment-emergent adverse events will be summarized by presenting, for each treatment group, the number and percentage of patients having any treatment-emergent AE, having an AE in each body system, and having each individual AE. (Note: In any given category [e.g. body system] a patient will only be counted once.) Similar displays will be provided for prior (conditions ending prior to the first exposure to sodium nitroprusside) and current (conditions present while on study medication) medical conditions. Adverse events will further be categorized by severity, relationship to study medication, and action taken. Other information collected will be listed, as appropriate. Any event starting more than 3 days after the final dose of study medication will be excluded from the above tables and only presented in a listing, unless the adverse event was serious or caused discontinuation from the study.

6.8.2 Clinical Laboratory Results

6.8.2.1 Overview

The primary presentation of the results by individual laboratory parameter will focus on the intra-patient changes from baseline (Pre-study Period). The presentation and analysis of laboratory data will be based on the observed data. All patients who have a baseline and at least one follow-up laboratory assessment will be included in the presentation of the clinical laboratory data. For each clinical laboratory test, there will be three sets of descriptive statistics that summarize the results at baseline, post-baseline assessment, and the change from baseline to post-baseline assessment. Descriptive statistics include N,

mean, standard deviation, median, and the minimum and maximum values. Within treatment group changes will be analyzed using a paired-difference t-test. Between treatment group differences will be compared using a one-factor analysis of variance test.

Shifts from baseline to each pre-specified post-baseline endpoint will also be summarized based on the laboratory categorization (*abnormally and clinically significant*, *abnormal but not clinically significant*, or *normal*) using the worst reported post-baseline observation that occurs within the pre-specified interval. The proportion of patients will be compared using a 2-tailed Fisher's exact test, pooling *abnormally and clinically significant* with *abnormally but not clinically significant.*

In the case that more than one laboratory is used, laboratory values will be transformed for mean change summaries to the same units and normal range as were provided by the central laboratory used in the study, using the formula:

$$y = (x - Li)\frac{Uc - Lc}{Ui - Li} + Lc$$

where x = original value, Li and Ui = lower and upper limits of normal for individual laboratory, Lc and Uc = lower and upper limit for central laboratory

In cases where the lower limit of central laboratory is 0, values that are below the lower limit of normal for a laboratory value prior to transformation will be assigned a value of 0.

6.8.3 Vital Signs

6.8.3.1 Overview

Vital signs of particular interested (blood pressure, MAP, heart rate) will be assessed during each phase of the study.

6.8.3.2 Presentation of Results

Descriptive statistics (n, mean, SD, median, minimum and maximum values) will be used to summarize systolic and diastolic blood pressure, MAP, and heart rate and compared between the randomized treatment groups using a one-factor analysis of variance test.

6.8.4 Physical Examination

6.8.4.1 Overview

The presentation of physical examination data is based on the dichotomous classification (normal or abnormal) of each of the 9 regions or body systems (General Appearance, HEENT, Cardiovascular, Respiratory, Abdomen, Extremities, Neurological, Hair and Skin, and Genitourinary). In addition to these 9 specific body systems, any other region recorded by the investigator under "other" will also be summarized and reported.

6.8.4.2 Presentation of Results

Results will be presented by treatment assignment using counts and percentages. Shift tables will be prepared containing the count and percentage of patients who transitioned from normal at baseline to abnormal at the end of the study. The number and percentage of patients that did not change (normal at baseline and normal at the end of the study, abnormal at baseline and abnormal at the end of the study) are also presented to frame the 2*2 contingency table. Shifts from baseline to each pre-specified post-baseline endpoint will be summarized using the worst reported post-baseline observation that occurred within the pre-specified interval. The count of the disagreements (normal to abnormal and abnormal to normal) by treatment assignment (active and placebo) will be compared for each parameter using McNemar's test.

7.0 Ethics

7.1 Independent Ethics Committee or Institutional Review Board

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator Brochure (IB), the informed consent and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an Independent Ethics Committee (IEC) or Institutional Review Board (IRB). IEC/IRB approval of the protocol, informed consent and subject information and/or advertising as relevant will be obtained prior to the authorization of drug shipment to a study site. Any amendments to the protocol will require IEC/IRB approval prior to implementation of any changes made to the study design.

7.2 Ethical Conduct of Study

This study will be conducted in accordance with the protocol, GCP, FDA regulations governing clinical study conduct, ethical principles that have their origin in the Declaration of Helsinki, 2000 revision (see Appendix E) and all applicable local regulations. The investigator must assure that the study is conducted in accordance with the provisions as stated in the FDA regulations and complies with prevailing local laws and customs. Responsibilities of the Investigator are specified in Appendix D.

7.3 Subject Information and Consent

The investigator or designated representative will explain the nature of the study to the subject, to the extent compatible with the subject's understanding, or the subject's parents or legal guardian, and answer all questions regarding this study. Prior to any study related screening procedures being performed on the subject, the informed consent statement will be reviewed, signed and dated by the subject, if capable, or the subject's parent or legal guardian, and by the person who administered the informed consent. A

copy of the informed consent form will be given to the subject and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that informed consent was obtained prior to any study-related procedures and that the subject received a signed copy.

8.0 Source Documents and CRF Completion

8.1 Source Documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

8.2 Case Report Forms

Data for individual subjects will be recorded on CRFs provided to the BPCA-CC. All entries must be complete. A case report form must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the CRF by the investigator. The principal investigators must review and approve each CRF.

Case report forms must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested during an FDA inspection, this log may be

shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

Because of the potential for errors and inaccuracies in entering data into CRFs, laboratory and other test results must be kept on file with the subject's study dossier. Case report forms and copies of test results must be available at all times for inspection by the CRA for the site and the FDA.

9.0 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA-CC, the investigators and their study coordinators and the CRAs for the study. This meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures by a CRA at a study initiation visit and will be given an CRF completion workbook for reference.

The CRAs will monitor each site throughout the study. At each visit, 100% source document review will be made against entries on the CRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and all applicable regulations.

After completion of the entry process, computer logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary correction will be made to the database and documented via addenda or audit trail. A manual review of selected line listings will also be performed at the end of the study.

10.0 Use of Information and Publication

10.1 Use of Information

This trial is sponsored by the NICHD. The NICHD endorses the sharing of final research data to expedite the translation of research results into new scientific knowledge in order to improve human health.

This contract is part of a collaborative program involving multiple sites. A data sharing dissemination plan will be developed jointly with the BPCA-CC, the NICHD, and the collaborating institutions following announcement of the award.

10.2 Publication

The BPCA-CC and steering committee for this study shall have the right to review and approve any paper for publication, including oral presentation and abstracts, which utilize data generated from this study. At least 90 days before any such paper or abstract is presented or submitted for publication, a complete copy shall be given to the BPA-CC for review by the steering committee. Any individual investigator agrees to defer publication of any such paper or abstract until the BPCA-CC and Steering Committee have reviewed and approved it.

11.0 Completion of Study

The investigator will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon in writing by the investigator, the NICHD, the BPCA-CC and the PODS. The investigator will provide a summary of the study's outcome to the IRB/IEC following the conclusion of the study.

The PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. The investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the NICHD and BPCA-CC within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

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12.0 Investigator Agreement

I have received and reviewed the investigator brochure for sodium nitroprusside (SNP).

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulation.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Signature of Principal Investigator Date

Name of Principal Investigator (printed or typed)

Appendices

Appendix A: Tanner Stages of Sexual Maturity

	Pubic Hair		Breasts	Penis	Testes
SMR Stage[1,2]	Boys	Girls	Girls	Boys	Boys
1	None	Preadolescent	Preadolescent	Preadolescent	Preadolescent
2	Scanty, long, slightly Pigmented	Sparse, lightly pigmented, straight, medial border of labia	Breast and papilla elevated as small mound; areolar diameter increased	Slight Enlargement	Enlarged scrotum, pink texture altered
3	Darker, starts to curl, small Amount	Darker, beginning to curl, increased amount	Breast and areola enlarged, no contour separation ,	Longer	Larger
4	Resembles adult type, but less in quantity; coarse, cu rly	Coarse, curly, abundant but amount less than in adult	Areola and papilla form secondary mound	Larger; glans and breadth increase in size	Larger, scrotum dark
5	Adult distribution, spread to medial surfaces of thighs	Adult feminine triangle, spread to medial surface of thighs	Mature; nipple projects, areola part of general breast contour	Adult size	Adult size

1. Adapted from Tanner, JM: Growth at Adolescence, 2 ed. Oxford, Blackwell Scientific Publications, 1962.
2. MR = Sexual Maturity

Appendix B: Research Consent Form with HIPAA

Protocol Title: A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects

Protocol Director: [PROGRAM PI]
IRB Approval Date: _____ IRB Expiration Date: _____

Is your child participating in any other research studies? _____Yes _____No

INTRODUCTION

You are being asked to agree to let your child be a part of a drug research study. He or she is scheduled for surgery, or needs to stay in an intensive care unit (ICU). During this operation or stay in the ICU, it will be necessary for the doctor to lower your child's blood pressure for a long period of time, up to 24 hours. We will tell you more about how he/she will do this and the drug that will be used later in this paper. Before you decide whether to let your child be involved in this study, *[Site PI]* wants you to read the following information. He wants you to ask him any questions you may think of. He wants to be sure that you understand what your child's participation will mean. You need to fully understand the type of treatment and its risks. Your doctor is responsible for providing you with the necessary information so that you understand the possible risks. Your child's participation in this study is entirely your choice.

Your child cannot participate in another research study at the same time as this research study [optional sentence]. Your child cannot be taking another experimental drug while enrolled in this research study, or within the previous 30 days. Your child will be a part of this study for approximately 30 days. There may be risks that we cannot predict. We will tell you about any new information that may affect your child's condition or affect your willingness to stay in this study.

NATURE AND PURPOSE OF THE RESEARCH STUDY

In certain kinds of surgeries, doctors often need to control the blood pressure of the patient. The doctor may also need to lower the patient's blood pressure below normal. This can reduce blood loss and avoid blood transfusions during stressful periods. Sodium nitroprusside is a drug that is approved by the Food and Drug Administration (FDA) for use in adults. Scientific studies show that this drug works well when doctors need to control blood pressure during surgeries in adult patients. Doctors also often use sodium nitroprusside in children. However, not many scientific studies tell us how best to use sodium nitroprusside in children.

In this study, we hope to learn the best dose of sodium nitroprusside to use in children who need it in the ICU for more than 12 hours. We also want to learn the same thing for children who need certain kinds of surgeries. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children. This research study is looking for 50-100 children at several hospitals in the United States.

Your child's participation in this study is entirely voluntary.

Your decision whether or not to allow your child to participate will not prejudice your child or his/her medical care. If you decide to allow your child to participate, you are free to withdraw your consent, and to discontinue participation at any time without prejudice to your child or effect on your child's medical care. If you decide to terminate your child's participation in this study, you should notify *(name)* at *(telephone number)*.

PROCEDURES

If you agree to your child's participation in this research study, he or she will undergo the following types of procedures:

- [Site PI] and his research staff will talk to you (and your child) about his or her health. They will ask you about your child's medical history. They will ask you about any medications your child is currently taking. They will give your child three physical examinations, including

measurement of blood pressure, pulse, and weight. The first one will be within 24 hours before study drug is given. The other two physical exams will be after your child receives study drug. A small amount of blood, much less than one teaspoon, will be drawn at these visits. We need this blood for our study, but the same blood work may also be needed as part of your child's regular medical care. If this is the case, we will use these results instead of having to take another blood sample.

- Your child will receive study treatment in stages:
  1. First, after your child is stable, sodium nitroprusside will be given into a vein. This will be done through tubing your child will already have in place. This drug may be the treatment of choice to control your child's blood pressure even if your child is not enrolled in this study. This first stage will last up to 24 hours. He or she will receive sodium nitroprusside at a pre-set initial rate. That rate will be changed until your child's blood pressure is in the range that his or her doctor has decided is the best.
  2. The second stage of study treatment will begin between 12 and 24 hours after the medication was first started. This stage will last up to 30 minutes. During this stage your child will receive one of two treatments. This phase of the research study is blinded. That means that the study doctor will not know which treatment group your child will be placed into. The choice of which treatment group your child will be in (placebo or sodium nitroprusside) will be random. The choice will be made in a way that is like flipping a coin. He or she might receive sodium nitroprusside at the rate that was being used before to keep his or her blood pressure at a stable level. Or, he or she might receive a placebo; this is a solution like salt water that is known to have no effect on your child's health. Your child's vital signs (blood pressure and heart rate) will be watched very closely. If your child's blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end immediately. Then your child will again be given sodium nitroprusside, or similar drug, to keep his or her blood pressure at a safe level.
  3. If your child stills needs sodium nitroprusside treatment to control his/her blood pressure, the treatment may continue after the research study ends.

During the research study, vital signs (blood pressure and heart rate) will be checked often at specific time points.

There will be a follow up evaluation. About 30 days after the research study is over, we will call you at home to ask questions about your child's health in the last month, since he or she participated in the research study. If your child is readmitted to the hospital before our call, please let us know.

Blood samples will be taken between 3 and 6 times (to total between 1 to 2.5 teaspoons) during the research study. This is to see how much of the drug is circulating in the blood at varying time points. If indicated, additional blood samples may be taken to help determine the amount of drug in your child's blood. We are very careful to minimize the amount of blood drawn from your child, and anticipate that 3 1/2 teaspoons is the most we will draw for these tests.

Whenever possible, blood will be taken from tubing already in place. The nurse will often take the study blood samples at the same time that routine blood samples are taken to check on your child's health. This is done to avoid any extra needle-sticks (drawing blood from a vein in the arm with a needle). It is very unlikely that there would not be a catheter (IV line) in place; however, if that were to happen, blood drawing would require a needle stick that might cause minor bruising. Blood drawing is done to assess safety and to measure the activity of the study drug.

The total amount of blood drawn during the entire research study for children less than 2 years of age is approximately 1 to 2 ½ teaspoons. For children more than 2 years of age, the total amount of blood is about 1 to 3 ½ teaspoons.

The information gained during this research study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help patients in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

YOUNG WOMEN OF CHILD-BEARING POTENTIAL

If your child is a young woman who is able to become pregnant, it is expected that she will use an effective method of birth control to prevent exposing a fetus to a potentially dangerous agent with unknown risk. If your child is pregnant or currently breast feeding, she may not participate in this study.

To confirm to the extent medically possible that your child is not pregnant, you agree that she will have a pregnancy test done before beginning this research study. This test will be carried out right before your child undergoes surgery.

PARTICIPANT RESPONSIBILITIES

You should:

- Follow the instructions of the Protocol Director and study staff.
- Tell the Protocol Director or research study staff about any side effects that your child may have.
- Tell the Protocol Director or research study staff if you believe your child might be pregnant.
- Ask questions as you think of them.
- Tell the Protocol Director or research staff if you change your mind about staying in the study.

While participating in this research study, your child should not take part in any other research project without approval from all of the Protocol Directors. This is to protect your child from possible injury arising from such things as extra blood drawing, the possible interaction(s) of research drugs, or other similar hazards.

WITHDRAWAL FROM STUDY

If you first agree to participate and then you change your mind, you are **free to withdraw** your consent and discontinue your child's participation at any time. Your decision will not affect your child's ability to receive medical care for his or her disease and your child will not lose any benefits to which he or she would otherwise be entitled.

If you decide to terminate your child's participation in this study, you should notify (*name)* at (*phone number)*.

The Protocol Director may also withdraw your child from the study and the study medication may be stopped without your consent for one or more of the following reasons:

- Failure to follow the instructions of the Protocol Director and/or study staff.
- The Protocol Director decides that continuing your child's participation could be harmful to him or her.
- Pregnancy (if applicable).
- Your child needs treatment not allowed in the study.

- The study is cancelled.
- Other administrative reasons.
- Unanticipated circumstances.

POSSIBLE RISKS, DISCOMFORTS, AND INCONVENIENCES

There are risks, discomforts, and inconveniences associated with any research study. These deserve careful thought. This is true whether it is a normal kind of treatment or an experimental type. You should talk with the Protocol Director if you have any questions.

In spite of all safety measures, your child might develop medical problems while taking part in this research study. These risks include elevated blood pressure. Treatment of these potential medical problems will not be limited or delayed by your child's participation in the study.

Sodium nitroprusside is a drug that lowers blood pressure. Because of this, there is a chance that your child could develop hypotension (low blood pressure). The doctor will monitor your child very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable blood pressure.

Another side effect that might happen is that your child's heart rate may increase in response to sodium nitroprusside. Again, the doctor and research nurse will monitor your child's heart rate very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable heart rate.

Minor side effects due to sodium nitroprusside may also occur but will not cause the study to be ended. Since sodium nitroprusside makes blood vessels bigger, the following side-effects may occur: nausea, headache, restlessness, abdominal pain, redness or flushing of the skin, nervousness, and perspiring.

Another side effect that happens when using sodium nitroprusside is that as it is used in the body, another chemical, cyanide, is released in small amounts. This can affect the amount of oxygen in the

blood and have a number of other effects. The doctors will carefully watch for any of these signs or symptoms and treat your child if needed.

POTENTIAL BENEFITS

The information gained during this study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help children in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOUR CHILD WILL RECEIVE ANY BENEFITS FROM THIS STUDY.

ALTERNATIVES

If you choose not to enroll your child in this study, your child may receive sodium nitroprusside anyway. This could happen if your child's doctor feels it is the best medicine to use to control blood pressure. Or, your child's doctor could choose to use other types of blood pressure medications, such as esmolol or fenoldopam, instead. Whether or not your child is enrolled, the medical team will, of course, do everything possible to ensure the safety and comfort of your child.

If you do not wish your child to take part in this study, other treatments can be used for your child's condition. If you withdraw your child's participation, the study doctor will recommend an alternative treatment for blood pressure control for your child, such as esmolol or fenoldopam. If this study is discontinued, your child will receive one of these alternative treatments.

PARTICIPANT'S RIGHTS

You should not feel obligated to agree that your child participate in this study. Your questions should be answered clearly and to your satisfaction.

If you decide not to participate, tell the Protocol Director. Your child will still receive care for his/her disease and will not lose any benefits to which he/she would otherwise be entitled.

You will be told of any important new information that is learned during the course of this research study, which might affect your child's condition or your willingness to continue participation in this study.

CONFIDENTIALITY

Your child's identity will be kept as confidential as possible as required by law. Except as required by law, your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your child's research records may be disclosed outside of Stanford, but in this case, your child will be identified only by a unique code number. Information about the code will be kept in a secure location and access limited to research study personnel.

The results of this research study may be presented at scientific or medical meetings or published in scientific journals. However, your child's identity will not be disclosed**.**

Patient information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your child's identity if this study falls within its jurisdiction.

- The purpose of this research study is to obtain data or information on the safety and effectiveness of sodium nitroprusside in children; the results will be provided to the sponsor, the Food and Drug Administration and other federal and regulatory agencies as required.

Authorization to Use Your Health Information for Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed consent form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?

In this study, we hope to learn the best dose of sodium nitroprusside to use in children of different ages who need it in the ICU for more than 12 hours. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children as determined by the NIH and FDA.

Do I have to sign this authorization form?

You do not have to sign this authorization form. But if you do not, your child will not be able to participate in this research study, including receiving any research-related treatment.

Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?

If you decide to allow your child to participate, you are free to withdraw your authorization regarding the use and disclosure of your child's health information (and to discontinue any other participation in the study) at any time. After any revocation, your child's health information will no longer be used or disclosed in the study, except to the extent that the law allows us to continue using your child's information (e.g., necessary to maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must contact: *(researcher's name and contact information, including telephone number).*

What Personal Information Will Be Used or Disclosed?

Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to, vital sign measurements, laboratory results of blood collections, physical exams, related medical records, and other data.

Who May Use or Disclose the Information?

The following parties are authorized to use and/or disclose your child's health information in connection with this research study:

- The Protocol Director *(Insert Name of PD)*
- The *(Insert name of Institution)* Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary
- Research Staff

(List every other class of persons or organization affiliated with the hospital/university who might need to use and/or disclose the participant's information in connection with this study.)

Who May Receive or Use the Information?

The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- The National Institutes of Health
- The Food and Drug Administration
- Collaborating Institutions
- The Coordinating Center, Premier Research

Your child's information may be re-disclosed by the recipients described above, if they are not required by law to protect the privacy of the information.

When will my authorization expire?

Your authorization for the use and/or disclosure of your child's health information will expire December 31, 2055.

Will access to my child's medical record be limited during the study?

To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or

billing decision about your child (e.g., if included in your child's official medical record).

_____

Signature of Participant

_____

Signature of Legally Authorized Representative

_____

Date

_____

Description of Representative's Authority to Act for Subject

FINANCIAL CONSIDERATIONS

PAYMENT

You and your child will not be paid to participate in this research study.

COSTS

The sponsor will pay for the cost of sodium nitroprusside and for the extra blood tests that will be used to monitor the amount of drug in your child's blood and for safety tests. You or your insurance company will be responsible for the medical procedures, surgery, anesthesia, and other normal costs associated with standard medical care for treatment of your child's condition.

SPONSOR

The National Institute of Child Health and Development of the National Institutes of Health (NIH) is providing financial support and/or material for this study.

COMPENSATION FOR RESEARCH-RELATED INJURY

All forms of medical diagnosis and treatment – whether routine or experimental – involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. **You will be responsible for any associated co-payments or deductibles as required by your insurance.**

If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

Additionally, *{Institution name}* is not responsible for research and medical care by other institutions or personnel participating in this study. You do not waive any liability rights for personal injury by signing this form.

CONTACT INFORMATION

- Questions, Concerns, or Complaints: If you have any questions, concerns or complaints about this **research study**, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director, *{name}*. You may contact *{him/her}* now or later at *{phone number}*.
- Emergency Contact: If you feel your child has been **hurt by being a part of this study**, or need immediate assistance please contact *{Protocol Director's name and phone number}*.
- Alternate Contact: If you cannot reach the Protocol Director, please page the research team at *{phone number}*.
- Independent of the Research Team Contact: If you are not satisfied with the manner in which this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a research study subject, please contact the *{Institution's name}* Institutional Review Board (IRB) to speak to an informed individual who is independent of the research team at *{phone number}*. Or write the *{Institution's name}* IRB, *{IRB full address}*. In addition, please call the *{Institution's name}* IRB at *{phone number}* if you wish to speak to someone other than the research team or if you cannot reach the research team.

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

As a human subject your child has the following rights. These rights include but are not limited to the subject's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
- be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise;
- be given an opportunity to ask questions concerning the experiment or the procedures involved;

- be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated consent form;
- and be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING CONSENT, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____

Signature of Parent or Legal Guardian

_____

Signature of Second Parent or Legal Guardian

_____

Date

_____

Name of Patient

PERSON OBTAINING CONSENT

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied - that the Parent or Legal Guardian has been provided with the Experimental Subject's Bill of Rights, if appropriate, that I have discussed the research project with the Parent or Legal Guardian and explained to him or her in nontechnical terms all of the information contained in this consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the Parent or Legal Guardian to ask questions and that all questions asked were answered.

_____ _____

Signature of Person Obtaining Consent Date

Appendix C: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles
for
Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when

providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The

responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's

freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case, the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in

the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix D: Responsibilities of the Investigator

Investigator Responsibility/Performance

Prior to starting enrollment at a site, all investigators must read and understand the Investigational Plan and must sign and complete the Investigator Agreement Form. This documents that they accept all conditions of the Investigational Plan and will conduct the study accordingly. The investigator must provide a current copy of his or her curriculum vitae that is not more than 2 years old.

Informed Consent and IRB Approval

The investigator must have written approval from the IRB prior to enrolling patients in the study. A copy of the written approval that includes the following must be provided to the DCRI:

- A statement of IRB approval for the proposed study and ICF at the institution
- The date the study was approved and the duration of approval
- A listing of any conditions attached to the approval
- Identification of the approved primary investigator
- The signature of the IRB chairperson

Any amendments to the protocol, as well as associated consent form changes, will be submitted to the IRB, and written approval must be obtained prior to implementation. Serious adverse event reports will be submitted as requested by the BPCA.

The study will be explained to the patients in lay language. Patients will sign and receive a copy of the IRB-approved informed consent form prior to study participation. Patients will be assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Source Documentation

Regulations require that investigators maintain information in the study patient's medical records that corroborate data collected on the CRFs. In order to comply with these regulatory requirements, at the minimum, the following information should be maintained:

1. Medical history/physical condition of the study patient before involvement in the study sufficient to verify protocol entry criteria (if not already present)
2. Dated and signed notes on the day of entry into the study including clinical site, patient number assigned and a statement that consent was obtained
3. Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams)
4. Notations on abnormal laboratory results
5. Serious adverse events reported through 30 days from the end of study drug administration
6. Notes regarding concomitant medications taken during the study (including start and stop dates)
7. Study patient's condition upon completion of or withdrawal from the study

Data Transmittal

Required data will be recorded on the CRFs as soon as possible after the patient is discharged, day 10, or death, whichever comes first. CRFs and any supporting documents must be sent to the BPCA and/or retrieved from the investigational site according to the outlined time windows. The 28-day follow-up CRF needs to be forwarded within 10 days of the follow-up visit.

Non-Protocol Research

BPCA has a legal responsibility to report fully to regulatory authorities all the results of clinical studies. No investigative procedures other than those in this protocol shall be undertaken on the enrolled patients without the agreement of the IRB and BPCA.

Publication Policies

At the conclusion of the study, a multicenter abstract reporting the primary results may be prepared and presented in an appropriate international forum. A multicenter, peer-reviewed manuscript will also be prepared for publication in a reputable scientific journal.

Appendix E: Treatment of Suspected Nitroprusside Toxicity

Signs of Toxicity:

- If the base deficit exceeds -8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the subject will be also be discontinued from study.
- If the lactate level rises by more than 4 mmol/L in a two to four hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output).
- If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes percent between arterial and mixed venous blood.
-

SUSPECTED CYANIDE TOXICITY SHOULD BE TREATED AS FOLLOWS:

1) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration.

2) Obtain blood for arterial and venous blood gases with co-oximetry, serum lactate, and cyanide and thiocyanate levels.

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10mL) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result)

Subject Hemoglobin g/dL	Initial dose Sodium NITRITE (3%) mL/kg IV	Initial dose Sodium Thiosulfate mL/kg IV
8 g/dL	0.22 mL/kg	1.10 mL/kg
(6.6 mg)/kg		
10 g/dL	0.27 mL/kg	1.35 mL/kg
(8.7 mg)/kg		
12 g/dL	0.33 mL/kg	1.65 mL/kg
(10 mg)/kg		
14 g/dL	0.39 mL/kg	1.95 mL/kg
X	(11.6 mg)/kg	

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals.

[Micromedex Poisondex www.micromedex.duhs.duke.edu , see also Berlin, 1970]

Appendix F: Assay of Nitroprusside Metabolites and Handling of Blood Samples for Assay of Nitroprusside Metabolites

A classical UV bioanalytical method is utilized for detection and quantitation of cyanide in whole blood. Cyanide concentrations are determined by measuring the absorbance of the chromophore formed by the interaction of the cyanide ion with 4-nitrobenzaldehyde and o-dinitrobenzene in 2-methoxyethanol (Rieders, 1971 and Guilbault, 1966). In summary, to a 1.0 ml aliquot of whole blood, 4-Nitrobenzaldehyde solution and o-Dinitrobenzene solution is added then made basic with sodium hydroxide. Following a specific incubation time a UV scan spectrum is obtained from 520 to 580 nm with a maximum reading at 555 nm. The exact details of the method are proprietary to NMS Labs. The method is sensitive to a LLOQ of 0.05 μg/mL which correlates to normal circulating levels. A toxic threshold is normally assessed as approximately 0.5 μg/mL and acute toxicity is observed at greater than 1 μg/mL. There are no known interferences with this assay method.

An ion chromatography method is used for detection and quantitation of thiocyanate in serum that is specific, accurate, precise and rugged. In summary, a 0.10 mL aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography (Vogel, 1981 and Vesey, 1976). The same procedure is employed to detect thiocyanate in urine. The normal range for non-smokers is 1-4 μg/mL in serum/plasma. For smokers, it is 3 – 12 μg/mL and the therapeutic range for sodium nitroprusside is generally between 6 and 29 μg/mL (Schulz, 1984).

Thiosulfate is detected in serum/plasma or urine also via a validated ion chromatography method with the analytes separated and detected via conductivity detection. For this method, a 0.5 ml aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography.

Sample Handling Procedures

At each specified blood collection for cyanide and thiocyanate, 2 mL of arterial blood is to be collected in a 2 mL gray top (BD reference number 367921). Samples should be inverted at least eight times to mix, then one half the whole blood in the gray top (1 mL) is removed and stored in a polypropylene screw capped container and stored on ice until it can be refrigerated. The remaining 1 mL of whole blood is centrifuged within 20 minutes of collection at approximately 1200 g for 10 - 12 minutes at ambient temperature to obtain 0.5 mL of plasma for thiocyanate analysis. Plasma and whole blood should be stored in a refrigerator before sending to National Medical Services as soon as possible. Samples should be shipped cold using "frozen cold packs" for overnight delivery. Whole blood samples should NEVER be frozen. NMS will accept deliveries on a Saturday so Friday shipments are possible.

For neonates or to spare the total amount of blood drawn for the analysis, the minimum blood draw is 1 mL of arterial blood. This translates to 0.5 mL of whole blood for cyanide analysis and 0.5 mLs to be centrifuged to obtain approximately 0.25 mLs of plasma for thiocyanate analysis.

Appendix G: Sedation Suggested Regimen

Intensive Care Unit:

For those patients who receive study drug in the intensive care unit for a surgical or medical procedure, the following guidelines may be utilized.

Sedation may be administered intravenously and initiated with a benzodiazepine and opiate agonist as follows:

Midazolam bolus 0.1-0.2 mg/kg followed by a continuous midazolam infusion of 0.06-0.3 mg/kg/hr or intermittent bolus of 0.1 mg/kg every 1-2 hours

Or

Lorazepam bolus 0.05-0.1 mg/kg followed by a continuous infusion of 0.025-0.05 mg/kg/hr or intermittent bolus of 0.05-0.10 mg/kg every 4-6 hours

And/or

Fentanyl bolus 1- 5 micrograms/kg (intubated, mechanically ventilated patients) followed by an infusion of fentanyl of 0.5 – 5 micrograms/kg/hour

Or

Morphine bolus 50-100 microgram/kg followed by a continuous infusion of 20-80 micrograms/kg/hour.

Sedation and analgesic medication may be titrated to patient response. Higher doses may be used in patients who exhibit benzodiazepine and/or narcotic habituation due to long term (> 4-7 day) usage. Where applicable, sedation assessment may be by clinical judgment of the responsible physician, and/or a quantitative scoring system such as the COMFORT score. Patients receiving neuromuscular blocking drugs such as vecuronium or rocuronium as part of their ICU management are eligible for study.

Appendix H: Measurement of Blood Pressure in Children

Excerpt from: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents

Correct measurement of BP in children requires use of a cuff that is appropriate to the size of the child's upper right arm. The equipment necessary to measure BP in children, ages 3 through adolescence, includes child cuffs of different sizes and must also include a standard adult cuff, a large adult cuff, and a thigh cuff. The latter 2 cuffs may be needed for use in adolescents.

By convention, an appropriate cuff size is a cuff with an inflatable bladder width that is at least 40% of the arm circumference at a point midway between the olecranon and the acromion. For such a cuff to be optimal for an arm, the cuff bladder length should cover 80% to 100% of the circumference of the arm. Such a requirement demands that the bladder width-to-length ratio be at least 1:2. Not all commercially available cuffs are manufactured with this ratio. Additionally, cuffs labeled for certain age populations (eg, infant or child cuffs) are constructed with widely disparate dimensions. Accordingly, the working group recommends that standard cuff dimensions for children be adopted (see Table 2).

TABLE 2. Recommended Dimensions for BP Cuff Bladders

Age Range	Width, cm	Length, cm	Maximum Arm Circumference, cm*
Newborn	4	8	10
Infant	6	12	15
Child	9	18	22
Small adult	10	24	26
Adult	13	30	34
Large adult	16	38	44
Thigh	20	42	52

* Calculated so that the largest arm would still allow the bladder to encircle arm by at least 80%.

BP measurements are overestimated to a greater degree with a cuff that is too small than they are underestimated by a cuff that is too large. If a cuff is too small, the next largest cuff should be used, even if it appears large. If the appropriate cuffs are used, the cuff-size effect is obviated

SBP is determined by the onset of the "tapping" Korotkoff sounds (K1). Population data in children and risk-associated epidemiologic data in adults have established the fifth Korotkoff sound (K5), or the disappearance of Korotkoff sounds, as the definition of DBP. In some children, Korotkoff sounds can be heard to 0 mm Hg. Under these circumstances, the BP measurement should be repeated with less pressure on the head of the stethoscope. Only if the very low K5 persists should K4 (muffling of the sounds) be recorded as the DBP.

Appendix I: Research Assent Form Template

Protocol Title: A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Director:

IRB Approval Date: _____IRB Expiration Date: _____

Are you taking part in any other research studies right now? ____ yes ____ no

Why Are We Doing this Research Study?

We are doing this study to learn more about a medicine called sodium nitroprusside. This study will help us find out more about how good this drug is in keeping blood pressure and heart rate under control in children who are in the intensive care unit (ICU). Altogether, there will be 60 kids under the age of 17 in this study.

What Will Happen During the Study?

You will be seen by a doctor, who will give you a physical exam and ask you and your parents some questions about your health history and medicines you take. At certain times during the study, blood will be drawn to check that you are okay and safe, and to see how the study drug is working in your body. Blood will be drawn either from a tube that is already connected to you to get blood, or from a needle. The amount of blood to be taken during the study is about 2½ teaspoons to 3½ teaspoons.

In the first part of the study, you will receive a study drug called sodium nitroprusside through a tube placed into a vein in your arm. Your doctor will make changes in the amount of drug you get to keep you safe and your vital signs (like your heart rate and blood pressure) stable. Your doctor will

change how much of the drug you get so you have the blood pressure the doctor thinks is right for you. This part of the study will last for at least 12 hours up to 24 hours during the time that your doctor needs to control your blood pressure. During the time you get study drug, your blood pressure and heart rate will be measured very often.

The second part of the study will last up to 30 minutes. During this part, you will get one of two treatments. This part of the research study is blinded. That means that the study doctor will not know which treatment group you are in. The choice of which treatment you receive will be made in a way that is like flipping a coin. You might receive the study drug at the same rate that was being used before to keep your blood pressure stable. Or, you might receive a placebo, which is a solution like salt water that doesn't have any effect on your blood pressure. Your blood pressure and heart rate will be watched very close. If your blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end right away. Then you will again be given sodium nitroprusside, or similar drug, at a rate that keeps your blood pressure at a safe level.

What if you don't want to be in the Study?

You can say "no" to being in the study if you want. You can also stop the study anytime you want by telling anyone that is caring for you. Your doctor can explain to you and your parents other treatments that could be used instead.

What You Should Know about the Medicine?

The medicine is used to keep your blood pressure where your doctor thinks is safe or needed for your operation or some other treatment or test. It has been okayed for use in adults but there is not a lot of information about how the drug works in kids. Like any medicine, it can cause unwanted things to happen. These unwanted things are called risks. Some of the risks from using this medicine are: getting sick to your stomach, headache, getting hot, having your blood pressure go down too much or your heart beat go up too high.

Things Girls Need to Know….

Some medicine can cause bad things to happen to an unborn baby. If you are able to get pregnant (if you have started having a period), you need to take a pregnancy test which your doctor will give you. If you are pregnant, you should not take part in this study.

What Else Do You Need to Know?

Sometimes doctors write about the research studies when they are done. If a paper is written about this research study, your name won't be used in it, but the medical information they find out about you may be used. We will keep your medical information private. People who work for *[site name]*, the people who are running the study, and some parts of the government (the part that takes care of medicines) will be able to look at your medical information.

There is no cost to you or your parents to be in this study.

If you have questions about the study you can call Dr. _____ at

_____.

I have read this form. I have had a chance to ask questions about things I don't understand. I want to be in this research study and understand what will happen to me.

_____ _____

Signature of Patient Date

_____

Name of Patient

_____ _____

Signature of the Person Obtaining Assent Date

NICHD-2003-09-LT

Summary of Changes of Protocol Amendment Dated 11 November 2008

1) Study Contact Information, Project Administrators

    a. Contact information has been updated

2) Inform Consent

    a. Throughout the protocol, the term "parental permission" is being substituted for "informed consent" (e.g., Section 7.3). Sites are being instructed that the parental-permission form must contain all of the elements of the informed consent, as stipulated in 21 CFR 50.25. We realize that some IRB's may have reservations using this term, and therefore we will allow sites to adopt language that meets their IRB requirements.

3) Pre-study drug vital signs, Table 1 and Section 3.4.1

    a. The timing of vital signs obtained prior to the start of study-drug administration (i.e., pre-study drug vital signs) has been more precisely defined to be: "immediately prior to induction if B1 will occur during general anesthesia; otherwise, as early as practical," where B1 is the MAP obtained immediately prior to the start of the open-label phase. Please note that we anticipate two types of patients will be eligible for this study—surgical patients, for whom B1 will be confounded by induction of general anesthesia, and medical patients, who will likely be first seen in the emergency department.

4) Collection of surgical information, Table 1 (footnote 16) and Section 3.4.6, #2

    a. The following statement has been added, "For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative

diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent)."

5) Minor changes

   a. Section 3.4.4, #4, revised wording to match Figure 1 and Section 6.3.1, last bullet point.

      i. To match the frequency of MAP assessments, the duration for defining lost of blood pressure control was changed from "sustained for 30 sec in the absence of stimulation" to "≥2 consecutive 1-minute measurements".

   b. Section 4.2, revised abbreviations for units and added minimum value for mild cyanide toxicity for erythrocytes (≥5 μmol/L).

   c. Appendix E, second bullet point under "signs of [cyanide] toxicity", the timing of the rise in lactate levels has been revised from "a two to four" hour period to "an eight" hour period in order to match the timing of lactate measurements provided in the protocol.

   d. Appendix F, the description of the sample handling procedures has been revised to indicate:

      i. RBC may be tested for cyanide levels.
         1. This investigational testing is being untaken as part of a counterterrorism research program at the National Institute of Neurological Disorders and Stroke
      ii. Because of this additional testing, references to the National Medical Services have been removed, as the testing laboratory for cyanide may be changed at a later date
      iii. Additional details regarding the sample handling can be found in the Manual of Procedures for this study

PROTOCOL

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Number: NICHD-2003-09-LT

Study Drug: Sodium Nitroprusside

IND: 71,979

Medical Monitor: Bernard Brownstein, M.D.

Principal Investigators:

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Sponsor: The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

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Confidential

APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

And I have fully discussed the objectives of this trial and the contents of this protocol with representatives of PODS Center and BPCA–Coordinating Center.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations.

I understand that, should the decision be made by the PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

PODS Center #1 Duke University Medical Center Box 3094 DUMC Durham, NC 27705	SUBCONTRACTOR: Institution: Address: Telephone: Fax: Email:
CO-PRINCIPAL INVESTIGATOR Scott Schulman, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol for sodium nitroprusside in the control of blood pressure entitled:

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CO-PRINCIPAL INVESTIGATOR Gregory Hammer, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

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TABLE OF CONTENTS

PROTOCOL SYNOPSIS

Protocol Title:	A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects
Protocol Number:	NICHD-2003-09-LT
Sponsor:	National Institute of Child Health and Human Development
Product:	Sodium Nitroprusside
Objectives:	1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours 2. To assess the potential for rebound hypertension following administration of sodium nitroprusside for 12 hours or more
Study Design:	This is a phase II, randomized, double blind, withdrawal to placebo study examining the efficacy, safety and tolerability of sodium nitroprusside in pediatric subjects.
Study Population:	Children up to 17 years of age who require long term (at least 12 hour) blood pressure control will be eligible for study.
Number of Subjects:	A target of approximately 60 patients will be enrolled.
Number of Sites:	Up to 15
Duration of Subject Participation:	Enrollment is anticipated to begin in 2008 and to be complete in approximately 12 months. Patients will be followed for up to 30 days following receipt of study drug.
Treatment:	Subjects who require vasodilator therapy for relatively long time periods will receive open-label infusion of sodium nitroprusside for at least 12 hours but not greater than 24 hours.
Dose Schedule:	Patients will be randomized to receive either placebo or sodium nitroprusside for 30 minutes following at least 12- hours but not more than 24 hours of open-label infusion of sodium nitroprusside.
Estimated Start:	Q3 2008
Estimated Finish:	Q3 2009
Ethics	All regulations stated in 21 CFR Parts 50, 56, and 312 and recommendations outlined in the ICH Guidelines for Good Clinical Practice, as well as all other applicable local and national laws and regulations, will be adhered to throughout this trial.

Safety:	The safety of the drug will be assessed by multiple subject assessments of vital signs, physical exams, clinical tests and laboratory evaluations. Adverse events will be monitored and tracked. All SAEs will be closely monitored throughout the course of the study.
Statistical Consideration:	The trial will be sized to detect the loss of as little as 50% of the expected blood pressure lowering effect of the chosen dose of sodium nitroprusside during the 30 minutes of withdrawal to placebo.

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BP	Blood Pressure
BPCA-CC	Best Pharmaceuticals for Children Act Coordinating Center
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
cGMP	cyclic Guanosine Monophosphate
CN^-	Cyanide
CRA	Clinical Research Associate
CRF	Case Report Form
d/c	Discontinuation
DCRI	Duke Clinical Research Institute
DBP	Diastolic Blood Pressure
DSMB	Data and Safety Monitoring Board
ECMO	Extracorporeal Membrane Oxygenation
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
hr	Hour

ACRONYMS AND ABBREVIATIONS

HR	Heart Rate
IB	Investigational Brochure
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
kg	Kilogram
MAP	Mean Arterial Pressure
mcg	Microgram
mEq/L	Milliequivalent per Liter
mcgs	Micrograms
min	Minute
mL	Milliliter
mm Hg	Millimeters of Mercury
mmol/L	Millimoles per Liter
NO	Nitric Oxide
NICHD	National Institute for Child Health and Human Development
NONMEM	Nonlinear Mixed Effect Model
NTG	Nitroglycerin
PD	Pharmacodynamic
PaO_2	Arterial Partial Pressure of Oxygen
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SCN	Thiocyanate
SNP	Sodium Nitroprusside
μM	Micromoles per liter, Micromolar

1.0 Background and Rationale

Blood pressure control in children is a significant concern in the intensive care unit (ICU), where management of arterial pressure is often necessary during periods of acute physiologic stress such as occurs after certain surgical and medical procedures. Examples of surgical procedures that require blood pressure control in the intensive care unit following surgery include aortic coarctation repair, Ross procedure (pulmonary valve autograft), and solid organ transplantation. Medical conditions requiring control of systemic arterial pressure include renal disease, drug therapy (corticosterioids and immunosuppression agents), and procedures such as extracorporeal membrane oxygenation (ECMO).

A wide variety of drugs of various therapeutic classes have been utilized for either controlled hypotension in the operating room or prevention of hypertension in the pediatric ICU. These drug classes include calcium channel blockers (Tobias et al, 1996), beta-adrenergic antagonists (Kay et al, 2001), ganglionic blockers (DuToit, 1970 and Gallagher and Milliken, 1979), inhalation anesthetics (Tobias, 1998) and direct acting vasodilators such as nitroglycerin and sodium nitroprusside (SNP) (Kaplan, 1980, and Tinker, 1976 Groshong, 1996, and Sinaiko, 1996). Although many vasodilator agents are available to lower blood pressure in the operating room and intensive care unit setting, few have been systematically studied in children.

SNP is a direct acting vasodilator commonly used for blood pressure control. It produces vascular smooth muscle relaxation when its metabolism in the red blood cell results in the liberation of nitric oxide (NO). NO then activates the enzyme guanylyl cyclase. This activation results in the formation of increased intracellular levels of cyclic guanosine monophosphate (cGMP). The result is vasodilation.

1.1 Metabolism

Five molecules of cyanide (CN^-) are released when SNP is metabolized in the red blood cell. The major metabolic pathway for CN^- is conversion to thiocyanate (SCN). This conversion occurs enzymatically via two sulfur transferase systems: 1) rhodenase (the primary pathway) and 2) beta-mercaptopyruvate-cyanide sulfurtransferase. Rhodenase is ubiquitous throughout the body, but it is highly concentrated in the liver. Rhodenase catalyzes the transfer of sulfur from a sulfur donor molecule such as thiosulfate (Na_2SO_3) to cyanide and thereby the formation of thiocyanate (SCN). SCN is subsequently eliminated in the urine and can therefore serve as a marker of cyanide exposure.

The ability of rhodenase to catalyze the conversion of cyanide to thiocyanate (SCN) is limited by the availability of sulfur donors in the body. Thus the provision of exogenous sulfur donors such as thiosulfate (Na_2SO_3) in the setting of acute cyanide intoxication is a potentially life-saving intervention (Pasch et al, 1983, Cole and Vesey, 1987).

One out of every five CN^- ions liberated by the metabolism of SNP binds to methemoglobin to form the non-toxic cyanomethemoglobin. The creation of additional quantities of methemoglobin by the intravenous infusion of sodium nitrite can thus provide additional CN^- buffering capacity. The resultant methemoglobinemia can then be treated with the administration of intravenous methylene blue.

Additional metabolic pathways for CN^- include the conversion of hydroxycobalamine (vitamin B12a) to cyanocobalamine, and conversion to 2-aminothiazoline 4-carboxylic acid.

If the above three pathways (rhodenase, methemoglobin, hydroxycobolamine) are overwhelmed, cyanide will bind to mitochondrial cytochrome oxidases and poison cellular oxidative phosphorylation. Cellular hypoxia is induced when cyanide inhibits the electron transport chain at cytochrome a_3. Oxygen cannot be utilized, mixed venous

oxygen tension rises and the generation of high-energy adenosine triphosphate (ATP) is blocked. The cell reverts from aerobic to anaerobic metabolism, with the subsequent generation of pyruvate and lactate. Acidosis ensues, and with it, deterioration in the organ systems most dependent on oxidative metabolism: the central nervous system and heart.

Clinical manifestations of cyanide toxicity to the central nervous system include headache, anxiety, agitation, confusion, lethargy, convulsions and coma. Cardiovascular manifestations include progressive heart failure with both loss of contractile force (negative inotropy) and slowing of rate (negative chronotropy). Bradycardia and hypotension are commonly observed pre-morbid events associated with cyanide toxicity.

In patients receiving SNP, the earliest, most sensitive signs of cyanide toxicity are acidosis, elevated mixed venous oxygen tension, and rising blood lactate levels. Venous blood that appears "bright" red due to the inability of the tissues to extract oxygen should suggest cyanide toxicity. Arterial and mixed venous blood gas analysis with co-oximetry can help confirm the diagnosis.

1.2 Previous Studies

SNP was first discovered in 1850. Its hypotensive effects were noticed in 1929, and its first therapeutic use was reported by Page et al. in 1955. Moraca et al. first described the clinical use of SNP for deliberate hypotension during surgical procedures in 1962. Since then, it has been widely used to control blood pressure in infants and children in the perioperative period.

Despite its widespread use, there is a paucity of information on its safety, efficacy, and pharmacokinetic/pharmacodynamic relationships in children. Davies et al (1975) and Bennett and Abbott (1977) described their retrospective experience with SNP used to induce deliberate hypotension in small cohorts of children. Both authors observed that younger patients required more SNP than older ones to achieve comparable degrees of

blood pressure control. In their small retrospective cohort, Bennett and Abbott recommended that doses of 10 micrograms/kilogram/minute were necessary to achieve satisfactory blood pressure response. Davies et al described three possible responses to SNP administration in children: 1) a constant response to "conventional" doses < 3 mg/kg; 2) a tachyphylactic response characterized by continuously escalating dose requirement (> 3 mg/kg) to achieve a satisfactory blood pressure; and 3) resistance to the blood pressure lowering effects of the drug. They cautioned against using total doses that exceeded 3 mg/kg or continuing administration of SNP in the latter two scenarios. Firm conclusions cannot be drawn because these small case series were not randomized controlled trials with specific pharmacodynamic endpoints.

Yaster et al (1986) compared SNP to nitroglycerin (NTG) for inducing hypotension in a group of 14 adolescents. They found doses of SNP between 6-8 micrograms/kg/minute superior to NTG at any dose in the reliable induction of hypotension for children and adolescents undergoing scoliosis, craniofacial or hepatic surgery.

Hersey et al (1997) performed a randomized trial comparing SNP to the dihydropyridine calcium channel antagonist nicardipine in 20 healthy adolescents with idiopathic scoliosis undergoing spinal fusion. Target blood pressures were easily obtainable in both groups and operating conditions were comparable. The time to restoration of baseline blood pressure after termination of the infusion was significantly longer in the nicardipine group. Interestingly, blood loss was significantly greater in the SNP group. Details on SNP dose requirements were not provided.

Przybylo et al (1995) described CN^- and SCN blood levels in ten children who received SNP at doses up to 10 micrograms/kg/min (mean infusion rate 6 microgram/kg/min) while undergoing cardiopulmonary bypass for repair of complex congenital cardiac defects. CN^- levels rose as a function of time while SNP was infused, and rapidly fell when SNP was discontinued. Despite the fact that some children demonstrated serum CN^- levels above the generally accepted threshold of 0.5 micrograms/ml, no patient

developed clinically apparent toxicity. Kazim et al (1996) questioned the validity of the results of this study because of the CN^- assay methods utilized.

Linakis et al (1991) retrospectively examined physician-ordering practice as it pertained to blood cyanide levels in children receiving SNP. They sought to determine how the laboratory determinations were used to monitor patients and if there was clinically apparent toxicity in children found to have cyanide concentrations exceeding the "normal" limit of 500 micrograms/liter. They found poor correlation between blood cyanide concentration and dose or duration of therapy in patients whose cyanide levels were "toxic." Thiocyanate determinations were normal and no child manifested signs or symptoms of cyanide toxicity. They concluded that further pediatric studies were needed.

2.0 Study Objectives

We propose a multicenter trial that will provide guidance for the use of SNP to reduce blood pressure in pediatric patients. The trial is a randomized, double-blinded withdrawal to placebo trial. The aims of the trial are:

1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours
2. To assess the potential for rebound hypertension during the 30-minute Blinded Phase following administration of sodium nitroprusside for 12 hours or more.

To meet these study aims, the following study phases, defined in Section 3.1.1, will have the following objectives:

- Open-Label Study Drug Administration (Dose-Titration) Phase
  The objective during this phase of the study is to determine the effectiveness and safety of SNP for controlling blood pressure during stable infusion lasting at least 12 hours.

- Blinded Study Drug Administration Phase

  The primary endpoint for the study will be determined during this phase of the study. The primary endpoint is the change in MAP recorded during the Blinded Study Drug Administration Phase in the absence of other stimuli. The primary objective is to determine the persistence of sodium nitroprusside versus placebo for reducing blood pressure in pediatric patients

  The secondary objectives during this phase of the study are as follows:

  1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience offset during the 30-minute blinded study drug period.
  2. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience rebound hypertension during the 30-minute blinded study drug period.
  3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the 30-minute blinded study drug period.
  4. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the 30-minute blinded study drug period.
  5. To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.

- Follow-up Phase

The following objectives to be evaluated during this phase of the study are as follows:

1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the Follow-up Period.
2. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the Follow-up Period.
3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience changes in individual physical examination parameters represented as either normal or abnormal from the Pre-Study Period to the end of the Follow-up Period.
4. To compare the changes (values recorded during the end of the Follow-up Period minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.
5. To compare the changes (values obtained during the two-hour period immediately following the stop of blinded study drug minus values obtained during the Pre-Study Drug Period) in individual laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo.

3.0 Investigational Plan

3.1 Overall Study Design and Plan: Description

This is a phase II, multicenter, randomized, double-blind placebo-controlled, parallel group study to determine the persistence of the effect of SNP on blood pressure and to assess the potential for rebound hypertension associated with prolonged infusion in pediatric subjects.

Target MAP is defined as the clinically appropriate MAP as determined by the investigator taking into account the clinical presentation and medical needs of the subject. The investigator may change the target MAP at his/her discretion based on clinical needs during the course of the study.

The initial baseline MAP (B1) is defined as the blood pressure measurement taken just prior to the initiation of SNP administration, either institutionally-supplied or open-label study drug after at least a 5-minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). See Figure 1 below.

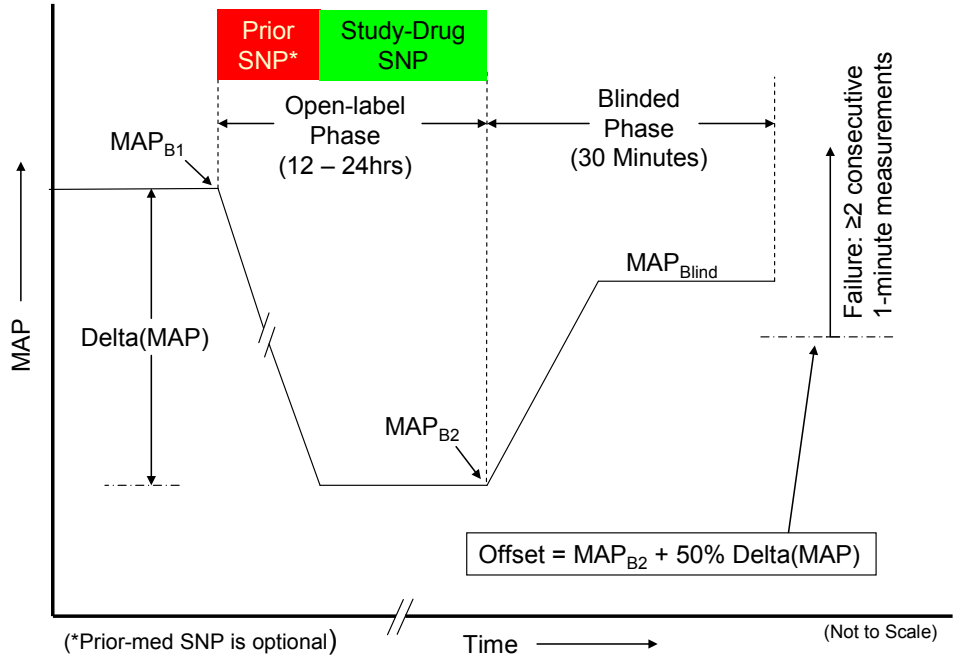
The subsequent baseline MAP (B2) is defined as the blood pressure measurement taken just prior to the initiation of blinded study drug after at least a 5 minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). Prior to establishing B2, there shall have been no changes in the SNP infusion rate for a period of at least 20 minutes.

Delta(MAP) is defined as the difference between MAP_{B1} and MAP_{B2}.

Offset is defined as MAP_{B2} plus 50% Delta(MAP)

Figure 1

Study Treatment Phases with MAPs and Delta(MAP)



- MAP_{B1} = MAP immediately prior to start of Open-label Study Drug Administration Phase
- MAP_{B2} = MAP immediately prior to start of Blinded Study Drug Administration Phase
- Delta(MAP) = $MAP_{B1} – MAP_{B2}$
- Offset = MAP_{B2} + 50% Delta(MAP)
- Treatment Failure: During the blinded phase, ≥2 consecutive 1-minute MAP measurements greater than Offset [MAP_{B2} + 50% Delta(MAP)]

Example: Subject's MAP immediately prior to start of the Open-label Study Drug Administration Phase is 100 mmHg (MAP_{B1}). At the end of the Open-label Study Drug Administration Phase, MAP is 60 mmHg (MAP_{B2}). Thus, Delta(MAP) = 40 mmHg. For *treatment success*, MAP during the Blinded Study Drug Administration Phase cannot exceed MAP_{B2} + 50% Delta(MAP), or 80 mmHg, for any 2 consecutive MAP_{Blind} measurements, obtained at 1-minute intervals.

Approximately 15 centers will participate in subject recruitment to complete the study.

Approximately sixty (60) patients who require long term (at least 12 hours) blood pressure control will be enrolled. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment.

Any patient who starts the blinded study drug administration period will be considered complete for analysis. Enrolled subjects will be randomized in equal proportions to receive either placebo or SNP for the duration of the blind-treatment period, which will immediately follow the open-label infusion of SNP.

3.1.1 Definition of Study Periods

Study periods are as follows:

- Pre-study drug administration: a period of up to 7 days preceding the start of study drug administration during which informed parental permission, and other enrollment procedures takes place.
- Open-label study drug administration (Dose-Titration): The period of open label study drug administration will be at least 12 hours but not greater than 24 hours. This period will begin at the start of SNP administration, either study drug or institutionally-supplied. Randomization will normally occur during this period.
- Blinded study drug administration: The period beginning with the start of blinded study drug administration and ending with the discontinuation of blinded study drug. It immediately follows the open-label period and will be no longer in duration than 30 minutes.
- Follow up: The period immediately following blinded study drug administration and ending 30 days after completion of study drug administration. AEs will be followed for 24 hours after termination of study drug. SAEs will be followed for 30 days.

Safety will be assessed via the evaluation of adverse events, pre- and post-treatment laboratory results, and vital sign data. The efficacy endpoints will mainly be assessed by examining blood pressure parameters.

3.2 Selection of Study Population

Children up to 17 years of age who require pharmacologic blood pressure control for at least 12 hours will be eligible for study. Blood pressure control is defined as the maintenance of the subject's mean arterial pressure to within 90-110% of a target MAP specified by the physician.

Five pediatric age groups will be enrolled in this trial:

Group A: Neonates from birth to less than 30 days of age
Group B: Infants and toddlers from 30 days to < 2 years
Group C: Preschool children from 2 years - < 6 years
Group D: School age children from 6 yrs - < Tanner stage III
Group E: Adolescents from Tanner stage III - < 17 years.

Tanner III refers to the onset of puberty and occurs at different ages in different individuals. The mean age at onset of Tanner III ranges from 12.4 to 13.1 years in males, and 11.9 to 12.6 years in females. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment (see Appendix A).

3.2.1 Inclusion Criteria

Subjects must meet all of the following criteria:

1. Subject is less than 17 years of age.
2. An in-dwelling arterial line is clinically indicated.
3. Subject's parent or legal guardian is willing and able to give informed parental permission signing and dating an IRB-approved informed parental permission containing all of the elements of informed consent, and subject provides assent, signing an IRB-approved and –required informed assent, if applicable.
4. Subject is anticipated to require a minimum of 20 mm Hg (15 mm Hg for subjects < 2 years old) reduction in MAP for at least 12 hours using SNP.

3.2.2 Exclusion Criteria

Subjects will be excluded from study if any of the following criteria exist:

1. Subject weighs < 3.0 kg.
2. Subject has a known allergy to SNP.
3. Subject has a known mitochondrial cytopathy with a disorder of oxidative phosphorylation or of respiratory chain enzymes.
4. Subject has a contraindication to vasodilator therapy for control of blood pressure during surgery or in the intensive care unit.
5. Subject has raised intracranial pressure.
6. Subject is anticipated to need anti-hypertensive drugs other than study drug either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) during the period of study drug administration. However, patients receiving stable doses of an anti-hypertensive drug(s) prior to the initiation of study drug may be enrolled, provided they will not have received IV vasodilator therapy for greater than 8 hours prior to receiving study drug.

7. Subject has any serious medical condition which, in the opinion of the investigator, is likely to interfere with study procedures.
8. Subject is moribund (death likely to occur within 48 hours).
9. Subject has a positive result for the urine or serum HCG test administered at screening.
10. Subject has participated in other clinical trials for investigational drugs and/or devices within 30 days prior to enrollment

3.2.3 Prior and Concomitant Therapy

Initiation of new anti-hypertensive drugs either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) other than the study drug during study drug administration is prohibited. However, in patients receiving stable doses of non-study anti-hypertensive drugs, these agents may be continued during study-drug administration. If open label study drug is initiated during anesthesia, the anesthetic medications will be recorded on the CRFs but are not considered as anti-hypertensive drugs. All concomitant medications and clinically meaningful, unexpected, and invasive procedures will be recorded for the period beginning 72 hours prior to study drug administration through 24 hours post study drug conclusion. The dates of administration, dosage and reason for use must be included. Concomitant medications will be collected for SAEs occurring within 30 days following study drug administration. Vaccines are considered a concomitant medication.

Subjects may receive institutionally-supplied SNP prior to the initiation of study drug administration; however, administration of institutionally-supplied SNP will be discontinued immediately prior to the initiation of study drug administration, and the initial infusion rate of study-drug SNP will be the same as the discontinued institutionally-supplied SNP (see Section 3.4.3 #6).

3.3 Efficacy and Safety Assessments

3.3.1 Efficacy and Safety Measurements

Table 1 is a schematic representation of study assessments and procedures.

3.3.2 Safety Assessments

Safety assessments will include monitoring the tolerability of the SNP infusion and assessing physical examinations, vital signs, clinical laboratory values, concomitant medications and procedures, and adverse events throughout the study. SAEs will be collected for 30 days following completion of study drug administration .

In cases of discharge from the hospital before 30 days, parent (or guardian) will be contacted to determine if any SAE's occurred following discharge but within 30 days of study drug discontinuation. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. See section 4.2 for specific adverse event parameters and actions to be taken.

3.3.3 Drug Concentration Measurements

Cyanide, thiocyanate, methemoglobin, lactic acid, and arterial blood gas analysis will be performed throughout the trial to indirectly query SNP levels and determine subject safety.

TABLE 1: Schedule of Assessments: Sodium Nitroprusside Long-Term Infusion Study

Procedure	Pre-study Drug Period (Up to 7 days prior to Study Drug administration)	Open-label Period (12 -24 hrs duration)	30 minute Blinded Study Drug Period	Study Drug d/c (Within 2 hours)	Follow-up (Up to 24 hours post blinded study drug)[1]
Assessments					
Review Entry Criteria	X				
Informed Parental Permission/ HIPAA Consent	X				
Collect Demographic Data	X				
Medical History	X				
Physical Examination	X			X	X[7]
Vital Signs (SBP, DBP, MAP, HR) [2]	X[15]	X	X	X	X
Growth Parameters[3]	X				
Urine Output[10]	X	X	X	X	X
Serious Adverse Events/Adverse Events		X	X	X	X[5]
Concomitant Medication/Proceedure[8]	X[9]	X	X	X	X[16]
Randomization of Blinded study drug		X			
Blinded Study Drug Administration			X		
Open-label Study Drug Administration		X			
Laboratory Assessments					
Pregnancy test (post-menarche females)	X[14]				
Electrolytes, BUN, creatinine	X			X	
Hematology (CBC & platelet count)	X			X	
Liver Enzymes (AST, ALT)	X			X	
Arterial Plasma Lactate level	X	Q 8 hours (± 30 min)		X	X[13]
Arterial Blood Gas with Co-oximetry (includes Methemoglobin)[4]	X	Q 8 hours (± 30 min)		X	X[13]
Mixed Venous Blood Gas with Co-oximetry (includes Methemoglobin)[, 12]	X	Q 8 hours (± 30 min)		X	X[13]
Plasma Thiocyanate and cyanide (central lab)	X	Q 8 hours (± 30 min)		X & 12 hr post d/c [11]	X[13]
Urine Thiocyanate (central lab)[6]	X	Q 8 hours (± 30 min)		X	Q8 hrs (± 30 min) X 3

1. End of Study assessment will be done at 24 hours post blinded study drug administration, except where noted
2. Vital sign measurements as described in protocol, sections 3.4.2 – 3.4.7.1. Vital signs will then be collected every 12 ± ½ hours for 24 hours post blinded study drug administration.
3. Growth parameters will include weight, height/ length, and Tanner stage, if ≥6 years old.
4. ABG sampling preferred, sample collected at drug d/c only if line is still in.
5. AEs will be followed for 24 hours & SAE will be followed for 30 days, after the completion of study drug administration.
6. Urine collection details are described in section 3.4.7.2 and the MOP.
7. To be performed 18-30 hours following the termination of study-drug administration
8. Clinically meaningful, unexpected, and invasive procedures only
9. Within 72 hours of study drug administration
10. Measurements to be performed at time of urine thiocyanate sample collection, if feasible

11. Blood for cyanide & thiocyanate at 12 hrs ± 30 min, post study drug d/c, only if arterial line in place
12. Mixed Venous Blood Gas done only if CVC is indwelling
13. Following concomitant SNP d/c, if feasible
14. To be performed within 48 hours of study drug administration
15. Immediately prior to induction if B1 will occur during general anesthesia; otherwise as early as practical
16. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).

