Title: Efficacy of Nitric Oxide Administration in Attenuating Ischemia / Reperfusion Injury During Neonatal Cardiopulmonary Bypass

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Protocol Title

Efficacy of Nitric Oxide Administration in Attenuating Ischemia / Reperfusion Injury During Neonatal Cardiopulmonary Bypass

1) IRB Review History

N/A

2) Objectives

Hypothesis: The introduction of nitric oxide (NO) directly into the cardiopulmonary bypass circuit, during neonatal surgical repair of congenital heart lesions, will attenuate ischemia reperfusion injury that can complicate the surgical procedure and postoperative recovery.

Specific Objectives: During the Norwood procedure for hypoplastic left heart syndrome (HLHS) or its variants, there is around 20 to 30 minutes of ischemia to the myocardium between cardioplegia doses, and 20 to 30 minutes of interruption of flow to the lower body during reconstruction of the aortic arch. The proposed study will help to know if NO can decrease ischemia reperfusion injury to the myocardium, as well as to the lower body organs (liver, kidney, gastro-intestinal tract).

Specific Aims:

1) Evaluate whether NO delivered through the neonatal CPB circuit can decrease the clinical signs of ischemia/reperfusion injury and/or cardiac dysfunction as reflected by fluid balances, inotropic support, diuretic requirement, length of ventilator support, length of ICU stay, and length of hospital stay.

2) Evaluate whether NO delivered through the neonatal CPB circuit can decrease various biochemical markers of ischemia/reperfusion injury and oxidative damage [cardiac troponin I, interleukens (IL), tumor necrosis factor (TNF), N-terminal prohormone for brain natriuretic peptide (NT-proBNP),lactate dehydrogenase (LDH), plasma anti-oxidant levels, plasma malondialdehyde (MDA) levels].

3) Evaluate the safety of NO delivered through the neonatal CPB circuit as measured by adverse events during the course of the study.

3) Background

Around 25,000-30,000 cardiac surgical operations are performed on pediatric patients each year, and around 7500 of them are performed on neonates. These operations are made possible by the use of cardiopulmonary bypass (CPB), which allows for circulatory and pulmonary support while the heart is being repaired. Although life-saving, the use of CPB is associated with a variety of adverse effects, such as systemic inflammatory responses, neurological insufficiencies, coagulopathies, and most importantly ischemia/reperfusion (I/R) injury. Adverse heart-related events after surgically induced

I/R continues to be a major contributor to the morbidity and mortality after pediatric cardiac surgery. With the absence of pulsatile flow of the cardiopulmonary bypass, the ischemia/reperfusion cellular injury is even more exaggerated in different vital organs, like the heart, lung, liver, and kidneys. This is exaggerated by episodes of circulatory arrest or low flow status necessitated by the complexity of the repair. This is also more prominent in neonates because of the complexity of the heart defects requiring longer time on cardiopulmonary bypass, and because of the immaturity of neonatal organ systems. According to the Society of Thoracic Surgeons database, most of the neonatal cardiac repairs belong to the Risk Adjustment for Congenital Heart Surgery (RACHS) 5 and 6 complexity categories, with a national hospital mortality of around 20% (1).

The morbidity associated with inadequate cardiac protection suggests that new strategies to enhance cardiac protection are needed. Recently, there has been much interest in the pharmacological conditioning of the heart to produce adequate protection during CPB-associated surgically induced I/R. More specifically, and at a cellular level, recent laboratory data suggests that certain agents, such as nitric oxide (NO), can protect cardiac cells against I/R injury by protecting the cardiac mitochondria. The mitochondria are the energy producing apparatus in human cells. Thus, by protecting the mitochondria, one can protect the cardiac cell and minimize the effects of surgically induced I/R injury during the repair of congenital heart defects.

Ischemia/reperfusion injury and systemic inflammatory response syndrome are linked. After a period of ischemia, reperfusion stimulates the production of additional pro-inflammatory mediators, in particular, reactive oxygen free radicals and cytokines by parenchymal, endothelial, platelet, and inflammatory cells, contributing to elevated serum levels of inflammatory mediators. Cell death results from coagulation necrosis and inflammatory mediator-induced apoptosis. Endothelial cell injury results in impairment of the NO-cyclic guanosine monophosphate signaling cascade and endothelial-dependent vasodilatation (2). Alternations in NO generation appear to underpin the interrelationship of endothelial function and inflammation (3). NO might play several protective roles in reperfusion injury (4). NO is an important regulator of apoptosis, capable of both inducing and inhibiting apoptosis (5). NO has been shown to inhibit platelet adhesion and aggregation (6) and is able to block monocyte adherence and migration (7). Furthermore, NO is an inhibitor of leukocyte activation, preventing neutrophil-endothelial adhesion and the generation of oxygen free radicals (8).