3.4 Study Visits and Procedures

3.4.1 Informed Parental Permission

Prior to the start of any study-related procedure, a signed and dated informed parental permission, containing all elements of informed consent and, if applicable, assent must be obtained and documented in the subject's medical record (See Appendix B).

3.4.2 Pre-study drug administration procedures

The following procedures will be completed prior to the administration of study drug:

1) Obtain signed and dated informed parental permission/HIPAA authorization/assent.
2) Collect demographic data and medical/surgical history.
3) Record diagnosis.
4) Perform a pertinent physical examination.
5) Obtain vital sign measurements--immediately prior to induction if B1 will occur during general anesthesia; otherwise, as early as practical.
6) Determine subject height in centimeters and subject weight in kilograms (for calculation of appropriate study drug dose).
7) Collect urine and blood samples for laboratory evaluations as per Table 1. Pregnancy test if required must be done within 48 hours of study drug administration. (If the screening pregnancy test will have been more than 48 hours prior to the start of the study drug administration, then the test will be repeated.)

To minimize the blood volume obtained under this protocol, laboratory evaluations performed prior to the consenting of the patient as part of the standard of care of the patient and within 7 days of the administration of study drug may be substituted for these procedures.

8) Document concomitant medications (including over-the-counter preparations).

3.4.3 Open-Label Study Drug Administration (Dose-Titration) Procedures:

The following procedures should be performed sequentially unless otherwise indicated.

1) Stabilize sedation/analgesia.
2) Insert arterial line if not already in place.
3) Obtain vital sign measurements immediately prior to the start of SNP administration, either institutionally-supplied or open-label study drug. This defines B1.
4) Determine and record target MAP.
5) If the difference between B1 and the target MAP is <20 mmHg (15 mmHg for subjects <2 years old), the patient will be withdrawn from the study and not given study drug.
6) Begin administration of open-label study drug at a dose not to exceed 0.3 mcg/kg/min, or, if applicable, at the infusion rate of the institutionally-supplied SNP.
7) The dose of open-label SNP will be titrated according to the subject's BP response such that the target MAP, chosen by the study physician, is achieved $\pm$10%. If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose. The duration of open-label study drug administration will be at least 12 hours but less than 24 hours.
8) Revise target MAP as clinically indicated; titrate SNP to achieve new target MAP ($\pm$10%).

9) Obtain vital sign measurements every one minute for the first 10 minutes then every 5 ± 1 minutes for an additional 20 minutes after initiation of open-label study drug infusion and after each dosage adjustment. After the initial 30 minutes, once a stable dose is achieved and BP control is satisfactory, vital sign measurements will be obtained every $\leq$ 20 minutes. Additionally, obtain vital sign measurements in a similar manner whenever it is necessary to change the open-label drug infusion rate.
10) Randomize patient
11) Collect blood samples for laboratory evaluations as per Table 1.
12) Record concomitant medications, procedures, and adverse events.
13) Whenever an adverse event occurs, obtain vital sign measurements. If clinically appropriate, a blood sample for safety including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations may be drawn.

3.4.4 Blinded Study Drug Administration Procedures

1) Obtain vital sign measurements immediately prior to the start of blinded study drug administration. This defines MAP_{B2}. There must be 5 min of stable conditions and 20 minutes of no changes in Open-label study drug prior to starting the blinded study drug administration phase.
2) Begin 30-minute blinded study drug administration as described in Section 3.4.8.
3) Record blood pressure and heart rate every one minute for the duration of blinded study drug administration.
4) If blood pressure control is lost (defined as loss of 50% of delta MAP for $\geq$2 consecutive 1-minute measurements) the blinded study drug is discontinued when there is a safety concern or the MAP reaches 120% of MAP_{B1}.
5) Record concomitant medications and procedures and adverse events. Whenever an adverse event occurs, obtain vital sign measurements and, if appropriate, a blood sample for safety analysis including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations.

3.4.5 Study Drug Discontinuation (Within 2 hours of discontinuing study drug)

1) Collect urine and blood samples for laboratory evaluations listed in Table 1
2) Conduct a pertinent physical examination and perform all other assessments listed in Table 1 for this study phase.

3.4.6 Follow up Procedures

The following will be performed after completion of study drug administration through 24 hours post study drug end:

1) If applicable, record the estimated blood loss and fluid intake, including blood and blood products and output during the trial period.
2) Record concomitant medications and clinically meaningful, unexpected, and invasive procedures. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
3) Record vital signs at 12 and 24 ± ½ hours after the end of study drug administration.
4) Collect blood sample at 12 hours (± 30 min) after study drug discontinuation for cyanide and thiocyanate analysis.
5) Perform a pertinent physical examination 18 –30 hours following discontinuation of study drug administration.
6) Collect adverse events for 24 hours following discontinuation of study drug administration.
7) Collect serious adverse events plus associated concomitant medications and clinically meaningful, and invasive procedures for 30 days following discontinuation of study drug administration.

8) Collect blood samples for laboratory evaluations as per Table 1.

   Blood samples for cyanide and thiocyanate analysis at the discontinuation of the non-study drug (concomitant) SNP to be done only if feasible (the informed parental permission form must specify this blood sample will be drawn & only if an indwelling catheter is present). Note: This blood draw may be several days following the discontinuation of study-drug SNP administration."

SAEs and associated concomitant medications and procedures will be collected for 30 days following the discontinuation of study drug administration, either through telephone contacts and/or study visits or spontaneously reported by the subjects.

3.4.7 Methods of Assessment

3.4.7.1 Vital Sign Measurements

Vital signs: systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate will be measured.

The principal method of obtaining blood pressure measurements will be from an intra-arterial catheter inserted in an upper or lower extremity artery. Manual blood pressures from a non-invasive blood pressure cuff will only be used prior to insertion of and during a malfunction of the arterial catheter. The blood pressure transducer is internally calibrated by the instrument upon performing the zeroing procedure. All blood pressure and heart rate data will be acquired electronically when possible.

3.4.7.2 Blood Draws and Urine Samples

Blood drawn for study related purposes will not exceed the maximum amounts specified by the American Association of Blood Banks for healthy infants, children, and

adolescents. Normally, this value is 7 ml/kg over an eight week period. This study is an inpatient trial of short duration, therefore, the amount of blood withdrawn for study related purposes will take into account the patient's pre-existing hemoglobin and hematocrit, and local Institutional Review Board limitations on maximum allowable blood draws for study-related purposes. A reasonable and conservative value is 3 ml/kg.

Urine will be sampled for thiocyanate concentrations every 8 hours, or fraction thereof, commencing with initial study drug administration to the end of study drug completion and then Q 8 hours times 3 after the discontinuation of study-drug administration, or until the urinary catheter is removed, whichever occurs first. All urine samples will be from a pooled urine collection from the 8-hour time period or fraction of 8-hour time period. The sample will be stored cold until shipment to the central lab. Details of urine collection procedures are described in the Manual of Procedures (MOP).

3.4.8 Dispensing of Study Drug

Study drug will be dispensed to the sites in 2 ml vials containing 25 mg/ml of SNP. The pharmacist will dispense two preparations of study drug, one for the open-label study drug period and one for the blinded study drug period.

The open-label study drug administration phase will utilize a fixed study drug concentration and variable infusion rate scheme. Syringes or bags will be prepared by the investigational drug pharmacy by adding 25 mg of SNP to 50 ml 5% dextrose. The syringe will have a label indicating the concentration of the solution (0.5 mg/ml SNP), and the infusion rate necessary to provide 1.0 microgram per kilogram per minute (1.0 mcg/kg/min) —for example, for a 25-kg subject, an infusion rate of 3.0 ml/hr will deliver 1.0 mcg/kg/min SNP. The infusion rate of 0.5 mg/ml SNP can be calculated using the conversion factor: Wt (kg) X 0.12 ml/kg/hr = 1.0 mcg/kg/min. Clinicians can then make the necessary dosage adjustment for adequate blood pressure control. All dosage adjustments will be captured on the case report forms (CRFs).

The pharmacist will supply blinded study drug for each subject according to a randomization assignment generated by the IVRS. The blinded study drug will be prepared by the pharmacist such that either the concentration of drug is the same as in the open label period or placebo, see Section 3.6.1. Subjects will receive blinded study drug at the same rate of infusion that was used at the conclusion of the initial open-label study drug administration period.

Syringes or bags will be wrapped in opaque or amber plastic to protect from light.

3.4.9 Delivery of Study Drug

Infusion pumps capable of reliable delivery at low infusion rates (to 0.1 ml/hr) will be used. All pumps will have free flow protection and will be internally calibrated for accuracy by the manufacturer. Accuracy will be verified at each site by the biomedical engineering department as part of their equipment management program. Quality assurance checks will be performed periodically according to manufacturer specifications.

Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. Microbore low compliance tubing, with volumes of approximately 1 mL will be used, where possible.

Study drug will be infused via a dedicated peripheral intravenous catheter or via a dedicated lumen of a multi-orifice central venous catheter. Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. The carrier flow rate will be 5.0 mL/hr.

3.5 Removal of Subjects from Therapy or Assessment

3.5.1 Early Discontinuation of Study Drug and Subject Withdrawal

If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose.

If the subject withdraws participation in the study for any reason, every effort will be made to collect safety data, vital sign measurements, samples for safety and laboratory analyses. The date, time and reason for discontinuation must be recorded on the case report form (CRF). Additionally, every attempt should be made to complete all other study related procedures on discontinued subjects who have received any amount of study drug as the data will be included in the safety and intention to treat analyses. Subjects who prematurely discontinue from the study will not be replaced.

Any subject who does not start the blinded study drug administration period will be considered prematurely withdrawn from the study. Any patient who starts the blinded study drug administration period will be considered complete for analysis.

Potential reasons for subject withdrawal from the study are as follows:

1) Subject's parent or legal guardian wishes to have the subject withdrawn for any reason;
2) Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the subject's safety;
3) The investigator feels that it is in the subject's best medical interest to be withdrawn.
4) Subject no longer needs blood pressure control.

3.5.2 Data Safety and Monitoring Board

To ensure that the welfare of trial patients receives appropriate consideration, an independent Data and Safety Monitoring Board (DSMB) has been organized by the BPCA-CC on behalf of the NICHD to review relevant safety and/or efficacy data during the course of the trial. The DSMB may recommend discontinuation of the study, or modifications to the study protocol for safety reasons.

The DSMB consists of four core members (Chair, ethicist, statistician, community representative) plus additional ad hoc members for the various medical subspecialties involved in the BPCA protocols.

Each DSMB will have a presenting statistician who will be responsible for presenting the interim data. This member will write the reports and will be one non-voting member of the DSMB. Except as their role in the DSMB, all DSMB members are not participating in the design or conduct of this study, as an investigator or otherwise, and lack any financial conflict that would introduce any bias.

3.5.2.1 DSMB Responsibilities

- Monitoring the safety of trial patients;
- Recommending discontinuation of the trial for safety reasons;
- Recommending changes to the study protocol for safety reasons;
- Providing written reports on an ongoing basis following scheduled and ad hoc meetings that will be archived and may be provided to regulatory agencies.

These responsibilities will be broadened to include decisions regarding efficacy if the trial is an efficacy trial. The DSMB will monitor the safety of trial patients by reviewing the occurrence of adverse events and deaths, on a real-time basis as SAE reports are transmitted. The DSMB may also monitor compliance with the protocol, and factors

affecting patient safety or the integrity of the trial. The DSMB may request any additional data that are not included in the report if deemed necessary for effective monitoring.

If the DSMB finds any major concerns about safety, it may recommend discontinuing the trial or modifying the study protocol. Following each data review, the DSMB will send a written recommendation regarding the trial, (e.g., to continue according to the protocol, or recommendations for specific actions) to the sponsor.

3.6 Investigational Product

3.6.1 Identity of Investigational Product

Sodium nitroprusside (sodium nitropentacyanoferrate (III) dihydrate) is a reddish-brown crystalline powder that is freely soluble in water. Its molecular formula is $Na_2[Fe(CN)_5NO] \bullet 2H_20$. Study drug will be supplied by the BPCA Coordinating Center to the Investigational Drug Service at each clinical center in a standard concentration of 25 mg/ml. The Investigational Drug Service at each clinical center will then prepare the drug in syringes or bags of sterile 5% dextrose for administration to randomized patients according to the guidelines provided above. Sterile 5% dextrose will be utilized as placebo.

3.6.1.1 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature from 15°- 30°C and protected from light in its carton until used. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug must be maintained under adequate security.

3.6.2 Methods of Assigning Subjects to Treatment Groups

After meeting all inclusion and exclusion criteria, subjects will be considered to be enrolled and will be randomly assigned to receive either placebo or active drug treatment groups during the blinded study drug administration phase of the trial using a single centralized randomization schedule. Randomization into the blinded portion of the trial will be performed via a centralized interactive voice response system (IVRS).

The BPCA-CC will provide a system for the pharmacist to obtain each subject's randomized treatment assignment in a timely manner prior to the administration of study drug.

3.6.3 Assigning Subject Numbers

Study participants will be assigned a subject number upon successful enrollment into the study. The subject number will consist of five digits. The first two digits will be the site number of the enrolling institution followed by the number "2"—for the second trial under this IND—followed by a two digit enrollment-sequence number. For example, Subject #10-2-23 would be the 23rd subject enrolled at Site #10.

3.6.4 Blinding

The subject, as well as all caregivers, will remain blinded to the treatment assignment throughout the course of the study. For subjects' safety, the pharmacist will be aware of the treatment group for each subject. The BPCA-CC will maintain the double-blinded randomization schedule.

The randomization for an individual subject may be revealed in an emergency; however, investigators are discouraged against requesting that the blind be broken for

an individual subject. Notification of any unblinding must be sent via facsimile to the BPCA-CC within 24 hours.

3.6.5 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms.

3.6.6 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the Clinical Supply Shipment Request and Verification or similar document. An accurate inventory of study drug will be kept by the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and returned to the BPCA-CC if requested. The investigator agrees not to supply study medication to any persons not enrolled in the study or not named as a sub-investigator for the trial.

4.0 Adverse Events

4.1 Definition

An adverse event is defined as any unintended and unfavorable medical occurrence in a clinical investigation subject, administered a pharmaceutical product, regardless of the causal relationship with treatment. An adverse event can therefore be any untoward sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not the event is considered causally related to the use of the product. Pre-existing conditions that remain stable

throughout the study period will not be considered adverse events. Any worsening of a pre-existing condition or illness is considered an adverse event.

Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional over-dosage, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events if they result in discontinuation from the study, necessitate therapeutic medical intervention, or the investigator considers them to be adverse events.

Adverse events will also include electronically-monitored vitals signs that meet the definitions of section 4.2; however, electronically-captured data excursions, which in the opinion of the investigator represent data artifacts, such as might be produced by the turning of the patient or brief interruption of the electronic circuit of the monitor device(s) or which do not likely reflect an actual untoward event will not be considered to adverse events.

New or worsening physical-exam findings that occur following the initiation of study-drug administration will be considered to be adverse events, without regard to causality.

4.1.1 Serious Adverse Events

A serious adverse event is one that meets the above criteria and also results in one of the following conditions:

1. Death
2. A threat to life
3. Requirement for inpatient hospitalization
4. Prolongation of hospitalization
5. Production of a congenital anomaly or birth defect

6. A persistent or significant disability or incapacity (excluding experiences of minor medical significance such as headache, nausea, vomiting, diarrhea, and accidental injury)
7. The requirement for a medical or surgical intervention in order to prevent a serious outcome.

If an adverse event meets any of the above criteria, it must be reported to the BPCA-CC as a serious adverse event (SAE) within 24 hours of the investigative site's awareness of its occurrence.

4.2 Adverse Event Severity

The criteria for rating adverse events are as follows:

Mild	The adverse event is transient and easily tolerated by the subject.
Moderate	The AE causes subject discomfort and interrupts usual activities.
Severe	The AE causes significant interference with normal activities and may be incapacitating or life threatening.

Safety data are a primary objective of this trial. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. Safety will be evaluated throughout the study and during the follow-up period by evaluating the tolerability of the study drug infusion and by monitoring clinical and laboratory signs of SNP toxicity such as hypotension, tachycardia, bradycardia, acid base status, serum lactate concentration, methemoglobin levels, cyanide levels, and when available, mixed venous oxygen tension.

- Co-oximetric arterial blood gas analysis with methemoglobin determination will be performed Q8. Arterial blood gas monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity.

- Lower than expected methemoglobin concentrations may reflect indirect evidence of cyanide toxicity because cyanide has a high affinity for methemoglobin, combining with it to form the non-toxic molecule cyanomethemoglobin.
- Other laboratory findings suggestive of cyanide toxicity that will be monitored in this trial include serum lactate levels and mixed venous oxygen saturation.

  Lactate levels will rise and mixed venous oxygen tension will increase when cyanide toxicity is occurring due to the reduced ability of the tissues to extract oxygen.
- Plasma and urine thiocyanate levels are indirect markers of cyanide exposure because the majority of the cyanide ions liberated by the metabolism of SNP in the red blood cell are converted to thiocyanate by the rhodenase enzyme system in the liver and excreted in the urine. Free cyanide levels will also be measured.

The adverse event of rebound hypertension shall be considered mild if the MAP rises >10% above the baseline (B1), moderate if the MAP rises >20% above baseline (MAP_{B1}), and severe if the MAP rises >30% above baseline (MAP_{B1}).

The adverse event of excessive hypotension shall be considered mild if the MAP falls >20% below target; fluid therapy may be administered. The adverse event of excessive hypotension shall be considered moderate if the MAP falls >25% below target, fluid therapy is required, and pharmacologic therapy is required. The adverse event of excessive hypotension shall be considered severe if the MAP falls >30% below target, fluid therapy is required, and repeated and/or continuous pharmacologic support is required.

The rating of the adverse event of tachycardia shall be defined for sustained rates exceeding the age adjusted maximums (Table 3) by using the mild, moderate, and severe ranges outlined in Table 4A.

TABLE 3: Age-Adjusted Maximums for Pediatric Heart Rate

Subject Age	Maximum Heart Rate (bpm)
1 month <6 months	180
6 months <3 years	160
3 <8 years	150
8 <17 years	130

TABLE 4A: Severity of Tachycardia (Heart Rate in Beats per Minute)

Age	Mild	Moderate	Severe
Age <6 months	180-199	200-219	220 and higher
6 months - <3 years	160-179	180-199	200 and higher
3 years to < 8 years	150-164	165-179	180 and higher
8 years to <17 years	130-149	150-169	170 and higher

The adverse event of lactic acidosis shall be considered mild if the serum lactate concentration is between 5 and 7.5 mmol/L, moderate if the serum lactate concentration is between 7.6 and 10 mmol/L, and severe if the serum lactate concentration exceeds 10 mmol/L.

The adverse event of cyanide toxicity will be considered mild if the serum cyanide concentration is 0.5 mcg/mL – 1.0 mcg/mL, moderate if the serum cyanide concentration is >1.0 – 1.5 mcg/mL, and severe if the serum cyanide concentration is > 1.5 mcg/mL. Corresponding erythrocyte (vs. serum) cyanide values are ≥5 µmol/L - < 10 µmol/L (mild), 10-20 µmol/L (moderate), and > 20 µmol/L (severe).

Severity ratings for these selected adverse events are displayed in Table 4B.

TABLE 4B: Severity of Selected Adverse Events

Event	Mild	Moderate	Severe
Rebound Hypertension (during blinded phase)	MAP rises >10% above baseline (MAP_{B1})	MAP rises >20% above baseline (MAP_{B1})	MAP rises >30% above baseline (MAP_{B1})
Hypotension	MAP falls >20% below target	MAP falls >25% below target IV therapy required; Pharmacological therapy required	MAP falls >30% below target IV therapy required; Repeated or continuous pharmacological therapy required
Lactic acidosis	5 -7.5 mmol/L	7.6-10 mmol/L	>10 mmol/L
Cyanide toxicity	serum cyanide 0.5 0.5 – 1.0 mcg/mL	serum cyanide >1.0-1.5 mcg/mL	serum cyanide >1.5 mcg/mL
Cyanide toxicity (erythrocyte)	Cyanide $\geq$5 - <10 μmol/L	cyanide $\geq$10–20 μmol/L	Cyanide >20 μmol/L

Frequent monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity. If the base deficit exceeds 8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the patient will be discontinued from study and the SNP infusion terminated. If the lactate level rises by more than 4 mmol/L in a 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output) SNP administration will be discontinued. If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes

percent between arterial and mixed venous blood in the absence of an explainable cause, SNP administration will be discontinued and treatment for suspected cyanide toxicity will be initiated.

Suspected cyanide toxicity will be further assessed and treated as follows:

1) Obtain blood for arterial and venous blood gases with co-oximetry, plasma lactate, and cyanide and thiocyanate levels.

2) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration (see below).

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10ml) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result).

TABLE 5: Dosage Chart for Children

Patient's Hemoglobin g/dL	Initial Dose of Sodium Nitrate (3%) mL/kg IV	Initial Dose of Sodium Thiosulfate mL/kg IV
8	0.22 mL/kg (6.6 mg/kg)	1.10 mL/kg
10	0.27 mL/kg (8.7 mg/kg)	1.35 mL/kg
12	0.33 mL/kg (10.0 mg/kg)	1.65 mL/kg
14	0.39 mL/kg (11.6 mg/kg)	1.95 mL/kg

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals. [Micromedex Poisondex www.micromedex.duhs.duke.edu , see also, Berlin, 1970]

4.3 Relationship to Study Drug

The criteria for determining the relationship of the AE to the study drug are as follows:

1) Probably related: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.
2) Possibly related: An AE that is likely to be related to the administration of the study drug and an alternative cause is equally or less likely when compared to the study drug.
3) Probably not related: An AE that has little or no relationship to the study drug and there exists a more likely, or equally likely, alternative cause.
4) Not related: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.

4.4 Adverse Event Collection Period

SAEs will be monitored and reported from the time the subject receives study drug through 30 days following termination of study drug. Knowledge of adverse events will be gained from direct monitoring of the study subject as well as from clinician observation, and self reporting by the study subject or his/her guardians. Adverse events that have not resolved, or are ongoing, will be monitored to resolution if felt to be related to study drug, or until it is felt that the subject has stabilized.

5.0 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual subject, the investigator or other responsible physician must contact one of the Co-Principal Investigators immediately so that a timely decision can be made as to whether or not the subject should be enrolled or continue in the study. If a deviation is being requested by one of the Co-Principal Investigators, he must contact the other Co-Principal Investigator

for a decision. The deviation from the protocol will be authorized only for that particular subject. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF.

6.0 Statistical Considerations

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a Statistical Analysis Plan, which will be finalized prior to unblinding. Any changes in the statistical methods described in this protocol that occur prior to unblinding will be documented in the Statistical Analysis Plan and will not result in a protocol amendment.

6.1 General Overview

The primary efficacy variable is the intra-patient change in Delta (MAP) during the blinded phase of the study. The primary null hypothesis to be tested is that there is no difference between the active study drug and placebo in the proportion of patients who experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP). Statistical analyses will be performed using two-sided tests. A 0.05 significance level will be used in all tests of treatment differences. Tests for interactions will utilize a 0.10 statistical significance level. Individual secondary endpoints will be evaluated using a hierarchical testing procedure. The Statistical Analysis Plan will include a detailed description of all statistical methods, testing procedures, and methods of data imputation. The Data Monitoring Committee charter will contain the specific details regarding the re-estimation of the target sample size.

Data will be summarized by treatment group with respect to demographic and baseline characteristics, efficacy variables, and safety variables. For parameters measured at baseline, the outcome variables of interest are the changes from baseline (Pre-Study Drug Period). Summary statistics will include the mean, N, standard deviation, median, minimum, and maximum values for continuous variables, and frequencies and

percentages for categorical variables. Prior to summarizing results by study center, or performing analyses that include center as a factor in the analysis, small centers will be pooled. All efficacy variables will be summarized by treatment and by visit. Analyses will be performed to explore whether there are treatment-by-center interactions. If a treatment-by-center interaction is detected, the interaction will be explored in an ad-hoc manner. Categorical variables will be analyzed using Cochran-Mantel-Haenszel (CMH) test blocking on centers. Details of the model and the analyses will be specified in the Statistical Analysis Plan and all statistical analyses will be performed using SAS, Version 8.2 or higher.

6.2 Study Objectives

The study objectives are as defined in Section 2.0 of this protocol.

6.3 Patient Population(s) for Analysis

6.3.1 Efficacy

The intent to treat (ITT) population will contain all patients who were exposed to the study drug during the Open-Label Study Drug Administration (Dose-Titration) Phase.

The Per-Protocol population will contain all patients randomized to the double-blind phase of the trial. The efficacy analysis will be based on the Per-Protocol population. A patient will be classified as a *treatment success* if they meet the following criteria:

• Complete the 30-minute double-blind phase without having an intra-patient increase greater than or equal to 50% Delta (MAP) [i.e., $MAP \geq (MAP_{B1} + MAP_{B2})/2$] and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

A patient will be classified as a *treatment failure* if they meet the following criteria:

• Fail to complete the entire 30-minute double-blind phase without receiving additional treatment to control their blood pressure in addition to the study drug.

• Fail to complete the entire 30-minute double-blind phase for any reason.

• Experience an intra-patient increase greater than or equal to 50% Delta (MAP) for ≥2 consecutive MAP measurements, obtained at one-minute intervals, during the 30-minute double-blind phase.

6.3.2 Safety

All patients who receive any study medication (ITT population) will be included in the safety analyses and summaries, independent of the patient actually reaching the double-blind phase of the study. All non-serious adverse events recorded within 24 hours of either completion of the double-blind phase, or within 24 hours of premature discontinuation of the study, will be reported.

All serious adverse events recorded within 30 days of either completion of the double-blind phase, or premature discontinuation of the study, will be reported.

6.4 Background and Demographic Characteristics

All baseline information, including demographic factors, physical examination parameters, vital signs, growth parameters (if applicable), laboratory and blood gas information will be summarized by treatment group for all enrolled patients (ITT population). Additionally, nonrandomized patients versus randomized patients will be summarized and compared by age, gender, and race to determine if there are any differences among the 2 subsets. Analyses will be conducted to determine differences in the demographic and baseline characteristics of the treatment groups. For continuous variables (e.g., age, weight), the number of non-missing and missing values and the

median, mean, standard deviation, minimum, and maximum will be displayed for each treatment group. For categorical variables (e.g., race, gender), the counts and proportions will be tabulated.

Baseline comparability will be evaluated based on the pooled data from all centers. To determine comparability of the treatment groups at baseline, continuous demographic and clinical variables will be analyzed using an analysis of variance test (with an appropriate transformation, if necessary). Baseline, demographic, and clinical variables that are ordinal will be analyzed using the Cochran Mantel Haenszel test; parameters that are dichotomous will be analyzed using a chi-square ($\chi 2$) test or Fisher's exact test, depending on the individual cell counts. If there are treatment group differences at the 0.10 level of significance in demographic or baseline clinical variables, these variables may be added as stratification variables or covariates to the efficacy analyses.

6.5 Study Medication

The duration of exposure to study medication will be summarized for all enrolled patients, and separately for all randomized patients.

6.6 Concomitant Therapy

Concomitant medications (medications present while on study medication) will be recorded throughout the study and at early termination. These medications will be coded using the WHO drug dictionary. The number of randomized patients using prior or concomitant medications will be categorized by the WHO drug category and preferred term, and presented for each treatment group. In any given category [e.g., drug category] a patient will be counted only once.

6.7 Statistical Design and Models for Analysis

This is a biphasic (open-label dose-titration phase, followed by a randomized phase), randomized, double-blind placebo-controlled study. Patients who are enrolled into the initial phase of the study will have their dose of sodium nitroprusside titrated and must receive a minimum of 12-hours of treatment to be eligible for the randomized phase of the study. Patients who cannot be adequately titrated during the initial 12-hour period will not proceed to the randomization phase of the study. Patients who reach the randomization phase of the study will be assigned to receive placebo, or continue to receive sodium nitroprusside based on a stratified permuted block central randomization scheme.

Five age groups (A through E) will be enrolled in this trial:

Age Group A: Age Group A: Neonates from birth to less than 30 days of age
Age Group B: Infants and toddlers from 30 days to < 2 years
Age Group C: Preschool children from 2 years - < 6 years
Age Group D: School age children from 6 yrs - < Tanner stage III
Age Group E: Adolescents from Tanner stage III - < 17 years.

In order to efficiently account for the effect of SNP on the different age groups, neonates from birth to less than 30 days of age (Age Group A) and infants and toddlers from 30 days to < 2 years (Age Group B) will be pooled for analysis. Based on the planning estimates of the study, patients from these two pooled age groups should represent approximately 25% of the target enrollment (~60 patients).

Preschool children from 2 years - < 6 years (Age Group C) and school age children from 6 yrs - < Tanner stage III (Age Group D) will also be pooled for analysis; patients from these two pooled age groups should also represent approximately 25% of the target enrollment (~60 patients).

In order to accurately determine the target number of patients required for enrollment, a Data Monitoring Committee will examine the results from the Blinded Study Drug Administration Phase after the following number of patients have been enrolled and randomized:

- The initial 12 patients in Age Groups A & B
- The initial 12 patients in Age Groups C & D
- The initial 16 patients in Age Group E

The specific objective of the Data Monitoring Committee is to determine the target sample size based on the observed magnitude of the effect, expressed as a proportion (*treatment success* (sodium nitroprusside) / Randomized to receive sodium nitroprusside vs. *treatment success* (Placebo) / Randomized to receive Placebo. Re-estimation of the sample size will be based on conditional power after 67% (40/60) of the target sample size has been enrolled.

6.7.1 Primary Efficacy Analysis

The primary efficacy analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} = \pi_{\text{ Patients randomized to receive placebo}}$
- H_A: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} \neq \pi_{\text{ Patients randomized to receive placebo}}$

where

- $\pi_{\text{Patients randomized to receive SNP}}$ = Proportion of *treatment successes* (sodium nitroprusside)
- $\pi_{\text{Patients randomized to receive placebo}}$ = Proportion of *treatment successes* (placebo)

6.7.2 Primary Safety Analysis

The primary safety analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} = \pi_{\text{ Patients randomized to receive placebo}}$
- H_A: $\pi_{\text{Patients randomized to receive sodium nitroprusside}} \neq \pi_{\text{ Patients randomized to receive placebo}}$

where

- $\pi_{\text{Patients randomized to receive sodium nitroprusside}}$ = Proportion of patients randomized to receive SNP who experience a serious adverse event

- $\pi_{\text{Patients randomized to receive placebo}}$ = Proportion of patients randomized to receive placebo who experience a serious adverse event

6.7.3 Interim Monitoring Based on Conditional Power

The interim assessment for sample size adjustment will be predicated on the primary efficacy endpoint and conducted using a Conditional Power (CP) approach as described by Chen (2004). The Data Monitoring Committee will conduct the analysis to determine if a sample size adjustment is required. This assessment will be conducted after 67% of the patients from the original target sample size have either completed or withdrawn from the study. The instructions to the Data Monitoring Committee for the sample size adjustment will be described in detail in the Data Monitoring Committee Charter. An alpha level adjustment will not be necessary for the procedure described below, based on the procedure proposed by Chen, DeMets and Lan (2004).

In order for the Data Monitoring Committee to calculate conditional power using the observed data, the treatment assignment codes will need to be provided to the Data Monitoring Committee statistician. The intra-patient MAP_{B2} values will be required, including listings of the intra-patient post-baseline values during the blinded phase of the trial. If an increase in the sample size is required, and the re-estimation is within the defined sample size limits pre-specified for the study, the Data Monitoring Committee will communicate the revised target sample size to the IVRS vender for the trial. Enrollment will continue towards the new target sample size, and the data will remain blinded to all involved parties with the exception of the Data Monitoring Committee. Additionally, the Data Monitoring Committee will not have any direct contact with the study Sponsor, or the clinical investigators.

The following steps will be used to evaluate the sample size after the initial randomized patients from each pooled age group (either 12 or 16) have been enrolled and have completed the Blinded Study Drug Administration Phase, or withdrawn prematurely.

Compute conditional power for the primary hypothesis, using data from the initial patients randomized from each pooled age group (either 12 or 16).

If the conditional power is ≥ 0.5, compute the sample size necessary to increase conditional power to 0.8.

The Data Monitoring Committee will compare the re-estimated sample size calculated using the observed data, relative to the initial estimates for the study. Based on this evidence, the Data Monitoring Committee will proceed with the following action:

If the conditional power is ≥ 0.5, the sample size will be increased up to the maximum sample size pre-specified for the study.

If the conditional power is < 0.5, an analysis will be performed based on the predictive probability of achieving the endpoint within the maximum target sample size allocated for the study.

6.7.4 Sample Size Estimation

The overall sample size was calculated based on performing an un-stratified analysis of the proportion of patients classified as a *treatment success* between the 2 randomized treatment groups. With a balance randomization (1:1, SNP:Placebo), a difference in the proportion of *treatment successes* ranging from 34% to 40% would have 80% power to reject the null hypothesis in favor of the alternative (ref. Sample Size Table No. 1.0).

Sample Size Table No. 1.0

Two group χ^2 Test of Equal Proportions

Scenario	**1**	**2**	**3**	**4**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
SNP, π_1	0.080	0.160	0.240	0.320
Placebo, π_2	0.420	0.530	0.630	0.710
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	8.328	5.920	5.392	5.203
Power (%)	80	80	81	80
n per group	30	30	30	30

Based on the target sample size and the distribution of enrollment relative to the pooled analysis groups, approximately 30 patients from Age Group E (Adolescents from Tanner stage III - < 17 years) are scheduled to be randomized. However, based on the difference in the proportion of patients who are classified as a *treatment success* between the

randomized treatment groups, 30 patients may not be required to detect a significant difference at the alpha = 0.05 level. For this reason, the proportion of patients classified as *treatment successes* will be compared after the initial 12 or 16 patients are randomized, depending on the pooled age group. If the difference in proportions has an odds ratio >12, then less than the 30 patients would be required to be randomized from Age Group E. Under this scenario, the randomization of patients from this specific age group would be stopped. Drawing from Age Groups A & B or Age Groups C & D, if the difference in the proportion of *treatment successes* between the randomized treatment groups requires more than 12 patients, the study could still meet its intended goal within the pre-defined total sample size of 60 patients by enrolling the minimum sample size, based on the conditional power calculated at the interim evaluation. Power estimates at the interim evaluation for 16 patients are presented in Sample Size Table No. 2.0. The re-estimated sample size is presented in Sample Size Table No. 3.0, again for 16 patients.

Sample Size Table No. 2.0

Two group χ^2 Test of Equal Proportions

Scenario	**5**	**6**	**7**	**8**	**9**	**10**
Test significance level, α	0.050	0.050	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2	2	2
Group 1 proportion, π_1	0.125	0.125	0.125	0.125	0.125	0.125
Group 2 proportion, π_2	0.250	0.375	0.500	0.625	0.750	0.875
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	2.333	4.200	7.000	11.667	21.000	49.000
Power (%)	9	20	35	54	76	94
n per group	8	8	8	8	8	8

Sample Size Table No. 3.0

Two group χ^2 Test of Equal Proportions

Scenario	**11**	**12**	**13**	**14**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
Group 1 proportion, π_1	0.125	0.125	0.125	0.125
Group 2 proportion, π_2	0.500	0.625	0.750	0.875
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	7.000	11.667	21.000	49.000
Power (%)	80	80	80	80
N per group	23	14	9	6

6.7.5 Strategy for the Statistical Analysis

The primary method for analysis will be a comparison of the proportion of *treatment success* between patients randomized to receive placebo compared to patients randomized to remain on sodium nitroprusside. Additional analysis will be described in the Statistical Analysis Plan that will include a comparison of the event time distribution functions for the time until an increase in MAP_{B2} + 50% Delta (MAP) is initially observed. During the Open-Label Study Drug Administration (Dose-Titration) Phase, the sustainability of the blood pressure will be graphed over time to determine the effectiveness of SNP to maintain the target MAP.

6.7.6 Handling Missing Data in the Analyses

The following method of imputation will be used:

Last observation carried forward (LOCF): The goal of this imputation scheme is to create an observation for a completely missing observation at the end of the study for every patient in the ITT population. If a patient evaluation for a post-baseline observation is missing, then the immediately preceding non-missing evaluation will be used.

Specific algorithms for imputing missing or partially missing dates will be discussed in the SAP. Imputed or derived data will be identified in the individual patient data listings. Imputed data will not be incorporated into the case report form datasets. Imputed data will be used in the preparation of the derived datasets.

6.7.7 Pooling of Small Sites for Analysis

Small sites (i.e., sites that have less than 4 patients per treatment arm) will be identified and the following method will be used for combining the data. Data from all small sites (< 4 patients) will be combined to form a single site in order to obviate non-estimable situations (i.e., at least 2 intra-group observations are needed to estimate variance) in the evaluation of site and site interaction effects. Once combined, the pooled site will remain as such for all analyses for which a site interaction effect is determined. If the pooled smaller sites represent a single site that has more than twice as many patients as the largest single site, however less than 3 times as many patients, the small sites will be ranked by size and divided into 2 pooled groups using an alternating sequence (ABABAB). If the pooled smaller sites represent a site that has more than three times as many patients as the largest single site, however less than 4 times as many patients, the small sites will be ranked by size and divided into 3 pooled groups using an alternating sequence (ABCABCABC). This methodology will be applied, based on the initial pooling of the smaller sites.

6.7.8 Dropouts, Protocol Violations, and Exclusions

Randomized patients who fail to complete the study will not be replaced. All protocol violations will be documented and categorized in the final study report.

The rate of attrition will be evaluated by the Data Monitoring Committee during the interim evaluation to re-estimate sample size. The reasons for withdraw will be classified into 3 mutually-exclusive classes:

- Withdraw due to tolerability of the study drug
- Withdraw due to lack of treatment effect
- Withdraw not due to tolerability or lack of treatment effect

The proportion of patients who withdraw prematurely will be compared between treatment groups to determine if there is a disproportionate rate of attrition. If the rates differ by a pre-specified amount, the reasons for withdraw will be examined to determine causation. The specific monitoring rules, boundaries, and actions will be described in detail in the Data Monitoring Committee Charter.

6.8 Safety Evaluation

The primary assessment of safety will be based on the frequency of treatment-emergent adverse events and on the frequency of clinically notable abnormal vital signs and laboratory values. The primary safety analysis will be based on a comparison of the proportion of patients receiving sodium nitroprusside vs. placebo who experience a serious adverse event during the Blinded Study Drug Administration Phase.

6.8.1 Adverse Events and Medical Conditions

Adverse events will be recorded throughout the study and at early termination. Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Treatment-emergent adverse events will be defined as those events, which are newly occurring or worsening from baseline. In all cases only treatment emergent adverse events will be summarized. Treatment-emergent adverse events will be summarized by presenting, for each treatment group, the number and percentage of patients having any treatment-emergent AE, having an AE in each body system, and having each individual AE. (Note: In any given category [e.g. body system] a patient will only be counted once.) Similar displays will be provided for prior (conditions ending prior to the first exposure to sodium nitroprusside) and current (conditions present while on study medication) medical conditions. Adverse events will further be categorized by severity, relationship to study medication, and action taken. Other information collected will be listed, as appropriate. Any event starting more than 3 days after the final dose of study medication will be excluded from the above tables and only presented in a listing, unless the adverse event was serious or caused discontinuation from the study.

6.8.2 Clinical Laboratory Results

6.8.2.1 Overview

The primary presentation of the results by individual laboratory parameter will focus on the intra-patient changes from baseline (Pre-study Period). The presentation and analysis of laboratory data will be based on the observed data. All patients who have a baseline and at least one follow-up laboratory assessment will be included in the presentation of the clinical laboratory data. For each clinical laboratory test, there will be three sets of descriptive statistics that summarize the results at baseline, post-baseline assessment, and the change from baseline to post-baseline assessment. Descriptive statistics include N,

mean, standard deviation, median, and the minimum and maximum values. Within treatment group changes will be analyzed using a paired-difference t-test. Between treatment group differences will be compared using a one-factor analysis of variance test.

Shifts from baseline to each pre-specified post-baseline endpoint will also be summarized based on the laboratory categorization (*abnormally and clinically significant*, *abnormal but not clinically significant*, or *normal*) using the worst reported post-baseline observation that occurs within the pre-specified interval. The proportion of patients will be compared using a 2-tailed Fisher's exact test, pooling *abnormally and clinically significant* with *abnormally but not clinically significant.*

In the case that more than one laboratory is used, laboratory values will be transformed for mean change summaries to the same units and normal range as were provided by the central laboratory used in the study, using the formula:

$$y = (x - Li)\frac{Uc - Lc}{Ui - Li} + Lc$$

where x = original value, Li and Ui = lower and upper limits of normal for individual laboratory, Lc and Uc = lower and upper limit for central laboratory

In cases where the lower limit of central laboratory is 0, values that are below the lower limit of normal for a laboratory value prior to transformation will be assigned a value of 0.

6.8.3 Vital Signs

6.8.3.1 Overview

Vital signs of particular interested (blood pressure, MAP, heart rate) will be assessed during each phase of the study.

6.8.3.2 Presentation of Results

Descriptive statistics (n, mean, SD, median, minimum and maximum values) will be used to summarize systolic and diastolic blood pressure, MAP, and heart rate and compared between the randomized treatment groups using a one-factor analysis of variance test.

6.8.4 Physical Examination

6.8.4.1 Overview

The presentation of physical examination data is based on the dichotomous classification (normal or abnormal) of each of the 9 regions or body systems (General Appearance, HEENT, Cardiovascular, Respiratory, Abdomen, Extremities, Neurological, Hair and Skin, and Genitourinary). In addition to these 9 specific body systems, any other region recorded by the investigator under "other" will also be summarized and reported.

6.8.4.2 Presentation of Results

Results will be presented by treatment assignment using counts and percentages. Shift tables will be prepared containing the count and percentage of patients who transitioned from normal at baseline to abnormal at the end of the study. The number and percentage of patients that did not change (normal at baseline and normal at the end of the study, abnormal at baseline and abnormal at the end of the study) are also presented to frame the 2*2 contingency table. Shifts from baseline to each pre-specified post-baseline endpoint will be summarized using the worst reported post-baseline observation that occurred within the pre-specified interval. The count of the disagreements (normal to abnormal and abnormal to normal) by treatment assignment (active and placebo) will be compared for each parameter using McNemar's test.

7.0 Ethics

7.1 Independent Ethics Committee or Institutional Review Board

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator Brochure (IB), the informed parental permission and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an Independent Ethics Committee (IEC) or Institutional Review Board (IRB). IEC/IRB approval of the protocol, informed parental permission and subject information and/or advertising as relevant will be obtained prior to the authorization of drug shipment to a study site. Any amendments to the protocol will require IEC/IRB approval prior to implementation of any changes made to the study design.

7.2 Ethical Conduct of Study

This study will be conducted in accordance with the protocol, GCP, FDA regulations governing clinical study conduct, ethical principles that have their origin in the Declaration of Helsinki, 2000 revision (see Appendix E) and all applicable local regulations. The investigator must assure that the study is conducted in accordance with the provisions as stated in the FDA regulations and complies with prevailing local laws and customs. Responsibilities of the Investigator are specified in Appendix D.

7.3 Subject Information and Parental Permissionn

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before protocol-specified procedures are carried out. Informed consent will be obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements.

Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation.

Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study.

The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed permission form, which will included all elements of informed consent as described in 21 CFR 50.25. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form and a copy of the signed assent (if applicable).

The parental/guardian permission form generated by the investigator with the assistance of BPCA-CC must be approved (along with the protocol) by the IRB and be acceptable to the Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative.

The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the ICH Guidelines and will comply with local regulations. This form may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated.

Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by BPCA-CC, Regulatory Authorities, and NICHD or its designees.

8.0 Source Documents and CRF Completion

8.1 Source Documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

8.2 Case Report Forms

Data for individual subjects will be recorded on CRFs provided to the BPCA-CC. All entries must be complete. A case report form must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the CRF by the investigator. The principal investigators must review and approve each CRF.

Case report forms must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested during an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

Because of the potential for errors and inaccuracies in entering data into CRFs, laboratory and other test results must be kept on file with the subject's study dossier. Case report

forms and copies of test results must be available at all times for inspection by the CRA for the site and the FDA.

9.0 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA-CC, the investigators and their study coordinators and the CRAs for the study. This meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures by a CRA at a study initiation visit and will be given an CRF completion workbook for reference.

The CRAs will monitor each site throughout the study. At each visit, 100% source document review will be made against entries on the CRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and all applicable regulations.

After completion of the entry process, computer logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary correction will be made to the database and documented via addenda or audit trail. A manual review of selected line listings will also be performed at the end of the study.

10.0 Use of Information and Publication

10.1 Use of Information

This trial is sponsored by the NICHD. The NICHD endorses the sharing of final research data to expedite the translation of research results into new scientific knowledge in order to improve human health.

This contract is part of a collaborative program involving multiple sites. A data sharing dissemination plan will be developed jointly with the BPCA-CC, the NICHD, and the collaborating institutions following announcement of the award.

10.2 Publication

The BPCA-CC and steering committee for this study shall have the right to review and approve any paper for publication, including oral presentation and abstracts, which utilize data generated from this study. At least 90 days before any such paper or abstract is presented or submitted for publication, a complete copy shall be given to the BPA-CC for review by the steering committee. Any individual investigator agrees to defer publication of any such paper or abstract until the BPCA-CC and Steering Committee have reviewed and approved it.

11.0 Completion of Study

The investigator will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon in writing by the investigator, the NICHD, the BPCA-CC and the PODS. The investigator will provide a summary of the study's outcome to the IRB/IEC following the conclusion of the study.

The PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. The investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the NICHD and BPCA-CC within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

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12.0 Investigator Agreement

I have received and reviewed the investigator brochure for sodium nitroprusside (SNP).

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulation.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Signature of Principal Investigator Date

Name of Principal Investigator (printed or typed)

Appendices

Appendix A: Tanner Stages of Sexual Maturity

	Pubic Hair		Breasts	Penis	Testes
SMR Stage[1,2]	Boys	Girls	Girls	Boys	Boys
1	None	Preadolescent	Preadolescent	Preadolescent	Preadolescent
2	Scanty, long, slightly Pigmented	Sparse, lightly pigmented, straight, medial border of labia	Breast and papilla elevated as small mound; areolar diameter increased	Slight Enlargement	Enlarged scrotum, pink texture altered
3	Darker, starts to curl, small Amount	Darker, beginning to curl, increased amount	Breast and areola enlarged, no contour separation ,	Longer	Larger
4	Resembles adult type, but less in quantity; coarse, cu rly	Coarse, curly, abundant but amount less than in adult	Areola and papilla form secondary mound	Larger; glans and breadth increase in size	Larger, scrotum dark
5	Adult distribution, spread to medial surfaces of thighs	Adult feminine triangle, spread to medial surface of thighs	Mature; nipple projects, areola part of general breast contour	Adult size	Adult size

1. Adapted from Tanner, JM: Growth at Adolescence, 2 ed. Oxford, Blackwell Scientific Publications, 1962.
2. MR = Sexual Maturity

Appendix B: Parental Permission Form with HIPAA

Protocol Title: A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects

Protocol Director: [PROGRAM PI]
IRB Approval Date: _____ IRB Expiration Date: _____

Is your child participating in any other research studies? _____Yes _____No

INTRODUCTION

You are being asked to agree to let your child be a part of a drug research study. He or she is scheduled for surgery, or needs to stay in an intensive care unit (ICU). During this operation or stay in the ICU, it will be necessary for the doctor to lower your child's blood pressure for a long period of time, up to 24 hours. We will tell you more about how he/she will do this and the drug that will be used later in this paper. Before you decide whether to let your child be involved in this study, *[Site PI]* wants you to read the following information. He wants you to ask him any questions you may think of. He wants to be sure that you understand what your child's participation will mean. You need to fully understand the type of treatment and its risks. Your doctor is responsible for providing you with the necessary information so that you understand the possible risks. Your child's participation in this study is entirely your choice.

Your child cannot participate in another research study at the same time as this research study [optional sentence]. Your child cannot be taking another experimental drug while enrolled in this research study, or within the previous 30 days. Your child will be a part of this study for approximately 30 days. There may be risks that we cannot predict. We will tell you about any new information that may affect your child's condition or affect your willingness to stay in this study.

NATURE AND PURPOSE OF THE RESEARCH STUDY

In certain kinds of surgeries, doctors often need to control the blood pressure of the patient. The doctor may also need to lower the patient's blood pressure below normal. This can reduce blood loss and avoid blood transfusions during stressful periods. Sodium nitroprusside is a drug that is approved by the Food and Drug Administration (FDA) for use in adults. Scientific studies show that this drug works well when doctors need to control blood pressure during surgeries in adult patients. Doctors also often use sodium nitroprusside in children. However, not many scientific studies tell us how best to use sodium nitroprusside in children.

In this study, we hope to learn the best dose of sodium nitroprusside to use in children who need it in the ICU for more than 12 hours. We also want to learn the same thing for children who need certain kinds of surgeries. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children. This research study is looking for 50-100 children at several hospitals in the United States.

Your child's participation in this study is entirely voluntary.

Your decision whether or not to allow your child to participate will not prejudice your child or his/her medical care. If you decide to allow your child to participate, you are free to withdraw your permission, and to discontinue participation at any time without prejudice to your child or effect on your child's medical care. If you decide to terminate your child's participation in this study, you should notify *(name)* at *(telephone number)*.

PROCEDURES

If you agree to your child's participation in this research study, he or she will undergo the following types of procedures:

- [Site PI] and his research staff will talk to you (and your child) about his or her health. They will ask you about your child's medical history. They will ask you about any medications your child is currently taking. They will give your child three physical examinations, including

measurement of blood pressure, pulse, and weight. The first one will be within 24 hours before study drug is given. The other two physical exams will be after your child receives study drug. A small amount of blood, much less than one teaspoon, will be drawn at these visits. We need this blood for our study, but the same blood work may also be needed as part of your child's regular medical care. If this is the case, we will use these results instead of having to take another blood sample.

- Your child will receive study treatment in stages:
  1. First, after your child is stable, sodium nitroprusside will be given into a vein. This will be done through tubing your child will already have in place. This drug may be the treatment of choice to control your child's blood pressure even if your child is not enrolled in this study. This first stage will last up to 24 hours. He or she will receive sodium nitroprusside at a pre-set initial rate. That rate will be changed until your child's blood pressure is in the range that his or her doctor has decided is the best.
  2. The second stage of study treatment will begin between 12 and 24 hours after the medication was first started. This stage will last up to 30 minutes. During this stage your child will receive one of two treatments. This phase of the research study is blinded. That means that the study doctor will not know which treatment group your child will be placed into. The choice of which treatment group your child will be in (placebo or sodium nitroprusside) will be random. The choice will be made in a way that is like flipping a coin. He or she might receive sodium nitroprusside at the rate that was being used before to keep his or her blood pressure at a stable level. Or, he or she might receive a placebo; this is a solution like salt water that is known to have no effect on your child's health. Your child's vital signs (blood pressure and heart rate) will be watched very closely. If your child's blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end immediately. Then your child will again be given sodium nitroprusside, or similar drug, to keep his or her blood pressure at a safe level.
  3. If your child stills needs sodium nitroprusside treatment to control his/her blood pressure, the treatment may continue after the research study ends.

During the research study, vital signs (blood pressure and heart rate) will be checked often at specific time points.

There will be a follow up evaluation. About 30 days after the research study is over, we will call you at home to ask questions about your child's health in the last month, since he or she participated in the research study. If your child is readmitted to the hospital before our call, please let us know.

Blood samples will be taken between 3 and 6 times (to total between 1 to 2.5 teaspoons) during the research study. This is to see how much of the drug is circulating in the blood at varying time points. If indicated, additional blood samples may be taken to help determine the amount of drug in your child's blood. We are very careful to minimize the amount of blood drawn from your child, and anticipate that 3 1/2 teaspoons is the most we will draw for these tests.

Whenever possible, blood will be taken from tubing already in place. The nurse will often take the study blood samples at the same time that routine blood samples are taken to check on your child's health. This is done to avoid any extra needle-sticks (drawing blood from a vein in the arm with a needle). It is very unlikely that there would not be a catheter (IV line) in place; however, if that were to happen, blood drawing would require a needle stick that might cause minor bruising. Blood drawing is done to assess safety and to measure the activity of the study drug.

The total amount of blood drawn during the entire research study for children less than 2 years of age is approximately 1 to 2 ½ teaspoons. For children more than 2 years of age, the total amount of blood is about 1 to 3 ½ teaspoons.

The information gained during this research study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help patients in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

YOUNG WOMEN OF CHILD-BEARING POTENTIAL

If your child is a young woman who is able to become pregnant, it is expected that she will use an effective method of birth control to prevent exposing a fetus to a potentially dangerous agent with unknown risk. If your child is pregnant or currently breast feeding, she may not participate in this study.

To confirm to the extent medically possible that your child is not pregnant, you agree that she will have a pregnancy test done before beginning this research study. This test will be carried out right before your child undergoes surgery.

PARTICIPANT RESPONSIBILITIES

You should:

- Follow the instructions of the Protocol Director and study staff.
- Tell the Protocol Director or research study staff about any side effects that your child may have.
- Tell the Protocol Director or research study staff if you believe your child might be pregnant.
- Ask questions as you think of them.
- Tell the Protocol Director or research staff if you change your mind about staying in the study.

While participating in this research study, your child should not take part in any other research project without approval from all of the Protocol Directors. This is to protect your child from possible injury arising from such things as extra blood drawing, the possible interaction(s) of research drugs, or other similar hazards.

WITHDRAWAL FROM STUDY

If you first agree to participate and then you change your mind, you are **free to withdraw** your permission and discontinue your child's participation at any time. Your decision will not affect your child's ability to receive medical care for his or her disease and your child will not lose any benefits to which he or she would otherwise be entitled.

If you decide to terminate your child's participation in this study, you should notify (*name)* at (*phone number)*.

The Protocol Director may also withdraw your child from the study and the study medication may be stopped without your permission for one or more of the following reasons:

- Failure to follow the instructions of the Protocol Director and/or study staff.
- The Protocol Director decides that continuing your child's participation could be harmful to him or her.
- Pregnancy (if applicable).
- Your child needs treatment not allowed in the study.

- The study is cancelled.
- Other administrative reasons.
- Unanticipated circumstances.

POSSIBLE RISKS, DISCOMFORTS, AND INCONVENIENCES

There are risks, discomforts, and inconveniences associated with any research study. These deserve careful thought. This is true whether it is a normal kind of treatment or an experimental type. You should talk with the Protocol Director if you have any questions.

In spite of all safety measures, your child might develop medical problems while taking part in this research study. These risks include elevated blood pressure. Treatment of these potential medical problems will not be limited or delayed by your child's participation in the study.

Sodium nitroprusside is a drug that lowers blood pressure. Because of this, there is a chance that your child could develop hypotension (low blood pressure). The doctor will monitor your child very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable blood pressure.

Another side effect that might happen is that your child's heart rate may increase in response to sodium nitroprusside. Again, the doctor and research nurse will monitor your child's heart rate very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable heart rate.

Minor side effects due to sodium nitroprusside may also occur but will not cause the study to be ended. Since sodium nitroprusside makes blood vessels bigger, the following side-effects may occur: nausea, headache, restlessness, abdominal pain, redness or flushing of the skin, nervousness, and perspiring.

Another side effect that happens when using sodium nitroprusside is that as it is used in the body, another chemical, cyanide, is released in small amounts. This can affect the amount of oxygen in the

blood and have a number of other effects. The doctors will carefully watch for any of these signs or symptoms and treat your child if needed.

POTENTIAL BENEFITS

The information gained during this study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help children in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOUR CHILD WILL RECEIVE ANY BENEFITS FROM THIS STUDY.

ALTERNATIVES

If you choose not to enroll your child in this study, your child may receive sodium nitroprusside anyway. This could happen if your child's doctor feels it is the best medicine to use to control blood pressure. Or, your child's doctor could choose to use other types of blood pressure medications, such as esmolol or fenoldopam, instead. Whether or not your child is enrolled, the medical team will, of course, do everything possible to ensure the safety and comfort of your child.

If you do not wish your child to take part in this study, other treatments can be used for your child's condition. If you withdraw your child's participation, the study doctor will recommend an alternative treatment for blood pressure control for your child, such as esmolol or fenoldopam. If this study is discontinued, your child will receive one of these alternative treatments.

PARTICIPANT'S RIGHTS

You should not feel obligated to agree that your child participate in this study. Your questions should be answered clearly and to your satisfaction.

If you decide not to participate, tell the Protocol Director. Your child will still receive care for his/her disease and will not lose any benefits to which he/she would otherwise be entitled.

You will be told of any important new information that is learned during the course of this research study, which might affect your child's condition or your willingness to continue participation in this study.

CONFIDENTIALITY

Your child's identity will be kept as confidential as possible as required by law. Except as required by law, your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your child's research records may be disclosed outside of Stanford, but in this case, your child will be identified only by a unique code number. Information about the code will be kept in a secure location and access limited to research study personnel.

The results of this research study may be presented at scientific or medical meetings or published in scientific journals. However, your child's identity will not be disclosed**.**

Patient information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your child's identity if this study falls within its jurisdiction.

- The purpose of this research study is to obtain data or information on the safety and effectiveness of sodium nitroprusside in children; the results will be provided to the sponsor, the Food and Drug Administration and other federal and regulatory agencies as required.

Authorization to Use Your Health Information for Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed parental premission form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?

In this study, we hope to learn the best dose of sodium nitroprusside to use in children of different ages who need it in the ICU for more than 12 hours. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children as determined by the NIH and FDA.

Do I have to sign this authorization form?

You do not have to sign this authorization form. But if you do not, your child will not be able to participate in this research study, including receiving any research-related treatment.

Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?

If you decide to allow your child to participate, you are free to withdraw your authorization regarding the use and disclosure of your child's health information (and to discontinue any other participation in the study) at any time. After any revocation, your child's health information will no longer be used or disclosed in the study, except to the extent that the law allows us to continue using your child's information (e.g., necessary to maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must contact: *(researcher's name and contact information, including telephone number).*

What Personal Information Will Be Used or Disclosed?

Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to, vital sign measurements, laboratory results of blood collections, physical exams, related medical records, and other data.

Who May Use or Disclose the Information?

The following parties are authorized to use and/or disclose your child's health information in connection with this research study:

- The Protocol Director *(Insert Name of PD)*
- The *(Insert name of Institution)* Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary
- Research Staff

(List every other class of persons or organization affiliated with the hospital/university who might need to use and/or disclose the participant's information in connection with this study.)

Who May Receive or Use the Information?

The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- The National Institutes of Health
- The Food and Drug Administration
- Collaborating Institutions
- The Coordinating Center, Premier Research

Your child's information may be re-disclosed by the recipients described above, if they are not required by law to protect the privacy of the information.

When will my authorization expire?

Your authorization for the use and/or disclosure of your child's health information will expire December 31, 2055.

Will access to my child's medical record be limited during the study?

To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or

billing decision about your child (e.g., if included in your child's official medical record).

_____

Signature of Participant

_____

Signature of Legally Authorized Representative

_____

Date

_____

Description of Representative's Authority to Act for Subject

FINANCIAL CONSIDERATIONS

PAYMENT

You and your child will not be paid to participate in this research study.

COSTS

The sponsor will pay for the cost of sodium nitroprusside and for the extra blood tests that will be used to monitor the amount of drug in your child's blood and for safety tests. You or your insurance company will be responsible for the medical procedures, surgery, anesthesia, and other normal costs associated with standard medical care for treatment of your child's condition.

SPONSOR

The National Institute of Child Health and Development of the National Institutes of Health (NIH) is providing financial support and/or material for this study.

COMPENSATION FOR RESEARCH-RELATED INJURY

All forms of medical diagnosis and treatment – whether routine or experimental – involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. **You will be responsible for any associated co-payments or deductibles as required by your insurance.**

If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

Additionally, *{Institution name}* is not responsible for research and medical care by other institutions or personnel participating in this study. You do not waive any liability rights for personal injury by signing this form.

CONTACT INFORMATION

- Questions, Concerns, or Complaints: If you have any questions, concerns or complaints about this **research study**, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director, *{name}*. You may contact *{him/her}* now or later at *{phone number}*.
- Emergency Contact: If you feel your child has been **hurt by being a part of this study**, or need immediate assistance please contact *{Protocol Director's name and phone number}*.
- Alternate Contact: If you cannot reach the Protocol Director, please page the research team at *{phone number}*.
- Independent of the Research Team Contact: If you are not satisfied with the manner in which this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a research study subject, please contact the *{Institution's name}* Institutional Review Board (IRB) to speak to an informed individual who is independent of the research team at *{phone number}*. Or write the *{Institution's name}* IRB, *{IRB full address}*. In addition, please call the *{Institution's name}* IRB at *{phone number}* if you wish to speak to someone other than the research team or if you cannot reach the research team.

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS *{Text can be revised to meet IRB requirements}*

As a human subject your child has the following rights. These rights include but are not limited to the subject's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
- be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise;
- be given an opportunity to ask questions concerning the experiment or the procedures involved;

- be instructed that parental permission to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated parental permission form;
- and be given the opportunity to decide to give parental permission or not to give parental permission to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING PARENTAL PERMISSION, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____

Signature of Parent or Legal Guardian

_____

Signature of Second Parent or Legal Guardian

_____

Date

_____

Name of Patient

PERSON OBTAINING PARENTAL PREMISSION

I attest that the requirements for informed parental permission for the medical research project described in this form have been satisfied - that the Parent or Legal Guardian has been provided with the Experimental Subject's Bill of Rights, if appropriate, that I have discussed the research project with the Parent or Legal Guardian and explained to him or her in nontechnical terms all of the information contained in this parental permission form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the Parent or Legal Guardian to ask questions and that all questions asked were answered.

_____ _____

Signature of Person Obtaining Parental Permission Date

Appendix C: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles
for
Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when

providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The

responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's

freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case, the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in

the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix D: Responsibilities of the Investigator

Investigator Responsibility/Performance

Prior to starting enrollment at a site, all investigators must read and understand the Investigational Plan and must sign and complete the Investigator Agreement Form. This documents that they accept all conditions of the Investigational Plan and will conduct the study accordingly. The investigator must provide a current copy of his or her curriculum vitae that is not more than 2 years old.

Informed Parental Permission and IRB Approval

The investigator must have written approval from the IRB prior to enrolling patients in the study. A copy of the written approval that includes the following must be provided to the DCRI:

- A statement of IRB approval for the proposed study and informed parental permission form at the institution
- The date the study was approved and the duration of approval
- A listing of any conditions attached to the approval
- Identification of the approved primary investigator
- The signature of the IRB chairperson

Any amendments to the protocol, as well as associated parental permission form changes, will be submitted to the IRB, and written approval must be obtained prior to implementation. Serious adverse event reports will be submitted as requested by the BPCA.

The study will be explained to the patients in lay language. Patients will sign and receive a copy of the IRB-approved informed parental permission form prior to study participation. Patients will be

assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Source Documentation

Regulations require that investigators maintain information in the study patient's medical records that corroborate data collected on the CRFs. In order to comply with these regulatory requirements, at the minimum, the following information should be maintained:

1. Medical history/physical condition of the study patient before involvement in the study sufficient to verify protocol entry criteria (if not already present)
2. Dated and signed notes on the day of entry into the study including clinical site, patient number assigned and a statement that parental permissin was obtained
3. Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams)
4. Notations on abnormal laboratory results
5. Serious adverse events reported through 30 days from the end of study drug administration
6. Notes regarding concomitant medications taken during the study (including start and stop dates)
7. Study patient's condition upon completion of or withdrawal from the study

Data Transmittal

Required data will be recorded on the CRFs as soon as possible after the patient is discharged, day 10, or death, whichever comes first. CRFs and any supporting documents must be sent to the BPCA and/or retrieved from the investigational site according to the outlined time windows. The 28-day follow-up CRF needs to be forwarded within 10 days of the follow-up visit.

Non-Protocol Research

BPCA has a legal responsibility to report fully to regulatory authorities all the results of clinical studies. No investigative procedures other than those in this protocol shall be undertaken on the enrolled patients without the agreement of the IRB and BPCA.

Publication Policies

At the conclusion of the study, a multicenter abstract reporting the primary results may be prepared and presented in an appropriate international forum. A multicenter, peer-reviewed manuscript will also be prepared for publication in a reputable scientific journal.

Appendix E: Treatment of Suspected Nitroprusside Toxicity

Signs of Toxicity:

- If the base deficit exceeds -8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the subject will be also be discontinued from study.
- If the lactate level rises by more than 4 mmol/L in an 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output).
- If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes percent between arterial and mixed venous blood.

SUSPECTED CYANIDE TOXICITY SHOULD BE TREATED AS FOLLOWS:

1) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration.

2) Obtain blood for arterial and venous blood gases with co-oximetry, serum lactate, and cyanide and thiocyanate levels.

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10mL) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result)

Subject Hemoglobin g/dL	Initial dose Sodium NITRITE (3%) mL/kg IV	Initial dose Sodium Thiosulfate mL/kg IV
8 g/dL	0.22 mL/kg	1.10 mL/kg
(6.6 mg)/kg		
10 g/dL	0.27 mL/kg	1.35 mL/kg
(8.7 mg)/kg		
12 g/dL	0.33 mL/kg	1.65 mL/kg
(10 mg)/kg		
14 g/dL	0.39 mL/kg	1.95 mL/kg
X	(11.6 mg)/kg	

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals.

[Micromedex Poisondex www.micromedex.duhs.duke.edu , see also Berlin, 1970]

Appendix F: Assay of Nitroprusside Metabolites and Handling of Blood Samples for Assay of Nitroprusside Metabolites

A classical UV bioanalytical method is utilized for detection and quantitation of cyanide in whole blood. Cyanide concentrations are determined by measuring the absorbance of the chromophore formed by the interaction of the cyanide ion with 4-nitrobenzaldehyde and o-dinitrobenzene in 2-methoxyethanol (Rieders, 1971 and Guilbault, 1966). In summary, to a 1.0 ml aliquot of whole blood, 4-Nitrobenzaldehyde solution and o-Dinitrobenzene solution is added then made basic with sodium hydroxide. Following a specific incubation time a UV scan spectrum is obtained from 520 to 580 nm with a maximum reading at 555 nm. The exact details of the method are proprietary to NMS Labs. The method is sensitive to a LLOQ of 0.05 μg/mL which correlates to normal circulating levels. A toxic threshold is normally assessed as approximately 0.5 μg/mL and acute toxicity is observed at greater than 1 μg/mL. There are no known interferences with this assay method.

An ion chromatography method is used for detection and quantitation of thiocyanate in serum that is specific, accurate, precise and rugged. In summary, a 0.10 mL aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography (Vogel, 1981 and Vesey, 1976). The same procedure is employed to detect thiocyanate in urine. The normal range for non-smokers is 1-4 μg/mL in serum/plasma. For smokers, it is 3 – 12 μg/mL and the therapeutic range for sodium nitroprusside is generally between 6 and 29 μg/mL (Schulz, 1984).

Thiosulfate is detected in serum/plasma or urine also via a validated ion chromatography method with the analytes separated and detected via conductivity detection. For this method, a 0.5 ml aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography.

Sample Handling Procedures

At each specified blood collection for cyanide and thiocyanate, 2 mL of arterial blood is to be collected in a 2 mL gray top. Samples should be mixed, then one half the whole blood in the gray top (1 mL) is removed and stored in a polypropylene screw capped container and stored on ice until it can be refrigerated. The remaining 1 mL of whole blood is centrifuged (within 20 minutes of collection at approximately 1200 g for 10 - 12 minutes at ambient or colder temperatures) to obtain 0.5 mL of plasma for thiocyanate analysis. The red bloods cells from this sample may be discarded, or separately tested for cyanide. All samples should be stored in a refrigerator before sending to the Central Lab(s), which should be done as soon as possible. Samples should be shipped COLD, using "frozen cold packs," for overnight delivery. Whole blood samples should NEVER be frozen.

For neonates or to spare the total amount of blood drawn for the analysis, the minimum blood draw is 1 mL of arterial blood. This translates to 0.5 mL of whole blood for cyanide analysis and 0.5 mLs to be centrifuged to obtain approximately 0.25 mLs of plasma for thiocyanate analysis.

The details of handling & shipping samples to the Central Lab(s) will be included in the MOP. This is a summary and not the complete instructions for sample handling.

Appendix G: Sedation Suggested Regimen

Intensive Care Unit:

For those patients who receive study drug in the intensive care unit for a surgical or medical procedure, the following guidelines may be utilized.

Sedation may be administered intravenously and initiated with a benzodiazepine and opiate agonist as follows:

Midazolam bolus 0.1-0.2 mg/kg followed by a continuous midazolam infusion of 0.06-0.3 mg/kg/hr or intermittent bolus of 0.1 mg/kg every 1-2 hours

Or

Lorazepam bolus 0.05-0.1 mg/kg followed by a continuous infusion of 0.025-0.05 mg/kg/hr or intermittent bolus of 0.05-0.10 mg/kg every 4-6 hours

And/or

Fentanyl bolus 1- 5 micrograms/kg (intubated, mechanically ventilated patients) followed by an infusion of fentanyl of 0.5 – 5 micrograms/kg/hour

Or

Morphine bolus 50-100 microgram/kg followed by a continuous infusion of 20-80 micrograms/kg/hour.

Sedation and analgesic medication may be titrated to patient response. Higher doses may be used in patients who exhibit benzodiazepine and/or narcotic habituation due to long term (> 4-7 day) usage. Where applicable, sedation assessment may be by clinical judgment of the responsible physician, and/or a quantitative scoring system such as the COMFORT score. Patients receiving neuromuscular blocking drugs such as vecuronium or rocuronium as part of their ICU management are eligible for study.

Appendix H: Measurement of Blood Pressure in Children

Excerpt from: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents

Correct measurement of BP in children requires use of a cuff that is appropriate to the size of the child's upper right arm. The equipment necessary to measure BP in children, ages 3 through adolescence, includes child cuffs of different sizes and must also include a standard adult cuff, a large adult cuff, and a thigh cuff. The latter 2 cuffs may be needed for use in adolescents.

By convention, an appropriate cuff size is a cuff with an inflatable bladder width that is at least 40% of the arm circumference at a point midway between the olecranon and the acromion. For such a cuff to be optimal for an arm, the cuff bladder length should cover 80% to 100% of the circumference of the arm. Such a requirement demands that the bladder width-to-length ratio be at least 1:2. Not all commercially available cuffs are manufactured with this ratio. Additionally, cuffs labeled for certain age populations (eg, infant or child cuffs) are constructed with widely disparate dimensions. Accordingly, the working group recommends that standard cuff dimensions for children be adopted (see Table 2).

TABLE 2. Recommended Dimensions for BP Cuff Bladders

Age Range	Width, cm	Length, cm	Maximum Arm Circumference, cm*
Newborn	4	8	10
Infant	6	12	15
Child	9	18	22
Small adult	10	24	26
Adult	13	30	34
Large adult	16	38	44
Thigh	20	42	52

* Calculated so that the largest arm would still allow the bladder to encircle arm by at least 80%.

BP measurements are overestimated to a greater degree with a cuff that is too small than they are underestimated by a cuff that is too large. If a cuff is too small, the next largest cuff should be used, even if it appears large. If the appropriate cuffs are used, the cuff-size effect is obviated

SBP is determined by the onset of the "tapping" Korotkoff sounds (K1). Population data in children and risk-associated epidemiologic data in adults have established the fifth Korotkoff sound (K5), or the disappearance of Korotkoff sounds, as the definition of DBP. In some children, Korotkoff sounds can be heard to 0 mm Hg. Under these circumstances, the BP measurement should be repeated with less pressure on the head of the stethoscope. Only if the very low K5 persists should K4 (muffling of the sounds) be recorded as the DBP.

Appendix I: Research Assent Form Template

Protocol Title: A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Director:

IRB Approval Date: _____IRB Expiration Date: _____

Are you taking part in any other research studies right now? ____ yes ____ no

Why Are We Doing this Research Study?

We are doing this study to learn more about a medicine called sodium nitroprusside. This study will help us find out more about how good this drug is in keeping blood pressure and heart rate under control in children who are in the intensive care unit (ICU). Altogether, there will be 60 kids under the age of 17 in this study.

What Will Happen During the Study?

You will be seen by a doctor, who will give you a physical exam and ask you and your parents some questions about your health history and medicines you take. At certain times during the study, blood will be drawn to check that you are okay and safe, and to see how the study drug is working in your body. Blood will be drawn either from a tube that is already connected to you to get blood, or from a needle. The amount of blood to be taken during the study is about 2½ teaspoons to 3½ teaspoons.

In the first part of the study, you will receive a study drug called sodium nitroprusside through a tube placed into a vein in your arm. Your doctor will make changes in the amount of drug you get to keep you safe and your vital signs (like your heart rate and blood pressure) stable. Your doctor will

change how much of the drug you get so you have the blood pressure the doctor thinks is right for you. This part of the study will last for at least 12 hours up to 24 hours during the time that your doctor needs to control your blood pressure. During the time you get study drug, your blood pressure and heart rate will be measured very often.

The second part of the study will last up to 30 minutes. During this part, you will get one of two treatments. This part of the research study is blinded. That means that the study doctor will not know which treatment group you are in. The choice of which treatment you receive will be made in a way that is like flipping a coin. You might receive the study drug at the same rate that was being used before to keep your blood pressure stable. Or, you might receive a placebo, which is a solution like salt water that doesn't have any effect on your blood pressure. Your blood pressure and heart rate will be watched very close. If your blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end right away. Then you will again be given sodium nitroprusside, or similar drug, at a rate that keeps your blood pressure at a safe level.

What if you don't want to be in the Study?

You can say "no" to being in the study if you want. You can also stop the study anytime you want by telling anyone that is caring for you. Your doctor can explain to you and your parents other treatments that could be used instead.

What You Should Know about the Medicine?

The medicine is used to keep your blood pressure where your doctor thinks is safe or needed for your operation or some other treatment or test. It has been okayed for use in adults but there is not a lot of information about how the drug works in kids. Like any medicine, it can cause unwanted things to happen. These unwanted things are called risks. Some of the risks from using this medicine are: getting sick to your stomach, headache, getting hot, having your blood pressure go down too much or your heart beat go up too high.

Things Girls Need to Know….

Some medicine can cause bad things to happen to an unborn baby. If you are able to get pregnant (if you have started having a period), you need to take a pregnancy test which your doctor will give you. If you are pregnant, you should not take part in this study.

What Else Do You Need to Know?

Sometimes doctors write about the research studies when they are done. If a paper is written about this research study, your name won't be used in it, but the medical information they find out about you may be used. We will keep your medical information private. People who work for *[site name]*, the people who are running the study, and some parts of the government (the part that takes care of medicines) will be able to look at your medical information.

There is no cost to you or your parents to be in this study.

If you have questions about the study you can call Dr. _____ at

_____.

I have read this form. I have had a chance to ask questions about things I don't understand. I want to be in this research study and understand what will happen to me.

_____ _____

Signature of Patient Date

_____

Name of Patient

_____ _____

Signature of the Person Obtaining Assent Date

Summary of Changes to Clinical Protocol NICHD-2003-09-LT, Version 4 (21 July 2009)

In addition to non-substantive corrections in spelling and punctuation, and abbreviations, the changes to the 29 June 2009 version of protocol NICHD-2003-09-LT are summarized below.

Clarifications and Changes in Definitions

1. Changes affecting VS measurements
   A. During the Open-label period, for both institutional and investigational SNP
      To clarify that VS measurements should be performed when the subject is receiving either institutional or investigational SNP
      i. Section 3.2.3.5 (SNP as Concomitant Medication)
         a. First paragraph, last sentence
            VS measurements described for the Open-label treatment period in Table 1 and Section 3.4.3 refer to both institutionally supplied SNP and investigational SNP (study drug).

            Note: For some subjects, VS measurements may not have been made at the intervals described in the protocol while they received institutionally-supplied SNP (because they had not yet been enrolled into the study), but sites will be instructed to provide those VS measurements that were obtained.

         b. New final paragraph for this section

            The start and stop times and dates, and dosage of institutionally-supplied SNP administration will be recorded on the appropriate CRF.

      ii. Section 3.4.3 (#9), first sentence
         Obtain vital sign measurements every one minute for the first 10 minutes then every 5 ± 1 minutes for an additional 20 minutes after initiation of open-label study drug infusion (and, if applicable, institutionally-supplied SNP) and after each dosage adjustment.
   B. Always obtain VS immediately prior to Open-label period, even if this is not MAP(B1)
      i. Section 3.4.3 (#3B)

         If B1 is not immediately prior to the initiation of SNP infusion, VS measurement will be obtained that this time.

2. Changes affecting MAPB1

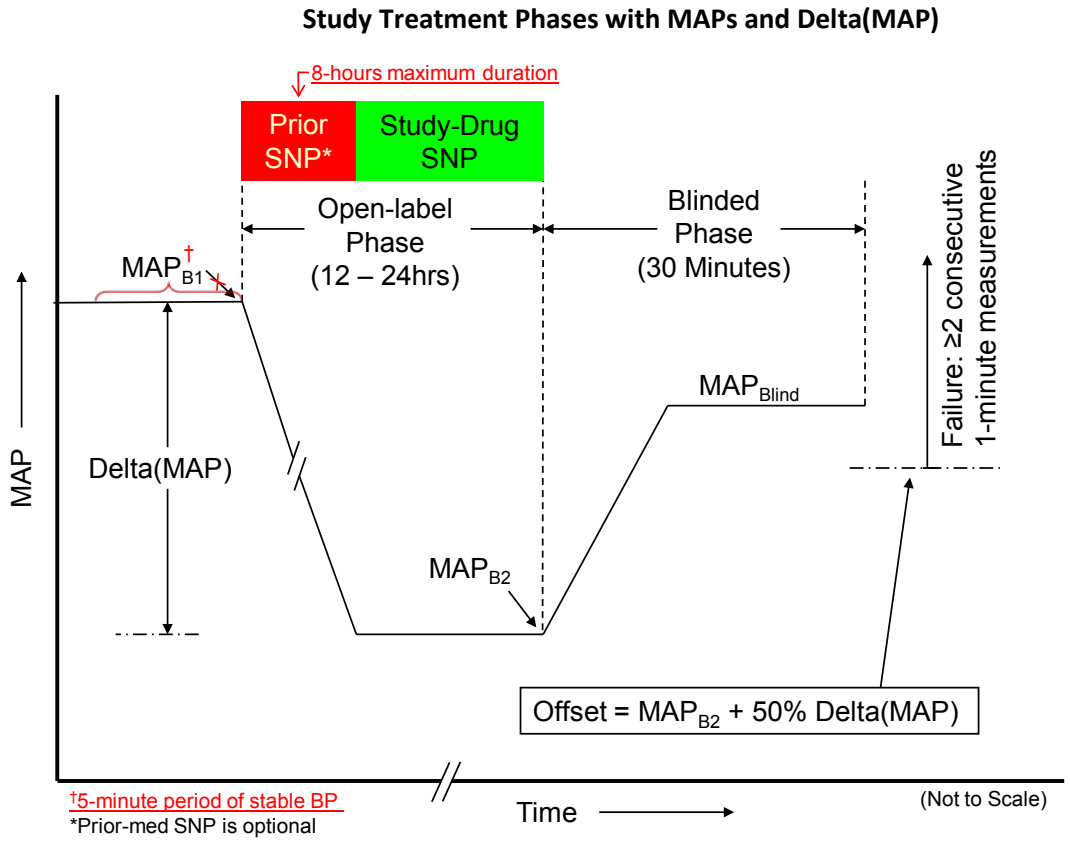
   The timing of MAP_{B1} has been revised as follows

   A. Section 3.1
      i. 3rd paragraph,

         The initial baseline MAP (B1) is defined as the blood pressure measurement taken ~~just~~ prior to the initiation of SNP administration, either institutionally-supplied or open-label study drug after at least a 5-minute period of stable conditions (e.g. no prn

doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP, See Figure 1 below.

Figure 1



ii. 1st bullet following Figure 1

- MAP_{B1} = MAP ~~immediately~~ prior to start of Open-label Study Drug Administration Phase

iii. 5th bullet following Figure 1 has been deleted

- ~~MAP_{B1} = MAP immediately prior to induction of general anesthesia~~

B. Section 3.4.2, Pre-study drug administration procedures, #5

5) Obtain vital sign measurements~~ immediately prior to induction if B1 will occur during general anesthesia; otherwise, as early as practical~~. Based upon clinical judgment, this may be MAP_{B1} for some patients.

C. Open-Label Study Drug Administration (Dose-Titration) Procedures, Section 3.4.3, #3

3. Obtain baseline vital sign measurements prior to the start of SNP administration, either institutionally-supplied or open-label study drug. This defines B1.

   A. The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in drug concentrations which may affect BP.

   B. If MAP(B1) is not obtained immediately prior to the initiation of infusion of SNP, a VS measurement will be obtained immediately prior to the initiation of the open-label SNP infusion (time=0).

3. Target MAP, Section 3.1, 2nd paragraph, last sentence

   For clarity, the following was added to the definition of Target MAP,

   The anticipated Target MAP must be at least 20 mmHg below MAP(B1)--(15 mm Hg for subjects < 2 years old), see next definition, for the patient to be eligible for enrollment, see Inclusion criterion #4.

4. Definition of Study Periods, Open-label study drug administration, Section 3.1.1

   To increase clarity, the following phrase was added to this definition:

   The period of open label study drug administration will be at least 12 hours but not greater than 24 hours, including the time of infusion of institutionally-supplied SNP, if any.

5. Selection of Study Population, Section 3.2

   To increase clarity, the following was added to the first sentence of this section: "Children up to 17 years of age who require pharmacologic blood pressure control for at least 12 hours will be eligible for enrollment into the study."

6. Inclusion Criteria #4, Section 3.2.1

   To increase clarity, the following has been added to Inclusion Criterion #4:

   4. Subject is anticipated to require a minimum of 20 mm Hg (15 mm Hg for subjects < 2 years old) reduction in MAP for at least 12 hours using SNP [i.e., $MAP_{B1} - MAP_{B2} \geq$ 20 mm Hg (15 mm Hg for subjects < 2 years old)]

7. Adverse Event Collection Period, Section 4.4

   To increase clarity, the following clause has been added to Section 4.4, "Non-serious adverse events will be monitored and reported from the time the subject receives study drug up to 24 hours following termination of study drug"

8. Protocol deviations and violations, Section 7.7.8

Because of a change in a Standard Operating Procedure of the study sponsor's CRO, "protocol violations" has been changed to "protocol deviations and violations" in the first paragraph of Section 7.7.8, "All protocol deviations and violations will be documented and categorized in the final study report."

9. SAE Definition, Section 4.1.1 (#8)

   To give investigators additional authority in classification of an adverse event as serious, an eighth condition has been added to the definition of Serious Adverse Events: "Any event deemed to be serious by the Investigator"

10. Pre-study drug administration procedures (#7), Section 3.4.2

    For clarity, the testing period for pregnancy has been revised as follows:

    "Pregnancy test if required must be done within 48 hours ~~of~~ prior to study drug administration."

11. Subjects who do not received blinded-study drug will not be considered to be withdrawn, Section 3.5.1

    Because it did not convey the desired intent (i.e., subjects who did not receive blinded-study drug would not be included in the testing of the primary efficacy endpoint), the first sentence of the third paragraph of Section 3.5.1 has been deleted. These subjects will not be withdrawn from study without cause (e.g, withdrawal of consent, see the fourth paragraph of this section).

    ~~Any subject who does not start the blinded study drug administration period will be considered prematurely withdrawn from the study.~~

Changes in Enrollment Criteria

12. Prohibition on use of Thiosulfate
    A. New exclusion criterion

       11. Subject has received or will have received Sodium Thiosulfate within 6 hours prior to the start of the open-label period

    B. New Section

       3.2.3.6 Use of Sodium Thiosulfate

       Administration of sodium thiosulfate will be prohibited from 6 hours prior to the start of the open-label period until the end of the blinded-study period, excepted in cases in which nitroprusside or cyanide toxicity is suspected, in which case, the

administration of sodium thiosulfate, as described in Appendix E, is recommended.

To facilitate the assessment of cyanide clearance, investigators are encouraged not to co-administer sodium thiosulfate with institutionally-supplied SNP, which may, at the investigator's discretion, be administered following the conclusion of the blinded-study period. Should sodium thiosulfate be co-administered with institutionally-supplied SNP following the conclusion of the blinded-study period, blood and urine samples will not be tested for cyanide and Thiocyanate in these subjects once the sodium thiosulfate administration has begun.

13. Exclusion of patient on ECMO

The following new exclusion criteria have been added:

12. Subject is either on, or anticipated to be on ECMO

14. No exclusion for use of an investigational devise

Exclusion criterion #10 has been revised to remove of the exclusion of patients who have participated in clinical trials involving investigational devices:

10) "Subject has participated in other clinical trials for investigational drugs ~~and/or devices~~ within 30 days prior to enrollment"

Protocol Templates

15. Consolidation of investigator's protocol signature page(s)

A. The following text was moved from Section 12 (Investigator Agreement) to "Approval Signatures", pages 3-6:

I have received and reviewed the investigator brochure for sodium nitroprusside (SNP).

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulation.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Signature of Principal Investigator Date

Name of Principal Investigator (printed or typed)

B. To Section 12 (Investigator Agreement), the following was added:

The investigator will sign and date a Study Protocol Agreement Form, provided earlier in this protocol. The Study Protocol Agreement will then be countersigned by the investigator's PODS Principal Investigator.

16. Template for the Parental Permission Form with HIPAA

A. The descriptions of the volume of blood draws has been updated in the informed consent template

2nd page of the template, three sentences before the bullet point—

A small amount of blood, ~~much~~ less than one teaspoon, will be drawn at ~~these~~ the first two visits.

Then, on the 4th page of the template—

"Blood samples will be taken between ~~3~~ 4 and to ~~6~~ 7 times (~~to total between 1 to 2.5 teaspoons~~ 1½ to 2½ mLs per each sample, or ½ teaspoon per sample) during the research study. This is to see how much of the metabolites (breakdown products) of the study drug is circulating in the blood at varying time points. If indicated, additional blood samples may be taken to help determine the amount of ~~drug~~ metabolites in your child's blood. We are very careful to minimize the amount of blood drawn from your child, and anticipate that 3½ teaspoons is the most we will draw for these scheduled tests."

Then, two paragraphs further, last sentence,

For children more than 2 years of age, the total amount of blood is about ~~1~~ 2 to 3 ½ teaspoons.

B. The description of cyanide testing has be revised to read:

~~Another side effect that happens when using sodium nitroprusside is that as it is used in the body, another chemical, cyanide, is released in small amounts. This can affect the amount of oxygen in the blood and have a number of other effects. The doctors will carefully watch for any of these signs or symptoms and treat your child if needed.~~

Sodium nitroprusside contains cyanide. Cyanide is present in all people and is important for normal body function. Extra cyanide can be present whenever sodium nitroprusside is used. Excess cyanide can affect the amount of oxygen in the blood. The doctors will carefully watch for any signs or symptoms of excess cyanide and treat your child if needed. Because safety is one of the most important aspects of this study, we will be testing your child's blood for cyanide. The results of this testing will not be immediately known. However, there are other means to detect ill effects from excess cyanide. If the doctors suspect it is in your child's best interest, additional unscheduled blood tests for cyanide and its breakdown product, thiocyanite, will be performed. Cyanide has been detected in patients who have participated in this study. However, no patient has shown any ill effects from the cyanide.

C. Safety data will be collected from withdrawn subjects:

To allow the collection of safety data from subject who are withdrawn from the study, the follow paragraph has been added.

If your child is withdrawn from the study after receiving any of the research drug, and if you agree, we will perform the following testing to ensure the safety of your child: physical examination, monitor vital signs, blood tests, as described earlier, as well as the follow-up phone call approximately 30 days later. You will be free to have only some of these done if you prefer.

17. The estimated beginning and end dates in the protocol synopsis have been revised

Estimated Start:	~~Q3~~ Q4 2008
Estimated Finish:	Q3 2010 ~~2009~~

Also, as a consequence of this change in study dates, the study duration has also been increased to 24 months

Concomitant Medications

18. Medications that affect blood pressure

Several changes to the protocol involve restrictions of medications that may affect blood pressure, "BP-affecting Drugs."

A. Section 3.2.2, (Exclusion Criteria),

i. Criterion #6 has been change as follows:

6. Subject is anticipated to need anti-hypertensive drugs other than Sodium Nitroprusside ~~study drug~~ either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) within three terminal half-lives (3X T½ β) of the blinded study drug period ~~during the period of study drug administration~~. However, patients receiving stable doses of an anti-hypertensive drug(s) prior to the initiation of study drug may be enrolled~~, provided they will not have received IV vasodilator therapy for greater than 8 hours prior to receiving study drug~~.

ii. Section 3.2.3 (Prior and Concomitant Therapy, including Medications and Procedures) now reads:

3.2.3 Prior and Concomitant Therapy, including Medications and Procedures

3.2.3.1 BP-Affecting Drugs

The following medications will be considered to affect blood pressure, and their use, both prior to and concomitant with, SNP infusions will be restricted as described in Sections 3.2.3.2 and 3.2.3.3. There will be no restrictions on the administration of non-study drug medications following the end of the blinded treatment period.

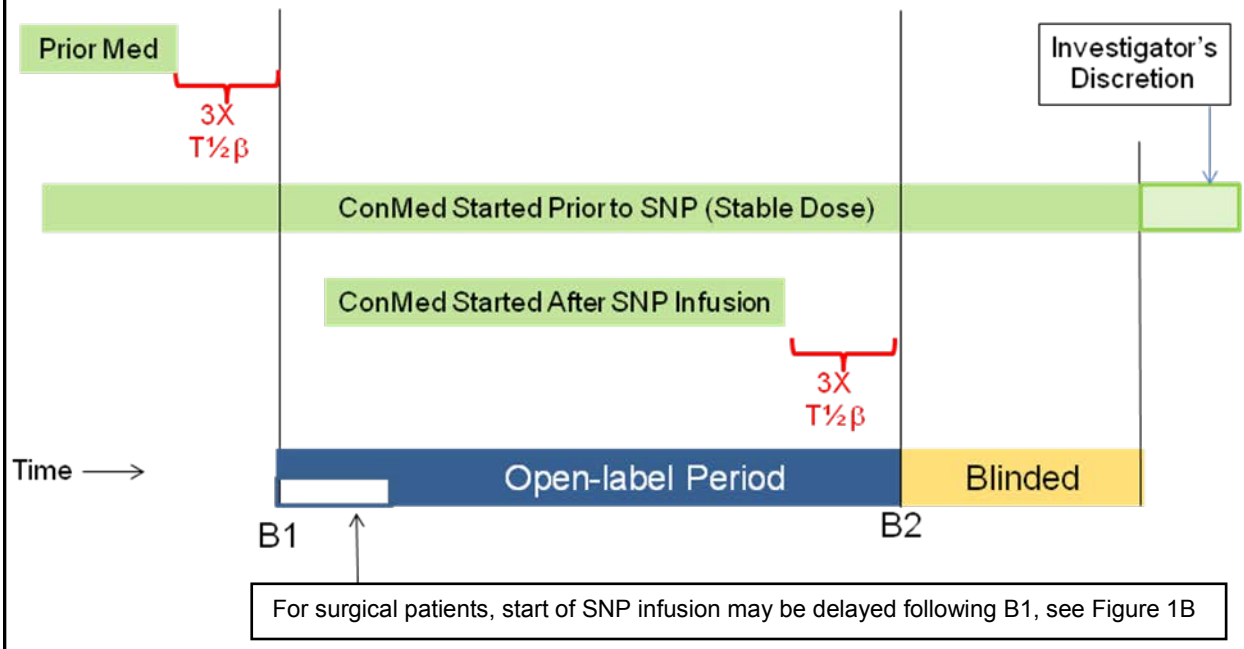
1) Anti-hypertensives and diuretics
2) Beta blocking agents
3) Ace-inhibitors, alone or in combinations
4) Calcium channel blockers and diuretics
5) Diuretics and potassium-sparing agents
6) Peripheral vasodilators
7) NSAIDs and narcotic analgesics
8) Parasympathomimetics
9) Antipsychotics and antineurotics
10) Psychostimulants and nootropics
11) Dopaminergic agents
12) Inotropic agents
13) Sedatives and hypnotics
14) Blood and blood products

3.2.3.2 Prior medications – administration started prior to B1

BP-affecting drugs that are started prior to B1 will be either (1) discontinued at least 3 terminal half-lives (3X T½ β) prior to B1, or (2) continued at a stable dose until at least the end of the blinded infusion period, see Figure 2.

Figure 2

Time Restriction for the Administration of Medications Affecting Blood Pressure



3.2.3.3 Concomitant medications – administration started after B1

BP-affecting drugs that are started after B1 will be discontinue at least 3 terminal half-lives (3X T½ β) prior to B2 (i.e., the start of the blinded treatment phase), see Figure 2.

3.2.3.4 Recording Prior and Concomitant Medications and Procedures

~~Initiation of new anti-hypertensive drugs either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g. local anesthetics, clonidine, etc.) other than the study drug during study drug administration is prohibited. However, in patients receiving stable doses of non-study anti-hypertensive drugs, these agents may be continued during study drug administration. If open label study drug is initiated during anesthesia, the anesthetic medications will be recorded on the CRFs but are not considered as anti-hypertensive drugs.~~ All concomitant medications that affect blood pressure, sodium thiosulfate, and clinically meaningful, unexpected, and invasive procedures will be recorded for the period beginning 72 hours prior to study drug administration through 24 hours post-study drug conclusion. The dates of administration, ~~dosage~~ and reason for use must be included. All concomitant medications will be collected for SAEs occurring within 30 days following study drug administration. ~~Vaccines are considered a concomitant medication~~.

3.2.3.5 SNP as Concomitant Medication

This subsection has the following new sentence plus the revisions described in above in Section 1(A)(i).

Subjects may receive institutionally-supplied SNP after the conclusion of the SNP Blinded Treatment Period at the discretion by the investigator.

19. Reporting of Prior and Concomitant Medications

In addition to restricting the usage of BP-affecting drugs, Section 3.2.3.4, provided above, also states that only the BP-affecting drugs and medications associated with SAEs will be documented on Case Report Forms although all medications will be recorded on the subjects' medical records, which will be reviewed by our monitors to ensure that all adverse events have been documented appropriately on a CRF. Other sections of the protocol describing the reporting of prior and concomitant medications include:

A. Section 3.4.2 (#8), "Document concomitant medications that may affect blood pressure ~~(including over-the-counter preparations)~~."

B. Section 3.4.3 (#12), "Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures, and adverse events."

C. Section 3.4.4 (#5), the first sentence of which now reads, "Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures, and adverse events."

D. Section 3.4.6 (#2), the first sentence of which now reads, "Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures."

E. Section 7.6, the first two sentences of which now read: "Concomitant medications (medications present while on study medication) will be recorded on source documents throughout the study and at early termination; however, only those concomitant mediations that (1) affect blood pressure, or (2) are associated with SAE's will be recorded on CRFs. ~~These~~ Medications listed on CRFs will be coded using the WHO drug dictionary."

Cyanide Testing

20. Additional Cyanide testing option for investigator

To ensure that the investigator has both guidance as well as discretion for additional cyanide and thiocyanate testing, should, in the investigator's judgment, additional safety testing be warranted, the following paragraph has been added to Section 3.4.7.2 (Blood draws and urine samples):

In addition to those cyanide and thiocyanate tests scheduled in Table 1 and Section 3.4, the investigator will be permitted to perform supplemental cyanide and thiocyanate tests provided that the investigator believes that the tests are in the patient's best interest and the

volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).

This section is also reference at the end of Sections 3.3.2 and Table 1, footnote #17.

21. Adverse Event Severity, Tables 1 and 4B, and Section 4.2

In Tables 1 and 4B as well as the text of Section 4.2, the description of cyanide testing has been revised from serum or plasma cyanide to "blood cyanide", as this more accurately describe the testing being performed in this study (i.e., determination of cyanide levels in whole blood and/or RBC's).

Administration of Study Drug

22. Duration of infusion of institutionally-supplied SNP to be ≤ 8 hours

The maximum duration of the administration of institutional-supplied Sodium Nitroprusside (SNP) during the Open-label treatment period is now specified. Section 3.1.1, 2nd bullet point, 3rd sentence now reads:

"The duration of infusion of the institutionally-supplied SNP will be no longer than 8 hours."

23. Carrier Flow Rate

To give investigators flexibility in adjusting the study drug's carrier flow rate, the last sentence of Section 3.4.9 (Delivery of Study Drug) has been modified to read: "The carrier flow rate will be ≥5.0 mL/hr from at least one hour prior to the start of the blinded study period until the end of the blinded treatment period. Clinical judgment should be exercised when adjusting the carrier flow rate to prevent unsafe spikes in the infusion rate."

24. Minimum infusion rate of SNP for Blinded-treatment period

A. The minimum infusion rate of SNP during the blinded-treatment period will be 0.5 mcg/kg/min. The last sentence of Section 3.1, 4th paragraph now reads:

"Prior to establishing B2, there shall have been no changes in the SNP infusion rate for a period of at least 20 minutes during which the infusion rate of SNP will be at least 0.5 mcg/kg/min."

B. Section 3.4.8, 3rd paragraph, last sentence states that the minimum infusion rate of the blinded-treatment period will also be 0.5 mcg/kg/min.

Subjects will receive blinded study drug at the same rate of infusion that was used at the conclusion of the initial open-label study drug administration period, which will be at least 0.5 mcg/kg/min.

DSMB and Statistical Methods

25. DSMB

The Data and Safety Monitoring Board is being renamed Data Monitoring Committee (DMC). The Section describing its function and duties has been revised as follows:

6.0 Data Monitoring Committee

To ensure that the welfare of trial patients receives appropriate consideration, an independent Data ~~and Safety~~ Monitoring Committee ~~Board~~ (DMC ~~DSMB~~) has been organized by the BPCA-CC on behalf of the NICHD to review relevant safety and~~/or~~ efficacy data during the course of the trial. The DMC ~~DSMB~~ may recommend discontinuation of the study, or modifications to the study protocol for safety reasons.

The DMC ~~DSMB~~ consists of four core members (Chair, ethicist, statistician, community representative) plus additional ad hoc members for the various medical subspecialties involved in the BPCA protocols.

Each DMC ~~DSMB~~ will have a presenting statistician who will be responsible for presenting the interim data. This member will write the reports and will be one non-voting member of the DMC ~~DSMB~~. Except as their role in the DMC ~~DSMB~~, all DMC ~~DSMB~~ members are not participating in the design or conduct of this study, as an investigator or otherwise, and lack any financial conflict that would introduce any bias.

6.1 DMC Responsibilities

- Monitoring the safety of trial patients;
- Recommending discontinuation of the trial for safety reasons;
- Recommending changes to the study protocol for safety reasons;
- Determining whether early stopping efficacy conditions are met;
- Providing written reports on an ongoing basis following scheduled and ad hoc meetings that will be archived and may be provided to regulatory agencies.

~~These responsibilities will be broadened to include decisions regarding efficacy if the trial is an efficacy trial.~~ The DMC ~~DSMB~~ will monitor the safety of trial patients by reviewing the occurrence of adverse events and deaths, on a real-time basis as SAE reports are transmitted. The DMC ~~DSMB~~ may also monitor compliance with the protocol, and factors affecting patient safety or the integrity of the trial. The DMC ~~DSMB~~ may request any additional data that are not included in the report if deemed necessary for effective monitoring.

If the DMC ~~DSMB~~ finds any major concerns about safety, it may recommend discontinuing the trial or modifying the study protocol. Following each data review, the DMC ~~DSMB~~ will send a written recommendation regarding the trial, (e.g., to continue according to the protocol, or recommendations for specific actions) to the sponsor.

26. Statistical Methods

The statistical methods section has been revised as follows:

7.1 General Overview

The primary efficacy variable is the intra-patient change in Delta (MAP) during the blinded phase of the study. The primary null hypothesis to be tested is that there is no difference between the active study drug and placebo in the proportion of patients who experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP). Statistical analyses will be performed using two-sided tests. A 0.05 significance level will be used in all tests of treatment differences. Tests for interactions will utilize a 0.10 statistical significance level. Individual secondary endpoints will be evaluated using a hierarchical testing procedure. The Statistical Analysis Plan will include a detailed description of all statistical methods, testing procedures, and methods of data imputation. One formal interim analysis is planned to evaluate the primary efficacy endpoint when 40 out of 60 patients have completed or withdrawn from the double-blind phase of the study. To control overall type I error at the nominal 0.05 level, critical values at interim and final analysis will be based on the Pocock stopping boundaries. The Data Monitoring Committee charter will contain the specific details regarding the early stopping rule at interim analysis ~~re-estimation of the target sample size~~.

Then continue at Section 7.3.1 (Efficacy), 2nd and 3rd paragraphs—

The Per-Protocol population will contain all patients randomized to and exposed to the study drug during the double-blind phase of the trial. The efficacy analysis will be based on the Per-Protocol population. A patient will be classified as a *treatment success* if they meet the following criteria:

• Complete the 30-minute double-blind phase without having ≥2 consecutive ~~an~~ intra-patient increases greater than or equal to 50% Delta (MAP) [i.e., MAP ≥ $(MAP_{B1} + MAP_{B2})/2$] and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

Then continuing at Section 7.7—

7.7 Statistical Design and Models for Analysis

This is a biphasic (open-label dose-titration phase, followed by a randomized phase), randomized, double-blind placebo-controlled study. Patients who are enrolled into the initial phase of the study will have their dose of sodium nitroprusside titrated and must receive a minimum of 12-hours of treatment to be eligible for the randomized phase of the study. Patients who cannot be adequately titrated during the initial 12-hour period will not proceed to the randomization phase of the study. Patients who reach the randomization phase of the study will be assigned to receive placebo, or continue to receive sodium nitroprusside based on a stratified permuted block central randomization scheme.

Five age groups (A through E) will be enrolled in this trial:

Age Group A: Age Group A: Neonates from birth to less than 30 days of age

Age Group B: Infants and toddlers from 30 days to < 2 years

Age Group C: Preschool children from 2 years - < 6 years

Age Group D: School age children from 6 yrs - < Tanner stage III

Age Group E: Adolescents from Tanner stage III - < 17 years.

In order to efficiently account for the effect of SNP on the different age groups, neonates from birth to less than 30 days of age (Age Group A) and infants and toddlers from 30 days to < 2 years (Age Group B) will be pooled for analysis. Based on the planning estimates of the study, patients from these two pooled age groups should represent approximately 25% of the target enrollment (~60 patients).

Preschool children from 2 years - < 6 years (Age Group C) and school age children from 6 yrs - < Tanner stage III (Age Group D) will also be pooled for analysis; patients from these two pooled age groups should also represent approximately 25% of the target enrollment (~60 patients).

In order to accurately determine whether a treatment difference is sufficient to stop the trial early ~~the target number of patients required for enrollment~~, the ~~a Data Monitoring Committee~~ will examine the results from the Blinded Study Drug Administration Phase after the first 40 patients have been enrolled, randomized and treated during the blinded treatment phase; the approximate expected distribution by age group is as follows ~~following number of patients have been enrolled and randomized~~:

- The initial 12 patients in Age Groups A & B
- The initial 12 patients in Age Groups C & D
- The initial 16 patients in Age Group E

The specific objective of the DMC ~~Data Monitoring Committee~~ is to determine the significance of the treatment difference ~~target sample size~~ based on the observed magnitude of the effect, expressed as a proportion (*treatment success* (sodium nitroprusside) / ~~Randomized~~ Treated with ~~to receive~~ sodium nitroprusside during the blinded treatment phase vs. *treatment success* (Placebo) / ~~Randomized~~ Treated with ~~to receive~~ Placebo during the blinded treatment phase. Determination of the treatment difference ~~Re-estimation of the sample size~~ will be performed ~~based on conditional power~~ after 67% (40/60) of the target sample size has been enrolled.

7.7.1 Primary Efficacy Analysis

The primary efficacy analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients ~~randomized to~~ received sodium nitroprusside}} = \pi_{\text{ Patients ~~randomized to~~ received receive placebo}}$
- H_A: $\pi_{\text{Patients ~~randomized to~~ received sodium nitroprusside}} \neq \pi_{\text{ Patients ~~randomized to~~ received receive placebo}}$

where

- $\pi_{\text{Patients ~~randomized to~~ received SNP}}$ = Proportion of *treatment successes* (sodium nitroprusside)
- $\pi_{\text{Patients ~~randomized to~~ received placebo}}$ = Proportion of *treatment successes* (placebo)

7.7.2 Primary Safety Analysis

The primary safety analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients ~~randomized to~~ received sodium nitroprusside}} = \pi_{\text{ Patients ~~randomized to~~ received placebo}}$
- H_A: $\pi_{\text{Patients ~~randomized to~~ received sodium nitroprusside}} \neq \pi_{\text{ Patients ~~randomized to~~ received placebo}}$

where

- $\pi_{\text{Patients ~~randomized to~~ received sodium nitroprusside}}$ = Proportion of patients ~~randomized to~~ who received SNP who experience a serious adverse event
- $\pi_{\text{Patients ~~randomized to~~ received placebo}}$ = Proportion of patients ~~randomized to~~ who received placebo who experience a serious adverse event

7.7.3 Interim Monitoring Based on Conditional Power Group Sequential Methods

The interim assessment of the treatment difference ~~for sample size adjustment~~ will be predicated on the primary efficacy endpoint and conducted using Group Sequential methods ~~a Conditional Power (CP) approach~~ as described by Pocock ~~Chen~~ (1977 ~~2004~~). The Data Monitoring Committee will conduct the analysis to determine whether the treatment difference is sufficient to stop the trial early ~~if a sample size adjustment is required~~. This assessment will be conducted after 67% of the patients from the original target sample size have either completed or withdrawn from the study. The instructions to the ~~Data Monitoring Committee~~ DMC for the treatment difference assessment ~~sample size adjustment~~ will be described in detail in the Data Monitoring Committee Charter. ~~An alpha level adjustment will not be necessary for the procedure described below, based on the procedure proposed by Chen, DeMets and Lan (2004).~~

In order for the ~~Data Monitoring Committee~~ DMC to calculate the treatment difference ~~conditional power~~ using the observed data, the treatment assignment codes will need to be provided to the ~~Data Monitoring Committee~~ DMC statistician. The intra-patient MAP_{B2} values will be required, including listings of the intra-patient post-baseline values during the blinded phase of the trial. ~~If an increase in the sample size is required, and the re-estimation is within the defined sample size limits pre-specified for the study, the Data Monitoring Committee will communicate the revised target sample size to the IVRS vender for the trial.~~ If the treatment difference does not meet the early stopping criteria, e~~E~~nrollment will continue towards the ~~new~~ target sample size, and the data will remain blinded to all involved parties with the exception of the ~~Data Monitoring Committee~~ DMC. Additionally, the ~~Data Monitoring Committee~~ DMC will not have any direct contact with the study Sponsor, or the clinical investigators.

The following steps will be used to evaluate the ~~sample size~~ treatment difference after the initial 40 randomized patients ~~from each pooled age group (either 12 or 16)~~ have been enrolled and have completed the Blinded Study Drug Administration Phase, or withdrawn prematurely.

The Group Sequential methods described by Pocock indicate that in order to control the type I error at 5%, the significance level at the interim analysis and the final analysis is as follows when the interim analysis is performed after 67% (40/60) of the target sample size has been enrolled.

Group Sequential Table 1.0

Significance Level Corresponding to the Pocock Stopping Boundary

Analysis	Subjects	Significance Level (α)
Interim	40/60	0.0407
Final	60/60	0.0215

At the interim analysis, test the null hypothesis at an α=0.0407 significance level using a two group χ^2 test of equal proportions. If the test is statistically significant with a p-value <0.0407, there will be sufficient evidence to reject the null hypothesis in favor of the alternative that a treatment difference exists between the two randomized treatment groups and the study can be stopped early for efficacy. Final decision will be based on recommendation from the DMC who will review overall efficacy and safety information. Alternatively, if a p-value <0.0407 is not achieved, the study will continue towards the target enrollment.

~~Compute conditional power for the primary hypothesis, using data from the initial patients randomized from each pooled age group (either 12 or 16).~~

~~If the conditional power is ≥ 0.5, compute the sample size necessary to increase conditional power to 0.8.~~

~~The Data Monitoring Committee will compare the re-estimated sample size calculated using the observed data, relative to the initial estimates for the study. Based on this evidence, the Data Monitoring Committee will proceed with the following action:~~

~~If the conditional power is ≥ 0.5, the sample size will be increased up to the maximum sample size pre-specified for the study.~~

~~If the conditional power is < 0.5, an analysis will be performed based on the predictive probability of achieving the endpoint within the maximum target sample size allocated for the study.~~

7.7.4 Sample Size Estimation

The overall sample size was calculated based on performing an un-stratified analysis of the proportion of patients classified as a *treatment success* between the 2 randomized treatment groups. With a balance randomization (1:1, SNP:Placebo), a difference in the proportion of *treatment successes* ranging from 34% to 40% would have 80% power to reject the null hypothesis in favor of the alternative (ref. Sample Size Table No. 1.0).

Sample Size Table No. 1.0

Two group χ^2 Test of Equal Proportions

Scenario	1	2	3	4
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
SNP, π_1	0.080	0.160	0.240	0.320
Placebo, π_2	0.420	0.530	0.630	0.710
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	8.328	5.920	5.392	5.203
Power (%)	80	80	81	80
n per group	30	30	30	30

Given the significance level corresponding to the Pocock stopping boundary as indicated in Group Sequential Table 1.0 above, the following scenarios identify the number of treatment successes that must be observed in the SNP treatment group to result in a statistically significant test of the null hypothesis given the placebo group success rate specified below.

Group Sequential Table 2.0

Required SNP Treatment Successes

Scenario	1	2	3	4
Analysis	Interim	Final	Interim	Final
n per group	20	30	20	30
Test significance level, α	0.0407	0.0215	0.0407	0.0215
Treatment Successes (Placebo)	2 (10%)	3 (10%)	4 (20%)	6 (20%)
Treatment Successes (SNP)	8 (40%)	11 (55%)	11 (37%)	15 (50%)

~~Scenario~~	~~5~~	~~6~~	~~7~~	~~8~~	~~9~~	~~10~~
~~**Test significance level, α**~~	~~0.050~~	~~0.050~~	~~0.050~~	~~0.050~~	~~0.050~~	~~0.050~~

1 or 2 sided test?	2	2	2	2	2	2
Group 1 proportion, π_1	0.125	0.125	0.125	0.125	0.125	0.125
Group 2 proportion, π_2	0.250	0.375	0.500	0.625	0.750	0.875
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	2.333	4.200	7.000	11.667	21.000	49.000
Power (%)	9	20	35	54	76	94
n per group	8	8	8	8	8	8

Based on the target sample size and the distribution of enrollment relative to the pooled analysis groups, approximately 30 patients from Age Group E (Adolescents from Tanner stage III - < 17 years) are scheduled to be randomized. However, based on the difference in the proportion of patients who are classified as a *treatment success* between the randomized treatment groups, 30 patients may not be required to detect a significant difference at the alpha = 0.05 level. For this reason, the proportion of patients classified as *treatment successes* will be compared after the initial 12 or 16 patients are randomized, depending on the pooled age group. If the difference in proportions has an odds ratio >12, then less than the 30 patients would be required to be randomized from Age Group E. Under this scenario, the randomization of patients from this specific age group would be stopped. Drawing from Age Groups A & B or Age Groups C & D, if the difference in the proportion of *treatment successes* between the randomized treatment groups requires more than 12 patients, the study could still meet its intended goal within the pre-defined total sample size of 60 patients by enrolling the minimum sample size, based on the conditional power calculated at the interim evaluation. Power estimates at the interim evaluation for 16 patients are presented in Sample Size Table No. 2.0. The re-estimated sample size is presented in Sample Size Table No. 3.0, again for 16 patients.

Sample Size Table No. 2.0

Two group χ^2 Test of Equal Proportions

Sample Size Table No. 3.0

Two group χ^2 Test of Equal Proportions

Scenario	**11**	**12**	**13**	**14**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2

~~**Group 1 proportion, π_1**~~	~~0.125~~	~~0.125~~	~~0.125~~	~~0.125~~
~~**Group 2 proportion, π_2**~~	~~0.500~~	~~0.625~~	~~0.750~~	~~0.875~~
~~**Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$**~~	~~7.000~~	~~11.667~~	~~21.000~~	~~49.000~~
~~**Power (%)**~~	~~80~~	~~80~~	~~80~~	~~80~~
~~**N per group**~~	~~23~~	~~14~~	~~9~~	~~6~~

Miscellaneous

27. References

A. The following reference was deleted

~~Chen, Y.H.J., DeMets, D.L. and Lan, K.K.G. (2004). Increasing the Sample Size When the Unblinded Interim Result is Promising, Statistics in Medicine, vol. 23, 1023-1038.~~

B. The following reference was added

Pocock, SJ (1977): Group sequential methods in the design and analysis of clinical trials, Biometrika 1977; 64(2): 191-199.

28. Table 1

Table 1 has been revised to be consistent with the changes described above. In addition, the protocol footnotes have been renumbered to increase readability.

TABLE 1: Schedule of Assessments: Sodium Nitroprusside Long-Term Infusion Study

Procedure	**Pre-study Drug Period (Up to 7 days prior to Study Drug administration)**	**Open-label Period (12 -24 hrs duration)**	**30 minute Blinded Study Drug Period**	**Study Drug d/c (Within 2 hours)**	**Follow-up (Up to 24 hours post blinded study drug)[1]**
Assessments					
Review Entry Criteria	X				
Informed Parental Permission/ HIPAA Consent	X				
Collect Demographic Data	X				
Medical History	X				
Physical Examination	X			X	X[2]
Vital Signs (SBP, DBP, MAP, HR)[3]	X[4]	X	X	X	X
B1	X[5]				
B2—Just Prior to Blinded Infusion		X			
Growth Parameters[6]	X				
Urine Output[7]	X	X	X	X	X
Serious Adverse Events/Adverse Events		X	X	X	X[8]
Concomitant Medication[9]	X[10]	X	X	X	X[11]
Concomitant Procedure[12]	X[10]	X	X	X	X[11]
Randomization of Blinded study drug		X			
Blinded Study Drug Administration			X		
Open-label Study Drug Administration		X			
Laboratory Assessments					
Pregnancy test (post-menarche females)	X[13]				
Electrolytes, BUN, creatinine	X			X	
Hematology (CBC & platelet count)	X			X	
Liver Enzymes (AST, ALT)	X			X	
Arterial Plasma Lactate level	X	Q 8 hours (± 30 min)		X	X[14]
Arterial Blood Gas with Co-oximetry (includes Methemoglobin)[15]	X	Q 8 hours (± 30 min)		X	X[14]
~~Mixed~~ Central Venous Blood Gas with Co-oximetry (includes Methemoglobin)[16]	X	Q 8 hours (± 30 min)		X	X[14]
~~Plasma~~ Blood for Thiocyanate and Cyanide[17] (central lab)	X	Q 8 hours (± 30 min)		X & 12 hr post d/c [18]	X[14]
Urine for Thiocyanate (central lab)[19]	X	Q 8 hours (± 30 min)		X	Q8 hrs (± 30 min) X 3

Table 1 footnotes:

1. End of Study assessment will be done at 24 hours post blinded study drug administration, except where noted.
2. To be performed 18-30 hours following the termination of study-drug administration.
3. Vital sign measurements as described in protocol, sections 3.4.2 – 3.4.7.1. Vital signs will then be collected every 12 ± ½ hours for 24 hours post blinded study drug administration. VS measurements described for the Open-label treatment period refer to both institutionally supplied SNP and investigational SNP (study drug), see also Section 3.4.3 (#9).
4. ~~Obtain one set of vital sign measurements as early as possible. For surgical patients, based upon clinical judgment, this may be MAP_{B1}.~~ Obtain vital sign measurements. ~~immediately prior to induction if B1 will occur during general anesthesia; otherwise, as early as practical~~. Based upon clinical judgment, this may be MAP_{B1} for some patients.
5. MAP_{B1} will be obtained prior to the start of the SNP open-label infusion and after a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP)
6. Growth parameters will include weight, height/ length, and Tanner stage, if ≥6 years old.
7. Measurements to be performed at time of urine thiocyanate sample collection, if feasible.
8. AEs will be followed for 24 hours and SAE will be followed for 30 days, after the completion of study drug administration.
9. CRFs will document administration of concomitant medications that (1) affect blood pressure, as defined in Section 3.2.3.1, or (2) are associated with SAE's. All concomitant medications will be recorded in source documents, such as the patients' medical charts.
10. Within 72 hours of study drug administration.
11. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
12. Clinically meaningful, unexpected, and invasive procedures only.
13. To be performed within 48 hours of study drug administration.
14. Following concomitant SNP d/c, if feasible.
15. ABG sampling preferred, sample collected at drug d/c only if line is still in.
16. ~~Mixed~~ Central Venous Blood Gas done only if CVC is indwelling.
17. Additional cyanide and thiocyanate tests are permitted provided that the investigator believes the tests to be in the patient's best interest and the volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).
18. Blood for cyanide & thiocyanate at 12 hrs ± 30 min, post study drug d/c, only if arterial line in place.
19. Urine collection details are described in section 3.4.7.2 and the MOP.
20. ~~Immediately prior to induction if B1 will occur during general anesthesia; otherwise as early as practical~~

PROTOCOL

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Number: NICHD-2003-09-LT

Study Drug: Sodium Nitroprusside

IND: 71,979

Medical Monitor: Bernard Brownstein, M.D.

Principal Investigators:

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Sponsor: The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

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APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol and its associated investigator brochure for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

And I have fully discussed the objectives of this trial and the contents of this protocol with representatives of PODS Center and BPCA–Coordinating Center.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and in accordance with all applicable local, state, and federal regulations.

I understand that, should the decision be made by the PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

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CO-PRINCIPAL INVESTIGATOR Scott Schulman, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol and its associated investigator brochure for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

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I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

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TABLE OF CONTENTS

PROTOCOL SYNOPSIS

Protocol Title:	A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects
Protocol Number:	NICHD-2003-09-LT
Sponsor:	National Institute of Child Health and Human Development
Product:	Sodium Nitroprusside
Objectives:	1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours 2. To assess the potential for rebound hypertension following administration of sodium nitroprusside for 12 hours or more
Study Design:	This is a phase II, randomized, double blind, withdrawal to placebo study examining the efficacy, safety and tolerability of sodium nitroprusside in pediatric subjects.
Study Population:	Children up to 17 years of age who require long term (at least 12 hour) blood pressure control will be eligible for study.
Number of Subjects:	A target of approximately 60 patients will be enrolled.
Number of Sites:	Up to 15
Duration of Subject Participation:	Enrollment is anticipated to begin in 2008 and to be complete in approximately 24 months. Patients will be followed for up to 30 days following receipt of study drug.
Treatment:	Subjects who require vasodilator therapy for relatively long time periods will receive open-label infusion of sodium nitroprusside for at least 12 hours but not greater than 24 hours.
Dose Schedule:	Patients will be randomized to receive either placebo or sodium nitroprusside for 30 minutes following at least 12- hours but not more than 24 hours of open-label infusion of sodium nitroprusside.
Estimated Start:	Q4 2008
Estimated Finish:	Q3 2010
Ethics	All regulations stated in 21 CFR Parts 50, 56, and 312 and recommendations outlined in the ICH Guidelines for Good Clinical Practice, as well as all other applicable local and national laws and regulations, will be adhered to throughout this trial.

Safety:	The safety of the drug will be assessed by multiple subject assessments of vital signs, physical exams, clinical tests and laboratory evaluations. Adverse events will be monitored and tracked. All SAEs will be closely monitored throughout the course of the study.
Statistical Consideration:	The trial will be sized to detect the loss of as little as 50% of the expected blood pressure lowering effect of the chosen dose of sodium nitroprusside during the 30 minutes of withdrawal to placebo.

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BP	Blood Pressure
BPCA-CC	Best Pharmaceuticals for Children Act Coordinating Center
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
cGMP	cyclic Guanosine Monophosphate
CN^-	Cyanide
CRA	Clinical Research Associate
CRF	Case Report Form
d/c	Discontinuation
DCRI	Duke Clinical Research Institute
DBP	Diastolic Blood Pressure
DMC	Data Monitoring Committee
ECMO	Extracorporeal Membrane Oxygenation
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
hr	Hour

ACRONYMS AND ABBREVIATIONS

HR	Heart Rate
IB	Investigational Brochure
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
kg	Kilogram
MAP	Mean Arterial Pressure
mcg	Microgram
mEq/L	Milliequivalent per Liter
mcgs	Micrograms
min	Minute
mL	Milliliter
mm Hg	Millimeters of Mercury
mmol/L	Millimoles per Liter
NO	Nitric Oxide
NICHD	National Institute for Child Health and Human Development
NONMEM	Nonlinear Mixed Effect Model
NTG	Nitroglycerin
PD	Pharmacodynamic
PaO_2	Arterial Partial Pressure of Oxygen
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SCN	Thiocyanate
SNP	Sodium Nitroprusside
μM	Micromoles per liter, Micromolar

1.0 Background and Rationale

Blood pressure control in children is a significant concern in the intensive care unit (ICU), where management of arterial pressure is often necessary during periods of acute physiologic stress such as occurs after certain surgical and medical procedures. Examples of surgical procedures that require blood pressure control in the intensive care unit following surgery include aortic coarctation repair, Ross procedure (pulmonary valve autograft), and solid organ transplantation. Medical conditions requiring control of systemic arterial pressure include renal disease, drug therapy (corticosterioids and immunosuppression agents), and procedures such as extracorporeal membrane oxygenation (ECMO).

A wide variety of drugs of various therapeutic classes have been utilized for either controlled hypotension in the operating room or prevention of hypertension in the pediatric ICU. These drug classes include calcium channel blockers (Tobias et al, 1996), beta-adrenergic antagonists (Kay et al, 2001), ganglionic blockers (DuToit, 1970 and Gallagher and Milliken, 1979), inhalation anesthetics (Tobias, 1998) and direct acting vasodilators such as nitroglycerin and sodium nitroprusside (SNP) (Kaplan, 1980, and Tinker, 1976 Groshong, 1996, and Sinaiko, 1996). Although many vasodilator agents are available to lower blood pressure in the operating room and intensive care unit setting, few have been systematically studied in children.

SNP is a direct acting vasodilator commonly used for blood pressure control. It produces vascular smooth muscle relaxation when its metabolism in the red blood cell results in the liberation of nitric oxide (NO). NO then activates the enzyme guanylyl cyclase. This activation results in the formation of increased intracellular levels of cyclic guanosine monophosphate (cGMP). The result is vasodilation.

1.1 Metabolism

Five molecules of cyanide (CN^-) are released when SNP is metabolized in the red blood cell. The major metabolic pathway for CN^- is conversion to thiocyanate (SCN). This conversion occurs enzymatically via two sulfur transferase systems: 1) rhodenase (the primary pathway) and 2) beta-mercaptopyruvate-cyanide sulfurtransferase. Rhodenase is ubiquitous throughout the body, but it is highly concentrated in the liver. Rhodenase catalyzes the transfer of sulfur from a sulfur donor molecule such as thiosulfate (Na_2SO_3) to cyanide and thereby the formation of thiocyanate (SCN). SCN is subsequently eliminated in the urine and can therefore serve as a marker of cyanide exposure.

The ability of rhodenase to catalyze the conversion of cyanide to thiocyanate (SCN) is limited by the availability of sulfur donors in the body. Thus the provision of exogenous sulfur donors such as thiosulfate (Na_2SO_3) in the setting of acute cyanide intoxication is a potentially life-saving intervention (Pasch et al, 1983, Cole and Vesey, 1987).

One out of every five CN^- ions liberated by the metabolism of SNP binds to methemoglobin to form the non-toxic cyanomethemoglobin. The creation of additional quantities of methemoglobin by the intravenous infusion of sodium nitrite can thus provide additional CN^- buffering capacity. The resultant methemoglobinemia can then be treated with the administration of intravenous methylene blue.

Additional metabolic pathways for CN^- include the conversion of hydroxycobalamine (vitamin B12a) to cyanocobalamine, and conversion to 2-aminothiazoline 4-carboxylic acid.

If the above three pathways (rhodenase, methemoglobin, hydroxycobolamine) are overwhelmed, cyanide will bind to mitochondrial cytochrome oxidases and poison cellular oxidative phosphorylation. Cellular hypoxia is induced when cyanide inhibits the electron transport chain at cytochrome a_3. Oxygen cannot be utilized, mixed venous

oxygen tension rises and the generation of high-energy adenosine triphosphate (ATP) is blocked. The cell reverts from aerobic to anaerobic metabolism, with the subsequent generation of pyruvate and lactate. Acidosis ensues, and with it, deterioration in the organ systems most dependent on oxidative metabolism: the central nervous system and heart.

Clinical manifestations of cyanide toxicity to the central nervous system include headache, anxiety, agitation, confusion, lethargy, convulsions and coma. Cardiovascular manifestations include progressive heart failure with both loss of contractile force (negative inotropy) and slowing of rate (negative chronotropy). Bradycardia and hypotension are commonly observed pre-morbid events associated with cyanide toxicity.

In patients receiving SNP, the earliest, most sensitive signs of cyanide toxicity are acidosis, elevated mixed venous oxygen tension, and rising blood lactate levels. Venous blood that appears "bright" red due to the inability of the tissues to extract oxygen should suggest cyanide toxicity. Arterial and mixed venous blood gas analysis with co-oximetry can help confirm the diagnosis.

1.2 Previous Studies

SNP was first discovered in 1850. Its hypotensive effects were noticed in 1929, and its first therapeutic use was reported by Page et al. in 1955. Moraca et al. first described the clinical use of SNP for deliberate hypotension during surgical procedures in 1962. Since then, it has been widely used to control blood pressure in infants and children in the perioperative period.

Despite its widespread use, there is a paucity of information on its safety, efficacy, and pharmacokinetic/pharmacodynamic relationships in children. Davies et al (1975) and Bennett and Abbott (1977) described their retrospective experience with SNP used to induce deliberate hypotension in small cohorts of children. Both authors observed that younger patients required more SNP than older ones to achieve comparable degrees of

blood pressure control. In their small retrospective cohort, Bennett and Abbott recommended that doses of 10 micrograms/kilogram/minute were necessary to achieve satisfactory blood pressure response. Davies et al described three possible responses to SNP administration in children: 1) a constant response to "conventional" doses < 3 mg/kg; 2) a tachyphylactic response characterized by continuously escalating dose requirement (> 3 mg/kg) to achieve a satisfactory blood pressure; and 3) resistance to the blood pressure lowering effects of the drug. They cautioned against using total doses that exceeded 3 mg/kg or continuing administration of SNP in the latter two scenarios. Firm conclusions cannot be drawn because these small case series were not randomized controlled trials with specific pharmacodynamic endpoints.

Yaster et al (1986) compared SNP to nitroglycerin (NTG) for inducing hypotension in a group of 14 adolescents. They found doses of SNP between 6-8 micrograms/kg/minute superior to NTG at any dose in the reliable induction of hypotension for children and adolescents undergoing scoliosis, craniofacial or hepatic surgery.

Hersey et al (1997) performed a randomized trial comparing SNP to the dihydropyridine calcium channel antagonist nicardipine in 20 healthy adolescents with idiopathic scoliosis undergoing spinal fusion. Target blood pressures were easily obtainable in both groups and operating conditions were comparable. The time to restoration of baseline blood pressure after termination of the infusion was significantly longer in the nicardipine group. Interestingly, blood loss was significantly greater in the SNP group. Details on SNP dose requirements were not provided.

Przybylo et al (1995) described CN^- and SCN blood levels in ten children who received SNP at doses up to 10 micrograms/kg/min (mean infusion rate 6 microgram/kg/min) while undergoing cardiopulmonary bypass for repair of complex congenital cardiac defects. CN^- levels rose as a function of time while SNP was infused, and rapidly fell when SNP was discontinued. Despite the fact that some children demonstrated serum CN^- levels above the generally accepted threshold of 0.5 micrograms/ml, no patient

developed clinically apparent toxicity. Kazim et al (1996) questioned the validity of the results of this study because of the CN^- assay methods utilized.