The use of inhaled NO as a pulmonary vasodilator agent in neonates is well established. In addition to its potent vasodilatory effects in the pulmonary vasculature, inhaled NO has also been shown to have significant peripheral anti-inflammatory effects. The safety of perioperative administration of NO via the CPB circuit has been described in animals and human beings (9,10,11). Inhaled NO during adult cardiac surgery patients has been reported to reduce systemic inflammatory markers, attenuate endothelial dysfunction, and reduce ischemia/reperfusion injury (12,13,14). Administration of NO to adult patients undergoing cardiac surgery has been shown to blunt the release of markers of cardiac injury and decreases ventricular dysfunction during and immediately after CPB.

A more recent study in infants has also shown that the NO administration via the CPB circuit can reduce various markers of inflammation and reduce postoperative complications (15). To date, there have been no such studies relating NO administration via the CPB circuit and ischemia reperfusion injury in neonates undergoing surgical repair of congenital heart lesions.

4) Inclusion and Exclusion Criteria

Inclusion criteria:

- Neonates, age 0-30 days
- Full term, > 37 weeks gestation
- Birth weight ≥ 2.6 kg

Exclusion criteria:

- Preoperative sepsis
- Renal dysfunction, (preoperative creatinine greater than 1.0)
- Intracranial hemorrhage (preoperative intraventricular hemorrhage more or equal to grade 1)
- Known Chromosomal abnormalities and/or known genetic syndromes. (The Fluorescent in situ hybridization (FISH) test is routinely done on all neonates with cardiac disease. If the test results are not received before surgery, the subject will be withdrawn as indicated in protocol section 14.)
- Prior cardiac intervention, catheter-based or surgical
- RV wall and endocardial fibroelastosis (as determined by pre-operative echocardiography).

6) Study-Wide Number of Subjects

We will enroll a total of 24 subjects with 12 subjects in the treatment group and 12 subjects in the control group.

7) Study-Wide Recruitment Methods

The study will be performed at ACH-OL. The pediatric cardiac surgery division performs approximately 400 surgeries each year at this site. Twenty-four neonates with HLHS or its variants requiring surgical repair in the first few days of life will be randomized into 2 groups.

All newborns with diagnosis of HLHS or its variant, who undergo Norwood procedure and satisfy the inclusion criteria will be invited to participate in the study. Most of these patients are diagnosed prenatally by fetal echocardiography and are born at ACH-OL. Few patients with no prenatal diagnosis or who are born at an outside institution are transferred to ACH-OL for further management. Parents will

be informed about the study by our team of Pediatric Cardiac surgeons when they see the child for a clinical preoperative exam. This exam typically occurs after the child is born and the diagnosis is confirmed by echocardiography, and before the scheduled surgical procedure.

8) Study Timelines

The estimated duration needed to recruit the 24 subjects for the study is around 2 years. Each subject will be enrolled in the study from the time of surgery (Norwood procedure) until discharge. After all the subjects have been enrolled and the data has been collected, we anticipate that 2 to 3 months to finish analyzing the data and write the manuscript.

9) Study Endpoints

The primary study endpoints are to evaluate whether NO delivered through the neonatal CPB circuit can decrease the clinical signs of ischemia/reperfusion injury and/or cardiac dysfunction. Clinical parameters include:

- fluid balance
- diuretics requirement
- inotropic support
- duration of mechanical ventilation (hours)
- ICU length of stay (LOS)
- Hospital LOS
- End organ failure (measured by BUN, creatinine, and liver function tests)

The secondary study endpoints are to evaluate whether NO delivered through the neonatal CPB circuit can decrease various biochemical markers of ischemia/reperfusion injury and oxidative damage. Markers to be analyzed will include:

- cardiac troponin I
- N-terminal prohormone for brain natriuretic peptide (NT-proBNP)
- plasma malondialdehyde (MDA) levels
- superoxide dismutase
- interleukens (IL)
- lactate dehydrogenase (LDH)
- procalcitonin
- tumor necrosis factor (TNF alpha)
- C-reactive protein (CRP)
- Creatine phosphokinase-MB (CPK-MB)

The primary safety measure is the level of methemoglobin while NO is being administered during cardiopulmonary bypass. The level will be checked every

½ hour per standard clinical practice, and NO administration will be adjusted accordingly, to keep the methemoglobin level below 3%.

10) **Procedures Involved**

Design
The study will employ a double-blind placebo-controlled randomized block design.

Sample
Twenty-four neonates with HLHS or its variants requiring surgical repair in the first few days of life will be randomized into 2 groups.

Group 1 will include 12 subjects who will receive inhaled NO via the CPB circuit for the duration of surgery. Group 2 will include 12 control subjects. These controls will receive an identical NO via CPB set-up, but will not receive NO during surgery. The subjects in both groups will undergo the standard Norwood procedure with a central systemic to pulmonary shunt or right ventricle to pulmonary artery shunt (Sano shunt). The only difference between groups will be the administration of NO via CPB.

Standard Intra-operative protocol:
After induction of general anesthesia using opiates (Fentanyl) and muscle relaxant, appropriate arterial line and central venous line access will be obtained. Intra-operative monitoring of arterial blood pressure, central venous pressure, systemic oxygen saturation, cerebral and somatic (kidney) near-infrared spectroscopy, will be done throughout surgery.