Linakis et al (1991) retrospectively examined physician-ordering practice as it pertained to blood cyanide levels in children receiving SNP. They sought to determine how the laboratory determinations were used to monitor patients and if there was clinically apparent toxicity in children found to have cyanide concentrations exceeding the "normal" limit of 500 micrograms/liter. They found poor correlation between blood cyanide concentration and dose or duration of therapy in patients whose cyanide levels were "toxic." Thiocyanate determinations were normal and no child manifested signs or symptoms of cyanide toxicity. They concluded that further pediatric studies were needed.

2.0 Study Objectives

We propose a multicenter trial that will provide guidance for the use of SNP to reduce blood pressure in pediatric patients. The trial is a randomized, double-blinded withdrawal to placebo trial. The aims of the trial are:

1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours
2. To assess the potential for rebound hypertension during the 30-minute Blinded Phase following administration of sodium nitroprusside for 12 hours or more.

To meet these study aims, the following study phases, defined in Section 3.1.1, will have the following objectives:

- Open-Label Study Drug Administration (Dose-Titration) Phase
  The objective during this phase of the study is to determine the effectiveness and safety of SNP for controlling blood pressure during stable infusion lasting at least 12 hours.

- Blinded Study Drug Administration Phase

  The primary endpoint for the study will be determined during this phase of the study. The primary endpoint is the change in mean arterial pressure (MAP) recorded during the Blinded Study Drug Administration Phase in the absence of other stimuli. The primary objective is to determine the persistence of sodium nitroprusside versus placebo for reducing blood pressure in pediatric patients

  The secondary objectives during this phase of the study are as follows:

  1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience offset during the 30-minute blinded study drug period.
  2. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience rebound hypertension during the 30-minute blinded study drug period.
  3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the 30-minute blinded study drug period.
  4. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the 30-minute blinded study drug period.
  5. To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.

- Follow-up Phase

The following objectives to be evaluated during this phase of the study are as follows:

1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the Follow-up Period.
2. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the Follow-up Period.
3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience changes in individual physical examination parameters represented as either normal or abnormal from the Pre-Study Period to the end of the Follow-up Period.
4. To compare the changes (values recorded during the end of the Follow-up Period minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.
5. To compare the changes (values obtained during the two-hour period immediately following the stop of blinded study drug minus values obtained during the Pre-Study Drug Period) in individual laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo.

3.0 Investigational Plan

3.1 Overall Study Design and Plan: Description

This is a phase II, multicenter, randomized, double-blind placebo-controlled, parallel group study to determine the persistence of the effect of SNP on blood pressure and to assess the potential for rebound hypertension associated with prolonged infusion in pediatric subjects.

Target MAP is defined as the clinically appropriate MAP as determined by the investigator taking into account the clinical presentation and medical needs of the subject. The investigator may change the target MAP at his/her discretion based on clinical needs during the course of the study. The anticipated Target MAP must be at least 20 mmHg below MAP_{B1}--(15 mm Hg for subjects < 2 years old), see next definition, for the patient to be eligible for enrollment, see Inclusion criterion #4.

The initial baseline MAP (B1) is defined as the blood pressure measurement taken prior to the initiation of SNP administration, either institutionally-supplied or open-label study drug after at least a 5-minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP). See Figure 1 below.

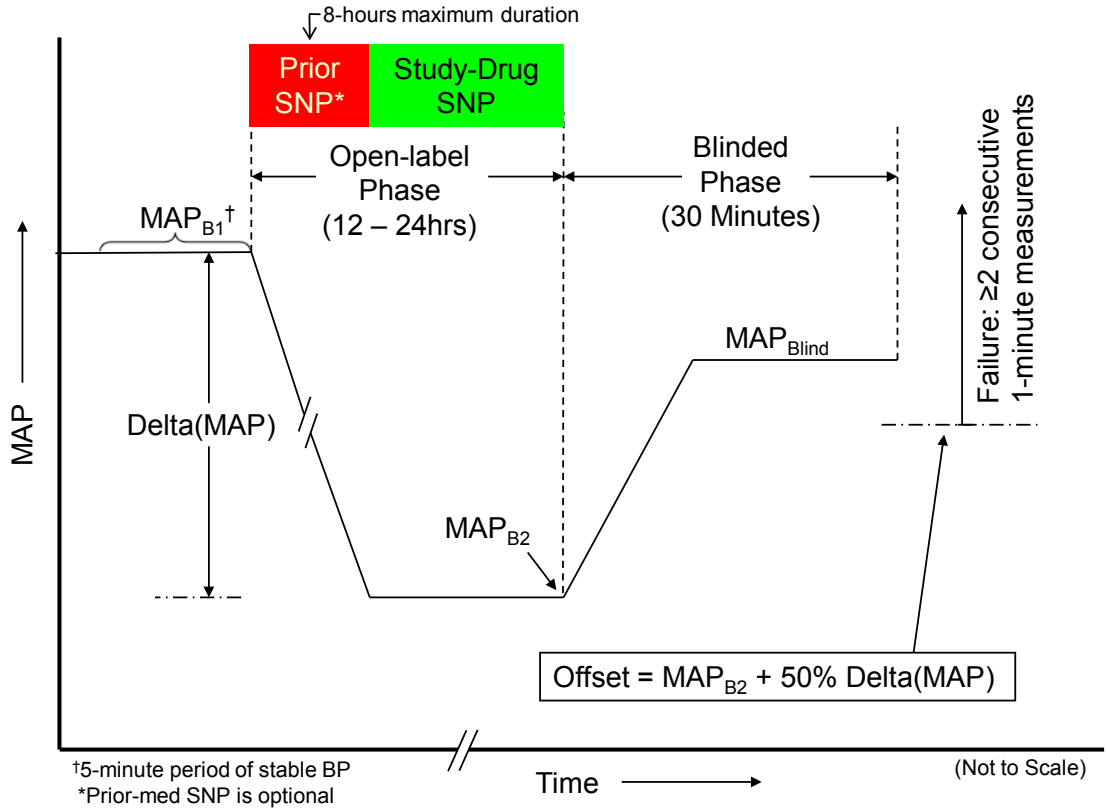
The subsequent baseline MAP (B2) is defined as the blood pressure measurement taken just prior to the initiation of blinded study drug after at least a 5 minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). Prior to establishing B2, there shall have been no changes in the SNP infusion rate for a period of at least 20 minutes during which the infusion rate of SNP will be at least 0.5 mcg/kg/min.

Delta(MAP) is defined as the difference between MAP_{B1} and $MAP_{B2.}$

Offset is defined as MAP_{B2} plus 50% Delta(MAP)

Figure 1

Study Treatment Phases with MAPs and Delta(MAP)



- MAP_{B1} = MAP prior to start of Open-label Study Drug Administration Phase
- MAP_{B2} = MAP immediately prior to start of Blinded Study Drug Administration Phase
- Delta(MAP) = $MAP_{B1} - MAP_{B2}$
- Offset = MAP_{B2} + 50% Delta(MAP)
- Treatment Failure: During the blinded phase, ≥2 consecutive 1-minute MAP measurements greater than Offset [MAP_{B2} + 50% Delta(MAP)]

Example: Subject's MAP immediately prior to start of the Open-label Study Drug Administration Phase is 100 mmHg (MAP_{B1}). At the end of the Open-label Study Drug Administration Phase, MAP is 60 mmHg (MAP_{B2}). Thus, Delta(MAP) = 40 mmHg. For *treatment success*, MAP during the Blinded Study Drug Administration Phase cannot exceed MAP_{B2} + 50% Delta(MAP), or 80 mmHg, for any 2 consecutive MAP_{Blind} measurements, obtained at 1-minute intervals.

Approximately 15 centers will participate in subject recruitment to complete the study.

Approximately sixty (60) patients who require long term (at least 12 hours) blood pressure control will be enrolled. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment.

Any patient who starts the blinded study drug administration period will be considered complete for analysis. Enrolled subjects will be randomized in equal proportions to receive either placebo or SNP for the duration of the blind-treatment period, which will immediately follow the open-label infusion of SNP.

3.1.1 Definition of Study Periods

Study periods are as follows:

- Pre-study drug administration: a period of up to 7 days preceding the start of study drug administration during which informed parental permission, and other enrollment procedures take place.
- Open-label study drug administration (Dose-Titration): The period of open label study drug administration will be at least 12 hours but not greater than 24 hours, including the time of infusion of institutionally-supplied SNP, if any. This period will begin at the start of SNP administration, either study drug or institutionally-

supplied. The duration of infusion of the institutionally-supplied SNP will be no longer than 8 hours. Randomization will normally occur during this period.

- Blinded study drug administration: The period beginning with the start of blinded study drug administration and ending with the discontinuation of blinded study drug. It immediately follows the open-label period and will be no longer in duration than 30 minutes.
- Follow up: The period immediately following blinded study drug administration and ending 30 days after completion of study drug administration. AEs will be followed for 24 hours after termination of study drug. SAEs will be followed for 30 days.

Safety will be assessed via the evaluation of adverse events, pre- and post-treatment laboratory results, and vital sign data. The efficacy endpoints will mainly be assessed by examining blood pressure parameters.

3.2 Selection of Study Population

Children up to 17 years of age who require pharmacologic blood pressure control for at least 12 hours will be eligible for enrollment into the study. Blood pressure control is defined as the maintenance of the subject's MAP to within 90-110% of a target MAP specified by the physician.

Five pediatric age groups will be enrolled in this trial:

Group A: Neonates from birth to less than 30 days of age
Group B: Infants and toddlers from 30 days to < 2 years
Group C: Preschool children from 2 years - < 6 years
Group D: School age children from 6 yrs - < Tanner stage III
Group E: Adolescents from Tanner stage III - < 17 years.

Tanner III refers to the onset of puberty and occurs at different ages in different individuals. The mean age at onset of Tanner III ranges from 12.4 to 13.1 years in males, and 11.9 to 12.6 years in females. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment (see Appendix A).

3.2.1 Inclusion Criteria

Subjects must meet all of the following criteria:

1. Subject is less than 17 years of age.
2. An in-dwelling arterial line is clinically indicated.
3. Subject's parent or legal guardian is willing and able to give informed parental permission signing and dating an IRB-approved informed parental permission containing all of the elements of informed consent, and subject provides assent, signing an IRB-approved and –required informed assent, if applicable.
4. Subject is anticipated to require a minimum of 20 mm Hg (15 mm Hg for subjects < 2 years old) reduction in MAP for at least 12 hours using SNP [i.e., $MAP_{B1} - MAP_{B2} \geq 20$ mm Hg (15 mm Hg for subjects < 2 years old)]

3.2.2 Exclusion Criteria

Subjects will be excluded from study if any of the following criteria exist:

1. Subject weighs < 3.0 kg.
2. Subject has a known allergy to SNP.
3. Subject has a known mitochondrial cytopathy with a disorder of oxidative phosphorylation or of respiratory chain enzymes.
4. Subject has a contraindication to vasodilator therapy for control of blood pressure during surgery or in the intensive care unit.
5. Subject has raised intracranial pressure.
6. Subject is anticipated to need anti-hypertensive drugs other than Sodium Nitroprusside either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g.

local anesthetics, clonidine, etc.) within three terminal half-lives (3X T½ β) of the blinded study drug period. However, patients receiving stable doses of an anti-hypertensive drug(s) prior to the initiation of study drug may be enrolled.

7. Subject has any serious medical condition which, in the opinion of the investigator, is likely to interfere with study procedures.
8. Subject is moribund (death likely to occur within 48 hours).
9. Subject has a positive result for the urine or serum HCG test administered at screening.
10. Subject has participated in other clinical trials for investigational drugs within 30 days prior to enrollment
11. Subject has received or will have received Sodium Thiosulfate within 6 hours prior to the start of the open-label period.
12. Subject is either on, or anticipated to be on, ECMO.

3.2.3 Prior and Concomitant Therapy, including Medications and Procedures

3.2.3.1 BP-Affecting Drugs

The following medications will be considered to affect blood pressure, and their use, both prior to and concomitant with, SNP infusions will be restricted as described in Sections 3.2.3.2 and 3.2.3.3. There will be no restrictions on the administration of non-study drug medications following the end of the blinded treatment period.

1) Anti-hypertensives and diuretics
2) Beta blocking agents
3) Ace-inhibitors, alone or in combinations
4) Calcium channel blockers and diuretics
5) Diuretics and potassium-sparing agents
6) Peripheral vasodilators
7) NSAIDs and narcotic analgesics
8) Parasympathomimetics

9) Antipsychotics and antineurotics
10) Psychostimulants and nootropics
11) Dopaminergic agents
12) Inotropic agents
13) Sedatives and hypnotics
14) Blood and blood products

3.2.3.2 Prior medications – administration started prior to B1

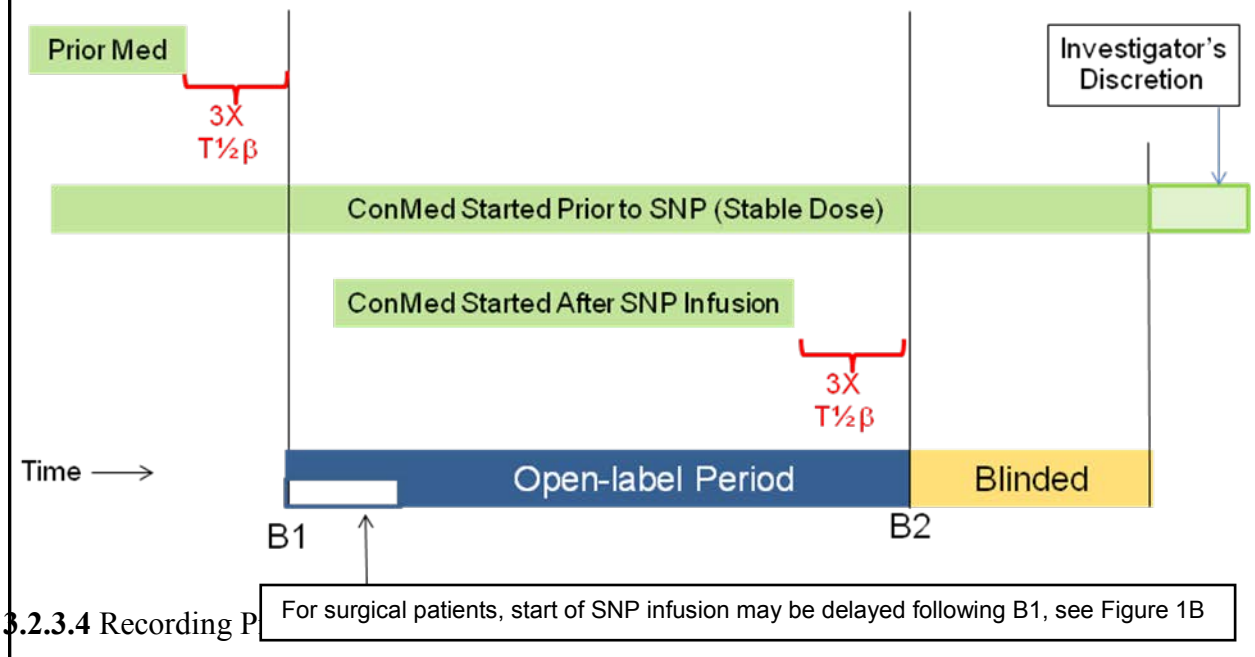
BP-affecting drugs that are started prior to B1 will be either (1) discontinued at least 3 terminal half-lives (3X T½ β) prior to B1, or (2) continued at a stable dose until at least the end of the blinded infusion period, see Figure 2.

3.2.3.3 Concomitant medications – administration started after B1

BP-affecting drugs that are started after B1 will be discontinue at least 3 terminal half-lives (3X T½ β) prior to B2 (i.e., the start of the blinded treatment phase), see Figure 2.

Figure 2

Time Restriction for the Administration of Medications Affecting Blood Pressure



3.2.3.4 Recording P

All concomitant medications that affect blood pressure, sodium thiosulfate, and clinically meaningful, unexpected, and invasive procedures will be recorded for the period beginning 72 hours prior to study drug administration through 24 hours post-study drug conclusion. The dates of administration and reason for use must be included. All concomitant medications will be collected for SAEs occurring within 30 days following study drug administration.

3.2.3.5 SNP as Concomitant Medication

Subjects may receive institutionally-supplied SNP prior to the initiation of study drug administration; however, administration of institutionally-supplied SNP will be discontinued immediately prior to the initiation of study drug administration, and the initial infusion rate of study-drug SNP will be the same as the discontinued institutionally-supplied SNP (see Section 3.4.3 #6). VS measurements described for the

Open-label treatment period in Table 1 and Section 3.4.3 refer to both institutionally supplied SNP and investigational SNP (study drug).

Subjects may receive institutionally-supplied SNP after the conclusion of the SNP Blinded Treatment Period at the discretion by the investigator.

The start and stop times and dates, and dosage of institutionally-supplied SNP administration will be recorded on the appropriate CRF.

3.2.3.6 Use of Sodium Thiosulfate

Administration of sodium thiosulfate will be prohibited from 6 hours prior to the start of the open-label period until the end of the blinded-study period, excepted in cases in which nitroprusside or cyanide toxicity is suspected, in which case, the administration of sodium thiosulfate, as described in Appendix E, is recommended.

To facilitate the assessment of cyanide clearance, investigators are encouraged not to co-administer sodium thiosulfate with institutionally-supplied SNP, which may, at the investigator's discretion, be administered following the conclusion of the blinded-study period. Should sodium thiosulfate be co-administered with institutionally-supplied SNP following the conclusion of the blinded-study period, blood and urine samples will not be tested for cyanide and Thiocyanate in these subjects once the sodium thiosulfate administration has begun.

3.3 Efficacy and Safety Assessments

3.3.1 Efficacy and Safety Measurements

Table 1 is a schematic representation of study assessments and procedures.

3.3.2 Safety Assessments

Safety assessments will include monitoring the tolerability of the SNP infusion and assessing physical examinations, vital signs, clinical laboratory values, concomitant medications and procedures, and adverse events throughout the study. SAEs will be collected for 30 days following completion of study drug administration.

In cases of discharge from the hospital before 30 days, parent (or guardian) will be contacted to determine if any SAE's occurred following discharge but within 30 days of study drug discontinuation. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. See section 4.2 for specific adverse event parameters and actions to be taken. See section 3.4.7.2 for supplemental cyanide and thiocyanate testing.

3.3.3 Drug Concentration Measurements

Cyanide, thiocyanate, methemoglobin, lactic acid, and arterial blood gas analysis will be performed throughout the trial to indirectly query SNP levels and determine subject safety.

TABLE 1: Schedule of Assessments: Sodium Nitroprusside Long-Term Infusion Study

Procedure	Pre-study Drug Period (Up to 7 days prior to Study Drug administration)	Open-label Period (12 -24 hrs duration)	30 minute Blinded Study Drug Period	Study Drug d/c (Within 2 hours)	Follow-up (Up to 24 hours post blinded study drug)[1]
Assessments					
Review Entry Criteria	X				
Informed Parental Permission/ HIPAA Consent	X				
Collect Demographic Data	X				
Medical History	X				
Physical Examination	X			X	X[2]
Vital Signs (SBP, DBP, MAP, HR)[3]	X[4]	X	X	X	X
B1	X[5]				
B2—Just Prior to Blinded Infusion		X			
Growth Parameters[6]	X				
Urine Output[7]	X	X	X	X	X
Serious Adverse Events/Adverse Events		X	X	X	X[8]
Concomitant Medication[9]	X[10]	X	X	X	X[11]
Concomitant Procedure[12]	X[10]	X	X	X	X[11]
Randomization of Blinded study drug		X			
Blinded Study Drug Administration			X		
Open-label Study Drug Administration		X			
Laboratory Assessments					
Pregnancy test (post-menarche females)	X[13]				
Electrolytes, BUN, creatinine	X			X	
Hematology (CBC & platelet count)	X			X	
Liver Enzymes (AST, ALT)	X			X	
Arterial Plasma Lactate level	X	Q 8 hours (± 30 min)		X	X[14]
Arterial Blood Gas with Co-oximetry (includes Methemoglobin)[15]	X	Q 8 hours (± 30 min)		X	X[14]
Central Venous Blood Gas with Co-oximetry (includes Methemoglobin)[16]	X	Q 8 hours (± 30 min)		X	X[14]
Blood for Thiocyanate and Cyanide[17] (central lab)	X	Q 8 hours (± 30 min)		X & 12 hr post d/c [18]	X[14]
Urine for Thiocyanate (central lab)[19]	X	Q 8 hours (± 30 min)		X	Q8 hrs (± 30 min) X 3

1. End of Study assessment will be done at 24 hours post blinded study drug administration, except where noted.
2. To be performed 18-30 hours following the termination of study-drug administration.
3. Vital sign measurements as described in protocol, sections 3.4.2 – 3.4.7.1. Vital signs will then be collected every 12 ± ½ hours for 24 hours post blinded study drug administration. VS measurements described for the Open-label treatment period refer to both institutionally supplied SNP and investigational SNP (study drug), see also Section 3.4.3 (#9).
4. Obtain vital sign measurements. Based upon clinical judgment, this may be MAP_{B1} for some patients.
5. MAP_{B1} will be obtained prior to the start of the SNP open-label infusion and after a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP)

6. Growth parameters will include weight, height/ length, and Tanner stage, if ≥6 years old.
7. Measurements to be performed at time of urine thiocyanate sample collection, if feasible.
8. AEs will be followed for 24 hours and SAE will be followed for 30 days, after the completion of study drug administration.
9. CRFs will document administration of concomitant medications that (1) affect blood pressure, as defined in Section 3.2.3.1, or (2) are associated with SAE's. All concomitant medications will be recorded in source documents, such as the patients' medical charts.
10. Within 72 hours of study drug administration.
11. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
12. Clinically meaningful, unexpected, and invasive procedures only.
13. To be performed within 48 hours of study drug administration.
14. Following concomitant SNP d/c, if feasible.
15. ABG sampling preferred, sample collected at drug d/c only if line is still in.
16. Central Venous Blood Gas done only if CVC is indwelling.
17. Additional cyanide and thiocyanate tests are permitted provided that the investigator believes the tests to be in the patient's best interest and the volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).
18. Blood for cyanide & thiocyanate at 12 hrs ± 30 min, post study drug d/c, only if arterial line in place.
19. Urine collection details are described in section 3.4.7.2 and the MOP.

3.4 Study Visits and Procedures

3.4.1 Informed Parental Permission

Prior to the start of any study-related procedure, a signed and dated informed parental permission, containing all elements of informed consent and, if applicable, assent must be obtained and documented in the subject's medical record (See Appendix B).

3.4.2 Pre-study drug administration procedures

The following procedures will be completed prior to the administration of study drug:

1) Obtain signed and dated informed parental permission/HIPAA authorization/assent.
2) Collect demographic data and medical/surgical history.
3) Record diagnosis.
4) Perform a pertinent physical examination.

5) Obtain vital sign measurements. Based upon clinical judgment, this may be MAP_{B1} for some patients.
6) Determine subject height in centimeters and subject weight in kilograms (for calculation of appropriate study drug dose).
7) Collect urine and blood samples for laboratory evaluations as per Table 1. Pregnancy test if required must be done within 48 hours prior to study drug administration. (If the screening pregnancy test will have been more than 48 hours prior to the start of the study drug administration, then the test will be repeated.)

   To minimize the blood volume obtained under this protocol, laboratory evaluations performed prior to the consenting of the patient as part of the standard of care of the patient and within 7 days of the administration of study drug may be substituted for these procedures.
8) Document concomitant medications that may affect blood.

3.4.3 Open-Label Study Drug Administration (Dose-Titration) Procedures:

The following procedures should be performed sequentially unless otherwise indicated.

1) Stabilize sedation/analgesia.
2) Insert arterial line if not already in place.
3) Obtain baseline vital sign measurements prior to the start of SNP administration, either institutionally-supplied or open-label study drug. This defines B1.
   A) The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in drug concentrations which may affect BP).
   B) If MAP_{B1} is not obtained immediately prior to the initiation of infusion of SNP, a VS measurement will be obtained immediately prior to the initiation of the open-label SNP infusion (time=0).
4) Determine and record target MAP.
5) If the difference between B1 and the target MAP is <20 mmHg (15 mmHg for subjects <2 years old), the patient will be withdrawn from the study and not given study drug.

6) Begin administration of open-label study drug at a dose not to exceed 0.3 mcg/kg/min, or, if applicable, at the infusion rate of the institutionally-supplied SNP.
7) The dose of open-label SNP will be titrated according to the subject's BP response such that the target MAP, chosen by the study physician, is achieved ±10%. If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose. The duration of open-label study drug administration will be at least 12 hours but less than 24 hours.
8) Revise target MAP as clinically indicated; titrate SNP to achieve new target MAP (±10%).
9) Obtain vital sign measurements every one minute for the first 10 minutes then every 5 ± 1 minutes for an additional 20 minutes after initiation of open-label study drug infusion (and, if applicable, institutionally-supplied SNP) and after each dosage adjustment. After the initial 30 minutes, once a stable dose is achieved and BP control is satisfactory, vital sign measurements will be obtained every ≤ 20 minutes. Additionally, obtain vital sign measurements in a similar manner whenever it is necessary to change the open-label drug infusion rate.
10) Randomize patient
11) Collect blood samples for laboratory evaluations as per Table 1.
12) Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures, and adverse events.
13) Whenever an adverse event occurs, obtain vital sign measurements. If clinically appropriate, a blood sample for safety including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations may be drawn.

3.4.4 Blinded Study Drug Administration Procedures

1) Obtain vital sign measurements immediately prior to the start of blinded study drug administration. This defines MAP_{B2}. There must be 5 minutes of stable conditions and 20 minutes of no changes in Open-label study drug prior to starting the blinded study drug administration phase.
2) Begin 30-minute blinded study drug administration as described in Section 3.4.8.
3) Record blood pressure and heart rate every one minute for the duration of blinded study drug administration.
4) If blood pressure control is lost (defined as loss of 50% of delta MAP for ≥2 consecutive 1-minute measurements) the blinded study drug is discontinued when there is a safety concern or the MAP reaches 120% of MAP_{B1}.
5) Record concomitant medications associated with an SAE or that may affect blood pressure, clinically meaningful, unexpected, and invasive procedures and adverse events. Whenever an adverse event occurs, obtain vital sign measurements and, if appropriate, a blood sample for safety analysis including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations.

3.4.5 Study Drug Discontinuation (Within 2 hours of discontinuing study drug)

1) Collect urine and blood samples for laboratory evaluations listed in Table 1
2) Conduct a pertinent physical examination and perform all other assessments listed in Table 1 for this study phase.

3.4.6 Follow up Procedures

The following will be performed after completion of study drug administration through 24 hours post study drug end:

1) If applicable, record the estimated blood loss and fluid intake, including blood and blood products and output during the trial period.
2) Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
3) Record vital signs at 12 and 24 ± ½ hours after the end of study drug administration.
4) Collect blood sample at 12 hours (± 30 min) after study drug discontinuation for cyanide and thiocyanate analysis.
5) Perform a pertinent physical examination 18 –30 hours following discontinuation of study drug administration.
6) Collect adverse events for 24 hours following discontinuation of study drug administration.
7) Collect serious adverse events plus associated concomitant medications and clinically meaningful, and invasive procedures for 30 days following discontinuation of study drug administration.

8) Collect blood samples for laboratory evaluations as per Table 1.

   Blood samples for cyanide and thiocyanate analysis at the discontinuation of the non-study drug (concomitant) SNP to be done only if feasible (the informed parental permission form must specify this blood sample will be drawn and only if an indwelling catheter is present). Note: This blood draw may be several days following the discontinuation of study-drug SNP administration."

SAEs and associated concomitant medications and procedures will be collected for 30 days following the discontinuation of study drug administration, either through telephone contacts and/or study visits or spontaneously reported by the subjects.

3.4.7 Methods of Assessment

3.4.7.1 Vital Sign Measurements

Vital signs: systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate will be measured.

The principal method of obtaining blood pressure measurements will be from an intra-arterial catheter inserted in an upper or lower extremity artery. Manual blood pressures from a non-invasive blood pressure cuff will only be used prior to insertion of and during a malfunction of the arterial catheter. The blood pressure transducer is internally calibrated by the instrument upon performing the zeroing procedure. All blood pressure and heart rate data will be acquired electronically when possible.

3.4.7.2 Blood Draws and Urine Samples

Blood drawn for study related purposes will not exceed the maximum amounts specified by the American Association of Blood Banks for healthy infants, children, and

adolescents. Normally, this value is 7 ml/kg over an eight week period. This study is an inpatient trial of short duration, therefore, the amount of blood withdrawn for study related purposes will take into account the patient's pre-existing hemoglobin and hematocrit, and local Institutional Review Board limitations on maximum allowable blood draws for study-related purposes. A reasonable and conservative value is 3 ml/kg.

In addition to those cyanide and thiocyanate tests scheduled in Table 1 and Section 3.4, the investigator will be permitted to perform supplemental cyanide and thiocyanate tests provided that the investigator believes that the tests are in the patient's best interest and the volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).

Urine will be sampled for thiocyanate concentrations every 8 hours, or fraction thereof, commencing with initial study drug administration to the end of study drug completion and then Q 8 hours times 3 after the discontinuation of study-drug administration, or until the urinary catheter is removed, whichever occurs first. All urine samples will be from a pooled urine collection from the 8-hour time period or fraction of 8-hour time period. The sample will be stored cold until shipment to the central lab. Details of urine collection procedures are described in the Manual of Procedures (MOP).

3.4.8 Dispensing of Study Drug

Study drug will be dispensed to the sites in 2 ml vials containing 25 mg/ml of SNP. The pharmacist will dispense two preparations of study drug, one for the open-label study drug period and one for the blinded study drug period.

The open-label study drug administration phase will utilize a fixed study drug concentration and variable infusion rate scheme. Syringes or bags will be prepared by the investigational drug pharmacy by adding 25 mg of SNP to 50 ml 5% dextrose. The syringe will have a label indicating the concentration of the solution (0.5 mg/ml SNP), and the infusion rate necessary to provide 1.0 microgram per kilogram per minute

(1.0 mcg/kg/min) —for example, for a 25-kg subject, an infusion rate of 3.0 ml/hr will deliver 1.0 mcg/kg/min SNP. The infusion rate of 0.5 mg/ml SNP can be calculated using the conversion factor: Wt (kg) × 0.12 ml/kg/hr = 1.0 mcg/kg/min. Clinicians can then make the necessary dosage adjustment for adequate blood pressure control. All dosage adjustments will be captured on the case report forms (CRFs).

The pharmacist will supply blinded study drug for each subject according to a randomization assignment generated by the IVRS. The blinded study drug will be prepared by the pharmacist such that either the concentration of drug is the same as in the open label period or placebo, see Section 3.6.1. Subjects will receive blinded study drug at the same rate of infusion that was used at the conclusion of the initial open-label study drug administration period, which will be at least 0.5 mcg/kg/min.

Syringes or bags will be wrapped in opaque or amber plastic to protect from light.

3.4.9 Delivery of Study Drug

Infusion pumps capable of reliable delivery at low infusion rates (to 0.1 ml/hr) will be used. All pumps will have free flow protection and will be internally calibrated for accuracy by the manufacturer. Accuracy will be verified at each site by the biomedical engineering department as part of their equipment management program. Quality assurance checks will be performed periodically according to manufacturer specifications.

Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. Microbore low compliance tubing, with volumes of approximately 1 mL will be used, where possible.

Study drug will be infused via a dedicated peripheral intravenous catheter or via a dedicated lumen of a multi-orifice central venous catheter. Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered

to this patient population. The carrier flow rate will be ≥5.0 mL/hr from at least one hour prior to the start of the blinded study period until the end of the blinded treatment period. Clinical judgment should be exercised when adjusting the carrier flow rate to prevent unsafe spikes in the infusion rate.

3.5 Removal of Subjects from Therapy or Assessment

3.5.1 Early Discontinuation of Study Drug and Subject Withdrawal

If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose.

If the subject withdraws participation in the study for any reason, every effort will be made to collect safety data, vital sign measurements, samples for safety and laboratory analyses. The date, time and reason for discontinuation must be recorded on the CRF. Additionally, every attempt should be made to complete all other study related procedures on discontinued subjects who have received any amount of study drug as the data will be included in the safety and intention to treat analyses. Subjects who prematurely discontinue from the study will not be replaced.

Any patient who starts the blinded study drug administration period will be considered complete for analysis.

Potential reasons for subject withdrawal from the study are as follows:

1) Subject's parent or legal guardian wishes to have the subject withdrawn for any reason;

2) Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the subject's safety;
3) The investigator feels that it is in the subject's best medical interest to be withdrawn.
4) Subject no longer needs blood pressure control.

3.6 Investigational Product

3.6.1 Identity of Investigational Product

Sodium nitroprusside (sodium nitropentacyanoferrate (III) dihydrate) is a reddish-brown crystalline powder that is freely soluble in water. Its molecular formula is $Na_2[Fe(CN)_5NO] \cdot 2H_20$. Study drug will be supplied by the BPCA Coordinating Center to the Investigational Drug Service at each clinical center in a standard concentration of 25 mg/ml. The Investigational Drug Service at each clinical center will then prepare the drug in syringes or bags of sterile 5% dextrose for administration to randomized patients according to the guidelines provided above. Sterile 5% dextrose will be utilized as placebo.

3.6.1.1 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature from 15°- 30°C and protected from light in its carton until used. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug must be maintained under adequate security.

3.6.2 Methods of Assigning Subjects to Treatment Groups

After meeting all inclusion and exclusion criteria, subjects will be considered to be enrolled and will be randomly assigned to receive either placebo or active drug treatment groups during the blinded study drug administration phase of the trial using a single centralized randomization schedule. Randomization into the blinded portion of the trial will be performed via a centralized interactive voice response system (IVRS).

The BPCA-CC will provide a system for the pharmacist to obtain each subject's randomized treatment assignment in a timely manner prior to the administration of study drug.

3.6.3 Assigning Subject Numbers

Study participants will be assigned a subject number upon successful enrollment into the study. The subject number will consist of five digits. The first two digits will be the site number of the enrolling institution followed by the number "2"—for the second trial under this IND—followed by a two digit enrollment-sequence number. For example, Subject #10-2-23 would be the 23rd subject enrolled at Site #10.

3.6.4 Blinding

The subject, as well as all caregivers, will remain blinded to the treatment assignment throughout the course of the study. For subjects' safety, the pharmacist will be aware of the treatment group for each subject. The BPCA-CC will maintain the double-blinded randomization schedule.

The randomization for an individual subject may be revealed in an emergency; however, investigators are discouraged against requesting that the blind be broken for

an individual subject. Notification of any unblinding must be sent via facsimile to the BPCA-CC within 24 hours.

3.6.5 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms.

3.6.6 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the Clinical Supply Shipment Request and Verification or similar document. An accurate inventory of study drug will be kept by the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and returned to the BPCA-CC if requested. The investigator agrees not to supply study medication to any persons not enrolled in the study or not named as a sub-investigator for the trial.

4.0 Adverse Events

4.1 Definition

An adverse event is defined as any unintended and unfavorable medical occurrence in a clinical investigation subject, administered a pharmaceutical product, regardless of the causal relationship with treatment. An adverse event can therefore be any untoward sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not the event is considered causally related to the use of the product. Pre-existing conditions that remain stable

throughout the study period will not be considered adverse events. Any worsening of a pre-existing condition or illness is considered an adverse event.

Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional over-dosage, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events if they result in discontinuation from the study, necessitate therapeutic medical intervention, or the investigator considers them to be adverse events.

Adverse events will also include electronically-monitored vital signs that meet the definitions of section 4.2; however, electronically-captured data excursions, which in the opinion of the investigator represent data artifacts, such as might be produced by the turning of the patient or brief interruption of the electronic circuit of the monitor device(s) or which do not likely reflect an actual untoward event will not be considered to adverse events.

New or worsening physical-exam findings that occur following the initiation of study-drug administration will be considered to be adverse events, without regard to causality.

4.1.1 Serious Adverse Events

A serious adverse event is one that meets the above criteria and also results in one of the following conditions:

1. Death
2. A threat to life
3. Requirement for inpatient hospitalization
4. Prolongation of hospitalization
5. Production of a congenital anomaly or birth defect

6. A persistent or significant disability or incapacity (excluding experiences of minor medical significance such as headache, nausea, vomiting, diarrhea, and accidental injury)
7. The requirement for a medical or surgical intervention in order to prevent a serious outcome.
8. Any event deemed to be serious by the Investigator

If an adverse event meets any of the above criteria, it must be reported to the BPCA-CC as a serious adverse event (SAE) within 24 hours of the investigative site's awareness of its occurrence.

4.2 Adverse Event Severity

The criteria for rating adverse events are as follows:

Mild	The adverse event is transient and easily tolerated by the subject.
Moderate	The AE causes subject discomfort and interrupts usual activities.
Severe	The AE causes significant interference with normal activities and may be incapacitating or life threatening.

Safety data are a primary objective of this trial. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. Safety will be evaluated throughout the study and during the follow-up period by evaluating the tolerability of the study drug infusion and by monitoring clinical and laboratory signs of SNP toxicity such as hypotension, tachycardia, bradycardia, acid base status, serum lactate concentration, methemoglobin levels, cyanide levels, and when available, mixed venous oxygen tension.

- Co-oximetric arterial blood gas analysis with methemoglobin determination will be performed Q8. Arterial blood gas monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity.

- Lower than expected methemoglobin concentrations may reflect indirect evidence of cyanide toxicity because cyanide has a high affinity for methemoglobin, combining with it to form the non-toxic molecule cyanomethemoglobin.
- Other laboratory findings suggestive of cyanide toxicity that will be monitored in this trial include serum lactate levels and mixed venous oxygen saturation.

  Lactate levels will rise and mixed venous oxygen tension will increase when cyanide toxicity is occurring due to the reduced ability of the tissues to extract oxygen.
- Plasma and urine thiocyanate levels are indirect markers of cyanide exposure because the majority of the cyanide ions liberated by the metabolism of SNP in the red blood cell are converted to thiocyanate by the rhodenase enzyme system in the liver and excreted in the urine. Free cyanide levels will also be measured.

The adverse event of rebound hypertension shall be considered mild if the MAP rises >10% above the baseline (B1), moderate if the MAP rises >20% above baseline (MAP_{B1}), and severe if the MAP rises >30% above baseline (MAP_{B1}).

The adverse event of excessive hypotension shall be considered mild if the MAP falls >20% below target; fluid therapy may be administered. The adverse event of excessive hypotension shall be considered moderate if the MAP falls >25% below target, fluid therapy is required, and pharmacologic therapy is required. The adverse event of excessive hypotension shall be considered severe if the MAP falls >30% below target, fluid therapy is required, and repeated and/or continuous pharmacologic support is required.

The rating of the adverse event of tachycardia shall be defined for sustained rates exceeding the age adjusted maximums (Table 3) by using the mild, moderate, and severe ranges outlined in Table 4A.

TABLE 3: Age-Adjusted Maximums for Pediatric Heart Rate

Subject Age	Maximum Heart Rate (bpm)
1 month <6 months	180
6 months <3 years	160
3 <8 years	150
8 <17 years	130

TABLE 4A: Severity of Tachycardia (Heart Rate in Beats per Minute)

Age	Mild	Moderate	Severe
Age <6 months	180-199	200-219	220 and higher
6 months - <3 years	160-179	180-199	200 and higher
3 years to < 8 years	150-164	165-179	180 and higher
8 years to <17 years	130-149	150-169	170 and higher

The adverse event of lactic acidosis shall be considered mild if the serum lactate concentration is between 5 and 7.5 mmol/L, moderate if the serum lactate concentration is between 7.6 and 10 mmol/L, and severe if the serum lactate concentration exceeds 10 mmol/L.

The adverse event of cyanide toxicity will be considered mild if the blood cyanide concentration is 0.5 mcg/mL – 1.0 mcg/mL, moderate if the blood cyanide concentration is >1.0 – 1.5 mcg/mL, and severe if the blood cyanide concentration is > 1.5 mcg/mL. Corresponding erythrocyte (vs. blood) cyanide values are ≥5 µmol/L - < 10 µmol/L (mild), 10-20 µmol/L (moderate), and > 20 µmol/L (severe).

Severity ratings for these selected adverse events are displayed in Table 4B.

TABLE 4B: Severity of Selected Adverse Events

Event	Mild	Moderate	Severe
Rebound Hypertension (during blinded phase)	MAP rises >10% above baseline (MAP_{B1})	MAP rises >20% above baseline (MAP_{B1})	MAP rises >30% above baseline (MAP_{B1})
Hypotension	MAP falls >20% below target	MAP falls >25% below target IV therapy required; Pharmacological therapy required	MAP falls >30% below target IV therapy required; Repeated or continuous pharmacological therapy required
Lactic acidosis	5 -7.5 mmol/L	7.6-10 mmol/L	>10 mmol/L
Cyanide toxicity (Blood)	Cyanide 0.5 – 1.0 mcg/mL	Cyanide >1.0-1.5 mcg/mL	Cyanide >1.5 mcg/mL
Cyanide toxicity (erythrocyte)	Cyanide $\geq$5 - <10 μmol/L	Cyanide $\geq$10–20 μmol/L	Cyanide >20 μmol/L

Frequent monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity. If the base deficit exceeds 8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the patient will be discontinued from study and the SNP infusion terminated. If the lactate level rises by more than 4 mmol/L in an 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output) SNP administration will be discontinued. If

the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes

percent between arterial and mixed venous blood in the absence of an explainable cause, SNP administration will be discontinued and treatment for suspected cyanide toxicity will be initiated.

Suspected cyanide toxicity will be further assessed and treated as follows:

1) Obtain blood for arterial and venous blood gases with co-oximetry, plasma lactate, and cyanide and thiocyanate levels.

2) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration (see below).

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10ml) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result).

TABLE 5: Dosage Chart for Children

Patient's Hemoglobin g/dL	Initial Dose of Sodium Nitrite (3%) mL/kg IV	Initial Dose of Sodium Thiosulfate mL/kg IV
8	0.22 mL/kg (6.6 mg/kg)	1.10 mL/kg
10	0.27 mL/kg (8.7 mg/kg)	1.35 mL/kg
12	0.33 mL/kg (10.0 mg/kg)	1.65 mL/kg
14	0.39 mL/kg (11.6 mg/kg)	1.95 mL/kg

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals. [Micromedex Poisondex www.micromedex.duhs.duke.edu , see also, Berlin, 1970]

4.3 Relationship to Study Drug

The criteria for determining the relationship of the AE to the study drug are as follows:

1) Probably related: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.
2) Possibly related: An AE that is likely to be related to the administration of the study drug and an alternative cause is equally or less likely when compared to the study drug.
3) Probably not related: An AE that has little or no relationship to the study drug and there exists a more likely, or equally likely, alternative cause.
4) Not related: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.

4.4 Adverse Event Collection Period

Non-serious adverse events will be monitored and reported from the time the subject receives study drug up to 24 hours following termination of study drug; SAEs will be monitored and reported from the time the subject receives study drug through 30 days following termination of study drug. Knowledge of adverse events will be gained from direct monitoring of the study subject as well as from clinician observation, and self reporting by the study subject or his/her guardians. Adverse events that have not resolved, or are ongoing, will be monitored to resolution if felt to be related to study drug, or until it is felt that the subject has stabilized.

5.0 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual subject, the investigator or other responsible physician must contact one of the Co-Principal Investigators immediately so that a timely decision can be made as to whether or not the

subject should be enrolled or continue in the study. If a deviation is being requested by one of the Co-Principal Investigators, he must contact the other Co-Principal Investigator for a decision. The deviation from the protocol will be authorized only for that particular subject. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF.

6.0 Data Monitoring Committee

To ensure that the welfare of trial patients receives appropriate consideration, an independent Data Monitoring Committee (DMC) has been organized by the BPCA-CC on behalf of the NICHD to review relevant safety and efficacy data during the course of the trial. The DMC may recommend discontinuation of the study, or modifications to the study protocol for safety reasons.

The DMC consists of four core members (Chair, ethicist, statistician, community representative) plus additional ad hoc members for the various medical subspecialties involved in the BPCA protocols.

Each DMC will have a presenting statistician who will be responsible for presenting the interim data. This member will write the reports and will be one non-voting member of the DMC. Except as their role in the DMC, all DMC members are not participating in the design or conduct of this study, as an investigator or otherwise, and lack any financial conflict that would introduce any bias.

6.1 DMC Responsibilities

- Monitoring the safety of trial patients;
- Recommending discontinuation of the trial for safety reasons;
- Recommending changes to the study protocol for safety reasons;
- Determining whether early stopping efficacy conditions are met;
- Providing written reports on an ongoing basis following scheduled and ad hoc

meetings that will be archived and may be provided to regulatory agencies.

The DMC will monitor the safety of trial patients by reviewing the occurrence of adverse events and deaths, on a real-time basis as SAE reports are transmitted. The DMC may also monitor compliance with the protocol, and factors affecting patient safety or the integrity of the trial. The DMC may request any additional data that are not included in the report if deemed necessary for effective monitoring.

If the DMC finds any major concerns about safety, it may recommend discontinuing the trial or modifying the study protocol. Following each data review, the DMC will send a written recommendation regarding the trial, (e.g., to continue according to the protocol, or recommendations for specific actions) to the sponsor.

7.0 Statistical Considerations

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a Statistical Analysis Plan, which will be finalized prior to unblinding. Any changes in the statistical methods described in this protocol that occur prior to unblinding will be documented in the Statistical Analysis Plan and will not result in a protocol amendment.

7.1 General Overview

The primary efficacy variable is the intra-patient change in Delta (MAP) during the blinded phase of the study. The primary null hypothesis to be tested is that there is no difference between the active study drug and placebo in the proportion of patients who experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP). Statistical analyses will be performed using two-sided tests. A 0.05 significance level will be used in all tests of treatment differences. Tests for interactions will utilize a 0.10 statistical significance level. Individual secondary endpoints will be evaluated using a hierarchical testing procedure. The Statistical Analysis Plan will include a detailed

description of all statistical methods, testing procedures, and methods of data imputation. One formal interim analysis is planned to evaluate the primary efficacy endpoint when 40 out of 60 patients have completed or withdrawn from the double-blind phase of the study. To control overall type I error at the nominal 0.05 level, critical values at interim and final analysis will be based on the Pocock stopping boundaries. The Data Monitoring Committee charter will contain the specific details regarding the early stopping rule at interim analysis.

Data will be summarized by treatment group with respect to demographic and baseline characteristics, efficacy variables, and safety variables. For parameters measured at baseline, the outcome variables of interest are the changes from baseline (Pre-Study Drug Period). Summary statistics will include the mean, N, standard deviation, median, minimum, and maximum values for continuous variables, and frequencies and percentages for categorical variables. Prior to summarizing results by study center, or performing analyses that include center as a factor in the analysis, small centers will be pooled. All efficacy variables will be summarized by treatment and by visit. Analyses will be performed to explore whether there are treatment-by-center interactions. If a treatment-by-center interaction is detected, the interaction will be explored in an ad-hoc manner. Categorical variables will be analyzed using Cochran-Mantel-Haenszel (CMH) test blocking on centers. Details of the model and the analyses will be specified in the Statistical Analysis Plan and all statistical analyses will be performed using SAS, Version 8.2 or higher.

7.2 Study Objectives

The study objectives are as defined in Section 2.0 of this protocol.

7.3 Patient Population(s) for Analysis

7.3.1 Efficacy

The intent to treat (ITT) population will contain all patients who were exposed to the study drug during the Open-Label Study Drug Administration (Dose-Titration) Phase.

The Per-Protocol population will contain all patients randomized to and exposed to the study drug during the double-blind phase of the trial. The efficacy analysis will be based on the Per-Protocol population. A patient will be classified as a *treatment success* if they meet the following criteria:

• Complete the 30-minute double-blind phase without having ≥2 consecutive intra-patient increases greater than or equal to 50% Delta (MAP) [i.e., MAP ≥ $(MAP_{B1} + MAP_{B2})/2$] and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

A patient will be classified as a *treatment failure* if they meet the following criteria:

• Fail to complete the entire 30-minute double-blind phase without receiving additional treatment to control their blood pressure in addition to the study drug.

• Fail to complete the entire 30-minute double-blind phase for any reason.

• Experience an intra-patient increase greater than or equal to 50% Delta (MAP) for ≥2 consecutive MAP measurements, obtained at one-minute intervals, during the 30-minute double-blind phase.

7.3.2 Safety

All patients who receive any study medication (ITT population) will be included in the safety analyses and summaries, independent of the patient actually reaching the double-blind phase of the study. All non-serious adverse events recorded within 24 hours of either completion of the double-blind phase, or within 24 hours of premature discontinuation of the study, will be reported.

All serious adverse events recorded within 30 days of either completion of the double-blind phase, or premature discontinuation of the study, will be reported.

7.4 Background and Demographic Characteristics

All baseline information, including demographic factors, physical examination parameters, vital signs, growth parameters (if applicable), laboratory and blood gas information will be summarized by treatment group for all enrolled patients (ITT population). Additionally, nonrandomized patients versus randomized patients will be summarized and compared by age, gender, and race to determine if there are any differences among the 2 subsets. Analyses will be conducted to determine differences in the demographic and baseline characteristics of the treatment groups. For continuous variables (e.g., age, weight), the number of non-missing and missing values and the median, mean, standard deviation, minimum, and maximum will be displayed for each treatment group. For categorical variables (e.g., race, gender), the counts and proportions will be tabulated.

Baseline comparability will be evaluated based on the pooled data from all centers. To determine comparability of the treatment groups at baseline, continuous demographic and clinical variables will be analyzed using an analysis of variance test (with an appropriate transformation, if necessary). Baseline, demographic, and clinical variables that are ordinal will be analyzed using the Cochran Mantel Haenszel test; parameters that are dichotomous will be analyzed using a chi-square ($\chi 2$) test or Fisher's exact test, depending on the individual cell counts. If there are treatment group differences at the

0.10 level of significance in demographic or baseline clinical variables, these variables may be added as stratification variables or covariates to the efficacy analyses.

7.5 Study Medication

The duration of exposure to study medication will be summarized for all enrolled patients, and separately for all randomized patients.

7.6 Concomitant Therapy

Concomitant medications (medications present while on study medication) will be recorded on source documents throughout the study and at early termination; however, only those concomitant mediations that (1) affect blood pressure, or (2) are associated with SAE's will be recorded on CRFs. Medications listed on CRFs will be coded using the WHO drug dictionary. The number of randomized patients using prior or concomitant medications will be categorized by the WHO drug category and preferred term, and presented for each treatment group. In any given category [e.g., drug category] a patient will be counted only once.

7.7 Statistical Design and Models for Analysis

This is a biphasic (open-label dose-titration phase, followed by a randomized phase), randomized, double-blind placebo-controlled study. Patients who are enrolled into the initial phase of the study will have their dose of sodium nitroprusside titrated and must receive a minimum of 12-hours of treatment to be eligible for the randomized phase of the study. Patients who cannot be adequately titrated during the initial 12-hour period will not proceed to the randomization phase of the study. Patients who reach the randomization phase of the study will be assigned to receive placebo, or continue to receive sodium nitroprusside based on a stratified permuted block central randomization scheme.

Five age groups (A through E) will be enrolled in this trial:

Age Group A: Age Group A: Neonates from birth to less than 30 days of age

Age Group B: Infants and toddlers from 30 days to < 2 years

Age Group C: Preschool children from 2 years - < 6 years

Age Group D: School age children from 6 yrs - < Tanner stage III

Age Group E: Adolescents from Tanner stage III - < 17 years.

In order to efficiently account for the effect of SNP on the different age groups, neonates from birth to less than 30 days of age (Age Group A) and infants and toddlers from 30 days to < 2 years (Age Group B) will be pooled for analysis. Based on the planning estimates of the study, patients from these two pooled age groups should represent approximately 25% of the target enrollment (~60 patients).

Preschool children from 2 years - < 6 years (Age Group C) and school age children from 6 yrs - < Tanner stage III (Age Group D) will also be pooled for analysis; patients from these two pooled age groups should also represent approximately 25% of the target enrollment (~60 patients).

In order to accurately determine whether a treatment difference is sufficient to stop the trial early, the DMC will examine the results from the Blinded Study Drug Administration Phase after the first 40 patients have been enrolled, randomized and treated during the blinded treatment phase; the approximate expected distribution by age group is as follows:

- The initial 12 patients in Age Groups A & B
- The initial 12 patients in Age Groups C & D
- The initial 16 patients in Age Group E

The specific objective of the DMC is to determine the significance of the treatment difference based on the observed magnitude of the effect, expressed as a proportion (*treatment success* (sodium nitroprusside) / Treated with sodium nitroprusside during the

blinded treatment phase vs. *treatment success* (Placebo) / Treated with Placebo during the blinded treatment phase. Determination of the treatment difference will be performed after 67% (40/60) of the target sample size has been enrolled.

7.7.1 Primary Efficacy Analysis

The primary efficacy analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients received sodium nitroprusside}} = \pi_{\text{ Patients received receive placebo}}$
- H_A: $\pi_{\text{Patients received sodium nitroprusside}} \neq \pi_{\text{ Patients received receive placebo}}$

where

- $\pi_{\text{Patients received SNP}}$ = Proportion of *treatment successes* (sodium nitroprusside)
- $\pi_{\text{Patients received placebo}}$ = Proportion of *treatment successes* (placebo)

7.7.2 Primary Safety Analysis

The primary safety analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients received sodium nitroprusside}} = \pi_{\text{ Patients received placebo}}$
- H_A: $\pi_{\text{Patients received sodium nitroprusside}} \neq \pi_{\text{ Patients received placebo}}$

where

- $\pi_{\text{Patients received sodium nitroprusside}}$ = Proportion of patients who received SNP who experience a serious adverse event

- $\pi_{\text{Patients received placebo}}$ = Proportion of patients who received placebo who experience a serious adverse event

7.7.3 Interim Monitoring Based on Group Sequential Methods

The interim assessment of the treatment difference will be predicated on the primary efficacy endpoint and conducted using Group Sequential methods as described by Pocock (1977). The Data Monitoring Committee will conduct the analysis to determine whether the treatment difference is sufficient to stop the trial early. This assessment will be conducted after 67% of the patients from the original target sample size have either completed or withdrawn from the study. The instructions to the DMC for the treatment difference assessment will be described in detail in the Data Monitoring Committee Charter.

In order for the DMC to calculate the treatment difference using the observed data, the treatment assignment codes will need to be provided to the DMC statistician. The intra-

patient MAP_{B2} values will be required, including listings of the intra-patient post-baseline values during the blinded phase of the trial. If the treatment difference does not meet the early stopping criteria, enrollment will continue towards the target sample size, and the data will remain blinded to all involved parties with the exception of the DMC. Additionally, the DMC will not have any direct contact with the study Sponsor, or the clinical investigators.

The following steps will be used to evaluate the treatment difference after the initial 40 randomized patients have been enrolled and have completed the Blinded Study Drug Administration Phase, or withdrawn prematurely.

The Group Sequential methods described by Pocock indicate that in order to control the type I error at 5%, the significance level at the interim analysis and the final analysis is as follows when the interim analysis is performed after 67% (40/60) of the target sample size has been enrolled.

Group Sequential Table 1.0

Significance Level Corresponding to the Pocock Stopping Boundary

Analysis	**Subjects**	**Significance Level (α)**
Interim	40/60	0.0407
Final	60/60	0.0215

At the interim analysis, test the null hypothesis at an α=0.0407 significance level using a two group χ^2 test of equal proportions. If the test is statistically significant with a p-value <0.0407, there will be sufficient evidence to reject the null hypothesis in favor of the alternative that a treatment difference exists between the two randomized treatment groups and the study can be stopped early for efficacy. Final decision will be based on recommendation from the DMC who will review overall efficacy and safety information. Alternatively, if a p-value <0.0407 is not achieved, the study will continue towards the target enrollment.

7.7.4 Sample Size Estimation

The overall sample size was calculated based on performing an un-stratified analysis of the proportion of patients classified as a *treatment success* between the 2 randomized treatment groups. With a balance randomization (1:1, SNP:Placebo), a difference in the proportion of *treatment successes* ranging from 34% to 40% would have 80% power to reject the null hypothesis in favor of the alternative (ref. Sample Size Table No. 1.0).

Sample Size Table No. 1.0

Two group χ^2 Test of Equal Proportions

Scenario	**1**	**2**	**3**	**4**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
SNP, π_1	0.080	0.160	0.240	0.320
Placebo, π_2	0.420	0.530	0.630	0.710
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	8.328	5.920	5.392	5.203
Power (%)	80	80	81	80
n per group	30	30	30	30

Given the significance level corresponding to the Pocock stopping boundary as indicated in Group Sequential Table 1.0 above, the following scenarios identify the number of treatment successes that must be observed in the SNP treatment group to result in a statistically significant test of the null hypothesis given the placebo group success rate specified below.

Group Sequential Table 2.0

Required SNP Treatment Successes

Scenario	**1**	**2**	**3**	**4**
Analysis	Interim	Final	Interim	Final
n per group	20	30	20	30
Test significance level, α	0.0407	0.0215	0.0407	0.0215
Treatment Successes (Placebo)	2 (10%)	3 (10%)	4 (20%)	6 (20%)
Treatment Successes (SNP)	8 (40%)	11 (55%)	11 (37%)	15 (50%)

7.7.5 Strategy for the Statistical Analysis

The primary method for analysis will be a comparison of the proportion of *treatment success* between patients randomized to receive placebo compared to patients randomized to remain on sodium nitroprusside. Additional analysis will be described in the Statistical Analysis Plan that will include a comparison of the event time distribution functions for the time until an increase in MAP_{B2} + 50% Delta (MAP) is initially observed. During the Open-Label Study Drug Administration (Dose-Titration) Phase, the sustainability of the blood pressure will be graphed over time to determine the effectiveness of SNP to maintain the target MAP.

7.7.6 Handling Missing Data in the Analyses

The following method of imputation will be used:

Last observation carried forward (LOCF): The goal of this imputation scheme is to create an observation for a completely missing observation at the end of the study for every patient in the ITT population. If a patient evaluation for a post-baseline observation is missing, then the immediately preceding non-missing evaluation will be used.

Specific algorithms for imputing missing or partially missing dates will be discussed in the SAP. Imputed or derived data will be identified in the individual patient data listings. Imputed data will not be incorporated into the case report form datasets. Imputed data will be used in the preparation of the derived datasets.

7.7.7 Pooling of Small Sites for Analysis

Small sites (i.e., sites that have less than 4 patients per treatment arm) will be identified and the following method will be used for combining the data. Data from all small sites (< 4 patients) will be combined to form a single site in order to obviate non-estimable situations (i.e., at least 2 intra-group observations are needed to estimate variance) in the evaluation of site and site interaction effects. Once combined, the pooled site will remain as such for all analyses for which a site interaction effect is determined. If the pooled smaller sites represent a single site that has more than twice as many patients as the largest single site, however less than 3 times as many patients, the small sites will be ranked by size and divided into 2 pooled groups using an alternating sequence (ABABAB). If the pooled smaller sites represent a site that has more than three times as many patients as the largest single site, however less than 4 times as many patients, the small sites will be ranked by size and divided into 3 pooled groups using an alternating sequence (ABCABCABC). This methodology will be applied, based on the initial pooling of the smaller sites.

7.7.8 Dropouts, Protocol Violations/Deviations, and Exclusions

Randomized patients who fail to complete the study will not be replaced. All protocol deviations and violations will be documented and categorized in the final study report.

The rate of attrition will be evaluated by the Data Monitoring Committee during the interim evaluation to re-estimate sample size. The reasons for withdraw will be classified into 3 mutually-exclusive classes:

- Withdraw due to tolerability of the study drug
- Withdraw due to lack of treatment effect
- Withdraw not due to tolerability or lack of treatment effect

The proportion of patients who withdraw prematurely will be compared between treatment groups to determine if there is a disproportionate rate of attrition. If the rates differ by a pre-specified amount, the reasons for withdraw will be examined to determine causation. The specific monitoring rules, boundaries, and actions will be described in detail in the Data Monitoring Committee Charter.

7.8 Safety Evaluation

The primary assessment of safety will be based on the frequency of treatment-emergent adverse events and on the frequency of clinically notable abnormal vital signs and laboratory values. The primary safety analysis will be based on a comparison of the proportion of patients receiving sodium nitroprusside vs. placebo who experience a serious adverse event during the Blinded Study Drug Administration Phase.

7.8.1 Adverse Events and Medical Conditions

Adverse events will be recorded throughout the study and at early termination. Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Treatment-emergent adverse events will be defined as those events, which are newly occurring or worsening from baseline. In all cases only treatment emergent adverse events will be summarized. Treatment-emergent adverse events will be summarized by presenting, for each treatment group, the number and percentage of patients having any treatment-emergent AE, having an AE in each body system, and having each individual AE. (Note: In any given category [e.g. body system] a patient will only be counted once.) Similar displays will be provided for prior (conditions ending prior to the first exposure to sodium nitroprusside) and current (conditions present while on study medication) medical conditions. Adverse events will further be categorized by severity, relationship to study medication, and action taken. Other information collected will be listed, as appropriate. Any event starting more than 3 days after the final dose of study medication will be excluded from the above tables and only presented in a listing, unless the adverse event was serious or caused discontinuation from the study.

7.8.2 Clinical Laboratory Results

7.8.2.1 Overview

The primary presentation of the results by individual laboratory parameter will focus on the intra-patient changes from baseline (Pre-study Period). The presentation and analysis of laboratory data will be based on the observed data. All patients who have a baseline and at least one follow-up laboratory assessment will be included in the presentation of the clinical laboratory data. For each clinical laboratory test, there will be three sets of descriptive statistics that summarize the results at baseline, post-baseline assessment, and the change from baseline to post-baseline assessment. Descriptive statistics include N,

mean, standard deviation, median, and the minimum and maximum values. Within treatment group changes will be analyzed using a paired-difference t-test. Between treatment group differences will be compared using a one-factor analysis of variance test.

Shifts from baseline to each pre-specified post-baseline endpoint will also be summarized based on the laboratory categorization (*abnormally and clinically significant*, *abnormal but not clinically significant*, or *normal*) using the worst reported post-baseline observation that occurs within the pre-specified interval. The proportion of patients will be compared using a 2-tailed Fisher's exact test, pooling *abnormally and clinically significant* with *abnormally but not clinically significant.*

In the case that more than one laboratory is used, laboratory values will be transformed for mean change summaries to the same units and normal range as were provided by the central laboratory used in the study, using the formula:

$$y = (x - Li)\frac{Uc - Lc}{Ui - Li} + Lc$$

where x = original value, Li and Ui = lower and upper limits of normal for individual laboratory, Lc and Uc = lower and upper limit for central laboratory

In cases where the lower limit of central laboratory is 0, values that are below the lower limit of normal for a laboratory value prior to transformation will be assigned a value of 0.

7.8.3 Vital Signs

7.8.3.1 Overview

Vital signs of particular interested (blood pressure, MAP, heart rate) will be assessed during each phase of the study.

7.8.3.2 Presentation of Results

Descriptive statistics (n, mean, SD, median, minimum and maximum values) will be used to summarize systolic and diastolic blood pressure, MAP, and heart rate and compared between the randomized treatment groups using a one-factor analysis of variance test.

7.8.4 Physical Examination

7.8.4.1 Overview

The presentation of physical examination data is based on the dichotomous classification (normal or abnormal) of each of the 9 regions or body systems (General Appearance, HEENT, Cardiovascular, Respiratory, Abdomen, Extremities, Neurological, Hair and Skin, and Genitourinary). In addition to these 9 specific body systems, any other region recorded by the investigator under "other" will also be summarized and reported.

7.8.4.2 Presentation of Results

Results will be presented by treatment assignment using counts and percentages. Shift tables will be prepared containing the count and percentage of patients who transitioned from normal at baseline to abnormal at the end of the study. The number and percentage of patients that did not change (normal at baseline and normal at the end of the study, abnormal at baseline and abnormal at the end of the study) are also presented to frame the 2*2 contingency table. Shifts from baseline to each pre-specified post-baseline endpoint will be summarized using the worst reported post-baseline observation that occurred within the pre-specified interval. The count of the disagreements (normal to abnormal and abnormal to normal) by treatment assignment (active and placebo) will be compared for each parameter using McNemar's test.

8.0 Ethics

8.1 Independent Ethics Committee or Institutional Review Board

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator Brochure (IB), the informed parental permission and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an Independent Ethics Committee (IEC) or Institutional Review Board (IRB). IEC/IRB approval of the protocol, informed parental permission and subject information and/or advertising as relevant will be obtained prior to the authorization of drug shipment to a study site. Any amendments to the protocol will require IEC/IRB approval prior to implementation of any changes made to the study design.

8.2 Ethical Conduct of Study

This study will be conducted in accordance with the protocol, GCP, FDA regulations governing clinical study conduct, ethical principles that have their origin in the Declaration of Helsinki, 2000 revision (see Appendix E) and all applicable local regulations. The investigator must assure that the study is conducted in accordance with the provisions as stated in the FDA regulations and complies with prevailing local laws and customs. Responsibilities of the Investigator are specified in Appendix D.

8.3 Subject Information and Parental Permission

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before protocol-specified procedures are carried out. Informed consent will be obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements.

Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation.

Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study.

The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed permission form, which will included all elements of informed consent as described in 21 CFR 50.25. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form and a copy of the signed assent (if applicable).

The parental/guardian permission form generated by the investigator with the assistance of BPCA-CC must be approved (along with the protocol) by the IRB and be acceptable to the Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative.

The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the ICH Guidelines and will comply with local regulations. This form

may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated.

Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by BPCA-CC, Regulatory Authorities, and NICHD or its designees.

9.0 Source Documents and CRF Completion

9.1 Source Documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

9.2 Case Report Forms

Data for individual subjects will be recorded on CRFs provided to the BPCA-CC. All entries must be complete. A case report form must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the CRF by the investigator. The principal investigators must review and approve each CRF.

Case report forms must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested during an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

Because of the potential for errors and inaccuracies in entering data into CRFs, laboratory and other test results must be kept on file with the subject's study dossier. Case report forms and copies of test results must be available at all times for inspection by the CRA for the site and the FDA.

10.0 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA-CC, the investigators and their study coordinators and the CRAs for the study. This meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures by a CRA at a study initiation visit and will be given an CRF completion workbook for reference.

The CRAs will monitor each site throughout the study. At each visit, 100% source document review will be made against entries on the CRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and all applicable regulations.

After completion of the entry process, computer logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary

correction will be made to the database and documented via addenda or audit trail. A manual review of selected line listings will also be performed at the end of the study.

11.0 Use of Information and Publication

11.1 Use of Information

This trial is sponsored by the NICHD. The NICHD endorses the sharing of final research data to expedite the translation of research results into new scientific knowledge in order to improve human health.

This contract is part of a collaborative program involving multiple sites. A data sharing dissemination plan will be developed jointly with the BPCA-CC, the NICHD, and the collaborating institutions following announcement of the award.

11.2 Publication

The BPCA-CC and steering committee for this study shall have the right to review and approve any paper for publication, including oral presentation and abstracts, which utilize data generated from this study. At least 90 days before any such paper or abstract is presented or submitted for publication, a complete copy shall be given to the BPA-CC for review by the steering committee. Any individual investigator agrees to defer publication of any such paper or abstract until the BPCA-CC and Steering Committee have reviewed and approved it.

12.0 Completion of Study

The investigator will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon in writing by the investigator, the NICHD, the BPCA-CC

and the PODS. The investigator will provide a summary of the study's outcome to the IRB/IEC following the conclusion of the study.

The PODS Center, BPCA-Coordinating Center, NICHD and/or the FDA may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. The investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the NICHD and BPCA-CC within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

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13.0 Investigator Agreement

The investigator will sign and date a Study Protocol Agreement Form, provided earlier in this protocol. The Study Protocol Agreement will then be countersigned by the investigator's PODS Principal Investigator.

Appendices

Appendix A: Tanner Stages of Sexual Maturity

	Pubic Hair		Breasts	Penis	Testes
SMR Stage[1,2]	Boys	Girls	Girls	Boys	Boys
1	None	Preadolescent	Preadolescent	Preadolescent	Preadolescent
2	Scanty, long, slightly Pigmented	Sparse, lightly pigmented, straight, medial border of labia	Breast and papilla elevated as small mound; areolar diameter increased	Slight Enlargement	Enlarged scrotum, pink texture altered
3	Darker, starts to curl, small Amount	Darker, beginning to curl, increased amount	Breast and areola enlarged, no contour separation ,	Longer	Larger
4	Resembles adult type, but less in quantity; coarse, cu rly	Coarse, curly, abundant but amount less than in adult	Areola and papilla form secondary mound	Larger; glans and breadth increase in size	Larger, scrotum dark
5	Adult distribution, spread to medial surfaces of thighs	Adult feminine triangle, spread to medial surface of thighs	Mature; nipple projects, areola part of general breast contour	Adult size	Adult size

1. Adapted from Tanner, JM: Growth at Adolescence, 2 ed. Oxford, Blackwell Scientific Publications, 1962.
2. MR = Sexual Maturity

Appendix B: Parental Permission Form with HIPAA

Protocol Title: A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects

Protocol Director: [PROGRAM PI]
IRB Approval Date: _____ IRB Expiration Date: _____

Is your child participating in any other research studies? _____Yes _____No

INTRODUCTION

You are being asked to agree to let your child be a part of a drug research study. He or she is scheduled for surgery, or needs to stay in an intensive care unit (ICU). During this operation or stay in the ICU, it will be necessary for the doctor to lower your child's blood pressure for a long period of time, up to 24 hours. We will tell you more about how he/she will do this and the drug that will be used later in this paper. Before you decide whether to let your child be involved in this study, *[Site PI]* wants you to read the following information. He wants you to ask him any questions you may think of. He wants to be sure that you understand what your child's participation will mean. You need to fully understand the type of treatment and its risks. Your doctor is responsible for providing you with the necessary information so that you understand the possible risks. Your child's participation in this study is entirely your choice.

Your child cannot participate in another research study at the same time as this research study [optional sentence]. Your child cannot be taking another experimental drug while enrolled in this research study, or within the previous 30 days. Your child will be a part of this study for approximately 30 days. There may be risks that we cannot predict. We will tell you about any new information that may affect your child's condition or affect your willingness to stay in this study.

NATURE AND PURPOSE OF THE RESEARCH STUDY

In certain kinds of surgeries, doctors often need to control the blood pressure of the patient. The doctor may also need to lower the patient's blood pressure below normal. This can reduce blood loss and avoid blood transfusions during stressful periods. Sodium nitroprusside is a drug that is approved by the Food and Drug Administration (FDA) for use in adults. Scientific studies show that this drug works well when doctors need to control blood pressure during surgeries in adult patients. Doctors also often use sodium nitroprusside in children. However, not many scientific studies tell us how best to use sodium nitroprusside in children.

In this study, we hope to learn the best dose of sodium nitroprusside (the study drug) to use in children who need it in the ICU for more than 12 hours. We also want to learn the same thing for children who need certain kinds of surgeries. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children. This research study is looking for 50-100 children at several hospitals in the United States.

Your child's participation in this study is entirely voluntary.

Your decision whether or not to allow your child to participate will not prejudice your child or his/her medical care. If you decide to allow your child to participate, you are free to withdraw your permission, and to discontinue participation at any time without prejudice to your child or effect on your child's medical care. If you decide to terminate your child's participation in this study, you should notify *(name)* at *(telephone number)*.

PROCEDURES

If you agree to your child's participation in this research study, he or she will undergo the following types of procedures:

- [Site PI] and his research staff will talk to you (and your child) about his or her health. They will ask you about your child's medical history. They will ask you about any medications your

child is currently taking. They will give your child three physical examinations, including measurement of blood pressure, pulse, and weight. The first one will be within 24 hours before study drug is given. The other two physical exams will be after your child receives study drug. A small amount of blood, less than one teaspoon, will be drawn at the first two visits. We need this blood for our study, but the same blood work may also be needed as part of your child's regular medical care. If this is the case, we will use these results instead of having to take another blood sample.

- Your child will receive study treatment in stages:
  1. First, after your child is stable, sodium nitroprusside will be given into a vein. This will be done through tubing your child will already have in place. This drug may be the treatment of choice to control your child's blood pressure even if your child is not enrolled in this study. This first stage will last up to 24 hours. He or she will receive sodium nitroprusside at a pre-set initial rate. That rate will be changed until your child's blood pressure is in the range that his or her doctor has decided is the best.
  2. The second stage of study treatment will begin between 12 and 24 hours after the medication was first started. This stage will last up to 30 minutes. During this stage your child will receive one of two treatments. This phase of the research study is blinded. That means that the study doctor will not know which treatment group your child will be placed into. The choice of which treatment group your child will be in (placebo or sodium nitroprusside) will be random. The choice will be made in a way that is like flipping a coin. He or she might receive sodium nitroprusside at the rate that was being used before to keep his or her blood pressure at a stable level. Or, he or she might receive a placebo; this is a solution like salt water that is known to have no effect on your child's health. Your child's vital signs (blood pressure and heart rate) will be watched very closely. If your child's blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end immediately. Then your child will again be given sodium nitroprusside, or similar drug, to keep his or her blood pressure at a safe level.
  3. If your child stills needs sodium nitroprusside treatment to control his/her blood pressure, the treatment may continue after the research study ends.

During the research study, vital signs (blood pressure and heart rate) will be checked often at specific time points.

There will be a follow up evaluation. About 30 days after the research study is over, we will call you at home to ask questions about your child's health in the last month, since he or she participated in the research study. If your child is readmitted to the hospital before our call, please let us know.

Blood samples will be taken between 4 to 7 times (1½ to 2½ mLs per each sample, or ½ teaspoon per sample) during the research study. This is to see how much of the metabolites (breakdown products) of the study drug is circulating in the blood at varying time points. If indicated, additional blood samples may be taken to help determine the amount of metabolites in your child's blood. We are very careful to minimize the amount of blood drawn from your child, and anticipate that 3½ teaspoons is the most we will draw for these scheduled tests.

Whenever possible, blood will be taken from tubing already in place. The nurse will often take the study blood samples at the same time that routine blood samples are taken to check on your child's health. This is done to avoid any extra needle-sticks (drawing blood from a vein in the arm with a needle). It is very unlikely that there would not be a catheter (IV line) in place; however, if that were to happen, blood drawing would require a needle stick that might cause minor bruising. Blood drawing is done to assess safety and to measure the activity of the study drug.

The total amount of blood drawn during the entire research study for children less than 2 years of age is approximately 1 to 2 ½ teaspoons. For children more than 2 years of age, the total amount of blood is about 2 to 3 ½ teaspoons.

The information gained during this research study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help patients in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

YOUNG WOMEN OF CHILD-BEARING POTENTIAL

If your child is a young woman who is able to become pregnant, it is expected that she will use an effective method of birth control to prevent exposing a fetus to a potentially dangerous agent with

unknown risk. If your child is pregnant or currently breast feeding, she may not participate in this study.

To confirm to the extent medically possible that your child is not pregnant, you agree that she will have a pregnancy test done before beginning this research study. This test will be carried out right before your child undergoes surgery.

PARTICIPANT RESPONSIBILITIES

You should:

- Follow the instructions of the Protocol Director and study staff.
- Tell the Protocol Director or research study staff about any side effects that your child may have.
- Tell the Protocol Director or research study staff if you believe your child might be pregnant.
- Ask questions as you think of them.
- Tell the Protocol Director or research staff if you change your mind about staying in the study.

While participating in this research study, your child should not take part in any other research project without approval from all of the Protocol Directors. This is to protect your child from possible injury arising from such things as extra blood drawing, the possible interaction(s) of research drugs, or other similar hazards.

WITHDRAWAL FROM STUDY

If you first agree to participate and then you change your mind, you are **free to withdraw** your permission and discontinue your child's participation at any time. Your decision will not affect your child's ability to receive medical care for his or her disease and your child will not lose any benefits to which he or she would otherwise be entitled.

If you decide to terminate your child's participation in this study, you should notify (*name)* at (*phone number)*.

The Protocol Director may also withdraw your child from the study and the study medication may be stopped without your permission for one or more of the following reasons:

- Failure to follow the instructions of the Protocol Director and/or study staff.
- The Protocol Director decides that continuing your child's participation could be harmful to him or her.

- Pregnancy (if applicable).
- Your child needs treatment not allowed in the study.
- The study is cancelled.
- Other administrative reasons.
- Unanticipated circumstances.

If your child is withdrawn from the study after receiving any of the research drug, and if you agree, we will perform the following testing to ensure the safety of your child: physical examination, monitor vital signs, blood tests, as described earlier, as well as the follow-up phone call approximately 30 days later. You will be free to have only some of these done if you prefer.

POSSIBLE RISKS, DISCOMFORTS, AND INCONVENIENCES

There are risks, discomforts, and inconveniences associated with any research study. These deserve careful thought. This is true whether it is a normal kind of treatment or an experimental type. You should talk with the Protocol Director if you have any questions.

In spite of all safety measures, your child might develop medical problems while taking part in this research study. These risks include elevated blood pressure. Treatment of these potential medical problems will not be limited or delayed by your child's participation in the study.

Sodium nitroprusside is a drug that lowers blood pressure. Because of this, there is a chance that your child could develop hypotension (low blood pressure). The doctor will monitor your child very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable blood pressure.

Another side effect that might happen is that your child's heart rate may increase in response to sodium nitroprusside. Again, the doctor and research nurse will monitor your child's heart rate very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable heart rate.

Minor side effects due to sodium nitroprusside may also occur but will not cause the study to be ended. Since sodium nitroprusside makes blood vessels bigger, the following side-effects may occur: nausea, headache, restlessness, abdominal pain, redness or flushing of the skin, nervousness, and perspiring.

Sodium nitroprusside contains cyanide. Cyanide is present in all people and is important for normal body function. Extra cyanide can be present whenever sodium nitroprusside is used. Excess cyanide can affect the amount of oxygen in the blood. The doctors will carefully watch for any signs or symptoms of excess cyanide and treat your child if needed. Because safety is one of the most important aspects of this study, we will be testing your child's blood for cyanide. The results of this testing will not be immediately known. However, there are other means to detect ill effects from excess cyanide. If the doctors suspect it is in your child's best interest, additional unscheduled blood tests for cyanide and its breakdown product, thiocyanite, will be performed. Cyanide has been detected in patients who have participated in this study. However, no patient has shown any ill effects from the cyanide.

POTENTIAL BENEFITS

The information gained during this study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help children in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOUR CHILD WILL RECEIVE ANY BENEFITS FROM THIS STUDY.

ALTERNATIVES

If you choose not to enroll your child in this study, your child may receive sodium nitroprusside anyway. This could happen if your child's doctor feels it is the best medicine to use to control blood pressure. Or, your child's doctor could choose to use other types of blood pressure medications, such as esmolol or fenoldopam, instead. Whether or not your child is enrolled, the medical team will, of course, do everything possible to ensure the safety and comfort of your child.

If you do not wish your child to take part in this study, other treatments can be used for your child's condition. If you withdraw your child's participation, the study doctor will recommend an alternative treatment for blood pressure control for your child, such as esmolol or fenoldopam. If this study is discontinued, your child will receive one of these alternative treatments.

PARTICIPANT'S RIGHTS

You should not feel obligated to agree that your child participate in this study. Your questions should be answered clearly and to your satisfaction.

If you decide not to participate, tell the Protocol Director. Your child will still receive care for his/her disease and will not lose any benefits to which he/she would otherwise be entitled.

You will be told of any important new information that is learned during the course of this research study, which might affect your child's condition or your willingness to continue participation in this study.

CONFIDENTIALITY

Your child's identity will be kept as confidential as possible as required by law. Except as required by law, your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your child's research records may be disclosed outside of Stanford, but in this case, your child will be identified only by a unique code number. Information about the code will be kept in a secure location and access limited to research study personnel.

The results of this research study may be presented at scientific or medical meetings or published in scientific journals. However, your child's identity will not be disclosed**.**

Patient information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your child's identity if this study falls within its jurisdiction.

- The purpose of this research study is to obtain data or information on the safety and effectiveness of sodium nitroprusside in children; the results will be provided to the sponsor, the Food and Drug Administration and other federal and regulatory agencies as required.

Authorization to Use Your Health Information for Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed parental permission form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?

In this study, we hope to learn the best dose of sodium nitroprusside to use in children of different ages who need it in the ICU for more than 12 hours. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children as determined by the NIH and FDA.

Do I have to sign this authorization form?

You do not have to sign this authorization form. But if you do not, your child will not be able to participate in this research study, including receiving any research-related treatment.

Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?

If you decide to allow your child to participate, you are free to withdraw your authorization regarding the use and disclosure of your child's health information (and to discontinue any other participation in the study) at any time. After any revocation, your child's health information will no longer be used or disclosed in the study, except to the extent that the law allows us to continue using your child's information (e.g., necessary to maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must contact: *(researcher's name and contact information, including telephone number).*

What Personal Information Will Be Used or Disclosed?

Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to, vital sign measurements, laboratory results of blood collections, physical exams, related medical records, and other data.

Who May Use or Disclose the Information?

The following parties are authorized to use and/or disclose your child's health information in connection with this research study:

- The Protocol Director *(Insert Name of PD)*
- The *(Insert name of Institution)* Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary
- Research Staff

(List every other class of persons or organization affiliated with the hospital/university who might need to use and/or disclose the participant's information in connection with this study.)

Who May Receive or Use the Information?

The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- The National Institutes of Health
- The Food and Drug Administration
- Collaborating Institutions
- The Coordinating Center, Premier Research

Your child's information may be re-disclosed by the recipients described above, if they are not required by law to protect the privacy of the information.

When will my authorization expire?

Your authorization for the use and/or disclosure of your child's health information will expire December 31, 2055.

Will access to my child's medical record be limited during the study?

To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or

billing decision about your child (e.g., if included in your child's official medical record).

_____

Signature of Participant

_____

Signature of Legally Authorized Representative

_____

Date

_____

Description of Representative's Authority to Act for Subject

FINANCIAL CONSIDERATIONS

PAYMENT

You and your child will not be paid to participate in this research study.

COSTS

The sponsor will pay for the cost of sodium nitroprusside and for the extra blood tests that will be used to monitor the amount of drug in your child's blood and for safety tests. You or your insurance company will be responsible for the medical procedures, surgery, anesthesia, and other normal costs associated with standard medical care for treatment of your child's condition.

SPONSOR

The National Institute of Child Health and Development of the National Institutes of Health (NIH) is providing financial support and/or material for this study.

COMPENSATION FOR RESEARCH-RELATED INJURY

All forms of medical diagnosis and treatment – whether routine or experimental – involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. **You will be responsible for any associated co-payments or deductibles as required by your insurance.**

If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

Additionally, *{Institution name}* is not responsible for research and medical care by other institutions or personnel participating in this study. You do not waive any liability rights for personal injury by signing this form.

CONTACT INFORMATION

- Questions, Concerns, or Complaints: If you have any questions, concerns or complaints about this **research study**, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director, *{name}*. You may contact *{him/her}* now or later at *{phone number}*.
- Emergency Contact: If you feel your child has been **hurt by being a part of this study**, or need immediate assistance please contact *{Protocol Director's name and phone number}*.
- Alternate Contact: If you cannot reach the Protocol Director, please page the research team at *{phone number}*.
- Independent of the Research Team Contact: If you are not satisfied with the manner in which this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a research study subject, please contact the *{Institution's name}* Institutional Review Board (IRB) to speak to an informed individual who is independent of the research team at *{phone number}*. Or write the *{Institution's name}* IRB, *{IRB full address}*. In addition, please call the *{Institution's name}* IRB at *{phone number}* if you wish to speak to someone other than the research team or if you cannot reach the research team.

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS *{Text can be revised to meet IRB requirements}*

As a human subject your child has the following rights. These rights include but are not limited to the subject's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
- be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise;
- be given an opportunity to ask questions concerning the experiment or the procedures involved;

- be instructed that parental permission to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated parental permission form;
- and be given the opportunity to decide to give parental permission or not to give parental permission to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING PARENTAL PERMISSION, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____

Signature of Parent or Legal Guardian

_____

Signature of Second Parent or Legal Guardian

_____

Date

_____

Name of Patient

PERSON OBTAINING PARENTAL PREMISSION

I attest that the requirements for informed parental permission for the medical research project described in this form have been satisfied - that the Parent or Legal Guardian has been provided with the Experimental Subject's Bill of Rights, if appropriate, that I have discussed the research project with the Parent or Legal Guardian and explained to him or her in nontechnical terms all of the information contained in this parental permission form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the Parent or Legal Guardian to ask questions and that all questions asked were answered.

_____ _____

Signature of Person Obtaining Parental Permission Date

Appendix C: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles
for
Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when

providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The

responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's

freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case, the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in

the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix D: Responsibilities of the Investigator

Investigator Responsibility/Performance

Prior to starting enrollment at a site, all investigators must read and understand the Investigational Plan and must sign and complete the Investigator Agreement Form. This documents that they accept all conditions of the Investigational Plan and will conduct the study accordingly. The investigator must provide a current copy of his or her curriculum vitae that is not more than 2 years old.

Informed Parental Permission and IRB Approval

The investigator must have written approval from the IRB prior to enrolling patients in the study. A copy of the written approval that includes the following must be provided to the DCRI:

- A statement of IRB approval for the proposed study and informed parental permission form at the institution
- The date the study was approved and the duration of approval
- A listing of any conditions attached to the approval
- Identification of the approved primary investigator
- The signature of the IRB chairperson

Any amendments to the protocol, as well as associated parental permission form changes, will be submitted to the IRB, and written approval must be obtained prior to implementation. Serious adverse event reports will be submitted as requested by the BPCA.

The study will be explained to the patients in lay language. Patients will sign and receive a copy of the IRB-approved informed parental permission form prior to study participation. Patients will be

assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Source Documentation

Regulations require that investigators maintain information in the study patient's medical records that corroborate data collected on the CRFs. In order to comply with these regulatory requirements, at the minimum, the following information should be maintained:

1. Medical history/physical condition of the study patient before involvement in the study sufficient to verify protocol entry criteria (if not already present)
2. Dated and signed notes on the day of entry into the study including clinical site, patient number assigned and a statement that parental permission was obtained
3. Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams)
4. Notations on abnormal laboratory results
5. Serious adverse events reported through 30 days from the end of study drug administration
6. Notes regarding concomitant medications taken during the study (including start and stop dates)
7. Study patient's condition upon completion of or withdrawal from the study

Data Transmittal

Required data will be recorded on the CRFs as soon as possible after the patient is discharged, day 10, or death, whichever comes first. CRFs and any supporting documents must be sent to the BPCA and/or retrieved from the investigational site according to the outlined time windows. The 28-day follow-up CRF needs to be forwarded within 10 days of the follow-up visit.

Non-Protocol Research

BPCA has a legal responsibility to report fully to regulatory authorities all the results of clinical studies. No investigative procedures other than those in this protocol shall be undertaken on the enrolled patients without the agreement of the IRB and BPCA.

Publication Policies

At the conclusion of the study, a multicenter abstract reporting the primary results may be prepared and presented in an appropriate international forum. A multicenter, peer-reviewed manuscript will also be prepared for publication in a reputable scientific journal.

Appendix E: Treatment of Suspected Nitroprusside Toxicity

Signs of Toxicity:

- If the base deficit exceeds -8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the subject will be also be discontinued from study.
- If the lactate level rises by more than 4 mmol/L in an 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output).
- If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes percent between arterial and mixed venous blood.

SUSPECTED CYANIDE TOXICITY SHOULD BE TREATED AS FOLLOWS:

1) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration.

2) Obtain blood for arterial and venous blood gases with co-oximetry, serum lactate, and cyanide and thiocyanate levels.

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10mL) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result)

Subject Hemoglobin g/dL	Initial dose Sodium NITRITE (3%) mL/kg IV	Initial dose Sodium Thiosulfate mL/kg IV
8 g/dL	0.22 mL/kg	1.10 mL/kg
(6.6 mg)/kg		
10 g/dL	0.27 mL/kg	1.35 mL/kg
(8.7 mg)/kg		
12 g/dL	0.33 mL/kg	1.65 mL/kg
(10 mg)/kg		
14 g/dL	0.39 mL/kg	1.95 mL/kg
X	(11.6 mg)/kg	

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals.

[Micromedex Poisondex www.micromedex.duhs.duke.edu , see also Berlin, 1970]

Appendix F: Assay of Nitroprusside Metabolites and Handling of Blood Samples for Assay of Nitroprusside Metabolites

A classical UV bioanalytical method is utilized for detection and quantitation of cyanide in whole blood. Cyanide concentrations are determined by measuring the absorbance of the chromophore formed by the interaction of the cyanide ion with 4-nitrobenzaldehyde and o-dinitrobenzene in 2-methoxyethanol (Rieders, 1971 and Guilbault, 1966). In summary, to a 1.0 ml aliquot of whole blood, 4-Nitrobenzaldehyde solution and o-Dinitrobenzene solution is added then made basic with sodium hydroxide. Following a specific incubation time a UV scan spectrum is obtained from 520 to 580 nm with a maximum reading at 555 nm. The exact details of the method are proprietary to NMS Labs. The method is sensitive to a LLOQ of 0.05 μg/mL which correlates to normal circulating levels. A toxic threshold is normally assessed as approximately 0.5 μg/mL and acute toxicity is observed at greater than 1 μg/mL. There are no known interferences with this assay method.

An ion chromatography method is used for detection and quantitation of thiocyanate in serum that is specific, accurate, precise and rugged. In summary, a 0.10 mL aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography (Vogel, 1981 and Vesey, 1976). The same procedure is employed to detect thiocyanate in urine. The normal range for non-smokers is 1-4 μg/mL in serum/plasma. For smokers, it is 3 – 12 μg/mL and the therapeutic range for sodium nitroprusside is generally between 6 and 29 μg/mL (Schulz, 1984).

Thiosulfate is detected in serum/plasma or urine also via a validated ion chromatography method with the analytes separated and detected via conductivity detection. For this method, a 0.5 ml aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography.

Sample Handling Procedures

At each specified blood collection for cyanide and thiocyanate, 2 mL of arterial blood is to be collected in a 2 mL gray top. Samples should be mixed, then one half the whole blood in the gray top (1 mL) is removed and stored in a polypropylene screw capped container and stored on ice until it can be refrigerated. The remaining 1 mL of whole blood is centrifuged (within 20 minutes of collection at approximately 1200 g for 10 - 12 minutes at ambient or colder temperatures) to obtain 0.5 mL of plasma for thiocyanate analysis. The red bloods cells from this sample may be discarded, or separately tested for cyanide. All samples should be stored in a refrigerator before sending to the Central Lab(s), which should be done as soon as possible. Samples should be shipped COLD, using "frozen cold packs," for overnight delivery. Whole blood samples should NEVER be frozen.

For neonates or to spare the total amount of blood drawn for the analysis, the minimum blood draw is 1 mL of arterial blood. This translates to 0.5 mL of whole blood for cyanide analysis and 0.5 mLs to be centrifuged to obtain approximately 0.25 mLs of plasma for thiocyanate analysis.

The details of handling & shipping samples to the Central Lab(s) will be included in the MOP. This is a summary and not the complete instructions for sample handling.

Appendix G: Sedation Suggested Regimen

Intensive Care Unit:

For those patients who receive study drug in the intensive care unit for a surgical or medical procedure, the following guidelines may be utilized.

Sedation may be administered intravenously and initiated with a benzodiazepine and opiate agonist as follows:

Midazolam bolus 0.1-0.2 mg/kg followed by a continuous midazolam infusion of 0.06-0.3 mg/kg/hr or intermittent bolus of 0.1 mg/kg every 1-2 hours

Or

Lorazepam bolus 0.05-0.1 mg/kg followed by a continuous infusion of 0.025-0.05 mg/kg/hr or intermittent bolus of 0.05-0.10 mg/kg every 4-6 hours

And/or

Fentanyl bolus 1- 5 micrograms/kg (intubated, mechanically ventilated patients) followed by an infusion of fentanyl of 0.5 – 5 micrograms/kg/hour

Or

Morphine bolus 50-100 microgram/kg followed by a continuous infusion of 20-80 micrograms/kg/hour.

Sedation and analgesic medication may be titrated to patient response. Higher doses may be used in patients who exhibit benzodiazepine and/or narcotic habituation due to long term (> 4-7 day) usage. Where applicable, sedation assessment may be by clinical judgment of the responsible physician, and/or a quantitative scoring system such as the COMFORT score. Patients receiving neuromuscular blocking drugs such as vecuronium or rocuronium as part of their ICU management are eligible for study.

Appendix H: Measurement of Blood Pressure in Children

Excerpt from: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents

Correct measurement of BP in children requires use of a cuff that is appropriate to the size of the child's upper right arm. The equipment necessary to measure BP in children, ages 3 through adolescence, includes child cuffs of different sizes and must also include a standard adult cuff, a large adult cuff, and a thigh cuff. The latter 2 cuffs may be needed for use in adolescents.

By convention, an appropriate cuff size is a cuff with an inflatable bladder width that is at least 40% of the arm circumference at a point midway between the olecranon and the acromion. For such a cuff to be optimal for an arm, the cuff bladder length should cover 80% to 100% of the circumference of the arm. Such a requirement demands that the bladder width-to-length ratio be at least 1:2. Not all commercially available cuffs are manufactured with this ratio. Additionally, cuffs labeled for certain age populations (eg, infant or child cuffs) are constructed with widely disparate dimensions. Accordingly, the working group recommends that standard cuff dimensions for children be adopted (see Table 2).

TABLE 2. Recommended Dimensions for BP Cuff Bladders

Age Range	Width, cm	Length, cm	Maximum Arm Circumference, cm*
Newborn	4	8	10
Infant	6	12	15
Child	9	18	22
Small adult	10	24	26
Adult	13	30	34
Large adult	16	38	44
Thigh	20	42	52

* Calculated so that the largest arm would still allow the bladder to encircle arm by at least 80%.

BP measurements are overestimated to a greater degree with a cuff that is too small than they are underestimated by a cuff that is too large. If a cuff is too small, the next largest cuff should be used, even if it appears large. If the appropriate cuffs are used, the cuff-size effect is obviated

SBP is determined by the onset of the "tapping" Korotkoff sounds (K1). Population data in children and risk-associated epidemiologic data in adults have established the fifth Korotkoff sound (K5), or the disappearance of Korotkoff sounds, as the definition of DBP. In some children, Korotkoff sounds can be heard to 0 mm Hg. Under these circumstances, the BP measurement should be repeated with less pressure on the head of the stethoscope. Only if the very low K5 persists should K4 (muffling of the sounds) be recorded as the DBP.

Appendix I: Research Assent Form Template

Protocol Title: A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Director:

IRB Approval Date: _____IRB Expiration Date: _____

Are you taking part in any other research studies right now? ____ yes ____ no

Why Are We Doing this Research Study?

We are doing this study to learn more about a medicine called sodium nitroprusside. This study will help us find out more about how good this drug is in keeping blood pressure and heart rate under control in children who are in the intensive care unit (ICU). Altogether, there will be 60 kids under the age of 17 in this study.

What Will Happen During the Study?

You will be seen by a doctor, who will give you a physical exam and ask you and your parents some questions about your health history and medicines you take. At certain times during the study, blood will be drawn to check that you are okay and safe, and to see how the study drug is working in your body. Blood will be drawn either from a tube that is already connected to you to get blood, or from a needle. The amount of blood to be taken during the study is about 2½ teaspoons to 3½ teaspoons.

In the first part of the study, you will receive a study drug called sodium nitroprusside through a tube placed into a vein in your arm. Your doctor will make changes in the amount of drug you get to keep you safe and your vital signs (like your heart rate and blood pressure) stable. Your doctor will

change how much of the drug you get so you have the blood pressure the doctor thinks is right for you. This part of the study will last for at least 12 hours up to 24 hours during the time that your doctor needs to control your blood pressure. During the time you get study drug, your blood pressure and heart rate will be measured very often.

The second part of the study will last up to 30 minutes. During this part, you will get one of two treatments. This part of the research study is blinded. That means that the study doctor will not know which treatment group you are in. The choice of which treatment you receive will be made in a way that is like flipping a coin. You might receive the study drug at the same rate that was being used before to keep your blood pressure stable. Or, you might receive a placebo, which is a solution like salt water that doesn't have any effect on your blood pressure. Your blood pressure and heart rate will be watched very close. If your blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end right away. Then you will again be given sodium nitroprusside, or similar drug, at a rate that keeps your blood pressure at a safe level.

What if you don't want to be in the Study?

You can say "no" to being in the study if you want. You can also stop the study anytime you want by telling anyone that is caring for you. Your doctor can explain to you and your parents other treatments that could be used instead.

What You Should Know about the Medicine?

The medicine is used to keep your blood pressure where your doctor thinks is safe or needed for your operation or some other treatment or test. It has been okayed for use in adults but there is not a lot of information about how the drug works in kids. Like any medicine, it can cause unwanted things to happen. These unwanted things are called risks. Some of the risks from using this medicine are: getting sick to your stomach, headache, getting hot, having your blood pressure go down too much or your heart beat go up too high.

Things Girls Need to Know….

Some medicine can cause bad things to happen to an unborn baby. If you are able to get pregnant (if you have started having a period), you need to take a pregnancy test which your doctor will give you. If you are pregnant, you should not take part in this study.

What Else Do You Need to Know?

Sometimes doctors write about the research studies when they are done. If a paper is written about this research study, your name won't be used in it, but the medical information they find out about you may be used. We will keep your medical information private. People who work for *[site name]*, the people who are running the study, and some parts of the government (the part that takes care of medicines) will be able to look at your medical information.

There is no cost to you or your parents to be in this study.

If you have questions about the study you can call Dr. _____ at

_____.

I have read this form. I have had a chance to ask questions about things I don't understand. I want to be in this research study and understand what will happen to me.

_____ _____

Signature of Patient Date

_____

Name of Patient

_____ _____

Signature of the Person Obtaining Assent Date

PROTOCOL

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Number: NICHD-2003-09-LT

Study Drug: Sodium Nitroprusside

IND: 71,979

Medical Monitor: Robert Lindblad, M.D.

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Sponsor: The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

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Confidential

APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol and its associated investigator brochure for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

And I have fully discussed the objectives of this trial and the contents of this protocol with representatives of PODS Center and BPCA Data Coordinating Center.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and in accordance with all applicable local, state, and federal regulations.

I understand that, should the decision be made by the PODS Center, BPCA Data Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

PODS Center #1 Duke University Medical Center Box 3094 DUMC Durham, NC 27705	SUBCONTRACTOR: Institution: Address: Telephone: Fax: Email:
CO-PRINCIPAL INVESTIGATOR Scott Schulman, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol and its associated investigator brochure for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

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I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

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CO-PRINCIPAL INVESTIGATOR Gregory Hammer, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

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TABLE OF CONTENTS

PROTOCOL SYNOPSIS

Protocol Title:	A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects
Protocol Number:	NICHD-2003-09-LT
Sponsor:	National Institute of Child Health and Human Development
Product:	Sodium Nitroprusside
Objectives:	1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours 2. To assess the potential for rebound hypertension following administration of sodium nitroprusside for 12 hours or more
Study Design:	This is a phase II, randomized, double blind, withdrawal to placebo study examining the efficacy, safety and tolerability of sodium nitroprusside in pediatric subjects.
Study Population:	Children less than 17 years of age who require long term (at least 12 hour) blood pressure control will be eligible for study.
Number of Subjects:	A target of approximately 60 patients will be enrolled.
Number of Sites:	Up to 15
Duration of Subject Participation:	Enrollment is anticipated to begin in 2008 and to be complete in approximately 24 months. Patients will be followed for up to 30 days following receipt of study drug.
Treatment:	Subjects who require vasodilator therapy for relatively long time periods will receive open-label infusion of sodium nitroprusside for at least 12 hours but not greater than 24 hours.
Dose Schedule:	Patients will be randomized to receive either placebo or sodium nitroprusside for 30 minutes following at least 12- hours but not more than 24 hours of open-label infusion of sodium nitroprusside.
Estimated Start:	Q4 2008
Estimated Finish:	Q3 2010
Ethics	All regulations stated in 21 CFR Parts 50, 56, and 312 and recommendations outlined in the ICH Guidelines for Good Clinical Practice, as well as all other applicable local and national laws and regulations, will be adhered to throughout this trial.

Safety:	The safety of the drug will be assessed by multiple subject assessments of vital signs, physical exams, clinical tests and laboratory evaluations. Adverse events will be monitored and tracked. All SAEs will be closely monitored throughout the course of the study.
Statistical Consideration:	The trial will be sized to detect the loss of as little as 50% of the expected blood pressure lowering effect of the chosen dose of sodium nitroprusside during the 30 minutes of withdrawal to placebo.

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BP	Blood Pressure
BPCA DCC	Best Pharmaceuticals for Children Act Data Coordinating Center
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
cGMP	cyclic Guanosine Monophosphate
CN^-	Cyanide
CRA	Clinical Research Associate
CRF	Case Report Form
d/c	Discontinuation
DCRI	Duke Clinical Research Institute
DBP	Diastolic Blood Pressure
DMC	Data Monitoring Committee
ECMO	Extracorporeal Membrane Oxygenation
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
hr	Hour
HR	Heart Rate

ACRONYMS AND ABBREVIATIONS

IB	Investigational Brochure
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
kg	Kilogram
MAP	Mean Arterial Pressure
mcg	Microgram
mEq/L	Milliequivalent per Liter
mcgs	Micrograms
min	Minute
mL	Milliliter
mm Hg	Millimeters of Mercury
mmol/L	Millimoles per Liter
NO	Nitric Oxide
NICHD	National Institute for Child Health and Human Development
NONMEM	Nonlinear Mixed Effect Model
NTG	Nitroglycerin
PD	Pharmacodynamic
PaO_2	Arterial Partial Pressure of Oxygen
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SCN	Thiocyanate
SNP	Sodium Nitroprusside
μM	Micromoles per liter, Micromolar

1.0 Background and Rationale

Blood pressure control in children is a significant concern in the intensive care unit (ICU), where management of arterial pressure is often necessary during periods of acute physiologic stress such as occurs after certain surgical and medical procedures. Examples of surgical procedures that require blood pressure control in the intensive care unit following surgery include aortic coarctation repair, Ross procedure (pulmonary valve autograft), and solid organ transplantation. Medical conditions requiring control of systemic arterial pressure include renal disease, drug therapy (corticosterioids and immunosuppression agents), and procedures such as extracorporeal membrane oxygenation (ECMO).

A wide variety of drugs of various therapeutic classes have been utilized for either controlled hypotension in the operating room or prevention of hypertension in the pediatric ICU. These drug classes include calcium channel blockers (Tobias et al, 1996), beta-adrenergic antagonists (Kay et al, 2001), ganglionic blockers (DuToit, 1970 and Gallagher and Milliken, 1979), inhalation anesthetics (Tobias, 1998) and direct acting vasodilators such as nitroglycerin and sodium nitroprusside (SNP) (Kaplan, 1980, and Tinker, 1976 Groshong, 1996, and Sinaiko, 1996). Although many vasodilator agents are available to lower blood pressure in the operating room and intensive care unit setting, few have been systematically studied in children.

SNP is a direct acting vasodilator commonly used for blood pressure control. It produces vascular smooth muscle relaxation when its metabolism in the red blood cell results in the liberation of nitric oxide (NO). NO then activates the enzyme guanylyl cyclase. This activation results in the formation of increased intracellular levels of cyclic guanosine monophosphate (cGMP). The result is vasodilation.

1.1 Metabolism

Five molecules of cyanide (CN^-) are released when SNP is metabolized in the red blood cell. The major metabolic pathway for CN^- is conversion to thiocyanate (SCN). This conversion occurs enzymatically via two sulfur transferase systems: 1) rhodenase (the primary pathway) and 2) beta-mercaptopyruvate-cyanide sulfurtransferase. Rhodenase is ubiquitous throughout the body, but it is highly concentrated in the liver. Rhodenase catalyzes the transfer of sulfur from a sulfur donor molecule such as thiosulfate (Na_2SO_3) to cyanide and thereby the formation of thiocyanate (SCN). SCN is subsequently eliminated in the urine and can therefore serve as a marker of cyanide exposure.

The ability of rhodenase to catalyze the conversion of cyanide to thiocyanate (SCN) is limited by the availability of sulfur donors in the body. Thus the provision of exogenous sulfur donors such as thiosulfate (Na_2SO_3) in the setting of acute cyanide intoxication is a potentially life-saving intervention (Pasch et al, 1983, Cole and Vesey, 1987).

One out of every five CN^- ions liberated by the metabolism of SNP binds to methemoglobin to form the non-toxic cyanomethemoglobin. The creation of additional quantities of methemoglobin by the intravenous infusion of sodium nitrite can thus provide additional CN^- buffering capacity. The resultant methemoglobinemia can then be treated with the administration of intravenous methylene blue.

Additional metabolic pathways for CN^- include the conversion of hydroxycobalamine (vitamin B12a) to cyanocobalamine, and conversion to 2-aminothiazoline 4-carboxylic acid.

If the above three pathways (rhodenase, methemoglobin, hydroxycobolamine) are overwhelmed, cyanide will bind to mitochondrial cytochrome oxidases and poison cellular oxidative phosphorylation. Cellular hypoxia is induced when cyanide inhibits the electron transport chain at cytochrome a_3. Oxygen cannot be utilized, mixed venous

oxygen tension rises and the generation of high-energy adenosine triphosphate (ATP) is blocked. The cell reverts from aerobic to anaerobic metabolism, with the subsequent generation of pyruvate and lactate. Acidosis ensues, and with it, deterioration in the organ systems most dependent on oxidative metabolism: the central nervous system and heart.

Clinical manifestations of cyanide toxicity to the central nervous system include headache, anxiety, agitation, confusion, lethargy, convulsions and coma. Cardiovascular manifestations include progressive heart failure with both loss of contractile force (negative inotropy) and slowing of rate (negative chronotropy). Bradycardia and hypotension are commonly observed pre-morbid events associated with cyanide toxicity.

In patients receiving SNP, the earliest, most sensitive signs of cyanide toxicity are acidosis, elevated mixed venous oxygen tension, and rising blood lactate levels. Venous blood that appears "bright" red due to the inability of the tissues to extract oxygen should suggest cyanide toxicity. Arterial and mixed venous blood gas analysis with co-oximetry can help confirm the diagnosis.

1.2 Previous Studies

SNP was first discovered in 1850. Its hypotensive effects were noticed in 1929, and its first therapeutic use was reported by Page et al. in 1955. Moraca et al. first described the clinical use of SNP for deliberate hypotension during surgical procedures in 1962. Since then, it has been widely used to control blood pressure in infants and children in the perioperative period.

Despite its widespread use, there is a paucity of information on its safety, efficacy, and pharmacokinetic/pharmacodynamic relationships in children. Davies et al (1975) and Bennett and Abbott (1977) described their retrospective experience with SNP used to induce deliberate hypotension in small cohorts of children. Both authors observed that younger patients required more SNP than older ones to achieve comparable degrees of

blood pressure control. In their small retrospective cohort, Bennett and Abbott recommended that doses of 10 micrograms/kilogram/minute were necessary to achieve satisfactory blood pressure response. Davies et al described three possible responses to SNP administration in children: 1) a constant response to "conventional" doses < 3 mg/kg; 2) a tachyphylactic response characterized by continuously escalating dose requirement (> 3 mg/kg) to achieve a satisfactory blood pressure; and 3) resistance to the blood pressure lowering effects of the drug. They cautioned against using total doses that exceeded 3 mg/kg or continuing administration of SNP in the latter two scenarios. Firm conclusions cannot be drawn because these small case series were not randomized controlled trials with specific pharmacodynamic endpoints.

Yaster et al (1986) compared SNP to nitroglycerin (NTG) for inducing hypotension in a group of 14 adolescents. They found doses of SNP between 6-8 micrograms/kg/minute superior to NTG at any dose in the reliable induction of hypotension for children and adolescents undergoing scoliosis, craniofacial or hepatic surgery.

Hersey et al (1997) performed a randomized trial comparing SNP to the dihydropyridine calcium channel antagonist nicardipine in 20 healthy adolescents with idiopathic scoliosis undergoing spinal fusion. Target blood pressures were easily obtainable in both groups and operating conditions were comparable. The time to restoration of baseline blood pressure after termination of the infusion was significantly longer in the nicardipine group. Interestingly, blood loss was significantly greater in the SNP group. Details on SNP dose requirements were not provided.

Przybylo et al (1995) described CN^- and SCN blood levels in ten children who received SNP at doses up to 10 micrograms/kg/min (mean infusion rate 6 microgram/kg/min) while undergoing cardiopulmonary bypass for repair of complex congenital cardiac defects. CN^- levels rose as a function of time while SNP was infused, and rapidly fell when SNP was discontinued. Despite the fact that some children demonstrated serum CN^- levels above the generally accepted threshold of 0.5 micrograms/ml, no patient

developed clinically apparent toxicity. Kazim et al (1996) questioned the validity of the results of this study because of the CN^- assay methods utilized.

Linakis et al (1991) retrospectively examined physician-ordering practice as it pertained to blood cyanide levels in children receiving SNP. They sought to determine how the laboratory determinations were used to monitor patients and if there was clinically apparent toxicity in children found to have cyanide concentrations exceeding the "normal" limit of 500 micrograms/liter. They found poor correlation between blood cyanide concentration and dose or duration of therapy in patients whose cyanide levels were "toxic." Thiocyanate determinations were normal and no child manifested signs or symptoms of cyanide toxicity. They concluded that further pediatric studies were needed.

2.0 Study Objectives

We propose a multicenter trial that will provide guidance for the use of SNP to reduce blood pressure in pediatric patients. The trial is a randomized, double-blinded withdrawal to placebo trial. The aims of the trial are:

1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours
2. To assess the potential for rebound hypertension during the 30-minute Blinded Phase following administration of sodium nitroprusside for 12 hours or more.

To meet these study aims, the following study phases, defined in Section 3.1.1, will have the following objectives:

- Open-Label Study Drug Administration (Dose-Titration) Phase
  The objective during this phase of the study is to determine the effectiveness and safety of SNP for controlling blood pressure during stable infusion lasting at least 12 hours.

- Blinded Study Drug Administration Phase

  The primary endpoint for the study will be determined during this phase of the study. The primary endpoint is the change in mean arterial pressure (MAP) recorded during the Blinded Study Drug Administration Phase in the absence of other stimuli. The primary objective is to determine the persistence of sodium nitroprusside versus placebo for reducing blood pressure in pediatric patients

  The secondary objectives during this phase of the study are as follows:

  1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience offset during the 30-minute blinded study drug period.
  2. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience rebound hypertension during the 30-minute blinded study drug period.
  3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the 30-minute blinded study drug period.
  4. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the 30-minute blinded study drug period.
  5. To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.

- Follow-up Phase

The following objectives to be evaluated during this phase of the study are as follows:

1. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the Follow-up Period.
2. To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the Follow-up Period.
3. To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience changes in individual physical examination parameters represented as either normal or abnormal from the Pre-Study Period to the end of the Follow-up Period.
4. To compare the changes (values recorded during the end of the Follow-up Period minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.
5. To compare the changes (values obtained during the two-hour period immediately following the stop of blinded study drug minus values obtained during the Pre-Study Drug Period) in individual laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo.

3.0 Investigational Plan

3.1 Overall Study Design and Plan: Description

This is a phase II, multicenter, randomized, double-blind placebo-controlled, parallel group study to determine the persistence of the effect of SNP on blood pressure and to assess the potential for rebound hypertension associated with prolonged infusion in pediatric subjects.

Target MAP is defined as the clinically appropriate MAP as determined by the investigator taking into account the clinical presentation and medical needs of the subject. The investigator may change the target MAP at his/her discretion based on clinical needs during the course of the study. The anticipated Target MAP must be at least 20 mmHg below MAP_{B1}--(15 mm Hg for subjects < 2 years old), see next definition, for the patient to be eligible for enrollment, see Inclusion criterion #4.

The initial baseline MAP (B1) is defined as the blood pressure measurement taken prior to the initiation of SNP administration, either institutionally-supplied or open-label study drug after at least a 5-minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP). See Figure 1 below.

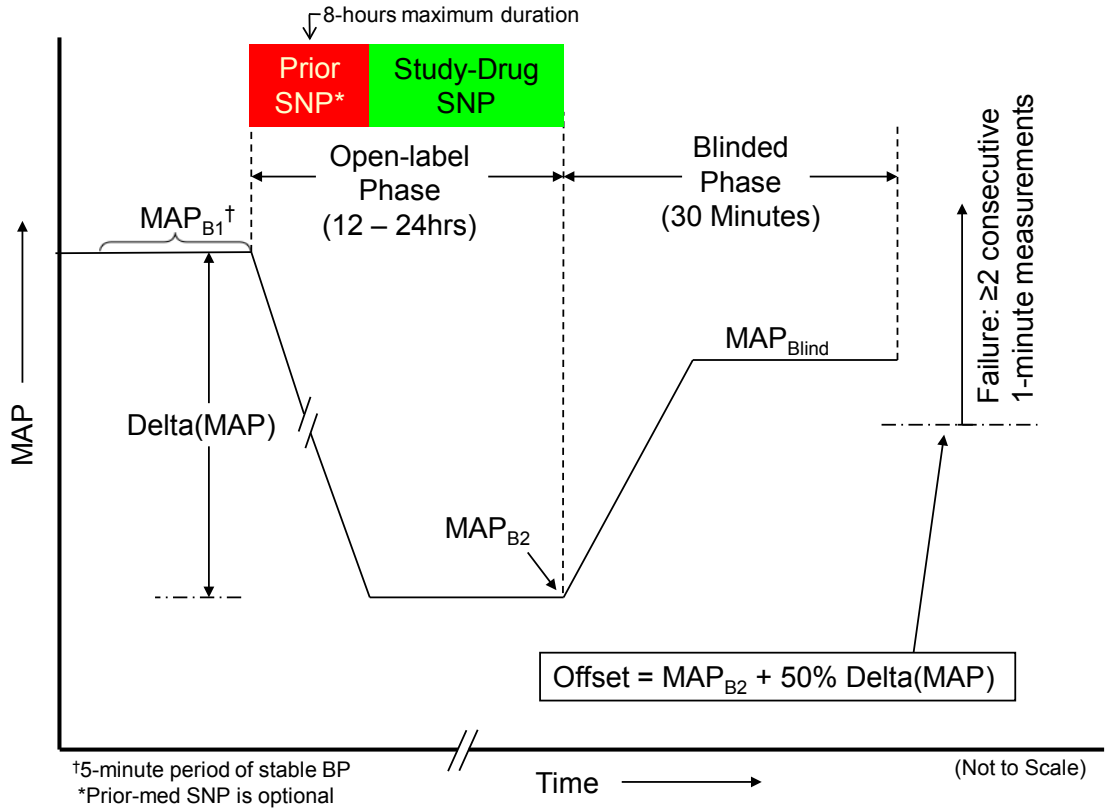
The subsequent baseline MAP (B2) is defined as the blood pressure measurement taken just prior to the initiation of blinded study drug after at least a 5 minute period of stable conditions (e.g. no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). Prior to establishing B2, there shall have been no changes in the SNP infusion rate for a period of at least 20 minutes during which the infusion rate of SNP will be at least 0.5 mcg/kg/min.

Delta(MAP) is defined as the difference between MAP_{B1} and MAP_{B2}.

Offset is defined as MAP_{B2} plus 50% Delta(MAP)

Figure 1

Study Treatment Phases with MAPs and Delta(MAP)



- MAP$_{B1}$ = MAP prior to start of Open-label Study Drug Administration Phase
- MAP$_{B2}$ = MAP immediately prior to start of Blinded Study Drug Administration Phase
- Delta(MAP) = MAP$_{B1}$ – MAP$_{B2}$
- Offset = MAP$_{B2}$ + 50% Delta(MAP)
- Treatment Failure: During the blinded phase, ≥2 consecutive 1-minute MAP measurements greater than Offset [MAP$_{B2}$ + 50% Delta(MAP)]

Example: Subject's MAP immediately prior to start of the Open-label Study Drug Administration Phase is 100 mmHg (MAP_{B1}). At the end of the Open-label Study Drug Administration Phase, MAP is 60 mmHg (MAP_{B2}). Thus, Delta(MAP) = 40 mmHg. For *treatment success*, MAP during the Blinded Study Drug Administration Phase cannot exceed MAP_{B2} + 50% Delta(MAP), or 80 mmHg, for any 2 consecutive MAP_{Blind} measurements, obtained at 1-minute intervals.

Approximately 15 centers will participate in subject recruitment to complete the study.

Approximately sixty (60) patients who require long term (at least 12 hours) blood pressure control will be enrolled. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment.

Any patient who starts the blinded study drug administration period will be considered complete for analysis. Enrolled subjects will be randomized in equal proportions to receive either placebo or SNP for the duration of the blind-treatment period, which will immediately follow the open-label infusion of SNP.

3.1.1 Definition of Study Periods

Study periods are as follows:

- Pre-study drug administration: a period of up to 7 days preceding the start of study drug administration during which informed parental permission, and other enrollment procedures take place.
- Open-label study drug administration (Dose-Titration): The period of open label study drug administration will be at least 12 hours but not greater than 24 hours, including the time of infusion of institutionally-supplied SNP, if any. This period will begin at the start of SNP administration, either study drug or institutionally-

supplied. The duration of infusion of the institutionally-supplied SNP will be no longer than 8 hours. Randomization will normally occur during this period.

- Blinded study drug administration: The period beginning with the start of blinded study drug administration and ending with the discontinuation of blinded study drug. It immediately follows the open-label period and will be no longer in duration than 30 minutes.
- Follow up: The period immediately following blinded study drug administration and ending 30 days after completion of study drug administration. AEs will be followed for 24 hours after termination of study drug. SAEs will be followed for 30 days.

Safety will be assessed via the evaluation of adverse events, pre- and post-treatment laboratory results, and vital sign data. The efficacy endpoints will mainly be assessed by examining blood pressure parameters.

3.2 Selection of Study Population

Children less than 17 years of age who require pharmacologic blood pressure control for at least 12 hours will be eligible for enrollment into the study. Blood pressure control is defined as the maintenance of the subject's MAP to within 90-110% of a target MAP specified by the physician.

Five pediatric age groups will be enrolled in this trial:

Group A: Neonates from birth to less than 30 days of age
Group B: Infants and toddlers from 30 days to < 2 years
Group C: Preschool children from 2 years - < 6 years
Group D: School age children from 6 yrs - < Tanner stage III
Group E: Adolescents from Tanner stage III - < 17 years.

Tanner III refers to the onset of puberty and occurs at different ages in different individuals. The mean age at onset of Tanner III ranges from 12.4 to 13.1 years in males, and 11.9 to 12.6 years in females. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment (see Appendix A).

3.2.1 Inclusion Criteria

Subjects must meet all of the following criteria:

1. Subject is less than 17 years of age.
2. An in-dwelling arterial line is clinically indicated.
3. Subject's parent or legal guardian is willing and able to give informed parental permission signing and dating an IRB-approved informed parental permission containing all of the elements of informed consent, and subject provides assent, signing an IRB-approved and –required informed assent, if applicable.
4. Subject is anticipated to require a minimum of 20 mm Hg (15 mm Hg for subjects < 2 years old) reduction in MAP for at least 12 hours using SNP [i.e., $MAP_{B1} - MAP_{B2} \geq 20$ mm Hg (15 mm Hg for subjects < 2 years old)]

3.2.2 Exclusion Criteria

Subjects will be excluded from study if any of the following criteria exist:

1. Subject weighs < 3.0 kg.
2. Subject has a known allergy to SNP.
3. Subject has a known mitochondrial cytopathy with a disorder of oxidative phosphorylation or of respiratory chain enzymes.
4. Subject has a contraindication to vasodilator therapy for control of blood pressure during surgery or in the intensive care unit.
5. Subject has raised intracranial pressure.
6. Subject is anticipated to need anti-hypertensive drugs other than Sodium Nitroprusside either IV (e.g. dexmedetomidine, esmolol, etc.) or epidural (e.g.

local anesthetics, clonidine, etc.) within three terminal half-lives (3X T½ β) of the blinded study drug period. However, patients receiving stable doses of an anti-hypertensive drug(s) prior to the initiation of study drug may be enrolled.

7. Subject has any serious medical condition which, in the opinion of the investigator, is likely to interfere with study procedures.
8. Subject is moribund (death likely to occur within 48 hours).
9. Subject has a positive result for the urine or serum HCG test administered at screening.
10. Subject has participated in other clinical trials for investigational drugs within 30 days prior to enrollment
11. Subject has received or will have received Sodium Thiosulfate within 6 hours prior to the start of the open-label period.
12. Subject is either on, or anticipated to be on, ECMO.

3.2.3 Prior and Concomitant Therapy, including Medications and Procedures

3.2.3.1 BP-Affecting Drugs

The following medications will be considered to affect blood pressure, and their use, both prior to and concomitant with, SNP infusions will be restricted as described in Sections 3.2.3.2 and 3.2.3.3. There will be no restrictions on the administration of non-study drug medications following the end of the blinded treatment period.

1) Anti-hypertensives and diuretics
2) Beta blocking agents
3) Ace-inhibitors, alone or in combinations
4) Calcium channel blockers and diuretics
5) Diuretics and potassium-sparing agents
6) Peripheral vasodilators
7) NSAIDs and narcotic analgesics
8) Parasympathomimetics

9) Antipsychotics and antineurotics
10) Psychostimulants and nootropics
11) Dopaminergic agents
12) Inotropic agents
13) Sedatives and hypnotics
14) Blood and blood products

3.2.3.2 Prior medications – administration started prior to B1

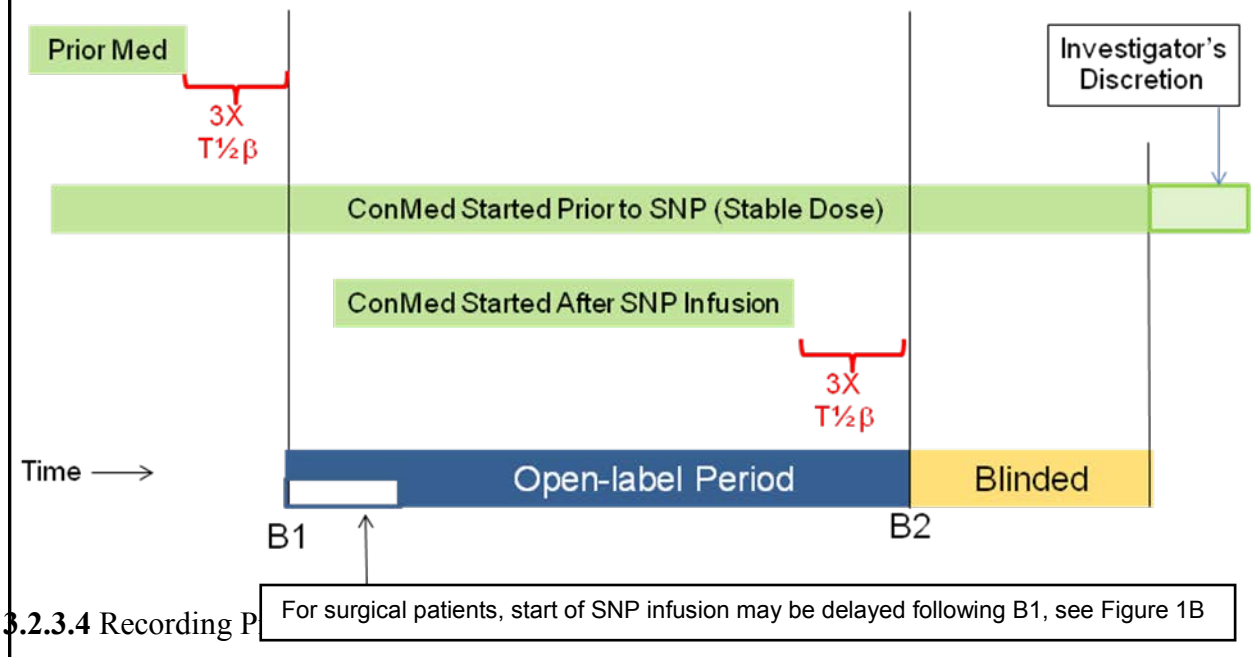
BP-affecting drugs that are started prior to B1 will be either (1) discontinued at least 3 terminal half-lives (3X T½ β) prior to B1, or (2) continued at a stable dose until at least the end of the blinded infusion period, see Figure 2.

3.2.3.3 Concomitant medications – administration started after B1

BP-affecting drugs that are started after B1 will be discontinue at least 3 terminal half-lives (3X T½ β) prior to B2 (i.e., the start of the blinded treatment phase), see Figure 2.

Figure 2

Time Restriction for the Administration of Medications Affecting Blood Pressure



3.2.3.4 Recording P

All concomitant medications that affect blood pressure, sodium thiosulfate, and clinically meaningful, unexpected, and invasive procedures will be recorded for the period beginning 72 hours prior to study drug administration through 24 hours post-study drug conclusion. The dates of administration and reason for use must be included. All concomitant medications will be collected for SAEs occurring within 30 days following study drug administration.

3.2.3.5 SNP as Concomitant Medication

Subjects may receive institutionally-supplied SNP prior to the initiation of study drug administration; however, administration of institutionally-supplied SNP will be discontinued immediately prior to the initiation of study drug administration, and the initial infusion rate of study-drug SNP will be the same as the discontinued institutionally-supplied SNP (see Section 3.4.3 #6). VS measurements described for the

Open-label treatment period in Table 1 and Section 3.4.3 refer to both institutionally supplied SNP and investigational SNP (study drug).

Subjects may receive institutionally-supplied SNP after the conclusion of the SNP Blinded Treatment Period at the discretion by the investigator.

The start and stop times and dates, and dosage of institutionally-supplied SNP administration will be recorded on the appropriate CRF.

3.2.3.6 Use of Sodium Thiosulfate

Administration of sodium thiosulfate will be prohibited from 6 hours prior to the start of the open-label period until the end of the blinded-study period, excepted in cases in which nitroprusside or cyanide toxicity is suspected, in which case, the administration of sodium thiosulfate, as described in Appendix E, is recommended.

To facilitate the assessment of cyanide clearance, investigators are encouraged not to co-administer sodium thiosulfate with institutionally-supplied SNP, which may, at the investigator's discretion, be administered following the conclusion of the blinded-study period. Should sodium thiosulfate be co-administered with institutionally-supplied SNP following the conclusion of the blinded-study period, blood and urine samples will not be tested for cyanide and Thiocyanate in these subjects once the sodium thiosulfate administration has begun.

3.3 Efficacy and Safety Assessments

3.3.1 Efficacy and Safety Measurements

Table 1 is a schematic representation of study assessments and procedures.

3.3.2 Safety Assessments

Safety assessments will include monitoring the tolerability of the SNP infusion and assessing physical examinations, vital signs, clinical laboratory values, concomitant medications and procedures, and adverse events throughout the study. SAEs will be collected for 30 days following completion of study drug administration.

In cases of discharge from the hospital before 30 days, parent (or guardian) will be contacted to determine if any SAE's occurred following discharge but within 30 days of study drug discontinuation. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. See section 4.2 for specific adverse event parameters and actions to be taken. See section 3.4.7.2 for supplemental cyanide and thiocyanate testing.

3.3.3 Drug Concentration Measurements

Cyanide, thiocyanate, methemoglobin, lactic acid, and arterial blood gas analysis will be performed throughout the trial to indirectly query SNP levels and determine subject safety.

TABLE 1: Schedule of Assessments: Sodium Nitroprusside Long-Term Infusion Study

Procedure	Pre-study Drug Period (Up to 7 days prior to Study Drug administration)	Open-label Period (12 -24 hrs duration)	30 minute Blinded Study Drug Period	Study Drug d/c (Within 2 hours)	Follow-up (Up to 24 hours post blinded study drug)[1]
Assessments					
Review Entry Criteria	X				
Informed Parental Permission/ HIPAA Consent	X				
Collect Demographic Data	X				
Medical History	X				
Physical Examination	X			X	X[2]
Vital Signs (SBP, DBP, MAP, HR)[3]	X[4]	X	X	X	X
B1	X[5]				
B2—Just Prior to Blinded Infusion		X			
Growth Parameters[6]	X				
Urine Output[7]	X	X	X	X	X
Serious Adverse Events/Adverse Events		X	X	X	X[8]
Concomitant Medication[9]	X[10]	X	X	X	X[11]
Concomitant Procedure[12]	X[10]	X	X	X	X[11]
Randomization of Blinded study drug		X			
Blinded Study Drug Administration			X		
Open-label Study Drug Administration		X			
Laboratory Assessments					
Pregnancy test (post-menarche females)	X[13]				
Electrolytes, BUN, creatinine	X			X	
Hematology (CBC & platelet count)	X			X	
Liver Enzymes (AST, ALT)	X			X	
Arterial Plasma Lactate level	X	Q 8 hours (± 30 min)		X	X[14]
Arterial Blood Gas with Co-oximetry (includes Methemoglobin)[15]	X	Q 8 hours (± 30 min)		X	X[14]
Central Venous Blood Gas with Co-oximetry (includes Methemoglobin)[16]	X	Q 8 hours (± 30 min)		X	X[14]
Blood for Thiocyanate and Cyanide[17] (central lab)	X	Q 8 hours (± 30 min)		X & 12 hr post d/c [18]	X[14]
Urine for Thiocyanate (central lab)[19]	X	Q 8 hours (± 30 min)		X	Q8 hrs (± 30 min) X 3

1. End of Study assessment will be done at 24 hours post blinded study drug administration, except where noted.
2. To be performed 18-30 hours following the termination of study-drug administration.
3. Vital sign measurements as described in protocol, sections 3.4.2 – 3.4.7.1. Vital signs will then be collected every 12 ± ½ hours for 24 hours post blinded study drug administration. VS measurements described for the Open-label treatment period refer to both institutionally supplied SNP and investigational SNP (study drug), see also Section 3.4.3 (#9).
4. Obtain vital sign measurements. Based upon clinical judgment, this may be MAP_{B1} for some patients.
5. MAP_{B1} will be obtained prior to the start of the SNP open-label infusion and after a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP)

6. Growth parameters will include weight, height/ length, and Tanner stage, if ≥6 years old.
7. Measurements to be performed at time of urine thiocyanate sample collection, if feasible.
8. AEs will be followed for 24 hours and SAE will be followed for 30 days, after the completion of study drug administration.
9. CRFs will document administration of concomitant medications that (1) affect blood pressure, as defined in Section 3.2.3.1, or (2) are associated with SAE's. All concomitant medications will be recorded in source documents, such as the patients' medical charts.
10. Within 72 hours of study drug administration.
11. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
12. Clinically meaningful, unexpected, and invasive procedures only.
13. To be performed within 48 hours of study drug administration.
14. Following concomitant SNP d/c, if feasible.
15. ABG sampling preferred, sample collected at drug d/c only if line is still in.
16. Central Venous Blood Gas done only if CVC is indwelling.
17. Additional cyanide and thiocyanate tests are permitted provided that the investigator believes the tests to be in the patient's best interest and the volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).
18. Blood for cyanide & thiocyanate at 12 hrs ± 30 min, post study drug d/c, only if arterial line in place.
19. Urine collection details are described in section 3.4.7.2 and the MOP.

3.4 Study Visits and Procedures

3.4.1 Informed Parental Permission

Prior to the start of any study-related procedure, a signed and dated informed parental permission, containing all elements of informed consent and, if applicable, assent must be obtained and documented in the subject's medical record (See Appendix B).

3.4.2 Pre-study drug administration procedures

The following procedures will be completed prior to the administration of study drug:

1) Obtain signed and dated informed parental permission/HIPAA authorization/assent.
2) Collect demographic data and medical/surgical history.
3) Record diagnosis.
4) Perform a pertinent physical examination.

5) Obtain vital sign measurements. Based upon clinical judgment, this may be MAP_{B1} for some patients.
6) Determine subject height in centimeters and subject weight in kilograms (for calculation of appropriate study drug dose).
7) Collect urine and blood samples for laboratory evaluations as per Table 1. Pregnancy test if required must be done within 48 hours prior to study drug administration. (If the screening pregnancy test will have been more than 48 hours prior to the start of the study drug administration, then the test will be repeated.)

   To minimize the blood volume obtained under this protocol, laboratory evaluations performed prior to the consenting of the patient as part of the standard of care of the patient and within 7 days of the administration of study drug may be substituted for these procedures.
8) Document concomitant medications that may affect blood.

3.4.3 Open-Label Study Drug Administration (Dose-Titration) Procedures:

The following procedures should be performed sequentially unless otherwise indicated.

1) Stabilize sedation/analgesia.
2) Insert arterial line if not already in place.
3) Obtain baseline vital sign measurements prior to the start of SNP administration, either institutionally-supplied or open-label study drug. This defines B1.
   A) The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in drug concentrations which may affect BP).
   B) If MAP_{B1} is not obtained immediately prior to the initiation of infusion of SNP, a VS measurement will be obtained immediately prior to the initiation of the open-label SNP infusion (time=0).
4) Determine and record target MAP.
5) If the difference between B1 and the target MAP is <20 mmHg (15 mmHg for subjects <2 years old), the patient will be withdrawn from the study and not given study drug.

6) Begin administration of open-label study drug at a dose not to exceed 0.3 mcg/kg/min, or, if applicable, at the infusion rate of the institutionally-supplied SNP.
7) The dose of open-label SNP will be titrated according to the subject's BP response such that the target MAP, chosen by the study physician, is achieved ±10%. If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose. The duration of open-label study drug administration will be at least 12 hours but less than 24 hours.
8) Revise target MAP as clinically indicated; titrate SNP to achieve new target MAP (±10%).
9) Obtain vital sign measurements every one minute for the first 10 minutes then every 5 ± 1 minutes for an additional 20 minutes after initiation of open-label study drug infusion (and, if applicable, institutionally-supplied SNP) and after each dosage adjustment. After the initial 30 minutes, once a stable dose is achieved and BP control is satisfactory, vital sign measurements will be obtained every ≤ 20 minutes. Additionally, obtain vital sign measurements in a similar manner whenever it is necessary to change the open-label drug infusion rate.
10) Randomize patient
11) Collect blood samples for laboratory evaluations as per Table 1.
12) Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures, and adverse events.
13) Whenever an adverse event occurs, obtain vital sign measurements. If clinically appropriate, a blood sample for safety including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations may be drawn.

3.4.4 Blinded Study Drug Administration Procedures

1) Obtain vital sign measurements immediately prior to the start of blinded study drug administration. This defines MAP_{B2}. There must be 5 minutes of stable conditions and 20 minutes of no changes in Open-label study drug prior to starting the blinded study drug administration phase.
2) Begin 30-minute blinded study drug administration as described in Section 3.4.8.
3) Record blood pressure and heart rate every one minute for the duration of blinded study drug administration.
4) If blood pressure control is lost (defined as loss of 50% of delta MAP for ≥2 consecutive 1-minute measurements) the blinded study drug is discontinued when there is a safety concern or the MAP reaches 120% of MAP_{B1}.
5) Record concomitant medications associated with an SAE or that may affect blood pressure, clinically meaningful, unexpected, and invasive procedures and adverse events. Whenever an adverse event occurs, obtain vital sign measurements and, if appropriate, a blood sample for safety analysis including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations.

3.4.5 Study Drug Discontinuation (Within 2 hours of discontinuing study drug)

1) Collect urine and blood samples for laboratory evaluations listed in Table 1
2) Conduct a pertinent physical examination and perform all other assessments listed in Table 1 for this study phase.

3.4.6 Follow up Procedures

The following will be performed after completion of study drug administration through 24 hours post study drug end:

1) If applicable, record the estimated blood loss and fluid intake, including blood and blood products and output during the trial period.
2) Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
3) Record vital signs at 12 and 24 ± ½ hours after the end of study drug administration.
4) Collect blood sample at 12 hours (± 30 min) after study drug discontinuation for cyanide and thiocyanate analysis.
5) Perform a pertinent physical examination 18 –30 hours following discontinuation of study drug administration.
6) Collect adverse events for 24 hours following discontinuation of study drug administration.
7) Collect serious adverse events plus associated concomitant medications and clinically meaningful, and invasive procedures for 30 days following discontinuation of study drug administration.

8) Collect blood samples for laboratory evaluations as per Table 1.

   Blood samples for cyanide and thiocyanate analysis at the discontinuation of the non-study drug (concomitant) SNP to be done only if feasible (the informed parental permission form must specify this blood sample will be drawn and only if an indwelling catheter is present). Note: This blood draw may be several days following the discontinuation of study-drug SNP administration."

SAEs and associated concomitant medications and procedures will be collected for 30 days following the discontinuation of study drug administration, either through telephone contacts and/or study visits or spontaneously reported by the subjects.

3.4.7 Methods of Assessment

3.4.7.1 Vital Sign Measurements

Vital signs: systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate will be measured.

The principal method of obtaining blood pressure measurements will be from an intra-arterial catheter inserted in an upper or lower extremity artery. Manual blood pressures from a non-invasive blood pressure cuff will only be used prior to insertion of and during a malfunction of the arterial catheter. The blood pressure transducer is internally calibrated by the instrument upon performing the zeroing procedure. All blood pressure and heart rate data will be acquired electronically when possible.

3.4.7.2 Blood Draws and Urine Samples

Blood drawn for study related purposes will not exceed the maximum amounts specified by the American Association of Blood Banks for healthy infants, children, and

adolescents. Normally, this value is 7 ml/kg over an eight week period. This study is an inpatient trial of short duration, therefore, the amount of blood withdrawn for study related purposes will take into account the patient's pre-existing hemoglobin and hematocrit, and local Institutional Review Board limitations on maximum allowable blood draws for study-related purposes. A reasonable and conservative value is 3 ml/kg.

In addition to those cyanide and thiocyanate tests scheduled in Table 1 and Section 3.4, the investigator will be permitted to perform supplemental cyanide and thiocyanate tests provided that the investigator believes that the tests are in the patient's best interest and the volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).

Urine will be sampled for thiocyanate concentrations every 8 hours, or fraction thereof, commencing with initial study drug administration to the end of study drug completion and then Q 8 hours times 3 after the discontinuation of study-drug administration, or until the urinary catheter is removed, whichever occurs first. All urine samples will be from a pooled urine collection from the 8-hour time period or fraction of 8-hour time period. The sample will be stored cold until shipment to the central lab. Details of urine collection procedures are described in the Manual of Procedures (MOP).

3.4.8 Dispensing of Study Drug

Study drug will be dispensed to the sites in 2 ml vials containing 25 mg/ml of SNP. The pharmacist will dispense two preparations of study drug, one for the open-label study drug period and one for the blinded study drug period.

The open-label study drug administration phase will utilize a fixed study drug concentration and variable infusion rate scheme. Syringes or bags will be prepared by the investigational drug pharmacy by adding 25 mg of SNP to 50 ml 5% dextrose. The syringe will have a label indicating the concentration of the solution (0.5 mg/ml SNP), and the infusion rate necessary to provide 1.0 microgram per kilogram per minute

(1.0 mcg/kg/min) —for example, for a 25-kg subject, an infusion rate of 3.0 ml/hr will deliver 1.0 mcg/kg/min SNP. The infusion rate of 0.5 mg/ml SNP can be calculated using the conversion factor: Wt (kg) × 0.12 ml/kg/hr = 1.0 mcg/kg/min. Clinicians can then make the necessary dosage adjustment for adequate blood pressure control. All dosage adjustments will be captured on the case report forms (CRFs).

The pharmacist will supply blinded study drug for each subject according to a randomization assignment generated by the IVRS. The blinded study drug will be prepared by the pharmacist such that either the concentration of drug is the same as in the open label period or placebo, see Section 3.6.1. Subjects will receive blinded study drug at the same rate of infusion that was used at the conclusion of the initial open-label study drug administration period, which will be at least 0.5 mcg/kg/min.

Syringes or bags will be wrapped in opaque or amber plastic to protect from light.

3.4.9 Delivery of Study Drug

Infusion pumps capable of reliable delivery at low infusion rates (to 0.1 ml/hr) will be used. All pumps will have free flow protection and will be internally calibrated for accuracy by the manufacturer. Accuracy will be verified at each site by the biomedical engineering department as part of their equipment management program. Quality assurance checks will be performed periodically according to manufacturer specifications.

Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. Microbore low compliance tubing, with volumes of approximately 1 mL will be used, where possible.

Study drug will be infused via a dedicated peripheral intravenous catheter or via a dedicated lumen of a multi-orifice central venous catheter. Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered

to this patient population. The carrier flow rate will be ≥5.0 mL/hr from at least one hour prior to the start of the blinded study period until the end of the blinded treatment period. Clinical judgment should be exercised when adjusting the carrier flow rate to prevent unsafe spikes in the infusion rate.

3.5 Removal of Subjects from Therapy or Assessment

3.5.1 Early Discontinuation of Study Drug and Subject Withdrawal

If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose.

If the subject withdraws participation in the study for any reason, every effort will be made to collect safety data, vital sign measurements, samples for safety and laboratory analyses. The date, time and reason for discontinuation must be recorded on the CRF. Additionally, every attempt should be made to complete all other study related procedures on discontinued subjects who have received any amount of study drug as the data will be included in the safety and intention to treat analyses. Subjects who prematurely discontinue from the study will not be replaced.

Any patient who starts the blinded study drug administration period will be considered complete for analysis.

Potential reasons for subject withdrawal from the study are as follows:

1) Subject's parent or legal guardian wishes to have the subject withdrawn for any reason;

2) Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the subject's safety;
3) The investigator feels that it is in the subject's best medical interest to be withdrawn.
4) Subject no longer needs blood pressure control.

3.6 Investigational Product

3.6.1 Identity of Investigational Product

Sodium nitroprusside (sodium nitropentacyanoferrate (III) dihydrate) is a reddish-brown crystalline powder that is freely soluble in water. Its molecular formula is $Na_2[Fe(CN)_5NO] \cdot 2H_20$. Study drug will be supplied by the BPCA Data Coordinating Center to the Investigational Drug Service at each clinical center in a standard concentration of 25 mg/ml. The Investigational Drug Service at each clinical center will then prepare the drug in syringes or bags of sterile 5% dextrose for administration to randomized patients according to the guidelines provided above. Sterile 5% dextrose will be utilized as placebo.

3.6.1.1 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature from 15°- 30°C and protected from light in its carton until used. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug must be maintained under adequate security.

3.6.2 Methods of Assigning Subjects to Treatment Groups

After meeting all inclusion and exclusion criteria, subjects will be considered to be enrolled and will be randomly assigned to receive either placebo or active drug treatment groups during the blinded study drug administration phase of the trial using a single centralized randomization schedule. Randomization into the blinded portion of the trial will be performed via a centralized interactive voice response system (IVRS).

The BPCA DCC will provide a system for the pharmacist to obtain each subject's randomized treatment assignment in a timely manner prior to the administration of study drug.

3.6.3 Assigning Subject Numbers

Study participants will be assigned a subject number upon successful enrollment into the study. The subject number will consist of five digits. The first two digits will be the site number of the enrolling institution followed by the number "2"—for the second trial under this IND—followed by a two digit enrollment-sequence number. For example, Subject #10-2-23 would be the 23rd subject enrolled at Site #10.

3.6.4 Blinding

The subject, as well as all caregivers, will remain blinded to the treatment assignment throughout the course of the study. For subjects' safety, the pharmacist will be aware of the treatment group for each subject. The BPCA DCC will maintain the double-blinded randomization schedule.

The randomization for an individual subject may be revealed in an emergency; however, investigators are discouraged against requesting that the blind be broken for

an individual subject. Notification of any unblinding must be sent via facsimile to the BPCA DCC within 24 hours.

3.6.5 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms.

3.6.6 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the Clinical Supply Shipment Request and Verification or similar document. An accurate inventory of study drug will be kept by the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and returned to the BPCA DCC if requested. The investigator agrees not to supply study medication to any persons not enrolled in the study or not named as a sub-investigator for the trial.

4.0 Adverse Events

4.1 Definition

An adverse event is defined as any unintended and unfavorable medical occurrence in a clinical investigation subject, administered a pharmaceutical product, regardless of the causal relationship with treatment. An adverse event can therefore be any untoward sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not the event is considered causally related to the use of the product. Pre-existing conditions that remain stable

throughout the study period will not be considered adverse events. Any worsening of a pre-existing condition or illness is considered an adverse event.

Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional over-dosage, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events if they result in discontinuation from the study, necessitate therapeutic medical intervention, or the investigator considers them to be adverse events.

Adverse events will also include electronically-monitored vital signs that meet the definitions of section 4.2; however, electronically-captured data excursions, which in the opinion of the investigator represent data artifacts, such as might be produced by the turning of the patient or brief interruption of the electronic circuit of the monitor device(s) or which do not likely reflect an actual untoward event will not be considered to adverse events.

New or worsening physical-exam findings that occur following the initiation of study-drug administration will be considered to be adverse events, without regard to causality.

4.1.1 Serious Adverse Events

A serious adverse event is one that meets the above criteria and also results in one of the following conditions:

1. Death
2. A threat to life
3. Requirement for inpatient hospitalization
4. Prolongation of hospitalization
5. Production of a congenital anomaly or birth defect

6. A persistent or significant disability or incapacity (excluding experiences of minor medical significance such as headache, nausea, vomiting, diarrhea, and accidental injury)
7. The requirement for a medical or surgical intervention in order to prevent a serious outcome.
8. Any event deemed to be serious by the Investigator

If an adverse event meets any of the above criteria, it must be reported to the BPCA DCC as a serious adverse event (SAE) within 24 hours of the investigative site's awareness of its occurrence.

4.2 Adverse Event Severity

The criteria for rating adverse events are as follows:

Mild	The adverse event is transient and easily tolerated by the subject.
Moderate	The AE causes subject discomfort and interrupts usual activities.
Severe	The AE causes significant interference with normal activities and may be incapacitating or life threatening.

Safety data are a primary objective of this trial. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. Safety will be evaluated throughout the study and during the follow-up period by evaluating the tolerability of the study drug infusion and by monitoring clinical and laboratory signs of SNP toxicity such as hypotension, tachycardia, bradycardia, acid base status, serum lactate concentration, methemoglobin levels, cyanide levels, and when available, mixed venous oxygen tension.

- Co-oximetric arterial blood gas analysis with methemoglobin determination will be performed Q8. Arterial blood gas monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity.

- Lower than expected methemoglobin concentrations may reflect indirect evidence of cyanide toxicity because cyanide has a high affinity for methemoglobin, combining with it to form the non-toxic molecule cyanomethemoglobin.
- Other laboratory findings suggestive of cyanide toxicity that will be monitored in this trial include serum lactate levels and mixed venous oxygen saturation.

  Lactate levels will rise and mixed venous oxygen tension will increase when cyanide toxicity is occurring due to the reduced ability of the tissues to extract oxygen.
- Plasma and urine thiocyanate levels are indirect markers of cyanide exposure because the majority of the cyanide ions liberated by the metabolism of SNP in the red blood cell are converted to thiocyanate by the rhodenase enzyme system in the liver and excreted in the urine. Free cyanide levels will also be measured.

The adverse event of rebound hypertension shall be considered mild if the MAP rises >10% above the baseline (B1), moderate if the MAP rises >20% above baseline (MAP_{B1}), and severe if the MAP rises >30% above baseline (MAP_{B1}).

The adverse event of excessive hypotension shall be considered mild if the MAP falls >20% below target; fluid therapy may be administered. The adverse event of excessive hypotension shall be considered moderate if the MAP falls >25% below target, fluid therapy is required, and pharmacologic therapy is required. The adverse event of excessive hypotension shall be considered severe if the MAP falls >30% below target, fluid therapy is required, and repeated and/or continuous pharmacologic support is required.

The rating of the adverse event of tachycardia shall be defined for sustained rates exceeding the age adjusted maximums (Table 3) by using the mild, moderate, and severe ranges outlined in Table 4A.

TABLE 3: Age-Adjusted Maximums for Pediatric Heart Rate

Subject Age	Maximum Heart Rate (bpm)
1 month <6 months	180
6 months <3 years	160
3 <8 years	150
8 <17 years	130

TABLE 4A: Severity of Tachycardia (Heart Rate in Beats per Minute)

Age	Mild	Moderate	Severe
Age <6 months	180-199	200-219	220 and higher
6 months - <3 years	160-179	180-199	200 and higher
3 years to < 8 years	150-164	165-179	180 and higher
8 years to <17 years	130-149	150-169	170 and higher

The adverse event of lactic acidosis shall be considered mild if the serum lactate concentration is between 5 and 7.5 mmol/L, moderate if the serum lactate concentration is between 7.6 and 10 mmol/L, and severe if the serum lactate concentration exceeds 10 mmol/L.

The adverse event of cyanide toxicity will be considered mild if the blood cyanide concentration is 0.5 mcg/mL – 1.0 mcg/mL, moderate if the blood cyanide concentration is >1.0 – 1.5 mcg/mL, and severe if the blood cyanide concentration is > 1.5 mcg/mL. Corresponding erythrocyte (vs. blood) cyanide values are ≥5 µmol/L - < 10 µmol/L (mild), 10-20 µmol/L (moderate), and > 20 µmol/L (severe).

Severity ratings for these selected adverse events are displayed in Table 4B.

TABLE 4B: Severity of Selected Adverse Events

Event	Mild	Moderate	Severe
Rebound Hypertension (during blinded phase)	MAP rises >10% above baseline (MAP_{B1})	MAP rises >20% above baseline (MAP_{B1})	MAP rises >30% above baseline (MAP_{B1})
Hypotension	MAP falls >20% below target	MAP falls >25% below target IV therapy required; Pharmacological therapy required	MAP falls >30% below target IV therapy required; Repeated or continuous pharmacological therapy required
Lactic acidosis	5 -7.5 mmol/L	7.6-10 mmol/L	>10 mmol/L
Cyanide toxicity (Blood)	Cyanide 0.5 – 1.0 mcg/mL	Cyanide >1.0-1.5 mcg/mL	Cyanide >1.5 mcg/mL
Cyanide toxicity (erythrocyte)	Cyanide $\geq$5 - <10 μmol/L	Cyanide $\geq$10–20 μmol/L	Cyanide >20 μmol/L

Frequent monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity. If the base deficit exceeds 8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the patient will be discontinued from study and the SNP infusion terminated. If the lactate level rises by more than 4 mmol/L in an 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output) SNP administration will be discontinued. If

the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes
percent between arterial and mixed venous blood in the absence of an explainable cause, SNP administration will be discontinued and treatment for suspected cyanide toxicity will be initiated.
Suspected cyanide toxicity will be further assessed and treated as follows:

1) Obtain blood for arterial and venous blood gases with co-oximetry, plasma lactate, and cyanide and thiocyanate levels.

2) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration (see below).

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10ml) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result).

TABLE 5: Dosage Chart for Children

Patient's Hemoglobin g/dL	Initial Dose of Sodium Nitrite (3%) mL/kg IV	Initial Dose of Sodium Thiosulfate mL/kg IV
8	0.22 mL/kg (6.6 mg/kg)	1.10 mL/kg
10	0.27 mL/kg (8.7 mg/kg)	1.35 mL/kg
12	0.33 mL/kg (10.0 mg/kg)	1.65 mL/kg
14	0.39 mL/kg (11.6 mg/kg)	1.95 mL/kg

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals. [Micromedex Poisondex www.micromedex.duhs.duke.edu , see also, Berlin, 1970]

4.3 Relationship to Study Drug

The criteria for determining the relationship of the AE to the study drug are as follows:

1) Probably related: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.
2) Possibly related: An AE that is likely to be related to the administration of the study drug and an alternative cause is equally or less likely when compared to the study drug.
3) Probably not related: An AE that has little or no relationship to the study drug and there exists a more likely, or equally likely, alternative cause.
4) Not related: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.

4.4 Adverse Event Collection Period

Non-serious adverse events will be monitored and reported from the time the subject receives study drug up to 24 hours following termination of study drug; SAEs will be monitored and reported from the time the subject receives study drug through 30 days following termination of study drug. Knowledge of adverse events will be gained from direct monitoring of the study subject as well as from clinician observation, and self reporting by the study subject or his/her guardians. Adverse events that have not resolved, or are ongoing, will be monitored to resolution if felt to be related to study drug, or until it is felt that the subject has stabilized.

5.0 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual subject, the investigator or other responsible physician must contact one of the Co-Principal Investigators immediately so that a timely decision can be made as to whether or not the

subject should be enrolled or continue in the study. If a deviation is being requested by one of the Co-Principal Investigators, he must contact the other Co-Principal Investigator for a decision. The deviation from the protocol will be authorized only for that particular subject. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF.

6.0 Data Monitoring Committee

To ensure that the welfare of trial patients receives appropriate consideration, an independent Data Monitoring Committee (DMC) has been organized by the BPCA DCC on behalf of the NICHD to review relevant safety and efficacy data during the course of the trial. The DMC may recommend discontinuation of the study, or modifications to the study protocol for safety reasons.

The DMC consists of four core members (Chair, ethicist, statistician, community representative) plus additional ad hoc members for the various medical subspecialties involved in the BPCA protocols.

Each DMC will have a presenting statistician who will be responsible for presenting the interim data. This member will write the reports and will be one non-voting member of the DMC. Except as their role in the DMC, all DMC members are not participating in the design or conduct of this study, as an investigator or otherwise, and lack any financial conflict that would introduce any bias.

6.1 DMC Responsibilities

- Monitoring the safety of trial patients;
- Recommending discontinuation of the trial for safety reasons;
- Recommending changes to the study protocol for safety reasons;
- Determining whether early stopping efficacy conditions are met;
- Providing written reports on an ongoing basis following scheduled and ad hoc

meetings that will be archived and may be provided to regulatory agencies.

The DMC will monitor the safety of trial patients by reviewing the occurrence of adverse events and deaths, on a real-time basis as SAE reports are transmitted. The DMC may also monitor compliance with the protocol, and factors affecting patient safety or the integrity of the trial. The DMC may request any additional data that are not included in the report if deemed necessary for effective monitoring.

If the DMC finds any major concerns about safety, it may recommend discontinuing the trial or modifying the study protocol. Following each data review, the DMC will send a written recommendation regarding the trial, (e.g., to continue according to the protocol, or recommendations for specific actions) to the sponsor.

7.0 Statistical Considerations

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a Statistical Analysis Plan, which will be finalized prior to unblinding. Any changes in the statistical methods described in this protocol that occur prior to unblinding will be documented in the Statistical Analysis Plan and will not result in a protocol amendment.

7.1 General Overview

The primary efficacy variable is the intra-patient change in Delta (MAP) during the blinded phase of the study. The primary null hypothesis to be tested is that there is no difference between the active study drug and placebo in the proportion of patients who experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP). Statistical analyses will be performed using two-sided tests. A 0.05 significance level will be used in all tests of treatment differences. Tests for interactions will utilize a 0.10 statistical significance level. Individual secondary endpoints will be evaluated using a hierarchical testing procedure. The Statistical Analysis Plan will include a detailed

description of all statistical methods, testing procedures, and methods of data imputation. One formal interim analysis is planned to evaluate the primary efficacy endpoint when 40 out of 60 patients have completed or withdrawn from the double-blind phase of the study. To control overall type I error at the nominal 0.05 level, critical values at interim and final analysis will be based on the Pocock stopping boundaries. The Data Monitoring Committee charter will contain the specific details regarding the early stopping rule at interim analysis.

Data will be summarized by treatment group with respect to demographic and baseline characteristics, efficacy variables, and safety variables. For parameters measured at baseline, the outcome variables of interest are the changes from baseline (Pre-Study Drug Period). Summary statistics will include the mean, N, standard deviation, median, minimum, and maximum values for continuous variables, and frequencies and percentages for categorical variables. Prior to summarizing results by study center, or performing analyses that include center as a factor in the analysis, small centers will be pooled. All efficacy variables will be summarized by treatment and by visit. Analyses will be performed to explore whether there are treatment-by-center interactions. If a treatment-by-center interaction is detected, the interaction will be explored in an ad-hoc manner. Categorical variables will be analyzed using Cochran-Mantel-Haenszel (CMH) test blocking on centers. Details of the model and the analyses will be specified in the Statistical Analysis Plan and all statistical analyses will be performed using SAS, Version 8.2 or higher.

7.2 Study Objectives

The study objectives are as defined in Section 2.0 of this protocol.

7.3 Patient Population(s) for Analysis

7.3.1 Efficacy

The intent to treat (ITT) population will contain all patients who were exposed to the study drug during the Open-Label Study Drug Administration (Dose-Titration) Phase.

The Per-Protocol population will contain all patients randomized to and exposed to the study drug during the double-blind phase of the trial. The efficacy analysis will be based on the Per-Protocol population. A patient will be classified as a *treatment success* if they meet the following criteria:

• Complete the 30-minute double-blind phase without having ≥2 consecutive intra-patient increases greater than or equal to 50% Delta (MAP) [i.e., MAP ≥ $(MAP_{B1} + MAP_{B2})/2$] and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

A patient will be classified as a *treatment failure* if they meet the following criteria:

• Fail to complete the entire 30-minute double-blind phase without receiving additional treatment to control their blood pressure in addition to the study drug.

• Fail to complete the entire 30-minute double-blind phase for any reason.

• Experience an intra-patient increase greater than or equal to 50% Delta (MAP) for ≥2 consecutive MAP measurements, obtained at one-minute intervals, during the 30-minute double-blind phase.

7.3.2 Safety

All patients who receive any study medication (ITT population) will be included in the safety analyses and summaries, independent of the patient actually reaching the double-blind phase of the study. All non-serious adverse events recorded within 24 hours of either completion of the double-blind phase, or within 24 hours of premature discontinuation of the study, will be reported.

All serious adverse events recorded within 30 days of either completion of the double-blind phase, or premature discontinuation of the study, will be reported.

7.4 Background and Demographic Characteristics

All baseline information, including demographic factors, physical examination parameters, vital signs, growth parameters (if applicable), laboratory and blood gas information will be summarized by treatment group for all enrolled patients (ITT population). Additionally, nonrandomized patients versus randomized patients will be summarized and compared by age, gender, and race to determine if there are any differences among the 2 subsets. Analyses will be conducted to determine differences in the demographic and baseline characteristics of the treatment groups. For continuous variables (e.g., age, weight), the number of non-missing and missing values and the median, mean, standard deviation, minimum, and maximum will be displayed for each treatment group. For categorical variables (e.g., race, gender), the counts and proportions will be tabulated.

Baseline comparability will be evaluated based on the pooled data from all centers. To determine comparability of the treatment groups at baseline, continuous demographic and clinical variables will be analyzed using an analysis of variance test (with an appropriate transformation, if necessary). Baseline, demographic, and clinical variables that are ordinal will be analyzed using the Cochran Mantel Haenszel test; parameters that are

dichotomous will be analyzed using a chi-square ($\chi 2$) test or Fisher's exact test, depending on the individual cell counts. If there are treatment group differences at the 0.10 level of significance in demographic or baseline clinical variables, these variables may be added as stratification variables or covariates to the efficacy analyses.

7.5 Study Medication

The duration of exposure to study medication will be summarized for all enrolled patients, and separately for all randomized patients.

7.6 Concomitant Therapy

Concomitant medications (medications present while on study medication) will be recorded on source documents throughout the study and at early termination; however, only those concomitant mediations that (1) affect blood pressure, or (2) are associated with SAE's will be recorded on CRFs. Medications listed on CRFs will be coded using the WHO drug dictionary. The number of randomized patients using prior or concomitant medications will be categorized by the WHO drug category and preferred term, and presented for each treatment group. In any given category [e.g., drug category] a patient will be counted only once.

7.7 Statistical Design and Models for Analysis

This is a biphasic (open-label dose-titration phase, followed by a randomized phase), randomized, double-blind placebo-controlled study. Patients who are enrolled into the initial phase of the study will have their dose of sodium nitroprusside titrated and must receive a minimum of 12-hours of treatment to be eligible for the randomized phase of the study. Patients who cannot be adequately titrated during the initial 12-hour period will not proceed to the randomization phase of the study. Patients who reach the randomization phase of the study will be assigned to receive placebo, or continue to

receive sodium nitroprusside based on a stratified permuted block central randomization scheme.

Five age groups (A through E) will be enrolled in this trial:

Age Group A: Age Group A: Neonates from birth to less than 30 days of age

Age Group B: Infants and toddlers from 30 days to < 2 years

Age Group C: Preschool children from 2 years - < 6 years

Age Group D: School age children from 6 yrs - < Tanner stage III

Age Group E: Adolescents from Tanner stage III - < 17 years.

In order to efficiently account for the effect of SNP on the different age groups, neonates from birth to less than 30 days of age (Age Group A) and infants and toddlers from 30 days to < 2 years (Age Group B) will be pooled for analysis. Based on the planning estimates of the study, patients from these two pooled age groups should represent approximately 25% of the target enrollment (~60 patients).

Preschool children from 2 years - < 6 years (Age Group C) and school age children from 6 yrs - < Tanner stage III (Age Group D) will also be pooled for analysis; patients from these two pooled age groups should also represent approximately 25% of the target enrollment (~60 patients).

In order to accurately determine whether a treatment difference is sufficient to stop the trial early, the DMC will examine the results from the Blinded Study Drug Administration Phase after the first 40 patients have been enrolled, randomized and treated during the blinded treatment phase; the approximate expected distribution by age group is as follows:

- The initial 12 patients in Age Groups A & B
- The initial 12 patients in Age Groups C & D
- The initial 16 patients in Age Group E

The specific objective of the DMC is to determine the significance of the treatment difference based on the observed magnitude of the effect, expressed as a proportion (*treatment success* (sodium nitroprusside) / Treated with sodium nitroprusside during the blinded treatment phase vs. *treatment success* (Placebo) / Treated with Placebo during the blinded treatment phase. Determination of the treatment difference will be performed after 67% (40/60) of the target sample size has been enrolled.

7.7.1 Primary Efficacy Analysis

The primary efficacy analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients received sodium nitroprusside}} = \pi_{\text{ Patients received receive placebo}}$
- H_A: $\pi_{\text{Patients received sodium nitroprusside}} \neq \pi_{\text{ Patients received receive placebo}}$

where

- $\pi_{\text{Patients received SNP}}$ = Proportion of *treatment successes* (sodium nitroprusside)
- $\pi_{\text{Patients received placebo}}$ = Proportion of *treatment successes* (placebo)

7.7.2 Primary Safety Analysis

The primary safety analysis will be conducted using the Per-Protocol Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients received sodium nitroprusside}} = \pi_{\text{ Patients received placebo}}$
- H_A: $\pi_{\text{Patients received sodium nitroprusside}} \neq \pi_{\text{ Patients received placebo}}$

where

- $\pi_{\text{Patients received sodium nitroprusside}}$ = Proportion of patients who received SNP who experience a serious adverse event

- $\pi_{\text{Patients received placebo}}$ = Proportion of patients who received placebo who experience a serious adverse event

7.7.3 Interim Monitoring Based on Group Sequential Methods

The interim assessment of the treatment difference will be predicated on the primary efficacy endpoint and conducted using Group Sequential methods as described by Pocock (1977). The Data Monitoring Committee will conduct the analysis to determine whether the treatment difference is sufficient to stop the trial early. This assessment will be conducted after 67% of the patients from the original target sample size have either completed or withdrawn from the study. The instructions to the DMC for the treatment difference assessment will be described in detail in the Data Monitoring Committee Charter.

In order for the DMC to calculate the treatment difference using the observed data, the treatment assignment codes will need to be provided to the DMC statistician. The intra-

patient MAP_{B2} values will be required, including listings of the intra-patient post-baseline values during the blinded phase of the trial. If the treatment difference does not meet the early stopping criteria, enrollment will continue towards the target sample size, and the data will remain blinded to all involved parties with the exception of the DMC. Additionally, the DMC will not have any direct contact with the study Sponsor, or the clinical investigators.

The following steps will be used to evaluate the treatment difference after the initial 40 randomized patients have been enrolled and have completed the Blinded Study Drug Administration Phase, or withdrawn prematurely.

The Group Sequential methods described by Pocock indicate that in order to control the type I error at 5%, the significance level at the interim analysis and the final analysis is as follows when the interim analysis is performed after 67% (40/60) of the target sample size has been enrolled.

Group Sequential Table 1.0

Significance Level Corresponding to the Pocock Stopping Boundary

Analysis	**Subjects**	**Significance Level (α)**
Interim	40/60	0.0407
Final	60/60	0.0215

At the interim analysis, test the null hypothesis at an α=0.0407 significance level using a two group χ^2 test of equal proportions. If the test is statistically significant with a p-value <0.0407, there will be sufficient evidence to reject the null hypothesis in favor of the alternative that a treatment difference exists between the two randomized treatment groups and the study can be stopped early for efficacy. Final decision will be based on recommendation from the DMC who will review overall efficacy and safety information. Alternatively, if a p-value <0.0407 is not achieved, the study will continue towards the target enrollment.

7.7.4 Sample Size Estimation

The overall sample size was calculated based on performing an un-stratified analysis of the proportion of patients classified as a *treatment success* between the 2 randomized treatment groups. With a balance randomization (1:1, SNP:Placebo), a difference in the proportion of *treatment successes* ranging from 34% to 40% would have 80% power to reject the null hypothesis in favor of the alternative (ref. Sample Size Table No. 1.0).

Sample Size Table No. 1.0

Two group χ^2 Test of Equal Proportions

Scenario	**1**	**2**	**3**	**4**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
SNP, π_1	0.080	0.160	0.240	0.320
Placebo, π_2	0.420	0.530	0.630	0.710
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	8.328	5.920	5.392	5.203
Power (%)	80	80	81	80
n per group	30	30	30	30

Given the significance level corresponding to the Pocock stopping boundary as indicated in Group Sequential Table 1.0 above, the following scenarios identify the number of treatment successes that must be observed in the SNP treatment group to result in a statistically significant test of the null hypothesis given the placebo group success rate specified below.

Group Sequential Table 2.0

Required SNP Treatment Successes

Scenario	**1**	**2**	**3**	**4**
Analysis	Interim	Final	Interim	Final
n per group	20	30	20	30
Test significance level, α	0.0407	0.0215	0.0407	0.0215
Treatment Successes (Placebo)	2 (10%)	3 (10%)	4 (20%)	6 (20%)
Treatment Successes (SNP)	8 (40%)	11 (55%)	11 (37%)	15 (50%)

7.7.5 Strategy for the Statistical Analysis

The primary method for analysis will be a comparison of the proportion of *treatment success* between patients randomized to receive placebo compared to patients randomized to remain on sodium nitroprusside. Additional analysis will be described in the Statistical Analysis Plan that will include a comparison of the event time distribution functions for the time until an increase in MAP_{B2} + 50% Delta (MAP) is initially observed. During the Open-Label Study Drug Administration (Dose-Titration) Phase, the sustainability of the blood pressure will be graphed over time to determine the effectiveness of SNP to maintain the target MAP.

7.7.6 Handling Missing Data in the Analyses

The following method of imputation will be used:

Last observation carried forward (LOCF): The goal of this imputation scheme is to create an observation for a completely missing observation at the end of the study for every patient in the ITT population. If a patient evaluation for a post-baseline observation is missing, then the immediately preceding non-missing evaluation will be used.

Specific algorithms for imputing missing or partially missing dates will be discussed in the SAP. Imputed or derived data will be identified in the individual patient data listings. Imputed data will not be incorporated into the case report form datasets. Imputed data will be used in the preparation of the derived datasets.

7.7.7 Pooling of Small Sites for Analysis

Small sites (i.e., sites that have less than 4 patients per treatment arm) will be identified and the following method will be used for combining the data. Data from all small sites (< 4 patients) will be combined to form a single site in order to obviate non-estimable situations (i.e., at least 2 intra-group observations are needed to estimate variance) in the evaluation of site and site interaction effects. Once combined, the pooled site will remain as such for all analyses for which a site interaction effect is determined. If the pooled smaller sites represent a single site that has more than twice as many patients as the largest single site, however less than 3 times as many patients, the small sites will be ranked by size and divided into 2 pooled groups using an alternating sequence (ABABAB). If the pooled smaller sites represent a site that has more than three times as many patients as the largest single site, however less than 4 times as many patients, the small sites will be ranked by size and divided into 3 pooled groups using an alternating sequence (ABCABCABC). This methodology will be applied, based on the initial pooling of the smaller sites.

7.7.8 Dropouts, Protocol Violations/Deviations, and Exclusions

Randomized patients who fail to complete the study will not be replaced. All protocol deviations and violations will be documented and categorized in the final study report.

The rate of attrition will be evaluated by the Data Monitoring Committee during the interim evaluation to re-estimate sample size. The reasons for withdraw will be classified into 3 mutually-exclusive classes:

- Withdraw due to tolerability of the study drug
- Withdraw due to lack of treatment effect
- Withdraw not due to tolerability or lack of treatment effect

The proportion of patients who withdraw prematurely will be compared between treatment groups to determine if there is a disproportionate rate of attrition. If the rates differ by a pre-specified amount, the reasons for withdraw will be examined to determine causation. The specific monitoring rules, boundaries, and actions will be described in detail in the Data Monitoring Committee Charter.

7.8 Safety Evaluation

The primary assessment of safety will be based on the frequency of treatment-emergent adverse events and on the frequency of clinically notable abnormal vital signs and laboratory values. The primary safety analysis will be based on a comparison of the proportion of patients receiving sodium nitroprusside vs. placebo who experience a serious adverse event during the Blinded Study Drug Administration Phase.

7.8.1 Adverse Events and Medical Conditions

Adverse events will be recorded throughout the study and at early termination. Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Treatment-emergent adverse events will be defined as those events, which are newly occurring or worsening from baseline. In all cases only treatment emergent adverse events will be summarized. Treatment-emergent adverse events will be summarized by presenting, for each treatment group, the number and percentage of patients having any treatment-emergent AE, having an AE in each body system, and having each individual AE. (Note: In any given category [e.g. body system] a patient will only be counted once.) Similar displays will be provided for prior (conditions ending prior to the first exposure to sodium nitroprusside) and current (conditions present while on study medication) medical conditions. Adverse events will further be categorized by severity, relationship to study medication, and action taken. Other information collected will be listed, as appropriate. Any event starting more than 3 days after the final dose of study medication will be excluded from the above tables and only presented in a listing, unless the adverse event was serious or caused discontinuation from the study.

7.8.2 Clinical Laboratory Results

7.8.2.1 Overview

The primary presentation of the results by individual laboratory parameter will focus on the intra-patient changes from baseline (Pre-study Period). The presentation and analysis of laboratory data will be based on the observed data. All patients who have a baseline and at least one follow-up laboratory assessment will be included in the presentation of the clinical laboratory data. For each clinical laboratory test, there will be three sets of descriptive statistics that summarize the results at baseline, post-baseline assessment, and the change from baseline to post-baseline assessment. Descriptive statistics include N,

mean, standard deviation, median, and the minimum and maximum values. Within treatment group changes will be analyzed using a paired-difference t-test. Between treatment group differences will be compared using a one-factor analysis of variance test.

Shifts from baseline to each pre-specified post-baseline endpoint will also be summarized based on the laboratory categorization (*abnormally and clinically significant*, *abnormal but not clinically significant*, or *normal*) using the worst reported post-baseline observation that occurs within the pre-specified interval. The proportion of patients will be compared using a 2-tailed Fisher's exact test, pooling *abnormally and clinically significant* with *abnormally but not clinically significant.*

In the case that more than one laboratory is used, laboratory values will be transformed for mean change summaries to the same units and normal range as were provided by the central laboratory used in the study, using the formula:

$$y = (x - Li)\frac{Uc - Lc}{Ui - Li} + Lc$$

where x = original value, Li and Ui = lower and upper limits of normal for individual laboratory, Lc and Uc = lower and upper limit for central laboratory

In cases where the lower limit of central laboratory is 0, values that are below the lower limit of normal for a laboratory value prior to transformation will be assigned a value of 0.

7.8.3 Vital Signs

7.8.3.1 Overview

Vital signs of particular interested (blood pressure, MAP, heart rate) will be assessed during each phase of the study.

7.8.3.2 Presentation of Results

Descriptive statistics (n, mean, SD, median, minimum and maximum values) will be used to summarize systolic and diastolic blood pressure, MAP, and heart rate and compared between the randomized treatment groups using a one-factor analysis of variance test.

7.8.4 Physical Examination

7.8.4.1 Overview

The presentation of physical examination data is based on the dichotomous classification (normal or abnormal) of each of the 9 regions or body systems (General Appearance, HEENT, Cardiovascular, Respiratory, Abdomen, Extremities, Neurological, Hair and Skin, and Genitourinary). In addition to these 9 specific body systems, any other region recorded by the investigator under "other" will also be summarized and reported.

7.8.4.2 Presentation of Results

Results will be presented by treatment assignment using counts and percentages. Shift tables will be prepared containing the count and percentage of patients who transitioned from normal at baseline to abnormal at the end of the study. The number and percentage of patients that did not change (normal at baseline and normal at the end of the study, abnormal at baseline and abnormal at the end of the study) are also presented to frame the 2*2 contingency table. Shifts from baseline to each pre-specified post-baseline endpoint will be summarized using the worst reported post-baseline observation that occurred within the pre-specified interval. The count of the disagreements (normal to abnormal and abnormal to normal) by treatment assignment (active and placebo) will be compared for each parameter using McNemar's test.

8.0 Ethics

8.1 Independent Ethics Committee or Institutional Review Board

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator Brochure (IB), the informed parental permission and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an Independent Ethics Committee (IEC) or Institutional Review Board (IRB). IEC/IRB approval of the protocol, informed parental permission and subject information and/or advertising as relevant will be obtained prior to the authorization of drug shipment to a study site. Any amendments to the protocol will require IEC/IRB approval prior to implementation of any changes made to the study design.

8.2 Ethical Conduct of Study

This study will be conducted in accordance with the protocol, GCP, FDA regulations governing clinical study conduct, ethical principles that have their origin in the Declaration of Helsinki, 2000 revision (see Appendix E) and all applicable local regulations. The investigator must assure that the study is conducted in accordance with the provisions as stated in the FDA regulations and complies with prevailing local laws and customs. Responsibilities of the Investigator are specified in Appendix D.

8.3 Subject Information and Parental Permission

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before protocol-specified procedures are carried out. Informed consent will be obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements.

Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation.

Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study.

The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed permission form, which will included all elements of informed consent as described in 21 CFR 50.25. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form and a copy of the signed assent (if applicable).

The parental/guardian permission form generated by the investigator with the assistance of BPCA DCC must be approved (along with the protocol) by the IRB and be acceptable to the Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative.

The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the ICH Guidelines and will comply with local regulations. This form

may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated.

Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by BPCA DCC, Regulatory Authorities, and NICHD or its designees.

9.0 Source Documents and CRF Completion

9.1 Source Documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

9.2 Case Report Forms

Data for individual subjects will be recorded on CRFs provided to the BPCA DCC. All entries must be complete. A case report form must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the CRF by the investigator. The principal investigators must review and approve each CRF.

Case report forms must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested during an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

Because of the potential for errors and inaccuracies in entering data into CRFs, laboratory and other test results must be kept on file with the subject's study dossier. Case report forms and copies of test results must be available at all times for inspection by the CRA for the site and the FDA.

10.0 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA DCC, the investigators and their study coordinators and the CRAs for the study. This meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures by a CRA at a study initiation visit and will be given an CRF completion workbook for reference.

The CRAs will monitor each site throughout the study. At each visit, 100% source document review will be made against entries on the CRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and all applicable regulations.

After completion of the entry process, computer logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary

correction will be made to the database and documented via addenda or audit trail. A manual review of selected line listings will also be performed at the end of the study.

11.0 Use of Information and Publication

11.1 Use of Information

This trial is sponsored by the NICHD. The NICHD endorses the sharing of final research data to expedite the translation of research results into new scientific knowledge in order to improve human health.

This contract is part of a collaborative program involving multiple sites. A data sharing dissemination plan will be developed jointly with the BPCA DCC, the NICHD, and the collaborating institutions following announcement of the award.

11.2 Publication

The BPCA DCC and steering committee for this study shall have the right to review and approve any paper for publication, including oral presentation and abstracts, which utilize data generated from this study. At least 90 days before any such paper or abstract is presented or submitted for publication, a complete copy shall be given to the BPCA DCC for review by the steering committee. Any individual investigator agrees to defer publication of any such paper or abstract until the BPCA DCC and Steering Committee have reviewed and approved it.

12.0 Completion of Study

The investigator will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon in writing by the investigator, the NICHD, the BPCA DCC

and the PODS. The investigator will provide a summary of the study's outcome to the IRB/IEC following the conclusion of the study.

The PODS Center, BPCA Data Coordinating Center, NICHD and/or the FDA may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. The investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the NICHD and BPCA DCC within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

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13.0 Investigator Agreement

The investigator will sign and date a Study Protocol Agreement Form, provided earlier in this protocol. The Study Protocol Agreement will then be countersigned by the investigator's PODS Principal Investigator.

Appendices

Appendix A: Tanner Stages of Sexual Maturity

	Pubic Hair		Breasts	Penis	Testes
SMR Stage[1,2]	Boys	Girls	Girls	Boys	Boys
1	None	Preadolescent	Preadolescent	Preadolescent	Preadolescent
2	Scanty, long, slightly Pigmented	Sparse, lightly pigmented, straight, medial border of labia	Breast and papilla elevated as small mound; areolar diameter increased	Slight Enlargement	Enlarged scrotum, pink texture altered
3	Darker, starts to curl, small Amount	Darker, beginning to curl, increased amount	Breast and areola enlarged, no contour separation ,	Longer	Larger
4	Resembles adult type, but less in quantity; coarse, cu rly	Coarse, curly, abundant but amount less than in adult	Areola and papilla form secondary mound	Larger; glans and breadth increase in size	Larger, scrotum dark
5	Adult distribution, spread to medial surfaces of thighs	Adult feminine triangle, spread to medial surface of thighs	Mature; nipple projects, areola part of general breast contour	Adult size	Adult size

1. Adapted from Tanner, JM: Growth at Adolescence, 2 ed. Oxford, Blackwell Scientific Publications, 1962.
2. MR = Sexual Maturity

Appendix B: Parental Permission Form with HIPAA

Protocol Title: A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects

Protocol Director: [PROGRAM PI]
IRB Approval Date: _____ IRB Expiration Date: _____

Is your child participating in any other research studies? _____Yes _____No

INTRODUCTION

You are being asked to agree to let your child be a part of a drug research study. He or she is scheduled for surgery, or needs to stay in an intensive care unit (ICU). During this operation or stay in the ICU, it will be necessary for the doctor to lower your child's blood pressure for a long period of time, up to 24 hours. We will tell you more about how he/she will do this and the drug that will be used later in this paper. Before you decide whether to let your child be involved in this study, *[Site PI]* wants you to read the following information. He wants you to ask him any questions you may think of. He wants to be sure that you understand what your child's participation will mean. You need to fully understand the type of treatment and its risks. Your doctor is responsible for providing you with the necessary information so that you understand the possible risks. Your child's participation in this study is entirely your choice.

Your child cannot participate in another research study at the same time as this research study [optional sentence]. Your child cannot be taking another experimental drug while enrolled in this research study, or within the previous 30 days. Your child will be a part of this study for approximately 30 days. There may be risks that we cannot predict. We will tell you about any new information that may affect your child's condition or affect your willingness to stay in this study.

NATURE AND PURPOSE OF THE RESEARCH STUDY

In certain kinds of surgeries, doctors often need to control the blood pressure of the patient. The doctor may also need to lower the patient's blood pressure below normal. This can reduce blood loss and avoid blood transfusions during stressful periods. Sodium nitroprusside is a drug that is approved by the Food and Drug Administration (FDA) for use in adults. Scientific studies show that this drug works well when doctors need to control blood pressure during surgeries in adult patients. Doctors also often use sodium nitroprusside in children. However, not many scientific studies tell us how best to use sodium nitroprusside in children.

In this study, we hope to learn the best dose of sodium nitroprusside (the study drug) to use in children who need it in the ICU for more than 12 hours. We also want to learn the same thing for children who need certain kinds of surgeries. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children. This research study is looking for 50-100 children at several hospitals in the United States.

Your child's participation in this study is entirely voluntary.

Your decision whether or not to allow your child to participate will not prejudice your child or his/her medical care. If you decide to allow your child to participate, you are free to withdraw your permission, and to discontinue participation at any time without prejudice to your child or effect on your child's medical care. If you decide to terminate your child's participation in this study, you should notify *(name)* at *(telephone number)*.

PROCEDURES

If you agree to your child's participation in this research study, he or she will undergo the following types of procedures:

- [Site PI] and his research staff will talk to you (and your child) about his or her health. They will ask you about your child's medical history. They will ask you about any medications your

child is currently taking. They will give your child three physical examinations, including measurement of blood pressure, pulse, and weight. The first one will be within 24 hours before study drug is given. The other two physical exams will be after your child receives study drug. A small amount of blood, less than one teaspoon, will be drawn at the first two visits. We need this blood for our study, but the same blood work may also be needed as part of your child's regular medical care. If this is the case, we will use these results instead of having to take another blood sample.

- Your child will receive study treatment in stages:
  1. First, after your child is stable, sodium nitroprusside will be given into a vein. This will be done through tubing your child will already have in place. This drug may be the treatment of choice to control your child's blood pressure even if your child is not enrolled in this study. This first stage will last up to 24 hours. He or she will receive sodium nitroprusside at a pre-set initial rate. That rate will be changed until your child's blood pressure is in the range that his or her doctor has decided is the best.
  2. The second stage of study treatment will begin between 12 and 24 hours after the medication was first started. This stage will last up to 30 minutes. During this stage your child will receive one of two treatments. This phase of the research study is blinded. That means that the study doctor will not know which treatment group your child will be placed into. The choice of which treatment group your child will be in (placebo or sodium nitroprusside) will be random. The choice will be made in a way that is like flipping a coin. He or she might receive sodium nitroprusside at the rate that was being used before to keep his or her blood pressure at a stable level. Or, he or she might receive a placebo; this is a solution like salt water that is known to have no effect on your child's health. Your child's vital signs (blood pressure and heart rate) will be watched very closely. If your child's blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end immediately. Then your child will again be given sodium nitroprusside, or similar drug, to keep his or her blood pressure at a safe level.
  3. If your child stills needs sodium nitroprusside treatment to control his/her blood pressure, the treatment may continue after the research study ends.

During the research study, vital signs (blood pressure and heart rate) will be checked often at specific time points.

There will be a follow up evaluation. About 30 days after the research study is over, we will call you at home to ask questions about your child's health in the last month, since he or she participated in the research study. If your child is readmitted to the hospital before our call, please let us know.

Blood samples will be taken between 4 to 7 times (1½ to 2½ mLs per each sample, or ½ teaspoon per sample) during the research study. This is to see how much of the metabolites (breakdown products) of the study drug is circulating in the blood at varying time points. If indicated, additional blood samples may be taken to help determine the amount of metabolites in your child's blood. We are very careful to minimize the amount of blood drawn from your child, and anticipate that 3½ teaspoons is the most we will draw for these scheduled tests.

Whenever possible, blood will be taken from tubing already in place. The nurse will often take the study blood samples at the same time that routine blood samples are taken to check on your child's health. This is done to avoid any extra needle-sticks (drawing blood from a vein in the arm with a needle). It is very unlikely that there would not be a catheter (IV line) in place; however, if that were to happen, blood drawing would require a needle stick that might cause minor bruising. Blood drawing is done to assess safety and to measure the activity of the study drug.

The total amount of blood drawn during the entire research study for children less than 2 years of age is approximately 1 to 2 ½ teaspoons. For children more than 2 years of age, the total amount of blood is about 2 to 3 ½ teaspoons.

The information gained during this research study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help patients in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

YOUNG WOMEN OF CHILD-BEARING POTENTIAL

If your child is a young woman who is able to become pregnant, it is expected that she will use an effective method of birth control to prevent exposing a fetus to a potentially dangerous agent with

unknown risk. If your child is pregnant or currently breast feeding, she may not participate in this study.

To confirm to the extent medically possible that your child is not pregnant, you agree that she will have a pregnancy test done before beginning this research study. This test will be carried out right before your child undergoes surgery.

PARTICIPANT RESPONSIBILITIES

You should:

- Follow the instructions of the Protocol Director and study staff.
- Tell the Protocol Director or research study staff about any side effects that your child may have.
- Tell the Protocol Director or research study staff if you believe your child might be pregnant.
- Ask questions as you think of them.
- Tell the Protocol Director or research staff if you change your mind about staying in the study.

While participating in this research study, your child should not take part in any other research project without approval from all of the Protocol Directors. This is to protect your child from possible injury arising from such things as extra blood drawing, the possible interaction(s) of research drugs, or other similar hazards.

WITHDRAWAL FROM STUDY

If you first agree to participate and then you change your mind, you are **free to withdraw** your permission and discontinue your child's participation at any time. Your decision will not affect your child's ability to receive medical care for his or her disease and your child will not lose any benefits to which he or she would otherwise be entitled.

If you decide to terminate your child's participation in this study, you should notify (*name)* at (*phone number)*.

The Protocol Director may also withdraw your child from the study and the study medication may be stopped without your permission for one or more of the following reasons:

- Failure to follow the instructions of the Protocol Director and/or study staff.
- The Protocol Director decides that continuing your child's participation could be harmful to him or her.

- Pregnancy (if applicable).
- Your child needs treatment not allowed in the study.
- The study is cancelled.
- Other administrative reasons.
- Unanticipated circumstances.

If your child is withdrawn from the study after receiving any of the research drug, and if you agree, we will perform the following testing to ensure the safety of your child: physical examination, monitor vital signs, blood tests, as described earlier, as well as the follow-up phone call approximately 30 days later. You will be free to have only some of these done if you prefer.

POSSIBLE RISKS, DISCOMFORTS, AND INCONVENIENCES

There are risks, discomforts, and inconveniences associated with any research study. These deserve careful thought. This is true whether it is a normal kind of treatment or an experimental type. You should talk with the Protocol Director if you have any questions.

In spite of all safety measures, your child might develop medical problems while taking part in this research study. These risks include elevated blood pressure. Treatment of these potential medical problems will not be limited or delayed by your child's participation in the study.

Sodium nitroprusside is a drug that lowers blood pressure. Because of this, there is a chance that your child could develop hypotension (low blood pressure). The doctor will monitor your child very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable blood pressure.

Another side effect that might happen is that your child's heart rate may increase in response to sodium nitroprusside. Again, the doctor and research nurse will monitor your child's heart rate very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable heart rate.

Minor side effects due to sodium nitroprusside may also occur but will not cause the study to be ended. Since sodium nitroprusside makes blood vessels bigger, the following side-effects may occur: nausea, headache, restlessness, abdominal pain, redness or flushing of the skin, nervousness, and perspiring.

Sodium nitroprusside contains cyanide. Cyanide is present in all people and is important for normal body function. Extra cyanide can be present whenever sodium nitroprusside is used. Excess cyanide can affect the amount of oxygen in the blood. The doctors will carefully watch for any signs or symptoms of excess cyanide and treat your child if needed. Because safety is one of the most important aspects of this study, we will be testing your child's blood for cyanide. The results of this testing will not be immediately known. However, there are other means to detect ill effects from excess cyanide. If the doctors suspect it is in your child's best interest, additional unscheduled blood tests for cyanide and its breakdown product, thiocyanite, will be performed. Cyanide has been detected in patients who have participated in this study. However, no patient has shown any ill effects from the cyanide.

POTENTIAL BENEFITS

The information gained during this study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help children in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOUR CHILD WILL RECEIVE ANY BENEFITS FROM THIS STUDY.

ALTERNATIVES

If you choose not to enroll your child in this study, your child may receive sodium nitroprusside anyway. This could happen if your child's doctor feels it is the best medicine to use to control blood pressure. Or, your child's doctor could choose to use other types of blood pressure medications, such as esmolol or fenoldopam, instead. Whether or not your child is enrolled, the medical team will, of course, do everything possible to ensure the safety and comfort of your child.

If you do not wish your child to take part in this study, other treatments can be used for your child's condition. If you withdraw your child's participation, the study doctor will recommend an alternative treatment for blood pressure control for your child, such as esmolol or fenoldopam. If this study is discontinued, your child will receive one of these alternative treatments.

PARTICIPANT'S RIGHTS

You should not feel obligated to agree that your child participate in this study. Your questions should be answered clearly and to your satisfaction.

If you decide not to participate, tell the Protocol Director. Your child will still receive care for his/her disease and will not lose any benefits to which he/she would otherwise be entitled.

You will be told of any important new information that is learned during the course of this research study, which might affect your child's condition or your willingness to continue participation in this study.

CONFIDENTIALITY

Your child's identity will be kept as confidential as possible as required by law. Except as required by law, your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your child's research records may be disclosed outside of Stanford, but in this case, your child will be identified only by a unique code number. Information about the code will be kept in a secure location and access limited to research study personnel.

The results of this research study may be presented at scientific or medical meetings or published in scientific journals. However, your child's identity will not be disclosed**.**

Patient information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your child's identity if this study falls within its jurisdiction.

- The purpose of this research study is to obtain data or information on the safety and effectiveness of sodium nitroprusside in children; the results will be provided to the sponsor, the Food and Drug Administration and other federal and regulatory agencies as required.

Authorization to Use Your Health Information for Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed parental permission form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?

In this study, we hope to learn the best dose of sodium nitroprusside to use in children of different ages who need it in the ICU for more than 12 hours. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children as determined by the NIH and FDA.

Do I have to sign this authorization form?

You do not have to sign this authorization form. But if you do not, your child will not be able to participate in this research study, including receiving any research-related treatment.

Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?

If you decide to allow your child to participate, you are free to withdraw your authorization regarding the use and disclosure of your child's health information (and to discontinue any other participation in the study) at any time. After any revocation, your child's health information will no longer be used or disclosed in the study, except to the extent that the law allows us to continue using your child's information (e.g., necessary to maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must contact: *(researcher's name and contact information, including telephone number).*

What Personal Information Will Be Used or Disclosed?

Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to, vital sign measurements, laboratory results of blood collections, physical exams, related medical records, and other data.

Who May Use or Disclose the Information?

The following parties are authorized to use and/or disclose your child's health information in connection with this research study:

- The Protocol Director *(Insert Name of PD)*
- The *(Insert name of Institution)* Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary
- Research Staff

(List every other class of persons or organization affiliated with the hospital/university who might need to use and/or disclose the participant's information in connection with this study.)

Who May Receive or Use the Information?

The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- The National Institutes of Health
- The Food and Drug Administration
- Collaborating Institutions
- The Data Coordinating Center, The EMMES Corporation or authorized agent

Your child's information may be re-disclosed by the recipients described above, if they are not required by law to protect the privacy of the information.

When will my authorization expire?

Your authorization for the use and/or disclosure of your child's health information will expire December 31, 2055.

Will access to my child's medical record be limited during the study?

To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or

billing decision about your child (e.g., if included in your child's official medical record).

_____

Signature of Participant

_____

Signature of Legally Authorized Representative

_____

Date

_____

Description of Representative's Authority to Act for Subject

FINANCIAL CONSIDERATIONS

PAYMENT

You and your child will not be paid to participate in this research study.

COSTS

The sponsor will pay for the cost of sodium nitroprusside and for the extra blood tests that will be used to monitor the amount of drug in your child's blood and for safety tests. You or your insurance company will be responsible for the medical procedures, surgery, anesthesia, and other normal costs associated with standard medical care for treatment of your child's condition.

SPONSOR

The National Institute of Child Health and Development of the National Institutes of Health (NIH) is providing financial support and/or material for this study.

COMPENSATION FOR RESEARCH-RELATED INJURY

All forms of medical diagnosis and treatment – whether routine or experimental – involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. **You will be responsible for any associated co-payments or deductibles as required by your insurance.**

If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

Additionally, *{Institution name}* is not responsible for research and medical care by other institutions or personnel participating in this study. You do not waive any liability rights for personal injury by signing this form.

CONTACT INFORMATION

- Questions, Concerns, or Complaints: If you have any questions, concerns or complaints about this **research study**, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director, *{name}*. You may contact *{him/her}* now or later at *{phone number}*.
- Emergency Contact: If you feel your child has been **hurt by being a part of this study**, or need immediate assistance please contact *{Protocol Director's name and phone number}*.
- Alternate Contact: If you cannot reach the Protocol Director, please page the research team at *{phone number}*.
- Independent of the Research Team Contact: If you are not satisfied with the manner in which this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a research study subject, please contact the *{Institution's name}* Institutional Review Board (IRB) to speak to an informed individual who is independent of the research team at *{phone number}*. Or write the *{Institution's name}* IRB, *{IRB full address}*. In addition, please call the *{Institution's name}* IRB at *{phone number}* if you wish to speak to someone other than the research team or if you cannot reach the research team.

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS *{Text can be revised to meet IRB requirements}*

As a human subject your child has the following rights. These rights include but are not limited to the subject's right to:

- be informed of the nature and purpose of the experiment;
- be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
- be given a description of any attendant discomforts and risks reasonably to be expected;
- be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
- be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
- be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise;
- be given an opportunity to ask questions concerning the experiment or the procedures involved;

- be instructed that parental permission to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
- be given a copy of the signed and dated parental permission form;
- and be given the opportunity to decide to give parental permission or not to give parental permission to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING PARENTAL PERMISSION, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____

Signature of Parent or Legal Guardian

_____

Signature of Second Parent or Legal Guardian

_____

Date

_____

Name of Patient

PERSON OBTAINING PARENTAL PREMISSION

I attest that the requirements for informed parental permission for the medical research project described in this form have been satisfied - that the Parent or Legal Guardian has been provided with the Experimental Subject's Bill of Rights, if appropriate, that I have discussed the research project with the Parent or Legal Guardian and explained to him or her in nontechnical terms all of the information contained in this parental permission form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the Parent or Legal Guardian to ask questions and that all questions asked were answered.

_____ _____

Signature of Person Obtaining Parental Permission Date

Appendix C: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles
for
Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when

providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The

responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's

freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case, the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in

the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix D: Responsibilities of the Investigator

Investigator Responsibility/Performance

Prior to starting enrollment at a site, all investigators must read and understand the Investigational Plan and must sign and complete the Investigator Agreement Form. This documents that they accept all conditions of the Investigational Plan and will conduct the study accordingly. The investigator must provide a current copy of his or her curriculum vitae that is not more than 2 years old.

Informed Parental Permission and IRB Approval

The investigator must have written approval from the IRB prior to enrolling patients in the study. A copy of the written approval that includes the following must be provided to the DCRI:

- A statement of IRB approval for the proposed study and informed parental permission form at the institution
- The date the study was approved and the duration of approval
- A listing of any conditions attached to the approval
- Identification of the approved primary investigator
- The signature of the IRB chairperson

Any amendments to the protocol, as well as associated parental permission form changes, will be submitted to the IRB, and written approval must be obtained prior to implementation. Serious adverse event reports will be submitted as requested by the BPCA.

The study will be explained to the patients in lay language. Patients will sign and receive a copy of the IRB-approved informed parental permission form prior to study participation. Patients will be

assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Source Documentation

Regulations require that investigators maintain information in the study patient's medical records that corroborate data collected on the CRFs. In order to comply with these regulatory requirements, at the minimum, the following information should be maintained:

1. Medical history/physical condition of the study patient before involvement in the study sufficient to verify protocol entry criteria (if not already present)
2. Dated and signed notes on the day of entry into the study including clinical site, patient number assigned and a statement that parental permission was obtained
3. Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams)
4. Notations on abnormal laboratory results
5. Serious adverse events reported through 30 days from the end of study drug administration
6. Notes regarding concomitant medications taken during the study (including start and stop dates)
7. Study patient's condition upon completion of or withdrawal from the study

Data Transmittal

Required data will be recorded on the CRFs as soon as possible after the patient is discharged, day 10, or death, whichever comes first. CRFs and any supporting documents must be sent to the BPCA and/or retrieved from the investigational site according to the outlined time windows. The 28-day follow-up CRF needs to be forwarded within 10 days of the follow-up visit.

Non-Protocol Research

BPCA has a legal responsibility to report fully to regulatory authorities all the results of clinical studies. No investigative procedures other than those in this protocol shall be undertaken on the enrolled patients without the agreement of the IRB and BPCA.

Publication Policies

At the conclusion of the study, a multicenter abstract reporting the primary results may be prepared and presented in an appropriate international forum. A multicenter, peer-reviewed manuscript will also be prepared for publication in a reputable scientific journal.

Appendix E: Treatment of Suspected Nitroprusside Toxicity

Signs of Toxicity:

- If the base deficit exceeds -8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the subject will be also be discontinued from study.
- If the lactate level rises by more than 4 mmol/L in an 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output).
- If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes percent between arterial and mixed venous blood.

SUSPECTED CYANIDE TOXICITY SHOULD BE TREATED AS FOLLOWS:

1) Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration.

2) Obtain blood for arterial and venous blood gases with co-oximetry, serum lactate, and cyanide and thiocyanate levels.

3) SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10mL) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result)

Subject Hemoglobin g/dL	Initial dose Sodium NITRITE (3%) mL/kg IV	Initial dose Sodium Thiosulfate mL/kg IV
8 g/dL	0.22 mL/kg	1.10 mL/kg
(6.6 mg)/kg		
10 g/dL	0.27 mL/kg	1.35 mL/kg
(8.7 mg)/kg		
12 g/dL	0.33 mL/kg	1.65 mL/kg
(10 mg)/kg		
14 g/dL	0.39 mL/kg	1.95 mL/kg
X	(11.6 mg)/kg	

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals.

[Micromedex Poisondex www.micromedex.duhs.duke.edu , see also Berlin, 1970]

Appendix F: Assay of Nitroprusside Metabolites and Handling of Blood Samples for Assay of Nitroprusside Metabolites

A classical UV bioanalytical method is utilized for detection and quantitation of cyanide in whole blood. Cyanide concentrations are determined by measuring the absorbance of the chromophore formed by the interaction of the cyanide ion with 4-nitrobenzaldehyde and o-dinitrobenzene in 2-methoxyethanol (Rieders, 1971 and Guilbault, 1966). In summary, to a 1.0 ml aliquot of whole blood, 4-Nitrobenzaldehyde solution and o-Dinitrobenzene solution is added then made basic with sodium hydroxide. Following a specific incubation time a UV scan spectrum is obtained from 520 to 580 nm with a maximum reading at 555 nm. The exact details of the method are proprietary to NMS Labs. The method is sensitive to a LLOQ of 0.05 μg/mL which correlates to normal circulating levels. A toxic threshold is normally assessed as approximately 0.5 μg/mL and acute toxicity is observed at greater than 1 μg/mL. There are no known interferences with this assay method.

An ion chromatography method is used for detection and quantitation of thiocyanate in serum that is specific, accurate, precise and rugged. In summary, a 0.10 mL aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography (Vogel, 1981 and Vesey, 1976). The same procedure is employed to detect thiocyanate in urine. The normal range for non-smokers is 1-4 μg/mL in serum/plasma. For smokers, it is 3 – 12 μg/mL and the therapeutic range for sodium nitroprusside is generally between 6 and 29 μg/mL (Schulz, 1984).

Thiosulfate is detected in serum/plasma or urine also via a validated ion chromatography method with the analytes separated and detected via conductivity detection. For this method, a 0.5 ml aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography.

Sample Handling Procedures

At each specified blood collection for cyanide and thiocyanate, 2 mL of arterial blood is to be collected in a 2 mL gray top. Samples should be mixed, then one half the whole blood in the gray top (1 mL) is removed and stored in a polypropylene screw capped container and stored on ice until it can be refrigerated. The remaining 1 mL of whole blood is centrifuged (within 20 minutes of collection at approximately 1200 g for 10 - 12 minutes at ambient or colder temperatures) to obtain 0.5 mL of plasma for thiocyanate analysis. The red bloods cells from this sample may be discarded, or separately tested for cyanide. All samples should be stored in a refrigerator before sending to the Central Lab(s), which should be done as soon as possible. Samples should be shipped COLD, using "frozen cold packs," for overnight delivery. Whole blood samples should NEVER be frozen.

For neonates or to spare the total amount of blood drawn for the analysis, the minimum blood draw is 1 mL of arterial blood. This translates to 0.5 mL of whole blood for cyanide analysis and 0.5 mLs to be centrifuged to obtain approximately 0.25 mLs of plasma for thiocyanate analysis.

The details of handling & shipping samples to the Central Lab(s) will be included in the MOP. This is a summary and not the complete instructions for sample handling.

Appendix G: Sedation Suggested Regimen

Intensive Care Unit:

For those patients who receive study drug in the intensive care unit for a surgical or medical procedure, the following guidelines may be utilized.

Sedation may be administered intravenously and initiated with a benzodiazepine and opiate agonist as follows:

Midazolam bolus 0.1-0.2 mg/kg followed by a continuous midazolam infusion of 0.06-0.3 mg/kg/hr or intermittent bolus of 0.1 mg/kg every 1-2 hours

Or

Lorazepam bolus 0.05-0.1 mg/kg followed by a continuous infusion of 0.025-0.05 mg/kg/hr or intermittent bolus of 0.05-0.10 mg/kg every 4-6 hours

And/or

Fentanyl bolus 1- 5 micrograms/kg (intubated, mechanically ventilated patients) followed by an infusion of fentanyl of 0.5 – 5 micrograms/kg/hour

Or

Morphine bolus 50-100 microgram/kg followed by a continuous infusion of 20-80 micrograms/kg/hour.

Sedation and analgesic medication may be titrated to patient response. Higher doses may be used in patients who exhibit benzodiazepine and/or narcotic habituation due to long term (> 4-7 day) usage. Where applicable, sedation assessment may be by clinical judgment of the responsible physician, and/or a quantitative scoring system such as the COMFORT score. Patients receiving neuromuscular blocking drugs such as vecuronium or rocuronium as part of their ICU management are eligible for study.

Appendix H: Measurement of Blood Pressure in Children

Excerpt from: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents

Correct measurement of BP in children requires use of a cuff that is appropriate to the size of the child's upper right arm. The equipment necessary to measure BP in children, ages 3 through adolescence, includes child cuffs of different sizes and must also include a standard adult cuff, a large adult cuff, and a thigh cuff. The latter 2 cuffs may be needed for use in adolescents.

By convention, an appropriate cuff size is a cuff with an inflatable bladder width that is at least 40% of the arm circumference at a point midway between the olecranon and the acromion. For such a cuff to be optimal for an arm, the cuff bladder length should cover 80% to 100% of the circumference of the arm. Such a requirement demands that the bladder width-to-length ratio be at least 1:2. Not all commercially available cuffs are manufactured with this ratio. Additionally, cuffs labeled for certain age populations (eg, infant or child cuffs) are constructed with widely disparate dimensions. Accordingly, the working group recommends that standard cuff dimensions for children be adopted (see Table 2).

TABLE 2. Recommended Dimensions for BP Cuff Bladders

Age Range	Width, cm	Length, cm	Maximum Arm Circumference, cm*
Newborn	4	8	10
Infant	6	12	15
Child	9	18	22
Small adult	10	24	26
Adult	13	30	34
Large adult	16	38	44
Thigh	20	42	52

* Calculated so that the largest arm would still allow the bladder to encircle arm by at least 80%.

BP measurements are overestimated to a greater degree with a cuff that is too small than they are underestimated by a cuff that is too large. If a cuff is too small, the next largest cuff should be used, even if it appears large. If the appropriate cuffs are used, the cuff-size effect is obviated

SBP is determined by the onset of the "tapping" Korotkoff sounds (K1). Population data in children and risk-associated epidemiologic data in adults have established the fifth Korotkoff sound (K5), or the disappearance of Korotkoff sounds, as the definition of DBP. In some children, Korotkoff sounds can be heard to 0 mm Hg. Under these circumstances, the BP measurement should be repeated with less pressure on the head of the stethoscope. Only if the very low K5 persists should K4 (muffling of the sounds) be recorded as the DBP.

Appendix I: Research Assent Form Template

Protocol Title: A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Director:

IRB Approval Date: _____IRB Expiration Date: _____

Are you taking part in any other research studies right now? ____ yes ____ no

Why Are We Doing this Research Study?

We are doing this study to learn more about a medicine called sodium nitroprusside. This study will help us find out more about how good this drug is in keeping blood pressure and heart rate under control in children who are in the intensive care unit (ICU). Altogether, there will be 60 kids under the age of 17 in this study.

What Will Happen During the Study?

You will be seen by a doctor, who will give you a physical exam and ask you and your parents some questions about your health history and medicines you take. At certain times during the study, blood will be drawn to check that you are okay and safe, and to see how the study drug is working in your body. Blood will be drawn either from a tube that is already connected to you to get blood, or from a needle. The amount of blood to be taken during the study is about 2½ teaspoons to 3½ teaspoons.

In the first part of the study, you will receive a study drug called sodium nitroprusside through a tube placed into a vein in your arm. Your doctor will make changes in the amount of drug you get to keep you safe and your vital signs (like your heart rate and blood pressure) stable. Your doctor will

change how much of the drug you get so you have the blood pressure the doctor thinks is right for you. This part of the study will last for at least 12 hours up to 24 hours during the time that your doctor needs to control your blood pressure. During the time you get study drug, your blood pressure and heart rate will be measured very often.

The second part of the study will last up to 30 minutes. During this part, you will get one of two treatments. This part of the research study is blinded. That means that the study doctor will not know which treatment group you are in. The choice of which treatment you receive will be made in a way that is like flipping a coin. You might receive the study drug at the same rate that was being used before to keep your blood pressure stable. Or, you might receive a placebo, which is a solution like salt water that doesn't have any effect on your blood pressure. Your blood pressure and heart rate will be watched very close. If your blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end right away. Then you will again be given sodium nitroprusside, or similar drug, at a rate that keeps your blood pressure at a safe level.

What if you don't want to be in the Study?

You can say "no" to being in the study if you want. You can also stop the study anytime you want by telling anyone that is caring for you. Your doctor can explain to you and your parents other treatments that could be used instead.

What You Should Know about the Medicine?

The medicine is used to keep your blood pressure where your doctor thinks is safe or needed for your operation or some other treatment or test. It has been okayed for use in adults but there is not a lot of information about how the drug works in kids. Like any medicine, it can cause unwanted things to happen. These unwanted things are called risks. Some of the risks from using this medicine are: getting sick to your stomach, headache, getting hot, having your blood pressure go down too much or your heart beat go up too high.

Things Girls Need to Know….

Some medicine can cause bad things to happen to an unborn baby. If you are able to get pregnant (if you have started having a period), you need to take a pregnancy test which your doctor will give you. If you are pregnant, you should not take part in this study.

What Else Do You Need to Know?

Sometimes doctors write about the research studies when they are done. If a paper is written about this research study, your name won't be used in it, but the medical information they find out about you may be used. We will keep your medical information private. People who work for *[site name]*, the people who are running the study, and some parts of the government (the part that takes care of medicines) will be able to look at your medical information.

There is no cost to you or your parents to be in this study.

If you have questions about the study you can call Dr. _____ at

_____.

I have read this form. I have had a chance to ask questions about things I don't understand. I want to be in this research study and understand what will happen to me.

_____ _____

Signature of Patient Date

_____

Name of Patient

_____ _____

Signature of the Person Obtaining Assent Date

Protocol: NICHD-2003-09-LT (SNP2)
A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Amendment 4

SUMMARY OF CHANGES
February 25, 2011

AMENDMENT CHANGE	SECTION(S) AFFECTED BY AMENDMENT CHANGE
Minor formatting performed.	All applicable sections
Modified estimated finish of study.	Protocol Synopsis
Section revised to recommend that vasoactive drugs should not be administered prior to or concomitant with sodium nitroprusside.	Section 3.2.2 Prior and Concomitant Therapy, Including Medications and Procedures
Clarified the definition of the start of the blinded study drug period.	Section 3.4.4 Blinded Study Drug Administration Procedures
Clarified the possible impact of dead space in infusion set-up on administration of blinded study drug.	Section 3.4.9 Delivery of Study Drug
Membership of the DMC updated to seven members with expertise highlighted for all members.	Section 6.0 Data Monitoring Committee
References to the interim analysis were removed. This will no longer be performed.	Section 7.1 General Overview
References to the interim analysis were removed. This will no longer be performed.	Section 7.7 Statistical Design and Models for Analysis
Clarified that the primary efficacy analysis will be conducted on the ITT population.	Section 7.7.1 Primary Efficacy Analysis
Clarified that the primary efficacy analysis will be conducted on the ITT population.	Section 7.7.2 Primary Safety Analysis
Corrections were made to the row labels in the sample size table.	Section 7.7.3 Sample Size Estimation
Clarified plan for ITT analysis and per protocol analysis.	Section 7.7.4 Strategy for Statistical Analysis
Section title modified from Case Report Forms to Data Collection. References to paper CRFs removed and replaced with electronic data capture system and electronic CRFs.	Section 9.2 Data Collection
References to paper CRFs removed and replaced with electronic data capture system and electronic CRFs.	Section 10.0 Data Quality Control and Assurance
References to paper CRFs removed and replaced with electronic data capture system and electronic CRFs.	Appendix D Responsibilities of the Investigator

PROTOCOL

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Number: NICHD-2003-09-LT

Study Drug: Sodium Nitroprusside

IND: 71,979

Medical Monitor: Robert Lindblad, M.D.

Principal Investigators:

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Sponsor: The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

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APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol and its associated investigator brochure for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

And I have fully discussed the objectives of this trial and the contents of this protocol with representatives of PODS Center and BPCA Data Coordinating Center.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and in accordance with all applicable local, state, and federal regulations.

I understand that, should the decision be made by the PODS Center, BPCA Data Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

PODS Center #1 Duke University Medical Center Box 3094 DUMC Durham, NC 27705	SUBCONTRACTOR: Institution: Address: Telephone: Fax: Email:
CO-PRINCIPAL INVESTIGATOR Scott Schulman, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

APPROVAL SIGNATURES

STUDY PROTOCOL AGREEMENT FORM

I, _____, Investigator, have examined this PODS Center Protocol and its associated investigator brochure for sodium nitroprusside in the control of blood pressure entitled:

A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

And I have fully discussed the objectives of this trial and the contents of this protocol with representatives of PODS Center and BPCA Data Coordinating Center.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and in accordance with all applicable local, state, and federal regulations.

I understand that, should the decision be made by the PODS Center, BPCA Data Coordinating Center, NICHD and/or the FDA to terminate prematurely or suspend the study at any time for whatever reason; such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate immediately such decision in writing to the PODS Center Principal Investigator.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

PODS Center #2 Stanford University Medical Center 300 Pasteur Drive, H3580 Stanford, CA 94305	SUBCONTRACTOR: Institution: Address: Telephone: Fax: Email:
CO-PRINCIPAL INVESTIGATOR Gregory Hammer, M.D.	SUBCONTRACTOR PRINCIPAL INVESTIGATOR:
SIGNATURE DATE:	SIGNATURE DATE:

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PROTOCOL SYNOPSIS

Protocol Title:	A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects
Protocol Number:	NICHD-2003-09-LT
Sponsor:	National Institute of Child Health and Human Development
Product:	Sodium Nitroprusside
Objectives:	1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours 2. To assess the potential for rebound hypertension following administration of sodium nitroprusside for 12 hours or more
Study Design:	This is a phase II, randomized, double blind, withdrawal to placebo study examining the efficacy, safety and tolerability of sodium nitroprusside in pediatric subjects.
Study Population:	Children less than 17 years of age who require long term (at least 12 hour) blood pressure control will be eligible for study.
Number of Subjects:	A target of approximately 60 patients will be enrolled.
Number of Sites:	Up to 15
Duration of Subject Participation:	Enrollment is anticipated to begin in 2008 and to be complete in approximately 24 months. Patients will be followed for up to 30 days following receipt of study drug.
Treatment:	Subjects who require vasodilator therapy for relatively long time periods will receive open-label infusion of sodium nitroprusside for at least 12 hours but not greater than 24 hours.
Dose Schedule:	Patients will be randomized to receive either placebo or sodium nitroprusside for 30 minutes following at least 12- hours but not more than 24 hours of open-label infusion of sodium nitroprusside.
Estimated Start:	Q4 2008
Estimated Finish:	Q1 2011
Ethics	All regulations stated in 21 CFR Parts 50, 56, and 312 and recommendations outlined in the ICH Guidelines for Good Clinical Practice, as well as all other applicable local and national laws and regulations, will be adhered to throughout this trial.

Safety:	The safety of the drug will be assessed by multiple subject assessments of vital signs, physical exams, clinical tests and laboratory evaluations. Adverse events will be monitored and tracked. All SAEs will be closely monitored throughout the course of the study.
Statistical Consideration:	The trial will be sized to detect the loss of as little as 50% of the expected blood pressure lowering effect of the chosen dose of sodium nitroprusside during the blinded phase of the study.

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BP	Blood Pressure
BPCA DCC	Best Pharmaceuticals for Children Act Data Coordinating Center
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
cGMP	cyclic Guanosine Monophosphate
CN^-	Cyanide
CRA	Clinical Research Associate
CRF	Case Report Form
d/c	Discontinuation
DCRI	Duke Clinical Research Institute
DBP	Diastolic Blood Pressure
DMC	Data Monitoring Committee
ECMO	Extracorporeal Membrane Oxygenation
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
hr	Hour
HR	Heart Rate
IB	Investigational Brochure
ICU	Intensive Care Unit

IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
kg	Kilogram
MAP	Mean Arterial Pressure
mcg	Microgram
mEq/L	Milliequivalent per Liter
mcgs	Micrograms
min	Minute
mL	Milliliter
mm Hg	Millimeters of Mercury
mmol/L	Millimoles per Liter
NO	Nitric Oxide
NICHD	National Institute for Child Health and Human Development
NONMEM	Nonlinear Mixed Effect Model
NTG	Nitroglycerin
PD	Pharmacodynamic
PaO_2	Arterial Partial Pressure of Oxygen
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SCN	Thiocyanate
SNP	Sodium Nitroprusside
μM	Micromoles per liter, Micromolar

1.0 Background and Rationale

Blood pressure control in children is a significant concern in the intensive care unit (ICU), where management of arterial pressure is often necessary during periods of acute physiologic stress such as occurs after certain surgical and medical procedures. Examples of surgical procedures that require blood pressure control in the intensive care unit following surgery include aortic coarctation repair, Ross procedure (pulmonary valve autograft), and solid organ transplantation. Medical conditions requiring control of systemic arterial pressure include renal disease, drug therapy (corticosterioids and immunosuppression agents), and procedures such as extracorporeal membrane oxygenation (ECMO).

A wide variety of drugs of various therapeutic classes have been utilized for either controlled hypotension in the operating room or prevention of hypertension in the pediatric ICU. These drug classes include calcium channel blockers (Tobias et al, 1996), beta-adrenergic antagonists (Kay et al, 2001), ganglionic blockers (DuToit, 1970 and Gallagher and Milliken, 1979), inhalation anesthetics (Tobias, 1998) and direct acting vasodilators such as nitroglycerin and sodium nitroprusside (SNP) (Kaplan, 1980, and Tinker, 1976 Groshong, 1996, and Sinaiko, 1996). Although many vasodilator agents are available to lower blood pressure in the operating room and intensive care unit setting, few have been systematically studied in children.

SNP is a direct acting vasodilator commonly used for blood pressure control. It produces vascular smooth muscle relaxation when its metabolism in the red blood cell results in the liberation of nitric oxide (NO). NO then activates the enzyme guanylyl cyclase. This activation results in the formation of increased intracellular levels of cyclic guanosine monophosphate (cGMP). The result is vasodilation.

1.1 Metabolism

Five molecules of cyanide (CN^-) are released when SNP is metabolized in the red blood cell. The major metabolic pathway for CN^- is conversion to thiocyanate (SCN). This conversion occurs enzymatically via two sulfur transferase systems: 1) rhodenase (the primary pathway) and 2) beta-mercaptopyruvate-cyanide sulfurtransferase. Rhodenase is ubiquitous throughout the body, but it is highly concentrated in the liver. Rhodenase catalyzes the transfer of sulfur from a sulfur donor molecule such as thiosulfate (Na_2SO_3) to cyanide and thereby the formation of thiocyanate (SCN). SCN is subsequently eliminated in the urine and can therefore serve as a marker of cyanide exposure.

The ability of rhodenase to catalyze the conversion of cyanide to thiocyanate (SCN) is limited by the availability of sulfur donors in the body. Thus the provision of exogenous sulfur donors such as thiosulfate (Na_2SO_3) in the setting of acute cyanide intoxication is a potentially life-saving intervention (Pasch et al, 1983, Cole and Vesey, 1987).

One out of every five CN^- ions liberated by the metabolism of SNP binds to methemoglobin to form the non-toxic cyanomethemoglobin. The creation of additional quantities of methemoglobin by the intravenous infusion of sodium nitrite can thus provide additional CN^- buffering capacity. The resultant methemoglobinemia can then be treated with the administration of intravenous methylene blue.

Additional metabolic pathways for CN^- include the conversion of hydroxycobalamine (vitamin B12a) to cyanocobalamine, and conversion to 2-aminothiazoline 4-carboxylic acid.

If the above three pathways (rhodenase, methemoglobin, hydroxycobolamine) are overwhelmed, cyanide will bind to mitochondrial cytochrome oxidases and poison cellular oxidative phosphorylation. Cellular hypoxia is induced when cyanide inhibits the electron transport chain at cytochrome a_3. Oxygen cannot be utilized, mixed venous oxygen tension rises and the generation of high-energy adenosine triphosphate (ATP) is

blocked. The cell reverts from aerobic to anaerobic metabolism, with the subsequent generation of pyruvate and lactate. Acidosis ensues, and with it, deterioration in the organ systems most dependent on oxidative metabolism: the central nervous system and heart.

Clinical manifestations of cyanide toxicity to the central nervous system include headache, anxiety, agitation, confusion, lethargy, convulsions and coma. Cardiovascular manifestations include progressive heart failure with both loss of contractile force (negative inotropy) and slowing of rate (negative chronotropy). Bradycardia and hypotension are commonly observed pre-morbid events associated with cyanide toxicity.

In patients receiving SNP, the earliest, most sensitive signs of cyanide toxicity are acidosis, elevated mixed venous oxygen tension, and rising blood lactate levels. Venous blood that appears "bright" red due to the inability of the tissues to extract oxygen should suggest cyanide toxicity. Arterial and mixed venous blood gas analysis with co-oximetry can help confirm the diagnosis.

1.2 Previous Studies

SNP was first discovered in 1850. Its hypotensive effects were noticed in 1929, and its first therapeutic use was reported by Page et al. in 1955. Moraca et al. first described the clinical use of SNP for deliberate hypotension during surgical procedures in 1962. Since then, it has been widely used to control blood pressure in infants and children in the perioperative period.

Despite its widespread use, there is a paucity of information on its safety, efficacy, and pharmacokinetic/pharmacodynamic relationships in children. Davies et al (1975) and Bennett and Abbott (1977) described their retrospective experience with SNP used to induce deliberate hypotension in small cohorts of children. Both authors observed that younger patients required more SNP than older ones to achieve comparable degrees of blood pressure control. In their small retrospective cohort, Bennett and Abbott

recommended that doses of 10 micrograms/kilogram/minute were necessary to achieve satisfactory blood pressure response. Davies et al described three possible responses to SNP administration in children: 1) a constant response to "conventional" doses < 3 mg/kg; 2) a tachyphylactic response characterized by continuously escalating dose requirement (> 3 mg/kg) to achieve a satisfactory blood pressure; and 3) resistance to the blood pressure lowering effects of the drug. They cautioned against using total doses that exceeded 3 mg/kg or continuing administration of SNP in the latter two scenarios. Firm conclusions cannot be drawn because these small case series were not randomized controlled trials with specific pharmacodynamic endpoints.

Yaster et al (1986) compared SNP to nitroglycerin (NTG) for inducing hypotension in a group of 14 adolescents. They found doses of SNP between 6-8 micrograms/kg/minute superior to NTG at any dose in the reliable induction of hypotension for children and adolescents undergoing scoliosis, craniofacial or hepatic surgery.

Hersey et al (1997) performed a randomized trial comparing SNP to the dihydropyridine calcium channel antagonist nicardipine in 20 healthy adolescents with idiopathic scoliosis undergoing spinal fusion. Target blood pressures were easily obtainable in both groups and operating conditions were comparable. The time to restoration of baseline blood pressure after termination of the infusion was significantly longer in the nicardipine group. Interestingly, blood loss was significantly greater in the SNP group. Details on SNP dose requirements were not provided.

Przybylo et al (1995) described CN^- and SCN blood levels in ten children who received SNP at doses up to 10 micrograms/kg/min (mean infusion rate 6 microgram/kg/min) while undergoing cardiopulmonary bypass for repair of complex congenital cardiac defects. CN^- levels rose as a function of time while SNP was infused, and rapidly fell when SNP was discontinued. Despite the fact that some children demonstrated serum CN^- levels above the generally accepted threshold of 0.5 micrograms/ml, no patient developed clinically apparent toxicity. Kazim et al (1996) questioned the validity of the results of this study because of the CN^- assay methods utilized.

Linakis et al (1991) retrospectively examined physician-ordering practice as it pertained to blood cyanide levels in children receiving SNP. They sought to determine how the laboratory determinations were used to monitor patients and if there was clinically apparent toxicity in children found to have cyanide concentrations exceeding the "normal" limit of 500 micrograms/liter. They found poor correlation between blood cyanide concentration and dose or duration of therapy in patients whose cyanide levels were "toxic." Thiocyanate determinations were normal and no child manifested signs or symptoms of cyanide toxicity. They concluded that further pediatric studies were needed.

2.0 Study Objectives

We propose a multicenter trial that will provide guidance for the use of SNP to reduce blood pressure in pediatric patients. The trial is a randomized, double-blinded withdrawal to placebo trial. The aims of the trial are:

1. To determine the persistence of the effect of sodium nitroprusside on blood pressure during stable infusion regimens lasting at least 12 hours
2. To assess the potential for rebound hypertension during the Blinded Phase following administration of sodium nitroprusside for 12 hours or more.

To meet these study aims, the following study phases, defined in Section 3.1.1, will have the following objectives:

1. Open-Label Study Drug Administration (Dose-Titration) Phase
   The objective during this phase of the study is to determine the effectiveness and safety of SNP for controlling blood pressure during stable infusion lasting at least 12 hours.
2. Blinded Study Drug Administration Phase
   - The primary endpoint for the study will be determined during this phase of the study. The primary endpoint is the change in mean arterial pressure (MAP) recorded during the Blinded Study Drug Administration Phase in the absence

of other stimuli. The primary objective is to determine the persistence of sodium nitroprusside versus placebo for reducing blood pressure in pediatric patients.

- The secondary objectives during this phase of the study are as follows:
  - To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience offset during the blinded study drug period.
  - To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience rebound hypertension during the blinded study drug period.
  - To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the blinded study drug period.
  - To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the blinded study drug period.
  - To compare the changes (values recorded during the Blinded Study Drug Administration Phase minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.

3. Follow-up Phase

   The following objectives to be evaluated during this phase of the study are as follows:

   - To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience a serious adverse event during the Follow-up Period.
   - To compare the distribution of patients randomized to receive either sodium nitroprusside or placebo who experience a treatment-emergent and related adverse event, by maximum severity grade, during the Follow-up Period.

- To compare the proportion of patients randomized to receive either sodium nitroprusside or placebo who experience changes in individual physical examination parameters represented as either normal or abnormal from the Pre-Study Period to the end of the Follow-up Period.
- To compare the changes (values recorded during the end of the Follow-up Period minus values recorded during the Pre-Study Drug Period) in vital signs (systolic blood pressure, diastolic blood pressure, MAP, and heart rate) between patients randomized to receive either sodium nitroprusside or placebo.
- To compare the changes (values obtained during the two-hour period immediately following the stop of blinded study drug minus values obtained during the Pre-Study Drug Period) in individual laboratory parameters between patients randomized to receive either sodium nitroprusside or placebo.

3.0 Investigational Plan

3.1 Overall Study Design and Plan: Description

This is a phase II, multicenter, randomized, double-blind placebo-controlled, parallel group study to determine the persistence of the effect of SNP on blood pressure and to assess the potential for rebound hypertension associated with prolonged infusion in pediatric subjects.

Target MAP is defined as the clinically appropriate MAP as determined by the investigator taking into account the clinical presentation and medical needs of the subject. The investigator may change the target MAP at his/her discretion based on clinical needs during the course of the study. The anticipated Target MAP must be at least 20 mmHg below MAP_{B1}--(15 mm Hg for subjects < 2 years old), see next definition, for the patient to be eligible for enrollment, see Inclusion criterion #4.

The initial baseline MAP (B1) is defined as the blood pressure measurement taken prior to the initiation of SNP administration, either institutionally-supplied or open-label study drug after at least a 5-minute period of stable conditions (e.g., no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP). See Figure 1 below.

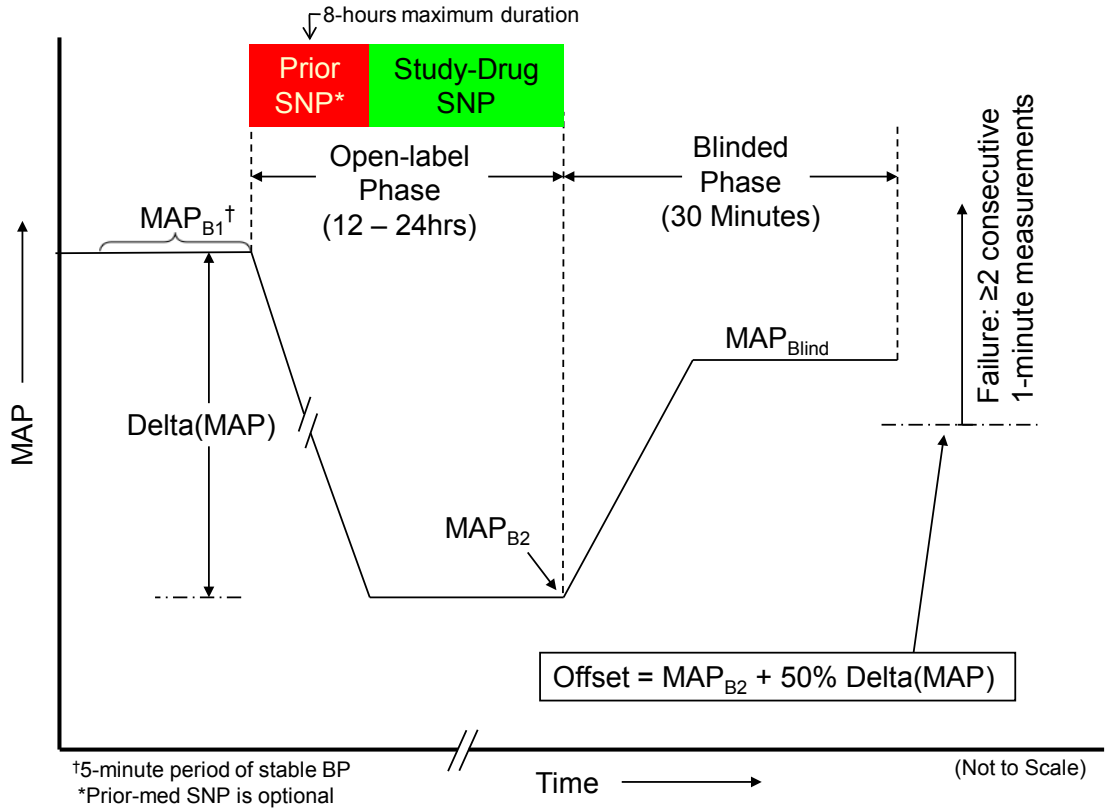
The subsequent baseline MAP (B2) is defined as the blood pressure measurement taken just prior to the initiation of blinded study drug after at least a 5 minute period of stable conditions (e.g., no prn doses or changes in sedative/analgesic drugs, tracheal suctioning, etc.). Prior to establishing B2, there shall have been no changes in the SNP infusion rate for a period of at least 20 minutes during which the infusion rate of SNP will be at least 0.5 mcg/kg/min.

Delta(MAP) is defined as the difference between MAP_{B1} and MAP_{B2}.

Offset is defined as MAP_{B2} plus 50% Delta(MAP)

Figure 1

Study Treatment Phases with MAPs and Delta(MAP)



- MAP_{B1} = MAP prior to start of Open-label Study Drug Administration Phase
- MAP_{B2} = MAP immediately prior to start of Blinded Study Drug Administration Phase
- Delta(MAP) = $MAP_{B1} - MAP_{B2}$
- Offset = MAP_{B2} + 50% Delta(MAP)
- Treatment Failure: During the blinded phase, ≥2 consecutive 1-minute MAP measurements greater than Offset [MAP_{B2} + 50% Delta(MAP)]

Example: Subject's MAP immediately prior to start of the Open-label Study Drug Administration Phase is 100 mmHg (MAP_{B1}). At the end of the Open-label Study Drug Administration Phase, MAP is 60 mmHg (MAP_{B2}). Thus, Delta(MAP) = 40 mmHg. For *treatment success*, MAP during the Blinded Study Drug Administration Phase cannot exceed MAP_{B2} + 50% Delta(MAP), or 80 mmHg, for any 2 consecutive MAP_{Blind} measurements, obtained at 1-minute intervals.

Approximately 15 centers will participate in subject recruitment to complete the study.

Approximately sixty (60) patients who require long term (at least 12 hours) blood pressure control will be enrolled. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment.

Any patient who starts the blinded study drug administration period will be considered complete for analysis. Enrolled subjects will be randomized in equal proportions to receive either placebo or SNP for the duration of the blind-treatment period, which will immediately follow the open-label infusion of SNP.

3.1.1 Definition of Study Periods

Study periods are as follows:

1. Pre-study drug administration: a period of up to 7 days preceding the start of study drug administration during which informed parental permission, and other enrollment procedures take place.
2. Open-label study drug administration (Dose-Titration): The period of open label study drug administration will be at least 12 hours but not greater than 24 hours, including the time of infusion of institutionally-supplied SNP, if any. This period will begin at the start of SNP administration, either study drug or institutionally-supplied. The duration of infusion of the institutionally-supplied

SNP will be no longer than 8 hours. Randomization will normally occur during this period.

3. Blinded study drug administration: The period beginning with the start of blinded study drug administration and ending with the discontinuation of blinded study drug. It immediately follows the open-label period and will be no longer in duration than 30 minutes.
4. Follow up: The period immediately following blinded study drug administration and ending 30 days after completion of study drug administration. AEs will be followed for 24 hours after termination of study drug. SAEs will be followed for 30 days.

Safety will be assessed via the evaluation of adverse events, pre- and post-treatment laboratory results, and vital sign data. The efficacy endpoints will mainly be assessed by examining blood pressure parameters.

3.2 Selection of Study Population

Children less than 17 years of age who require pharmacologic blood pressure control for at least 12 hours will be eligible for enrollment into the study. Blood pressure control is defined as the maintenance of the subject's MAP to within 90-110% of a target MAP specified by the physician.

Five pediatric age groups will be enrolled in this trial:

Group A: Neonates from birth to less than 30 days of age
Group B: Infants and toddlers from 30 days to < 2 years
Group C: Preschool children from 2 years - < 6 years
Group D: School age children from 6 yrs - < Tanner stage III
Group E: Adolescents from Tanner stage III - < 17 years.

Tanner III refers to the onset of puberty and occurs at different ages in different individuals. The mean age at onset of Tanner III ranges from 12.4 to 13.1 years in males,

and 11.9 to 12.6 years in females. At least 50% of the patients will be pre-pubertal, and at least 50% of these pre-pubertal patients will be neonates or toddlers at the time of enrollment (see Appendix A).

3.2.1 Inclusion Criteria

Subjects must meet all of the following criteria:

1. Subject is less than 17 years of age.
2. An in-dwelling arterial line is clinically indicated.
3. Subject's parent or legal guardian is willing and able to give informed parental permission signing and dating an IRB-approved informed parental permission containing all of the elements of informed consent, and subject provides assent, signing an IRB-approved and –required informed assent, if applicable.
4. Subject is anticipated to require a minimum of 20 mm Hg (15 mm Hg for subjects < 2 years old) reduction in MAP for at least 12 hours using SNP [e.g., MAP_{B1} - $MAP_{B2} \geq$ 20 mm Hg (15 mm Hg for subjects < 2 years old)]

3.2.2 Exclusion Criteria

Subjects will be excluded from study if any of the following criteria exist:

1. Subject weighs < 3.0 kg.
2. Subject has a known allergy to SNP.
3. Subject has a known mitochondrial cytopathy with a disorder of oxidative phosphorylation or of respiratory chain enzymes.
4. Subject has a contraindication to vasodilator therapy for control of blood pressure during surgery or in the intensive care unit.
5. Subject has raised intracranial pressure.
6. Subject is anticipated to need anti-hypertensive drugs other than Sodium Nitroprusside either IV (e.g., dexmedetomidine, esmolol, etc.) or epidural (e.g., local anesthetics, clonidine, etc.) within three terminal half-lives (3X T½ β) of the

blinded study drug period. However, patients receiving stable doses of an anti-hypertensive drug(s) prior to the initiation of study drug may be enrolled.

7. Subject has any serious medical condition which, in the opinion of the investigator, is likely to interfere with study procedures.
8. Subject is moribund (death likely to occur within 48 hours).
9. Subject has a positive result for the urine or serum HCG test administered at screening.
10. Subject has participated in other clinical trials for investigational drugs within 30 days prior to enrollment
11. Subject has received or will have received Sodium Thiosulfate within 6 hours prior to the start of the open-label period.
12. Subject is either on, or anticipated to be on, ECMO.

3.2.3 Prior and Concomitant Therapy, including Medications and Procedures

3.2.3.1 BP-Affecting Drugs

Administration of vasoactive drugs such as dopamine, epinephrine, and milrinone either prior to or concomitant with the administration of sodium nitroprusside is discouraged. However, if vasoactive drugs are clinically indicated, it is recommended that they are administered as defined in sections 3.2.3.2 and 3.2.3.3. There are not any restrictions on the administration of vasoactive medications following the end of the blinded treatment period.

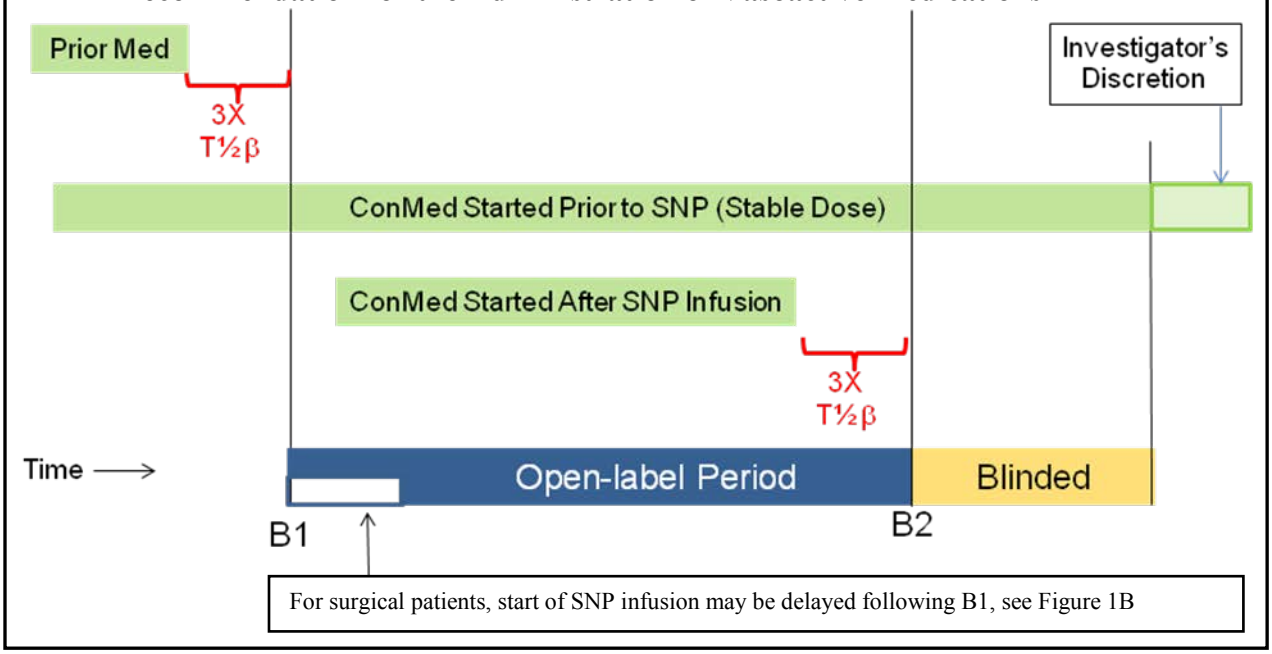
3.2.3.2 Prior Medications – Administration started prior to B1

It is recommended that vasoactive drugs started prior to B1 be either (1) discontinued at least 3 terminal half-lives (3X T½ β) prior to B1, or (2) continued at a stable dose until at least the end of the blinded infusion period, see Figure 2.

3.2.3.3 Concomitant Medications – Administration started after B1

It is recommended that vasoactive drugs started after B1 be discontinued at least 3 terminal half-lives (3X T½ β) prior to B2 (e.g., the start of the blinded treatment phase), see Figure 2.

Figure 2
Recommendation for the Administration of Vasoactive Medications



3.2.3.4 Recording Prior and Concomitant Medications and Procedures

All concomitant medications that affect blood pressure, sodium thiosulfate, and clinically meaningful, unexpected, and invasive procedures will be recorded for the period beginning 72 hours prior to study drug administration through 24 hours post-study drug conclusion. The dates of administration and reason for use must be included. All concomitant medications will be collected for SAEs occurring within 30 days following study drug administration.

3.2.3.5 SNP as Concomitant Medication

Subjects may receive institutionally-supplied SNP prior to the initiation of study drug administration; however, administration of institutionally-supplied SNP will be discontinued immediately prior to the initiation of study drug administration, and the initial infusion rate of study-drug SNP will be the same as the discontinued institutionally-supplied SNP (see Section 3.4.3 #6. VS measurements described for the Open-label treatment period in Table 1 and Section 3.4.3 refer to both institutionally supplied SNP and investigational SNP (study drug).

Subjects may receive institutionally-supplied SNP after the conclusion of the SNP Blinded Treatment Period at the discretion by the investigator.

The start and stop times and dates, and dosage of institutionally-supplied SNP administration will be recorded on the appropriate CRF.

3.2.3.6 Use of Sodium Thiosulfate

Administration of sodium thiosulfate will be prohibited from 6 hours prior to the start of the open-label period until the end of the blinded-study period, excepted in cases in which nitroprusside or cyanide toxicity is suspected, in which case, the administration of sodium thiosulfate, as described in Appendix E, is recommended.

To facilitate the assessment of cyanide clearance, investigators are encouraged not to co-administer sodium thiosulfate with institutionally-supplied SNP, which may, at the investigator's discretion, be administered following the conclusion of the blinded-study period. Should sodium thiosulfate be co-administered with institutionally-supplied SNP following the conclusion of the blinded-study period, blood and urine samples will not be tested for cyanide and Thiocyanate in these subjects once the sodium thiosulfate administration has begun.

3.3 Efficacy and Safety Assessments

3.3.1 Efficacy and Safety Measurements

Table 1 is a schematic representation of study assessments and procedures.

3.3.2 Safety Assessments

Safety assessments will include monitoring the tolerability of the SNP infusion and assessing physical examinations, vital signs, clinical laboratory values, concomitant medications and procedures, and adverse events throughout the study. SAEs will be collected for 30 days following completion of study drug administration.

In cases of discharge from the hospital before 30 days, parent (or guardian) will be contacted to determine if any SAE's occurred following discharge but within 30 days of study drug discontinuation. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. See section 4.2 for specific adverse event parameters and actions to be taken. See section 3.4.7.2 for supplemental cyanide and thiocyanate testing.

3.3.3 Drug Concentration Measurements

Cyanide, thiocyanate, methemoglobin, lactic acid, and arterial blood gas analysis will be performed throughout the trial to indirectly query SNP levels and determine subject safety.

TABLE 1: Schedule of Assessments: Sodium Nitroprusside Long-Term Infusion Study

Procedure	Pre-study Drug Period (Up to 7 days prior to Study Drug administration)	Open-label Period (12 -24 hrs duration)	Blinded Study Drug Period	Study Drug d/c (Within 2 hours)	Follow-up (Up to 24 hours post blinded study drug)[1]
Assessments					
Review Entry Criteria	X				
Informed Parental Permission/ HIPAA Consent	X				
Collect Demographic Data	X				
Medical History	X				
Physical Examination	X			X	X[2]
Vital Signs (SBP, DBP, MAP, HR)[3]	X[4]	X	X	X	X
B1	X[5]				
B2—Just Prior to Blinded Infusion		X			
Growth Parameters[6]	X				
Urine Output[7]	X	X	X	X	X
Serious Adverse Events/Adverse Events		X	X	X	X[8]
Concomitant Medication[9]	X[10]	X	X	X	X[11]
Concomitant Procedure[12]	X[10]	X	X	X	X[11]
Randomization of Blinded study drug		X			
Blinded Study Drug Administration			X		
Open-label Study Drug Administration		X			
Laboratory Assessments					
Pregnancy test (post-menarche females)	X[13]				
Electrolytes, BUN, creatinine	X			X	
Hematology (CBC & platelet count)	X			X	
Liver Enzymes (AST, ALT)	X			X	
Arterial Plasma Lactate level	X	Q 8 hours (± 30 min)		X	X[14]
Arterial Blood Gas with Co-oximetry (includes Methemoglobin)[15]	X	Q 8 hours (± 30 min)		X	X[14]
Central Venous Blood Gas with Co-oximetry (includes Methemoglobin)[16]	X	Q 8 hours (± 30 min)		X	X[14]
Blood for Thiocyanate and Cyanide[17] (central lab)	X	Q 8 hours (± 30 min)		X & 12 hr post d/c [18]	X[14]
Urine for Thiocyanate (central lab)[19]	X	Q 8 hours (± 30 min)		X	Q8 hrs (± 30 min) X 3

1. End of Study assessment will be done at 24 hours post blinded study drug administration, except where noted.
2. To be performed 18-30 hours following the termination of study-drug administration.
3. Vital sign measurements as described in protocol, sections 3.4.2 – 3.4.7.1. Vital signs will then be collected every 12 ± ½ hours for 24 hours post blinded study drug administration. VS measurements described for the Open-label treatment period refer to both institutionally supplied SNP and investigational SNP (study drug), see also Section 3.4.3 (#9.
4. Obtain vital sign measurements. Based upon clinical judgment, this may be MAP_{B1} for some patients.
5. MAP_{B1} will be obtained prior to the start of the SNP open-label infusion and after a 5-minute period of stable BP (e.g., no changes in administration rates of drugs that may affect BP)
6. Growth parameters will include weight, height/ length, and Tanner stage, if ≥6 years old.
7. Measurements to be performed at time of urine thiocyanate sample collection, if feasible.

8. AEs will be followed for 24 hours and SAE will be followed for 30 days, after the completion of study drug administration.
9. CRFs will document administration of concomitant medications that (1) affect blood pressure, as defined in Section 3.2.3.1, or (2) are associated with SAE's. All concomitant medications will be recorded in source documents, such as the patients' medical charts.
10. Within 72 hours of study drug administration.
11. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
12. Clinically meaningful, unexpected, and invasive procedures only.
13. To be performed within 48 hours of study drug administration.
14. Following concomitant SNP d/c, if feasible.
15. ABG sampling preferred, sample collected at drug d/c only if line is still in.
16. Central Venous Blood Gas done only if CVC is indwelling.
17. Additional cyanide and thiocyanate tests are permitted provided that the investigator believes the tests to be in the patient's best interest and the volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).
18. Blood for cyanide & thiocyanate at 12 hrs ± 30 min, post study drug d/c, only if arterial line in place.
19. Urine collection details are described in section 3.4.7.2 and the MOP.

3.4 Study Visits and Procedures

3.4.1 Informed Parental Permission

Prior to the start of any study-related procedure, a signed and dated informed parental permission, containing all elements of informed consent and, if applicable, assent must be obtained and documented in the subject's medical record (See Appendix B).

3.4.2 Pre-study drug administration procedures

The following procedures will be completed prior to the administration of study drug:

1. Obtain signed and dated informed parental permission/HIPAA authorization/assent.
2. Collect demographic data and medical/surgical history.
3. Record diagnosis.
4. Perform a pertinent physical examination.
5. Obtain vital sign measurements. Based upon clinical judgment, this may be MAP_{B1} for some patients.

6. Determine subject height in centimeters and subject weight in kilograms (for calculation of appropriate study drug dose).
7. Collect urine and blood samples for laboratory evaluations as per Table 1. Pregnancy test if required must be done within 48 hours prior to study drug administration. (If the screening pregnancy test will have been more than 48 hours prior to the start of the study drug administration, then the test will be repeated.)
   - To minimize the blood volume obtained under this protocol, laboratory evaluations performed prior to the consenting of the patient as part of the standard of care of the patient and within 7 days of the administration of study drug may be substituted for these procedures.
8. Document concomitant medications that may affect blood.

3.4.3 Open-Label Study Drug Administration (Dose-Titration) Procedures:

The following procedures should be performed sequentially unless otherwise indicated.

1. Stabilize sedation/analgesia.
2. Insert arterial line if not already in place.
3. Obtain baseline vital sign measurements prior to the start of SNP administration, either institutionally-supplied or open-label study drug. This defines B1.
   - The blood pressure measurement establishing MAP_{B1} will follow a 5-minute period of stable BP (e.g., no changes in drug concentrations which may affect BP).
   - If MAP_{B1} is not obtained immediately prior to the initiation of infusion of SNP, a VS measurement will be obtained immediately prior to the initiation of the open-label SNP infusion (time=0).
4. Determine and record target MAP.
5. If the difference between B1 and the target MAP is <20 mmHg (15 mmHg for subjects <2 years old), the patient will be withdrawn from the study and not given study drug.

6. Begin administration of open-label study drug at a dose not to exceed 0.3 mcg/kg/min, or, if applicable, at the infusion rate of the institutionally-supplied SNP.
7. The dose of open-label SNP will be titrated according to the subject's BP response such that the target MAP, chosen by the study physician, is achieved ±10%. If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose. The duration of open-label study drug administration will be at least 12 hours but less than 24 hours.
8. Revise target MAP as clinically indicated; titrate SNP to achieve new target MAP (±10%).
9. Obtain vital sign measurements every one minute for the first 10 minutes then every 5 ± 1 minutes for an additional 20 minutes after initiation of open-label study drug infusion (and, if applicable, institutionally-supplied SNP) and after each dosage adjustment. After the initial 30 minutes, once a stable dose is achieved and BP control is satisfactory, vital sign measurements will be obtained every ≤ 20 minutes. Additionally, obtain vital sign measurements in a similar manner whenever it is necessary to change the open-label drug infusion rate.
10. Randomize patient
11. Collect blood samples for laboratory evaluations as per Table 1.
12. Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures, and adverse events.
13. Whenever an adverse event occurs, obtain vital sign measurements. If clinically appropriate, a blood sample for safety including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations may be drawn.

3.4.4 Blinded Study Drug Administration Procedures

1. Obtain vital sign measurements immediately prior to the start of blinded study drug administration. This defines MAP_{B2}. There must be 5 minutes of stable conditions and 20 minutes of no changes in Open-label study drug prior to starting the blinded study drug administration phase. The start of blinded study period is defined as the start of the infusion pump containing the blinded study drug.
2. Begin 30 minute blinded study drug administration as described in Section 3.4.8.
3. Record blood pressure and heart rate every one minute for the duration of blinded study drug administration.
4. If blood pressure control is lost (defined as loss of 50% of delta MAP for ≥2 consecutive 1-minute measurements) the blinded study drug is discontinued when there is a safety concern or the MAP reaches 120% of MAP_{B1}.
5. Record concomitant medications associated with an SAE or that may affect blood pressure, clinically meaningful, unexpected, and invasive procedures and adverse events. Whenever an adverse event occurs, obtain vital sign measurements and, if appropriate, a blood sample for safety analysis including CN^-, thiocyanate, lactate, and arterial blood gas with co-oximetry and methemoglobin determinations.

3.4.5 Study Drug Discontinuation (Within 2 hours of discontinuing study drug)

1. Collect urine and blood samples for laboratory evaluations listed in Table 1
2. Conduct a pertinent physical examination and perform all other assessments listed in Table 1 for this study phase.

3.4.6 Follow up Procedures

The following will be performed after completion of study drug administration through 24 hours post study drug end:

1. If applicable, record the estimated blood loss and fluid intake, including blood and blood products and output during the trial period.
2. Record concomitant medications associated with an SAE or that may affect blood pressure, and clinically meaningful, unexpected, and invasive procedures. For patients who underwent surgery while under protocol, record surgical information, including name of surgical procedure, whether the patient was intubated, post-operative diagnosis, start and stop times of general anesthesia, and time of surgical incision and closure (or their equivalent).
3. Record vital signs at 12 and 24 ± ½ hours after the end of study drug administration.
4. Collect blood sample at 12 hours (± 30 min) after study drug discontinuation for cyanide and thiocyanate analysis.
5. Perform a pertinent physical examination 18 –30 hours following discontinuation of study drug administration.
6. Collect adverse events for 24 hours following discontinuation of study drug administration.
7. Collect serious adverse events plus associated concomitant medications and clinically meaningful, and invasive procedures for 30 days following discontinuation of study drug administration.
8. Collect blood samples for laboratory evaluations as per Table 1.

   Blood samples for cyanide and thiocyanate analysis at the discontinuation of the non-study drug (concomitant) SNP to be done only if feasible (the informed parental permission form must specify this blood sample will be drawn and only if an indwelling catheter is present). Note: This blood draw may be several days following the discontinuation of study-drug SNP administration.

SAEs and associated concomitant medications and procedures will be collected for 30 days following the discontinuation of study drug administration, either through telephone contacts and/or study visits or spontaneously reported by the subjects.

3.4.7 Methods of Assessment

3.4.7.1 Vital Sign Measurements

Vital signs: systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate will be measured.

The principal method of obtaining blood pressure measurements will be from an intra-arterial catheter inserted in an upper or lower extremity artery. Manual blood pressures from a non-invasive blood pressure cuff will only be used prior to insertion of and during a malfunction of the arterial catheter. The blood pressure transducer is internally calibrated by the instrument upon performing the zeroing procedure. All blood pressure and heart rate data will be acquired electronically when possible.

3.4.7.2 Blood Draws and Urine Samples

Blood drawn for study related purposes will not exceed the maximum amounts specified by the American Association of Blood Banks for healthy infants, children, and adolescents. Normally, this value is 7 ml/kg over an eight week period. This study is an inpatient trial of short duration, therefore, the amount of blood withdrawn for study related purposes will take into account the patient's pre-existing hemoglobin and hematocrit, and local Institutional Review Board limitations on maximum allowable blood draws for study-related purposes. A reasonable and conservative value is 3 ml/kg.

In addition to those cyanide and thiocyanate tests scheduled in Table 1 and Section 3.4, the investigator will be permitted to perform supplemental cyanide and thiocyanate tests provided that the investigator believes that the tests are in the patient's best interest and

the volume of blood required for the additional tests is not considered to be clinically significant (e.g., results in anemia or homologous blood transfusion).

Urine will be sampled for thiocyanate concentrations every 8 hours, or fraction thereof, commencing with initial study drug administration to the end of study drug completion and then Q 8 hours times 3 after the discontinuation of study-drug administration, or until the urinary catheter is removed, whichever occurs first. All urine samples will be from a pooled urine collection from the 8-hour time period or fraction of 8-hour time period. The sample will be stored cold until shipment to the central lab. Details of urine collection procedures are described in the Manual of Procedures (MOP).

3.4.8 Dispensing of Study Drug

Study drug will be dispensed to the sites in 2 ml vials containing 25 mg/ml of SNP. The pharmacist will dispense two preparations of study drug, one for the open-label study drug period and one for the blinded study drug period.

The open-label study drug administration phase will utilize a fixed study drug concentration and variable infusion rate scheme. Syringes or bags will be prepared by the investigational drug pharmacy by adding 25 mg of SNP to 50 ml 5% dextrose. The syringe will have a label indicating the concentration of the solution (0.5 mg/ml SNP), and the infusion rate necessary to provide 1.0 microgram per kilogram per minute (1.0 mcg/kg/min) —for example, for a 25-kg subject, an infusion rate of 3.0 ml/hr will deliver 1.0 mcg/kg/min SNP. The infusion rate of 0.5 mg/ml SNP can be calculated using the conversion factor: Wt (kg) × 0.12 ml/kg/hr = 1.0 mcg/kg/min. Clinicians can then make the necessary dosage adjustment for adequate blood pressure control. All dosage adjustments will be captured on the case report forms (CRFs).

The pharmacist will supply blinded study drug for each subject according to a randomization assignment generated by the IVRS. The blinded study drug will be prepared by the pharmacist such that either the concentration of drug is the same as in the

open label period or placebo, see Section 3.6.1. Subjects will receive blinded study drug at the same rate of infusion that was used at the conclusion of the initial open-label study drug administration period, which will be at least 0.5 mcg/kg/min.

Syringes or bags will be wrapped in opaque or amber plastic to protect from light.

3.4.9 Delivery of Study Drug

Infusion pumps capable of reliable delivery at low infusion rates (to 0.1 ml/hr) will be used. All pumps will have free flow protection and will be internally calibrated for accuracy by the manufacturer. Accuracy will be verified at each site by the biomedical engineering department as part of their equipment management program. Quality assurance checks will be performed periodically according to manufacturer specifications.

Study drug will be infused via a dedicated peripheral intravenous catheter or via a dedicated lumen of a multi-orifice central venous catheter. Catheters will be chosen to minimize dead space in order to ensure accuracy of drug concentrations being delivered to this patient population. Microbore low compliance tubing, with volumes of approximately 1 mL will be used, where possible. Some amount of dead space is expected and this may vary across study participants. The dead space in the catheters could cause delays in delivery of blinded study drug. The carrier flow rate will be ≥5.0 mL/hr from at least one hour prior to the start of the blinded study period until the end of the blinded treatment period. Clinical judgment should be exercised when adjusting the carrier flow rate to prevent unsafe spikes in the infusion rate.

3.5 Removal of Subjects from Therapy or Assessment

3.5.1 Early Discontinuation of Study Drug and Subject Withdrawal

If MAP falls below 50 mmHg (40 mmHg for subjects less than 1 month of age) or HR exceeds the age adjusted maximum with no other explainable cause (e.g., concomitant medication), open-label study drug should be discontinued until MAP and HR return to within protocol limits. Open-label study drug can then be restarted at a dose lower than the previous dose.

If the subject withdraws participation in the study for any reason, every effort will be made to collect safety data, vital sign measurements, samples for safety and laboratory analyses. The date, time and reason for discontinuation must be recorded on the CRF. Additionally, every attempt should be made to complete all other study related procedures on discontinued subjects who have received any amount of study drug as the data will be included in the safety and intention to treat analyses. Subjects who prematurely discontinue from the study will not be replaced.

Any patient who starts the blinded study drug administration period will be considered complete for analysis.

Potential reasons for subject withdrawal from the study are as follows:

1. Subject's parent or legal guardian wishes to have the subject withdrawn for any reason;
2. Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the subject's safety;
3. The investigator feels that it is in the subject's best medical interest to be withdrawn.
4. Subject no longer needs blood pressure control.

3.6 Investigational Product

3.6.1 Identity of Investigational Product

Sodium nitroprusside (sodium nitropentacyanoferrate (III) dihydrate) is a reddish-brown crystalline powder that is freely soluble in water. Its molecular formula is $Na_2[Fe(CN)_5NO] \bullet 2H_20$. Study drug will be supplied by the BPCA Data Coordinating Center to the Investigational Drug Service at each clinical center in a standard concentration of 25 mg/ml. The Investigational Drug Service at each clinical center will then prepare the drug in syringes or bags of sterile 5% dextrose for administration to randomized patients according to the guidelines provided above. Sterile 5% dextrose will be utilized as placebo.

3.6.1.1 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature from 15°- 30°C and protected from light in its carton until used. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug must be maintained under adequate security.

3.6.2 Methods of Assigning Subjects to Treatment Groups

After meeting all inclusion and exclusion criteria, subjects will be considered to be enrolled and will be randomly assigned to receive either placebo or active drug treatment groups during the blinded study drug administration phase of the trial using a single centralized randomization schedule. Randomization into the blinded portion of the trial will be performed via a centralized interactive voice response system (IVRS).

The BPCA DCC will provide a system for the pharmacist to obtain each subject's randomized treatment assignment in a timely manner prior to the administration of study drug.

3.6.3 Assigning Subject Numbers

Study participants will be assigned a subject number upon successful enrollment into the study. The subject number will consist of five digits. The first two digits will be the site number of the enrolling institution followed by the number "2"—for the second trial under this IND—followed by a two digit enrollment-sequence number. For example, Subject #10-2-23 would be the 23rd subject enrolled at Site #10.

3.6.4 Blinding

The subject, as well as all caregivers, will remain blinded to the treatment assignment throughout the course of the study. For subjects' safety, the pharmacist will be aware of the treatment group for each subject. The BPCA DCC will maintain the double-blinded randomization schedule.

The randomization for an individual subject may be revealed in an emergency; however, investigators are discouraged against requesting that the blind be broken for an individual subject. Notification of any unblinding must be sent via facsimile to the BPCA DCC within 24 hours.

3.6.5 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms.

3.6.6 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the Clinical Supply Shipment Request and Verification or similar document. An accurate inventory of study drug will be kept by

the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and returned to the BPCA DCC if requested. The investigator agrees not to supply study medication to any persons not enrolled in the study or not named as a sub-investigator for the trial.

4.0 Adverse Events

4.1 Definition

An adverse event is defined as any unintended and unfavorable medical occurrence in a clinical investigation subject, administered a pharmaceutical product, regardless of the causal relationship with treatment. An adverse event can therefore be any untoward sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not the event is considered causally related to the use of the product. Pre-existing conditions that remain stable throughout the study period will not be considered adverse events. Any worsening of a pre-existing condition or illness is considered an adverse event.

Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional over-dosage, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events if they result in discontinuation from the study, necessitate therapeutic medical intervention, or the investigator considers them to be adverse events.

Adverse events will also include electronically-monitored vital signs that meet the definitions of section 4.2; however, electronically-captured data excursions, which in the opinion of the investigator represent data artifacts, such as might be produced by the turning of the patient or brief interruption of the electronic circuit of the monitor

device(s) or which do not likely reflect an actual untoward event will not be considered to adverse events.

New or worsening physical-exam findings that occur following the initiation of study-drug administration will be considered to be adverse events, without regard to causality.

4.1.1 Serious Adverse Events

A serious adverse event is one that meets the above criteria and also results in one of the following conditions:

1. Death
2. A threat to life
3. Requirement for inpatient hospitalization
4. Prolongation of hospitalization
5. Production of a congenital anomaly or birth defect
6. A persistent or significant disability or incapacity (excluding experiences of minor medical significance such as headache, nausea, vomiting, diarrhea, and accidental injury)
7. The requirement for a medical or surgical intervention in order to prevent a serious outcome.
8. Any event deemed to be serious by the Investigator

If an adverse event meets any of the above criteria, it must be reported to the BPCA DCC as a serious adverse event (SAE) within 24 hours of the investigative site's awareness of its occurrence.

4.2 Adverse Event Severity

The criteria for rating adverse events are as follows:

Mild	The adverse event is transient and easily tolerated by the subject.
Moderate	The AE causes subject discomfort and interrupts usual activities.
Severe	The AE causes significant interference with normal activities and may be incapacitating or life threatening.

Safety data are a primary objective of this trial. Safety issues regarding the possible development of cyanide toxicity during SNP infusions will be a primary focus for monitoring study subjects for drug-related adverse events. Safety will be evaluated throughout the study and during the follow-up period by evaluating the tolerability of the study drug infusion and by monitoring clinical and laboratory signs of SNP toxicity such as hypotension, tachycardia, bradycardia, acid base status, serum lactate concentration, methemoglobin levels, cyanide levels, and when available, mixed venous oxygen tension.

1. Co-oximetric arterial blood gas analysis with methemoglobin determination will be performed Q8. Arterial blood gas monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity.
2. Lower than expected methemoglobin concentrations may reflect indirect evidence of cyanide toxicity because cyanide has a high affinity for methemoglobin, combining with it to form the non-toxic molecule cyanomethemoglobin.
3. Other laboratory findings suggestive of cyanide toxicity that will be monitored in this trial include serum lactate levels and mixed venous oxygen saturation. Lactate levels will rise and mixed venous oxygen tension will increase when cyanide toxicity is occurring due to the reduced ability of the tissues to extract oxygen.
4. Plasma and urine thiocyanate levels are indirect markers of cyanide exposure because the majority of the cyanide ions liberated by the metabolism of SNP in the red blood cell are converted to thiocyanate by the rhodenase enzyme system in the liver and excreted in the urine. Free cyanide levels will also be measured.

The adverse event of rebound hypertension shall be considered mild if the MAP rises >10% above the baseline (B1), moderate if the MAP rises >20% above baseline (MAP_{B1}), and severe if the MAP rises >30% above baseline (MAP_{B1}).

The adverse event of excessive hypotension shall be considered mild if the MAP falls >20% below target; fluid therapy may be administered. The adverse event of excessive hypotension shall be considered moderate if the MAP falls >25% below target, fluid therapy is required, and pharmacologic therapy is required. The adverse event of excessive hypotension shall be considered severe if the MAP falls >30% below target, fluid therapy is required, and repeated and/or continuous pharmacologic support is required.

The rating of the adverse event of tachycardia shall be defined for sustained rates exceeding the age adjusted maximums (Table 3) by using the mild, moderate, and severe ranges outlined in Table 4A.

TABLE 3: Age-Adjusted Maximums for Pediatric Heart Rate

Subject Age	Maximum Heart Rate (bpm)
1 month <6 months	180
6 months <3 years	160
3 <8 years	150
8 <17 years	130

TABLE 4A: Severity of Tachycardia (Heart Rate in Beats per Minute)

Age	Mild	Moderate	Severe
Age <6 months	180-199	200-219	220 and higher
6 months - <3 years	160-179	180-199	200 and higher
3 years to < 8 years	150-164	165-179	180 and higher
8 years to <17 years	130-149	150-169	170 and higher

The adverse event of lactic acidosis shall be considered mild if the serum lactate concentration is between 5 and 7.5 mmol/L, moderate if the serum lactate concentration is between 7.6 and 10 mmol/L, and severe if the serum lactate concentration exceeds 10 mmol/L.

The adverse event of cyanide toxicity will be considered mild if the blood cyanide concentration is 0.5 mcg/mL – 1.0 mcg/mL, moderate if the blood cyanide concentration is >1.0 – 1.5 mcg/mL, and severe if the blood cyanide concentration is > 1.5 mcg/mL. Corresponding erythrocyte (vs. blood) cyanide values are ≥5 µmol/L - < 10 µmol/L (mild), 10-20 µmol/L (moderate), and > 20 µmol/L (severe).

Severity ratings for these selected adverse events are displayed in Table 4B.

TABLE 4B: Severity of Selected Adverse Events

Event	Mild	Moderate	Severe
Rebound Hypertension (during blinded phase)	MAP rises >10% above baseline (MAP_{B1})	MAP rises >20% above baseline (MAP_{B1})	MAP rises >30% above baseline (MAP_{B1})
Hypotension	MAP falls >20% below target	MAP falls >25% below target IV therapy required; Pharmacological therapy required	MAP falls >30% below target IV therapy required; Repeated or continuous pharmacological therapy required
Lactic acidosis	5 -7.5 mmol/L	7.6-10 mmol/L	>10 mmol/L
Cyanide toxicity (Blood)	Cyanide 0.5 – 1.0 mcg/mL	Cyanide >1.0-1.5 mcg/mL	Cyanide >1.5 mcg/mL
Cyanide toxicity (erythrocyte)	Cyanide ≥5 - <10 µmol/L	Cyanide ≥10–20 µmol/L	Cyanide >20 µmol/L

Frequent monitoring of acid-base status will help identify patients with metabolic acidosis, the earliest sign of SNP toxicity. If the base deficit exceeds 8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the patient will be discontinued from study and the SNP infusion terminated. If the lactate level rises by more than 4 mmol/L in an 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output) SNP administration will be discontinued. If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes percent between arterial and mixed venous blood in the absence of an explainable cause, SNP administration will be discontinued and treatment for suspected cyanide toxicity will be initiated.

Suspected cyanide toxicity will be further assessed and treated as follows:

1. Obtain blood for arterial and venous blood gases with co-oximetry, plasma lactate, and cyanide and thiocyanate levels.
2. Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration (see below).
3. SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10ml) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result).

TABLE 5: Dosage Chart for Children

Patient's Hemoglobin g/dL	Initial Dose of Sodium Nitrite (3%) mL/kg IV	Initial Dose of Sodium Thiosulfate mL/kg IV
8	0.22 mL/kg (6.6 mg/kg)	1.10 mL/kg
10	0.27 mL/kg (8.7 mg/kg)	1.35 mL/kg
12	0.33 mL/kg (10.0 mg/kg)	1.65 mL/kg
14	0.39 mL/kg (11.6 mg/kg)	1.95 mL/kg

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals. [Micromedex Poisondex www.micromedex.duhs.duke.edu , see also, Berlin, 1970]

4.3 Relationship to Study Drug

The criteria for determining the relationship of the AE to the study drug are as follows:

1. Probably related: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.
2. Possibly related: An AE that is likely to be related to the administration of the study drug and an alternative cause is equally or less likely when compared to the study drug.
3. Probably not related: An AE that has little or no relationship to the study drug and there exists a more likely, or equally likely, alternative cause.
4. Not related: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.

4.4 Adverse Event Collection Period

Non-serious adverse events will be monitored and reported from the time the subject receives study drug up to 24 hours following termination of study drug; SAEs will be monitored and reported from the time the subject receives study drug through 30 days following termination of study drug. Knowledge of adverse events will be gained from direct monitoring of the study subject as well as from clinician observation, and self reporting by the study subject or his/her guardians. Adverse events that have not resolved, or are ongoing, will be monitored to resolution if felt to be related to study drug, or until it is felt that the subject has stabilized.

5.0 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual subject, the investigator or other responsible physician must contact one of the Co-Principal Investigators immediately so that a timely decision can be made as to whether or not the subject should be enrolled or continue in the study. If a deviation is being requested by one of the Co-Principal Investigators, he must contact the other Co-Principal Investigator for a decision. The deviation from the protocol will be authorized only for that particular subject. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF.

6.0 Data Monitoring Committee

To ensure that the welfare of trial patients receives appropriate consideration, an independent Data Monitoring Committee (DMC) has been organized by the BPCA DCC on behalf of the NICHD to review relevant safety and efficacy data during the course of the trial. The DMC may recommend discontinuation of the study, or modifications to the study protocol for safety reasons.

The DMC consists of seven core members (Chair, ethicist, two statisticians, pediatric clinical pharmacologist, generalist pediatrician, community representative) plus additional ad hoc members for the various medical subspecialties involved in the BPCA protocols.

Each DMC will have a presenting statistician who will be responsible for presenting the interim data. This member will write the reports and will be one non-voting member of the DMC. Except as their role in the DMC, all DMC members are not participating in the design or conduct of this study, as an investigator or otherwise, and lack any financial conflict that would introduce any bias.

6.1 DMC Responsibilities

1. Monitoring the safety of trial patients;
2. Recommending discontinuation of the trial for safety reasons;
3. Recommending changes to the study protocol for safety reasons;
4. Providing written reports on an ongoing basis following scheduled and ad hoc meetings that will be archived and may be provided to regulatory agencies.

The DMC will monitor the safety of trial patients by reviewing the occurrence of adverse events and deaths, on a real-time basis as SAE reports are transmitted. The DMC may also monitor compliance with the protocol, and factors affecting patient safety or the integrity of the trial. The DMC may request any additional data that are not included in the report if deemed necessary for effective monitoring.

If the DMC finds any major concerns about safety, it may recommend discontinuing the trial or modifying the study protocol. Following each data review, the DMC will send a written recommendation regarding the trial, (e.g., to continue according to the protocol, or recommendations for specific actions) to the sponsor.

7.0 Statistical Considerations

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a Statistical Analysis Plan, which will be finalized prior to unblinding. Any changes in the statistical methods described in this protocol that occur prior to unblinding will be documented in the Statistical Analysis Plan and will not result in a protocol amendment.

7.1 General Overview

The primary efficacy variable is the intra-patient change in Delta (MAP) during the blinded phase of the study. The primary null hypothesis to be tested is that there is no difference between the active study drug and placebo in the proportion of patients who experience an intra-patient increase greater than or equal to MAP_{B2} + 50% Delta (MAP). Statistical analyses will be performed using two-sided tests. A 0.05 significance level will be used in all tests of treatment differences. Tests for interactions will utilize a 0.10 statistical significance level. Individual secondary endpoints will be evaluated using a hierarchical testing procedure. The Statistical Analysis Plan will include a detailed description of all statistical methods, testing procedures, and methods of data imputation.

Data will be summarized by treatment group with respect to demographic and baseline characteristics, efficacy variables, and safety variables. For parameters measured at baseline, the outcome variables of interest are the changes from baseline (Pre-Study Drug Period). Summary statistics will include the mean, N, standard deviation, median, minimum, and maximum values for continuous variables, and frequencies and percentages for categorical variables. Prior to summarizing results by study center, or performing analyses that include center as a factor in the analysis, small centers will be pooled. All efficacy variables will be summarized by treatment and by visit. Analyses will be performed to explore whether there are treatment-by-center interactions. If a treatment-by-center interaction is detected, the interaction will be explored in an ad-hoc manner. Categorical variables will be analyzed using Cochran-Mantel-Haenszel (CMH) test blocking on centers. Details of the model and the analyses will be specified in the Statistical Analysis Plan and all statistical analyses will be performed using SAS, Version 9.2 or higher.

7.2 Study Objectives

The study objectives are as defined in Section 2.0 of this protocol.

7.3 Patient Population(s) for Analysis

7.3.1 Efficacy

The intent to treat (ITT) population will contain all patients who were randomized and exposed to the study drug during the double-blind phase of the trial. The efficacy analysis will be based on the ITT population. A patient will be classified as a *treatment success* if they meet the following criteria:

1. Complete double-blind phase without having ≥2 consecutive intra-patient increases greater than or equal to 50% Delta (MAP)
   [e.g., MAP ≥ $(MAP_{B1} + MAP_{B2})/2$] and without receiving any treatment to control their blood pressure, except the study drug they were randomized to receive.

A patient will be classified as a *treatment failure* if they meet the following criteria:

1. Fail to complete the entire 30-minute double-blind phase without receiving additional treatment to control their blood pressure in addition to the study drug.
2. Fail to complete the entire 30-minute double-blind phase for any reason.
3. Experience an intra-patient increase greater than or equal to 50% Delta (MAP) for ≥2 consecutive MAP measurements, obtained at one-minute intervals, during the 30-minute double-blind phase.

7.3.2 Safety

All patients who receive any study medication (safety population) will be included in the safety analyses and summaries, independent of the patient actually reaching the double-blind phase of the study. All non-serious adverse events recorded within 24 hours of

either completion of the double-blind phase, or within 24 hours of premature discontinuation of the study, will be reported.

All serious adverse events recorded within 30 days of either completion of the double-blind phase, or premature discontinuation of the study, will be reported.

7.4 Background and Demographic Characteristics

All baseline information, including demographic factors, physical examination parameters, vital signs, growth parameters (if applicable), laboratory and blood gas information will be summarized by treatment group for all enrolled patients (Safety population). Additionally, nonrandomized patients versus randomized patients will be summarized and compared by age, gender, and race to determine if there are any differences among the 2 subsets. Analyses will be conducted to determine differences in the demographic and baseline characteristics of the treatment groups. For continuous variables (e.g., age, weight), the number of non-missing and missing values and the median, mean, standard deviation, minimum, and maximum will be displayed for each treatment group. For categorical variables (e.g., race, gender), the counts and proportions will be tabulated.

Baseline comparability will be evaluated based on the pooled data from all centers. To determine comparability of the treatment groups at baseline, continuous demographic and clinical variables will be analyzed using an analysis of variance test (with an appropriate transformation, if necessary). Baseline, demographic, and clinical variables that are ordinal will be analyzed using the Cochran Mantel Haenszel test; parameters that are dichotomous will be analyzed using a chi-square ($\chi 2$) test or Fisher's exact test, depending on the individual cell counts. If there are treatment group differences at the 0.10 level of significance in demographic or baseline clinical variables, these variables may be added as stratification variables or covariates to the efficacy analyses.

7.5 Study Medication

The duration of exposure to study medication will be summarized for all enrolled patients, and separately for all randomized patients.

7.6 Concomitant Therapy

Concomitant medications (medications present while on study medication) will be recorded on source documents throughout the study and at early termination; however, only those concomitant mediations that (1) affect blood pressure, or (2) are associated with SAE's will be recorded on CRFs. Medications listed on CRFs will be coded using the WHO drug dictionary. The number of randomized patients using prior or concomitant medications will be categorized by the WHO drug category and preferred term, and presented for each treatment group. In any given category [e.g., drug category] a patient will be counted only once.

7.7 Statistical Design and Models for Analysis

This is a biphasic (open-label dose-titration phase, followed by a randomized phase), randomized, double-blind placebo-controlled study. Patients who are enrolled into the initial phase of the study will have their dose of sodium nitroprusside titrated and must receive a minimum of 12-hours of treatment to be eligible for the randomized phase of the study. Patients who cannot be adequately titrated during the initial 12-hour period will not proceed to the randomization phase of the study. Patients who reach the randomization phase of the study will be assigned to receive placebo, or continue to receive sodium nitroprusside based on a stratified permuted block central randomization scheme.

Five age groups (A through E) will be enrolled in this trial:

Age Group A: Age Group A: Neonates from birth to less than 30 days of age

Age Group B: Infants and toddlers from 30 days to < 2 years

Age Group C: Preschool children from 2 years - < 6 years

Age Group D: School age children from 6 yrs - < Tanner stage III

Age Group E: Adolescents from Tanner stage III - < 17 years.

In order to efficiently account for the effect of SNP on the different age groups, neonates from birth to less than 30 days of age (Age Group A) and infants and toddlers from 30 days to < 2 years (Age Group B) will be pooled for analysis. Based on the planning estimates of the study, patients from these two pooled age groups should represent approximately 25% of the target enrollment (~60 patients).

Preschool children from 2 years - < 6 years (Age Group C) and school age children from 6 yrs - < Tanner stage III (Age Group D) will also be pooled for analysis; patients from these two pooled age groups should also represent approximately 25% of the target enrollment (~60 patients).

7.7.1 Primary Efficacy Analysis

The primary efficacy analysis will be conducted using the ITT Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients received sodium nitroprusside}} = \pi_{\text{ Patients received receive placebo}}$
- H_A: $\pi_{\text{Patients received sodium nitroprusside}} \neq \pi_{\text{ Patients received receive placebo}}$

where:

- $\pi_{\text{Patients received SNP}}$ = Proportion of *treatment successes* (sodium nitroprusside)
- $\pi_{\text{Patients received placebo}}$ = Proportion of *treatment successes* (placebo)

7.7.2 Primary Safety Analysis

The primary safety analysis will be conducted using the ITT Population to evaluate the following hypothesis:

- H_0: $\pi_{\text{Patients received sodium nitroprusside}} = \pi_{\text{Patients received placebo}}$
- H_A: $\pi_{\text{Patients received sodium nitroprusside}} \neq \pi_{\text{Patients received placebo}}$

where:

- $\pi_{\text{Patients received sodium nitroprusside}}$ = Proportion of patients who received SNP who experience a serious adverse event
- $\pi_{\text{Patients received placebo}}$ = Proportion of patients who received placebo who experience a serious adverse event

7.7.3 Sample Size Estimation

The overall sample size was calculated based on performing an un-stratified analysis of the proportion of patients classified as a *treatment success* between the 2 randomized treatment groups. With a balance randomization (1:1, SNP:Placebo), a difference in the proportion of *treatment successes* ranging from 34% to 40% would have 80% power to reject the null hypothesis in favor of the alternative (ref. Sample Size Table No. 1.0).

Sample Size Table No. 1.0

Two group χ^2 Test of Equal Proportions

Scenario	**1**	**2**	**3**	**4**
Test significance level, α	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2
Placebo, π_1	0.080	0.160	0.240	0.320
SNP, π_2	0.420	0.530	0.630	0.710
Odds ratio, $\psi = \pi_2 (1 - \pi_1) / [\pi_1 (1 - \pi_2)]$	8.328	5.920	5.392	5.203
Power (%)	80	80	81	80
n per group	30	30	30	30

7.7.4 Strategy for the Statistical Analysis

The primary method for analysis will be a comparison of the proportion of *treatment success* between patients randomized to receive placebo compared to patients randomized to remain on sodium nitroprusside (Intent-to-treat analysis). Additional analysis will be described in the Statistical Analysis Plan that will include a comparison of the event time distribution functions for the time until an increase in MAP_{B2} + 50% Delta (MAP) is initially observed and per-protocol analysis where comparisons will be made between treatment groups defined by treatment received. During the Open-Label Study Drug Administration (Dose-Titration) Phase, the sustainability of the blood pressure will be graphed over time to determine the effectiveness of SNP to maintain the target MAP.

7.7.5 Handling Missing Data in the Analyses

The following method of imputation will be used:

Last observation carried forward (LOCF): The goal of this imputation scheme is to create an observation for a completely missing observation at the end of the study for every patient in the safety population. If a patient evaluation for a post-baseline observation is missing, then the immediately preceding non-missing evaluation will be used.

Specific algorithms for imputing missing or partially missing dates will be discussed in the SAP. Imputed or derived data will be identified in the individual patient data listings. Imputed data will not be incorporated into the case report form datasets. Imputed data will be used in the preparation of the derived datasets.

7.7.6 Pooling of Small Sites for Analysis

Small sites (e.g., sites that have less than 4 patients per treatment arm) will be identified and the following method will be used for combining the data. Data from all small sites (< 4 patients) will be combined to form a single site in order to obviate non-estimable

situations (e.g., at least 2 intra-group observations are needed to estimate variance) in the evaluation of site and site interaction effects. Once combined, the pooled site will remain as such for all analyses for which a site interaction effect is determined. If the pooled smaller sites represent a single site that has more than twice as many patients as the largest single site, however less than 3 times as many patients, the small sites will be ranked by size and divided into 2 pooled groups using an alternating sequence (ABABAB). If the pooled smaller sites represent a site that has more than three times as many patients as the largest single site, however less than 4 times as many patients, the small sites will be ranked by size and divided into 3 pooled groups using an alternating sequence (ABCABCABC). This methodology will be applied, based on the initial pooling of the smaller sites.

7.7.7 Dropouts, Protocol Violations/Deviations, and Exclusions

Randomized patients who fail to complete the study will not be replaced. All protocol deviations and violations will be documented and categorized in the final study report.

The reasons for withdraw will be classified into 3 mutually-exclusive classes:

1. Withdraw due to tolerability of the study drug
2. Withdraw due to lack of treatment effect
3. Withdraw not due to tolerability or lack of treatment effect

The proportion of patients who withdraw prematurely will be compared between treatment groups to determine if there is a disproportionate rate of attrition. If the rates differ by a pre-specified amount, the reasons for withdraw will be examined to determine causation. The specific monitoring rules, boundaries, and actions will be described in detail in the Data Monitoring Committee Charter.

7.8 Safety Evaluation

The primary assessment of safety will be based on the frequency of treatment-emergent adverse events and on the frequency of clinically notable abnormal vital signs and laboratory values. The primary safety analysis will be based on a comparison of the proportion of patients randomized to receive sodium nitroprusside vs. placebo who experience a serious adverse event during the Blinded Study Drug Administration Phase.

7.8.1 Adverse Events and Medical Conditions

Adverse events will be recorded throughout the study and at early termination. Adverse events and medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Treatment-emergent adverse events will be defined as those events, which are newly occurring or worsening from baseline. In all cases only treatment emergent adverse events will be summarized. Treatment-emergent adverse events will be summarized by presenting, for each treatment group, the number and percentage of patients having any treatment-emergent AE, having an AE in each body system, and having each individual AE. (Note: In any given category [e.g., body system] a patient will only be counted once.) Similar displays will be provided for prior (conditions ending prior to the first exposure to sodium nitroprusside) and current (conditions present while on study medication) medical conditions. Adverse events will further be categorized by severity, relationship to study medication, and action taken. Other information collected will be listed, as appropriate. Any event starting more than 3 days after the final dose of study medication will be excluded from the above tables and only presented in a listing, unless the adverse event was serious or caused discontinuation from the study.

7.8.2 Clinical Laboratory Results

7.8.2.1 Overview

The primary presentation of the results by individual laboratory parameter will focus on the intra-patient changes from baseline (Pre-study Period). The presentation and analysis of laboratory data will be based on the observed data. All patients who have a baseline and at least one follow-up laboratory assessment will be included in the presentation of the clinical laboratory data. For each clinical laboratory test, there will be three sets of descriptive statistics that summarize the results at baseline, post-baseline assessment, and the change from baseline to post-baseline assessment. Descriptive statistics include N, mean, standard deviation, median, and the minimum and maximum values. Within treatment group changes will be analyzed using a paired-difference t-test. Between treatment group differences will be compared using a one-factor analysis of variance test.

Shifts from baseline to each pre-specified post-baseline endpoint will also be summarized based on the laboratory categorization (*abnormally and clinically significant*, *abnormal but not clinically significant*, or *normal*) using the worst reported post-baseline observation that occurs within the pre-specified interval. The proportion of patients will be compared using a 2-tailed Fisher's exact test, pooling *abnormally and clinically significant* with *abnormally but not clinically significant.*

In the case that more than one laboratory is used, laboratory values will be transformed for mean change summaries to the same units and normal range as were provided by the central laboratory used in the study, using the formula:

$$y = (x - Li)\frac{Uc - Lc}{Ui - Li} + Lc$$

where x = original value, Li and Ui = lower and upper limits of normal for individual laboratory, Lc and Uc = lower and upper limit for central laboratory

In cases where the lower limit of central laboratory is 0, values that are below the lower limit of normal for a laboratory value prior to transformation will be assigned a value of 0.

7.8.3 Vital Signs

7.8.3.1 Overview

Vital signs of particular interested (blood pressure, MAP, heart rate) will be assessed during each phase of the study.

7.8.3.2 Presentation of Results

Descriptive statistics (n, mean, SD, median, minimum and maximum values) will be used to summarize systolic and diastolic blood pressure, MAP, and heart rate and compared between the randomized treatment groups using a one-factor analysis of variance test.

7.8.4 Physical Examination

7.8.4.1 Overview

The presentation of physical examination data is based on the dichotomous classification (normal or abnormal) of each of the 9 regions or body systems (General Appearance, HEENT, Cardiovascular, Respiratory, Abdomen, Extremities, Neurological, Hair and Skin, and Genitourinary). In addition to these 9 specific body systems, any other region recorded by the investigator under "other" will also be summarized and reported.

7.8.4.2 Presentation of Results

Results will be presented by treatment assignment using counts and percentages. Shift tables will be prepared containing the count and percentage of patients who transitioned from normal at baseline to abnormal at the end of the study. The number and percentage of patients that did not change (normal at baseline and normal at the end of the study, abnormal at baseline and abnormal at the end of the study) are also presented to frame the 2*2 contingency table. Shifts from baseline to each pre-specified post-baseline endpoint will be summarized using the worst reported post-baseline observation that occurred within the pre-specified interval. The count of the disagreements (normal to abnormal and abnormal to normal) by treatment assignment (active and placebo) will be compared for each parameter using McNemar's test.

8.0 Ethics

8.1 Independent Ethics Committee or Institutional Review Board

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator Brochure (IB), the informed parental permission and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an Independent Ethics Committee (IEC) or Institutional Review Board (IRB). IEC/IRB approval of the protocol, informed parental permission and subject information and/or advertising as relevant will be obtained prior to the authorization of drug shipment to a study site. Any amendments to the protocol will require IEC/IRB approval prior to implementation of any changes made to the study design.

8.2 Ethical Conduct of Study

This study will be conducted in accordance with the protocol, GCP, FDA regulations governing clinical study conduct, ethical principles that have their origin in the Declaration of Helsinki, 2000 revision (see Appendix E) and all applicable local regulations. The investigator must assure that the study is conducted in accordance with the provisions as stated in the FDA regulations and complies with prevailing local laws and customs. Responsibilities of the Investigator are specified in Appendix D.

8.3 Subject Information and Parental Permission

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before protocol-specified procedures are carried out. Informed consent will be obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements.

Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation.

Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study.

The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed

permission form, which will included all elements of informed consent as described in 21 CFR 50.25. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form and a copy of the signed assent (if applicable).

The parental/guardian permission form generated by the investigator with the assistance of BPCA DCC must be approved (along with the protocol) by the IRB and be acceptable to the Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative.

The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the ICH Guidelines and will comply with local regulations. This form may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated.

Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by BPCA DCC, Regulatory Authorities, and NICHD or its designees.

9.0 Source Documents and CRF Completion

9.1 Source Documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

9.2 Data Collection

Data for individual subjects will be recorded in the electronic data capture (EDC) system provided by the BPCA DCC. Electronic case report forms must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the eCRF by the investigator. The principal investigators must review and approve each eCRF.

Data must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified by name; appropriate coded identification must be used. The investigator must keep a separate log of subject names and addresses. If requested during an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

Because of the potential for errors and inaccuracies in entering data into eCRFs, laboratory and other test results must be kept on file with the subject's study dossier. Source documentation must be available at all times for inspection by the CRA for the site and the FDA.

10.0 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA DCC, the investigators and their study coordinators and the CRAs for the study. This meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures by a CRA at a study initiation visit and will be given a CRF completion workbook for reference.

The CRAs will monitor each site throughout the study. At each visit, data recorded in the EDC system will be compared to source documentation to ensure accuracy. Quality assurance checks will be performed to ensure that the investigator is complying with the protocol and all applicable regulations.

In addition to the quality assurance checks incorporated into the EDC system, additional logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary correction will be made to the database and documented via audit trail.

11.0 Use of Information and Publication

11.1 Use of Information

This trial is sponsored by the NICHD. The NICHD endorses the sharing of final research data to expedite the translation of research results into new scientific knowledge in order to improve human health.

This contract is part of a collaborative program involving multiple sites. A data sharing dissemination plan will be developed jointly with the BPCA DCC, the NICHD, and the collaborating institutions following announcement of the award.

11.2 Publication

The BPCA DCC and steering committee for this study shall have the right to review and approve any paper for publication, including oral presentation and abstracts, which utilize data generated from this study. At least 90 days before any such paper or abstract is presented or submitted for publication, a complete copy shall be given to the BPCA DCC for review by the steering committee. Any individual investigator agrees to defer publication of any such paper or abstract until the BPCA DCC and Steering Committee have reviewed and approved it.

12.0 Completion of Study

The investigator will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon in writing by the investigator, the NICHD, the BPCA DCC and the PODS. The investigator will provide a summary of the study's outcome to the IRB/IEC following the conclusion of the study.

The PODS Center, BPCA Data Coordinating Center, NICHD and/or the FDA may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. The investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the NICHD and BPCA DCC within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

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13.0 Investigator Agreement

The investigator will sign and date a Study Protocol Agreement Form, provided earlier in this protocol. The Study Protocol Agreement will then be countersigned by the investigator's PODS Principal Investigator.

Appendices

Appendix A: Tanner Stages of Sexual Maturity

	Pubic Hair		Breasts	Penis	Testes
SMR Stage[1,2]	Boys	Girls	Girls	Boys	Boys
1	None	Preadolescent	Preadolescent	Preadolescent	Preadolescent
2	Scanty, long, slightly Pigmented	Sparse, lightly pigmented, straight, medial border of labia	Breast and papilla elevated as small mound; areolar diameter increased	Slight Enlargement	Enlarged scrotum, pink texture altered
3	Darker, starts to curl, small Amount	Darker, beginning to curl, increased amount	Breast and areola enlarged, no contour separation ,	Longer	Larger
4	Resembles adult type, but less in quantity; coarse, curly	Coarse, curly, abundant but amount less than in adult	Areola and papilla form secondary mound	Larger; glans and breadth increase in size	Larger, scrotum dark
5	Adult distribution, spread to medial surfaces of thighs	Adult feminine triangle, spread to medial surface of thighs	Mature; nipple projects, areola part of general breast contour	Adult size	Adult size

1. Adapted from Tanner, JM: Growth at Adolescence, 2 ed. Oxford, Blackwell Scientific Publications, 1962.

2. MR = Sexual Maturity

Appendix B: Parental Permission Form with HIPAA

Protocol Title: A Phase II, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study To Determine The Pharmacodynamics Of Sodium Nitroprusside During Prolonged Infusion In Pediatric Subjects

Protocol Director: [PROGRAM PI]
IRB Approval Date: _____ IRB Expiration Date: _____

Is your child participating in any other research studies? _____Yes _____No

Introduction

You are being asked to agree to let your child be a part of a drug research study. He or she is scheduled for surgery, or needs to stay in an intensive care unit (ICU). During this operation or stay in the ICU, it will be necessary for the doctor to lower your child's blood pressure for a long period of time, up to 24 hours. We will tell you more about how he/she will do this and the drug that will be used later in this paper. Before you decide whether to let your child be involved in this study, *[Site PI]* wants you to read the following information. He wants you to ask him any questions you may think of. He wants to be sure that you understand what your child's participation will mean. You need to fully understand the type of treatment and its risks. Your doctor is responsible for providing you with the necessary information so that you understand the possible risks. Your child's participation in this study is entirely your choice.

Your child cannot participate in another research study at the same time as this research study [optional sentence]. Your child cannot be taking another experimental drug while enrolled in this research study, or within the previous 30 days. Your child will be a part of this study for approximately 30 days. There may be risks that we cannot predict. We will tell you about any new information that may affect your child's condition or affect your willingness to stay in this study.

Nature and Purpose of the Research Study

In certain kinds of surgeries, doctors often need to control the blood pressure of the patient. The doctor may also need to lower the patient's blood pressure below normal. This can reduce blood loss and avoid blood transfusions during stressful periods. Sodium nitroprusside is a drug that is approved by the Food and Drug Administration (FDA) for use in adults. Scientific studies show that this drug works well when doctors need to control blood pressure during surgeries in adult patients. Doctors also often use sodium nitroprusside in children. However, not many scientific studies tell us how best to use sodium nitroprusside in children.

In this study, we hope to learn the best dose of sodium nitroprusside (the study drug) to use in children who need it in the ICU for more than 12 hours. We also want to learn the same thing for children who need certain kinds of surgeries. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children. This research study is looking for 50-100 children at several hospitals in the United States.

Your child's participation in this study is entirely voluntary.

Your decision whether or not to allow your child to participate will not prejudice your child or his/her medical care. If you decide to allow your child to participate, you are free to withdraw your permission, and to discontinue participation at any time without prejudice to your child or effect on your child's medical care. If you decide to terminate your child's participation in this study, you should notify *(name)* at *(telephone number)*.

Procedures

If you agree to your child's participation in this research study, he or she will undergo the following types of procedures:

1. [Site PI] and his research staff will talk to you (and your child) about his or her health. They will ask you about your child's medical history. They will ask you about any medications your child is currently taking. They will give your child three physical examinations, including measurement of blood pressure, pulse, and weight. The first one will be within 24 hours before study drug is given. The other two physical exams will be after your child receives study drug. A small amount of blood, less than one teaspoon, will be drawn at the first two visits. We need this blood for our study, but the same blood work may also be needed as part of your child's regular medical care. If this is the case, we will use these results instead of having to take another blood sample.

2. Your child will receive study treatment in stages:
   a. First, after your child is stable, sodium nitroprusside will be given into a vein. This will be done through tubing your child will already have in place. This drug may be the treatment of choice to control your child's blood pressure even if your child is not enrolled in this study. This first stage will last up to 24 hours. He or she will receive sodium nitroprusside at a pre-set initial rate. That rate will be changed until your child's blood pressure is in the range that his or her doctor has decided is the best.

   b. The second stage of study treatment will begin between 12 and 24 hours after the medication was first started. This stage will last up to 30 minutes. During this stage your child will receive one of two treatments. This phase of the research study is blinded. That means that the study doctor will not know which treatment group your child will be placed into. The choice of which treatment group your child will be in (placebo or sodium nitroprusside) will be random. The choice will be made in a way that is like flipping a coin. He or she might receive sodium nitroprusside at the rate that was being used before to keep his or her blood pressure at a stable level. Or, he or she might receive a placebo; this is a solution like salt water that is known to have no effect on

your child's health. Your child's vital signs (blood pressure and heart rate) will be watched very closely. If your child's blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end immediately. Then your child will again be given sodium nitroprusside, or similar drug, to keep his or her blood pressure at a safe level.

c. If your child stills needs sodium nitroprusside treatment to control his/her blood pressure, the treatment may continue after the research study ends.

During the research study, vital signs (blood pressure and heart rate) will be checked often at specific time points.

There will be a follow up evaluation. About 30 days after the research study is over, we will call you at home to ask questions about your child's health in the last month, since he or she participated in the research study. If your child is readmitted to the hospital before our call, please let us know.

Blood samples will be taken between 4 to 7 times (1½ to 2½ mLs per each sample, or ½ teaspoon per sample) during the research study. This is to see how much of the metabolites (breakdown products) of the study drug is circulating in the blood at varying time points. If indicated, additional blood samples may be taken to help determine the amount of metabolites in your child's blood. We are very careful to minimize the amount of blood drawn from your child, and anticipate that 3½ teaspoons is the most we will draw for these scheduled tests.

Whenever possible, blood will be taken from tubing already in place. The nurse will often take the study blood samples at the same time that routine blood samples are taken to check on your child's health. This is done to avoid any extra needle-sticks (drawing blood from a vein in the arm with a needle). It is very unlikely that there would not be a catheter (IV line) in place; however, if that were to happen, blood drawing would require a needle stick that might cause minor bruising. Blood drawing is done to assess safety and to measure the activity of the study drug.

The total amount of blood drawn during the entire research study for children less than 2 years of age is approximately 1 to 2 ½ teaspoons. For children more than 2 years of age, the total amount of blood is about 2 to 3 ½ teaspoons.

The information gained during this research study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help patients in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

Young Women of Child-Bearing Potential

If your child is a young woman who is able to become pregnant, it is expected that she will use an effective method of birth control to prevent exposing a fetus to a potentially dangerous agent with unknown risk. If your child is pregnant or currently breast feeding, she may not participate in this study.

To confirm to the extent medically possible that your child is not pregnant, you agree that she will have a pregnancy test done before beginning this research study. This test will be carried out right before your child undergoes surgery.

Participant Responsibilities

You should:

- Follow the instructions of the Protocol Director and study staff.
- Tell the Protocol Director or research study staff about any side effects that your child may have.
- Tell the Protocol Director or research study staff if you believe your child might be pregnant.
- Ask questions as you think of them.
- Tell the Protocol Director or research staff if you change your mind about staying in the study.

While participating in this research study, your child should not take part in any other research project without approval from all of the Protocol Directors. This is to protect your child from possible injury arising from such things as extra blood drawing, the possible interaction(s) of research drugs, or other similar hazards.

Withdrawal from Study

If you first agree to participate and then you change your mind, you are **free to withdraw** your permission and discontinue your child's participation at any time. Your decision will not affect your child's ability to receive medical care for his or her disease and your child will not lose any benefits to which he or she would otherwise be entitled.

If you decide to terminate your child's participation in this study, you should notify (*name)* at (*phone number)*.

The Protocol Director may also withdraw your child from the study and the study medication may be stopped without your permission for one or more of the following reasons:

- Failure to follow the instructions of the Protocol Director and/or study staff.
- The Protocol Director decides that continuing your child's participation could be harmful to him or her.
- Pregnancy (if applicable).
- Your child needs treatment not allowed in the study.
- The study is cancelled.
- Other administrative reasons.
- Unanticipated circumstances.

If your child is withdrawn from the study after receiving any of the research drug, and if you agree, we will perform the following testing to ensure the safety of your child: physical examination, monitor vital signs, blood tests, as described earlier, as well as the follow-up phone call approximately 30 days later. You will be free to have only some of these done if you prefer.

Possible Risks, Discomforts, and Inconveniences

There are risks, discomforts, and inconveniences associated with any research study. These deserve careful thought. This is true whether it is a normal kind of treatment or an experimental type. You should talk with the Protocol Director if you have any questions.

In spite of all safety measures, your child might develop medical problems while taking part in this research study. These risks include elevated blood pressure. Treatment of these potential medical problems will not be limited or delayed by your child's participation in the study.

Sodium nitroprusside is a drug that lowers blood pressure. Because of this, there is a chance that your child could develop hypotension (low blood pressure). The doctor will monitor your child very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable blood pressure.

Another side effect that might happen is that your child's heart rate may increase in response to sodium nitroprusside. Again, the doctor and research nurse will monitor your child's heart rate very closely. He or she will change the amount of the sodium nitroprusside as needed to maintain a safe and stable heart rate.

Minor side effects due to sodium nitroprusside may also occur but will not cause the study to be ended. Since sodium nitroprusside makes blood vessels bigger, the following side-effects may occur: nausea, headache, restlessness, abdominal pain, redness or flushing of the skin, nervousness, and perspiring.

Sodium nitroprusside contains cyanide. Cyanide is present in all people and is important for normal body function. Extra cyanide can be present whenever sodium nitroprusside is used. Excess cyanide can affect the amount of oxygen in the blood. The doctors will carefully watch for any signs or symptoms of excess cyanide and treat your child if needed. Because safety is one of the most important aspects of this study, we will be testing your child's blood for cyanide. The results of this testing will not be immediately known. However, there are other means to detect ill effects

from excess cyanide. If the doctors suspect it is in your child's best interest, additional unscheduled blood tests for cyanide and its breakdown product, thiocyanite, will be performed. Cyanide has been detected in patients who have participated in this study. However, no patient has shown any ill effects from the cyanide.

Potential Benefits

The information gained during this study may help your child's doctors learn more about control of blood pressure in children. This knowledge may help children in the future. If the treatment that your child is given is later shown to be effective, he or she may benefit directly from it.

WE CANNOT AND DO NOT GUARANTEE OR PROMISE THAT YOUR CHILD WILL RECEIVE ANY BENEFITS FROM THIS STUDY.

Alternatives

If you choose not to enroll your child in this study, your child may receive sodium nitroprusside anyway. This could happen if your child's doctor feels it is the best medicine to use to control blood pressure. Or, your child's doctor could choose to use other types of blood pressure medications, such as esmolol or fenoldopam, instead. Whether or not your child is enrolled, the medical team will, of course, do everything possible to ensure the safety and comfort of your child.

If you do not wish your child to take part in this study, other treatments can be used for your child's condition. If you withdraw your child's participation, the study doctor will recommend an alternative treatment for blood pressure control for your child, such as esmolol or fenoldopam. If this study is discontinued, your child will receive one of these alternative treatments.

Participant's Rights

You should not feel obligated to agree that your child participate in this study. Your questions should be answered clearly and to your satisfaction.

If you decide not to participate, tell the Protocol Director. Your child will still receive care for his/her disease and will not lose any benefits to which he/she would otherwise be entitled.

You will be told of any important new information that is learned during the course of this research study, which might affect your child's condition or your willingness to continue participation in this study.

Confidentiality

Your child's identity will be kept as confidential as possible as required by law. Except as required by law, your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier. Your child's research records may be disclosed outside of Stanford, but in this case, your child will be identified only by a unique code number. Information about the code will be kept in a secure location and access limited to research study personnel.

The results of this research study may be presented at scientific or medical meetings or published in scientific journals. However, your child's identity will not be disclosed**.**
Patient information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your child's identity if this study falls within its jurisdiction.

- The purpose of this research study is to obtain data or information on the safety and effectiveness of sodium nitroprusside in children; the results will be provided to the sponsor, the Food and Drug Administration and other federal and regulatory agencies as required.

Authorization to Use Your Health Information for Research Purposes

Because information about you and your health is personal and private, it generally cannot be used in this research study without your written authorization. If you sign this form, it will provide that authorization. The form is intended to inform you about how your health information will be used or disclosed in the study. Your information will only be used in accordance with this authorization form and the informed parental permission form and as required or allowed by law. Please read it carefully before signing it.

What is the purpose of this research study and how will my health information be utilized in the study?

In this study, we hope to learn the best dose of sodium nitroprusside to use in children of different ages who need it in the ICU for more than 12 hours. We will study how fast this drug starts to work and how fast it stops working to control blood pressure. We would also like to find out how different amounts of sodium nitroprusside in blood affect blood pressure and heart rate in children. When the study is over, we will be able to use the information to work out accurate instructions for doses to be used in children as determined by the NIH and FDA.

Do I have to sign this authorization form?

You do not have to sign this authorization form. But if you do not, your child will not be able to participate in this research study, including receiving any research-related treatment. Signing the form is not a condition for receiving any medical care outside the study.

If I sign, can I revoke it or withdraw from the research later?

If you decide to allow your child to participate, you are free to withdraw your authorization regarding the use and disclosure of your child's health information (and to discontinue any other participation in the study) at any time. After any revocation, your child's health information will no

longer be used or disclosed in the study, except to the extent that the law allows us to continue using your child's information (e.g., necessary to maintain integrity of research). If you wish to revoke your authorization for the research use or disclosure of your health information in this study, you must contact: *(researcher's name and contact information, including telephone number).*

What Personal Information Will Be Used or Disclosed?

Your health information related to this study, may be used or disclosed in connection with this research study, including, but not limited to, vital sign measurements, laboratory results of blood collections, physical exams, related medical records, and other data.

Who May Use or Disclose the Information?

The following parties are authorized to use and/or disclose your child's health information in connection with this research study:

- The Protocol Director *(Insert Name of PD)*
- The *(Insert name of Institution)* Administrative Panel on Human Subjects in Medical Research and any other unit of Stanford University as necessary
- Research Staff

(List every other class of persons or organization affiliated with the hospital/university who might need to use and/or disclose the participant's information in connection with this study.)

Who May Receive or Use the Information?

The parties listed in the preceding paragraph may disclose your health information to the following persons and organizations for their use in connection with this research study:

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- The National Institutes of Health
- The Food and Drug Administration

- Collaborating Institutions
- The Data Coordinating Center, The EMMES Corporation or authorized agent

Your child's information may be re-disclosed by the recipients described above, if they are not required by law to protect the privacy of the information.

When will my authorization expire?

Your authorization for the use and/or disclosure of your child's health information will expire December 31, 2055.

Will access to my child's medical record be limited during the study?

To maintain the integrity of this research study, you may not have access to any health information developed as part of this study until it is completed. At that point, you would have access to such health information if it was used to make a medical or billing decision about your child (e.g., if included in your child's official medical record).

_____

Signature of Participant

_____

Signature of Legally Authorized Representative

_____

Date

_____

Description of Representative's Authority to Act for Subject

Financial Considerations

Payment

You and your child will not be paid to participate in this research study.

Costs

The sponsor will pay for the cost of sodium nitroprusside and for the extra blood tests that will be used to monitor the amount of drug in your child's blood and for safety tests. You or your insurance company will be responsible for the medical procedures, surgery, anesthesia, and other normal costs associated with standard medical care for treatment of your child's condition.

Sponsor

The National Institute of Child Health and Development of the National Institutes of Health (NIH) is providing financial support and/or material for this study.

Compensation For Research-Related Injury

All forms of medical diagnosis and treatment – whether routine or experimental – involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the Protocol Director and the research study staff will assist you in obtaining appropriate medical treatment. In the event that you have an injury or illness that is directly caused by your participation in this study, reimbursement for all related costs of care first will be sought from your insurer, managed care plan, or other benefits program. **You will be responsible for any associated co-payments or deductibles as required by your insurance.**

If costs of care related to such an injury are not covered by your insurer, managed care plan or other benefits program, you may be responsible for these costs. If you are unable to pay for such costs, the Protocol Director will assist you in applying for supplemental benefits and explain how to apply for patient financial assistance from the hospital.

Additionally, *{Institution name}* is not responsible for research and medical care by other institutions or personnel participating in this study. You do not waive any liability rights for personal injury by signing this form.

Contact Information

- Questions, Concerns, or Complaints: If you have any questions, concerns or complaints about this **research study**, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Protocol Director, *{name}*. You may contact *{him/her}* now or later at *{phone number}*.
- Emergency Contact: If you feel your child has been **hurt by being a part of this study**, or need immediate assistance please contact *{Protocol Director's name and phone number}*.
- Alternate Contact: If you cannot reach the Protocol Director, please page the research team at *{phone number}*.
- Independent of the Research Team Contact: If you are not satisfied with the manner in which this study is being conducted, or if you have any concerns, complaints, or general questions about the research or your rights as a research study subject, please contact the *{Institution's name}* Institutional Review Board (IRB) to speak to an informed individual who is independent of the research team at *{phone number}*. Or write the *{Institution's name}* IRB, *{IRB full address}*. In addition, please call the *{Institution's name}* IRB at *{phone number}* if you wish to speak to someone other than the research team or if you cannot reach the research team.

Experimental Subject's Bill Of Rights *{Text can be revised to meet IRB requirements}*

As a human subject your child has the following rights. These rights include but are not limited to the subject's right to:

1. be informed of the nature and purpose of the experiment;
2. be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;
3. be given a description of any attendant discomforts and risks reasonably to be expected;

4. be given an explanation of any benefits to the subject reasonably to be expected, if applicable;
5. be given a disclosure of any appropriate alternatives, drugs or devices that might be advantageous to the subject, their relative risks and benefits;
6. be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise;
7. be given an opportunity to ask questions concerning the experiment or the procedures involved;
8. be instructed that parental permission to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;
9. be given a copy of the signed and dated parental permission form;
10. and be given the opportunity to decide to give parental permission or not to give parental permission to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTAND THE ABOVE INFORMATION, THAT YOU HAVE DISCUSSED THIS STUDY WITH THE PERSON OBTAINING PARENTAL PERMISSION, THAT YOU HAVE DECIDED TO PARTICIPATE BASED ON THE INFORMATION PROVIDED, AND THAT A COPY OF THIS FORM HAS BEEN GIVEN TO YOU.

_____

Signature of Parent or Legal Guardian

_____

Signature of Second Parent or Legal Guardian

_____

Date

_____

Name of Patient

Person Obtaining Parental Permission

I attest that the requirements for informed parental permission for the medical research project described in this form have been satisfied - that the Parent or Legal Guardian has been provided with the Experimental Subject's Bill of Rights, if appropriate, that I have discussed the research project with the Parent or Legal Guardian and explained to him or her in nontechnical terms all of the information contained in this parental permission form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the Parent or Legal Guardian to ask questions and that all questions asked were answered.

_____ _____

Signature of Person Obtaining Parental Permission Date

Appendix C: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles
for
Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. Introduction

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. Basic Principles for All Medical Research

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case, the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. Additional Principles for Medical Research Combined With Medical Care

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix D: Responsibilities of the Investigator

Investigator Responsibility/Performance

Prior to starting enrollment at a site, all investigators must read and understand the Investigational Plan and must sign and complete the Investigator Agreement Form. This documents that they accept all conditions of the Investigational Plan and will conduct the study accordingly. The investigator must provide a current copy of his or her curriculum vitae that is not more than 2 years old.

Informed Parental Permission and IRB Approval

The investigator must have written approval from the IRB prior to enrolling patients in the study. A copy of the written approval that includes the following must be provided to the DCRI:

- A statement of IRB approval for the proposed study and informed parental permission form at the institution
- The date the study was approved and the duration of approval
- A listing of any conditions attached to the approval
- Identification of the approved primary investigator
- The signature of the IRB chairperson

Any amendments to the protocol, as well as associated parental permission form changes, will be submitted to the IRB, and written approval must be obtained prior to implementation. Serious adverse event reports will be submitted as requested by the BPCA.

The study will be explained to the patients in lay language. Patients will sign and receive a copy of the IRB-approved informed parental permission form prior to study participation. Patients will be assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Source Documentation

Regulations require that investigators maintain information in the study patient's medical records that corroborate data collected on the CRFs. In order to comply with these regulatory requirements, at the minimum, the following information should be maintained:

1. Medical history/physical condition of the study patient before involvement in the study sufficient to verify protocol entry criteria (if not already present)
2. Dated and signed notes on the day of entry into the study including clinical site, patient number assigned and a statement that parental permission was obtained
3. Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams)
4. Notations on abnormal laboratory results
5. Serious adverse events reported through 30 days from the end of study drug administration
6. Notes regarding concomitant medications taken during the study (including start and stop dates)
7. Study patient's condition upon completion of or withdrawal from the study

Data Transmittal

Required data will be recorded in the electronic data capture (EDC) system as soon as possible after the patient is discharged, day 10, or death, whichever comes first. Data must be entered into the EDC system according to the outlined time windows. The 30-day follow-up eCRF needs to be entered within 10 days of the follow-up visit.

Non-Protocol Research

BPCA has a legal responsibility to report fully to regulatory authorities all the results of clinical studies. No investigative procedures other than those in this protocol shall be undertaken on the enrolled patients without the agreement of the IRB and BPCA.

Publication Policies

At the conclusion of the study, a multicenter abstract reporting the primary results may be prepared and presented in an appropriate international forum. A multicenter, peer-reviewed manuscript will also be prepared for publication in a reputable scientific journal.

Appendix E: Treatment of Suspected Nitroprusside Toxicity

Signs of Toxicity:

1. If the base deficit exceeds -8 meq/L in the absence of an explainable cause (blood loss, low cardiac output), the subject will be also be discontinued from study.
2. If the lactate level rises by more than 4 mmol/L in an 8 hour period in the absence of an explainable cause (blood loss leading to anemia or low cardiac output).
3. If the arteriovenous oxygen saturation narrows such that the difference is less than 10 volumes percent between arterial and mixed venous blood.

Suspected Cyanide Toxicity Should Be Treated As Follows:

1. Administer 100 percent oxygen to maintain an elevated PaO_2. Oxygen may reverse the cyanide-cytochrome oxidase complex and facilitate the conversion to thiocyanate following thiosulfate administration.
2. Obtain blood for arterial and venous blood gases with co-oximetry, serum lactate, and cyanide and thiocyanate levels.
3. SODIUM NITRITE - Should be drawn up from the ampule (300 mg/10mL) and injected. Use the following DOSAGE CHART FOR CHILDREN (sodium nitrite should not exceed that listed below; fatal methemoglobinemia may result)

Subject Hemoglobin g/dL	Initial dose Sodium NITRITE (3%) mL/kg IV	Initial dose Sodium Thiosulfate mL/kg IV
8 g/dL	0.22 mL/kg	1.10 mL/kg
(6.6 mg)/kg		
10 g/dL	0.27 mL/kg	1.35 mL/kg
(8.7 mg)/kg		
12 g/dL	0.33 mL/kg	1.65 mL/kg
(10 mg)/kg		
14 g/dL	0.39 mL/kg	1.95 mL/kg
X	(11.6 mg)/kg	

Sodium nitrite should be followed by sodium thiosulfate in the doses described in the above table. One half of the above doses may be repeated at 30 minute intervals.

[Micromedex Poisondex www.micromedex.duhs.duke.edu , see also Berlin, 1970]

Appendix F: Assay of Nitroprusside Metabolites and Handling of Blood Samples for Assay of Nitroprusside Metabolites

A classical UV bioanalytical method is utilized for detection and quantitation of cyanide in whole blood. Cyanide concentrations are determined by measuring the absorbance of the chromophore formed by the interaction of the cyanide ion with 4-nitrobenzaldehyde and o-dinitrobenzene in 2-methoxyethanol (Rieders, 1971 and Guilbault, 1966). In summary, to a 1.0 ml aliquot of whole blood, 4-Nitrobenzaldehyde solution and o-Dinitrobenzene solution is added then made basic with sodium hydroxide. Following a specific incubation time a UV scan spectrum is obtained from 520 to 580 nm with a maximum reading at 555 nm. The exact details of the method are proprietary to NMS Labs. The method is sensitive to a LLOQ of 0.05 μg/mL which correlates to normal circulating levels. A toxic threshold is normally assessed as approximately 0.5 μg/mL and acute toxicity is observed at greater than 1 μg/mL. There are no known interferences with this assay method.

An ion chromatography method is used for detection and quantitation of thiocyanate in serum that is specific, accurate, precise and rugged. In summary, a 0.10 mL aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography (Vogel, 1981 and Vesey, 1976). The same procedure is employed to detect thiocyanate in urine. The normal range for non-smokers is 1-4 μg/mL in serum/plasma. For smokers, it is 3 – 12 μg/mL and the therapeutic range for sodium nitroprusside is generally between 6 and 29 μg/mL (Schulz, 1984).

Thiosulfate is detected in serum/plasma or urine also via a validated ion chromatography method with the analytes separated and detected via conductivity detection. For this method, a 0.5 ml aliquot of specimen is diluted with deionized water and filtered through an ultrafiltration membrane to remove particulate, followed by analysis by ion chromatography.

Sample Handling Procedures

At each specified blood collection for cyanide and thiocyanate, 2 mL of arterial blood is to be collected in a 2 mL gray top. Samples should be mixed, then one half the whole blood in the gray top (1 mL) is removed and stored in a polypropylene screw capped container and stored on ice until it can be refrigerated. The remaining 1 mL of whole blood is centrifuged (within 20 minutes of collection at approximately 1200 g for 10 - 12 minutes at ambient or colder temperatures) to obtain 0.5 mL of plasma for thiocyanate analysis. The red bloods cells from this sample may be discarded, or separately tested for cyanide. All samples should be stored in a refrigerator before sending to the Central Lab(s), which should be done as soon as possible. Samples should be shipped COLD, using "frozen cold packs," for overnight delivery. Whole blood samples should NEVER be frozen.

For neonates or to spare the total amount of blood drawn for the analysis, the minimum blood draw is 1 mL of arterial blood. This translates to 0.5 mL of whole blood for cyanide analysis and 0.5 mLs to be centrifuged to obtain approximately 0.25 mLs of plasma for thiocyanate analysis.

The details of handling & shipping samples to the Central Lab(s) will be included in the MOP. This is a summary and not the complete instructions for sample handling.

Appendix G: Sedation Suggested Regimen

Intensive Care Unit:

For those patients who receive study drug in the intensive care unit for a surgical or medical procedure, the following guidelines may be utilized.

Sedation may be administered intravenously and initiated with a benzodiazepine and opiate agonist as follows:

Midazolam bolus 0.1-0.2 mg/kg followed by a continuous midazolam infusion of 0.06-0.3 mg/kg/hr or intermittent bolus of 0.1 mg/kg every 1-2 hours

Or

Lorazepam bolus 0.05-0.1 mg/kg followed by a continuous infusion of 0.025-0.05 mg/kg/hr or intermittent bolus of 0.05-0.10 mg/kg every 4-6 hours

And/or

Fentanyl bolus 1- 5 micrograms/kg (intubated, mechanically ventilated patients) followed by an infusion of fentanyl of 0.5 – 5 micrograms/kg/hour

Or

Morphine bolus 50-100 microgram/kg followed by a continuous infusion of 20-80 micrograms/kg/hour.

Sedation and analgesic medication may be titrated to patient response. Higher doses may be used in patients who exhibit benzodiazepine and/or narcotic habituation due to long term (> 4-7 day) usage. Where applicable, sedation assessment may be by clinical judgment of the responsible physician, and/or a quantitative scoring system such as the COMFORT score. Patients receiving neuromuscular blocking drugs such as vecuronium or rocuronium as part of their ICU management are eligible for study.

Appendix H: Measurement of Blood Pressure in Children

Excerpt from: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents

Correct measurement of BP in children requires use of a cuff that is appropriate to the size of the child's upper right arm. The equipment necessary to measure BP in children, ages 3 through adolescence, includes child cuffs of different sizes and must also include a standard adult cuff, a large adult cuff, and a thigh cuff. The latter 2 cuffs may be needed for use in adolescents.

By convention, an appropriate cuff size is a cuff with an inflatable bladder width that is at least 40% of the arm circumference at a point midway between the olecranon and the acromion. For such a cuff to be optimal for an arm, the cuff bladder length should cover 80% to 100% of the circumference of the arm. Such a requirement demands that the bladder width-to-length ratio be at least 1:2. Not all commercially available cuffs are manufactured with this ratio. Additionally, cuffs labeled for certain age populations (eg, infant or child cuffs) are constructed with widely disparate dimensions. Accordingly, the working group recommends that standard cuff dimensions for children be adopted (see Table 2).

TABLE 2. Recommended Dimensions for BP Cuff Bladders

Age Range	Width, cm	Length, cm	Maximum Arm Circumference, cm*
Newborn	4	8	10
Infant	6	12	15
Child	9	18	22
Small adult	10	24	26
Adult	13	30	34
Large adult	16	38	44
Thigh	20	42	52

* Calculated so that the largest arm would still allow the bladder to encircle arm by at least 80%.

BP measurements are overestimated to a greater degree with a cuff that is too small than they are underestimated by a cuff that is too large. If a cuff is too small, the next largest cuff should be used, even if it appears large. If the appropriate cuffs are used, the cuff-size effect is obviated

SBP is determined by the onset of the "tapping" Korotkoff sounds (K1). Population data in children and risk-associated epidemiologic data in adults have established the fifth Korotkoff sound (K5), or the disappearance of Korotkoff sounds, as the definition of DBP. In some children, Korotkoff sounds can be heard to 0 mm Hg. Under these circumstances, the BP measurement should be repeated with less pressure on the head of the stethoscope. Only if the very low K5 persists should K4 (muffling of the sounds) be recorded as the DBP.

Appendix I: Research Assent Form Template

Protocol Title: A PHASE II, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY TO DETERMINE THE PHARMACODYNAMICS OF SODIUM NITROPRUSSIDE DURING PROLONGED INFUSION IN PEDIATRIC SUBJECTS

Protocol Director:

IRB Approval Date: _____IRB Expiration Date: _____

Are you taking part in any other research studies right now? ____ yes ____ no

Why Are We Doing this Research Study?

We are doing this study to learn more about a medicine called sodium nitroprusside. This study will help us find out more about how good this drug is in keeping blood pressure and heart rate under control in children who are in the intensive care unit (ICU). Altogether, there will be 60 kids under the age of 17 in this study.

What Will Happen During the Study?

You will be seen by a doctor, who will give you a physical exam and ask you and your parents some questions about your health history and medicines you take. At certain times during the study, blood will be drawn to check that you are okay and safe, and to see how the study drug is working in your body. Blood will be drawn either from a tube that is already connected to you to get blood, or from a needle. The amount of blood to be taken during the study is about 2½ teaspoons to 3½ teaspoons.

In the first part of the study, you will receive a study drug called sodium nitroprusside through a tube placed into a vein in your arm. Your doctor will make changes in the amount of drug you get to

keep you safe and your vital signs (like your heart rate and blood pressure) stable. Your doctor will change how much of the drug you get so you have the blood pressure the doctor thinks is right for you. This part of the study will last for at least 12 hours up to 24 hours during the time that your doctor needs to control your blood pressure. During the time you get study drug, your blood pressure and heart rate will be measured very often.

The second part of the study will last up to 30 minutes. During this part, you will get one of two treatments. This part of the research study is blinded. That means that the study doctor will not know which treatment group you are in. The choice of which treatment you receive will be made in a way that is like flipping a coin. You might receive the study drug at the same rate that was being used before to keep your blood pressure stable. Or, you might receive a placebo, which is a solution like salt water that doesn't have any effect on your blood pressure. Your blood pressure and heart rate will be watched very close. If your blood pressure does not stay at the level that his or her doctor thinks is safe, this second stage will end right away. Then you will again be given sodium nitroprusside, or similar drug, at a rate that keeps your blood pressure at a safe level.

What if you don't want to be in the Study?

You can say "no" to being in the study if you want. You can also stop the study anytime you want by telling anyone that is caring for you. Your doctor can explain to you and your parents other treatments that could be used instead.

What You Should Know about the Medicine?

The medicine is used to keep your blood pressure where your doctor thinks is safe or needed for your operation or some other treatment or test. It has been okayed for use in adults but there is not a lot of information about how the drug works in kids. Like any medicine, it can cause unwanted things to happen. These unwanted things are called risks. Some of the risks from using this medicine are: getting sick to your stomach, headache, getting hot, having your blood pressure go down too much or your heart beat go up too high.

Things Girls Need to Know

Some medicine can cause bad things to happen to an unborn baby. If you are able to get pregnant (if you have started having a period), you need to take a pregnancy test which your doctor will give you. If you are pregnant, you should not take part in this study.

What Else Do You Need to Know?

Sometimes doctors write about the research studies when they are done. If a paper is written about this research study, your name won't be used in it, but the medical information they find out about you may be used. We will keep your medical information private. People who work for *[site name]*, the people who are running the study, and some parts of the government (the part that takes care of medicines) will be able to look at your medical information.

There is no cost to you or your parents to be in this study.

If you have questions about the study you can call Dr. _____ at _____.

I have read this form. I have had a chance to ask questions about things I don't understand. I want to be in this research study and understand what will happen to me.

_____ _____

Signature of Patient Date

_____

Name of Patient

_____ _____

Signature of the Person Obtaining Assent Date