After adequate positioning, a regular median sternotomy incision will be done. The thymus gland is resected for better exposure. Under full heparinization (activated clotting time >300sec), CPB is instituted using an arterial cannula inserted in the ascending aorta (or ductus arteriosus in patients with HLHS), and a venous cannula inserted in the right atrium or 2 separate superior and inferior vena cava cannulas.

Once on CPB, the patient's temperature is cooled down to 28 degrees Celsius. Cooling and warming the patient are done with pH stat strategy, during which the carbon dioxide level is allowed to rise in the CPB circuit to maintain a normal temperature corrected pH. All patients receive inhaled NO through the endotracheal tube after weaning from CPB.

Research procedures
The subjects will be randomized by the research perfusionist to either groups of the study. Only the perfusionist will be aware of the randomization process and NO delivery. The surgeon will be blinded to group assignment. Group 1 (treatment group) will receive NO via the CPB for the duration of surgery. Once the patient is off CPB, NO administration via CPB will be discontinued and switched to endotracheal tube administration. Group 2 (control group) will be treated with a strategy identical to that in the treatment group, with the exception that they will not receive NO via CPB.

For subjects in Group 1, NO will be delivered using the Inovent Transport delivery device (Ikaria, Hampton, NJ) after calibrating the machine according to the manufacturer's instruction. While setting up and priming the CPB circuit, the NO delivery tubing will be incorporated into the oxygenator inlet gas tubing using special reducing connectors. NO sampling will be performed by adding a luer lock connector to the inlet gas tubing proximal to the oxygenator and attaching the sampling line to it.

Once CPB is initiated, NO delivery will be started at 40ppm as measured by the sampling line, and maintained at 40ppm during the procedure unless the serum methemoglobin level increases above 3%. Once the patient is off CPB, NO delivery will be discontinued, and the subject will be managed similar to controls.

Post-surgical care

After finishing surgery, all subjects will recover in the Pediatric Surgical Heart Unit (PSHU) under the direct care of the pediatric cardiac intensivist and the surgical team. Neither physician nor nurse involved in the post-operative care will be aware of the subject's randomization assignment.

Data collection (see schedule of events below)

All patients will have the following pre-operative labs drawn before surgery as part of our standard of care: complete blood count (CBC), complete metabolic panel, coagulation profile, and blood type and cross match.

On the day of surgery, the following research related markers will be drawn in the operating room after the patient is under anesthesia and after insertion of arterial and central venous access (Time 0): cardiac troponin I, NT pro-BNP, plasma (MDA) level, superoxide dismutase, IL-6, IL-8, lactate dehydrogenase (LDH), procalcitonin, TNF alpha, CRP and CPK-MB. These markers will be rechecked at the end of CPB and before modified ultrafiltration (Time 1), after modified ultrafiltration (Time 2), 12 hours (+/- one hour) after CPB (Time 3) and 24 hours (+/- one hour) after CPB (in the PSHU) (Time 4). The amount of blood needed for each research blood draw will be 2 ml for a total of 10 ml of blood to be drawn for research purposes.

All patients will have an echocardiogram, chest radiography and the following blood tests drawn in the PSHU on a daily basis that includes: complete blood count (CBC), complete metabolic panel with kidney function and liver function tests, and coagulation profile. These tests are conducted as part of our standard of care.

All standard of care laboratory tests will be performed and billed in accordance with usual hospital procedures. All research only labs will be shipped in batch to Immunetech labs and processed according to the appropriate procedures.

Clinical data that will be collected will include: CPB time and aortic cross clamp time, fluid requirements, diuretic requirement inotropic support, ventilator hours, need to leave sternum open, ICU LOS and total hospital LOS, transfusions, blood and coagulation

factors, total amount of chest tube drainage, post-operative complications like bleeding, infection or seizures, renal dysfunction, arrhythmias, and post-operative need for extracorporeal membrane oxygenation (ECMO) support.

Schedule of Events

	Screening								**End of Study**	**Post study follow-up**
Visit description	**Preop**	**Time 0 (Preop)**	**Time 1 (end of CPB and before MUF)**	**Time 2 (end of CPB, after MUF)**	**Time 3 (12 hrs +/- one hour Post CPB)**	**Time 4 (24 hrs +/- one hour Post CPB)**	**Time 5 Day 2 Postop**	**Time 6 Day 3 Postop**	**Time 7 D/C date**	**Time 8**
Informed Consent	X									
Demographic information	X									
Concomitant medications	X								X	X
Laboratory tests										
Cardiac Troponin I		X	X	X	X	X				
NT pro-BNP		X	X	X	X	X				
Plasma MDA		X	X	X	X	X				
Superoxide Dismutase		X	X	X	X	X				
IL-6		X	X	X	X	X				
IL-8		X	X	X	X	X				
LDH		X	X	X	X	X				
Procalcitonin		X	X	X	X	X				
TNF alpha		X	X	X	X	X				
CRP		X	X	X	X	X				
CPK-MB		X	X	X	X	X				
BUN and Cr		SOC			SOC		SOC	SOC		
Liver function tests		SOC			SOC		SOC	SOC		
Diagnostic tests										
CXR	SOC				SOC	SOC	SOC	SOC		
Echocardiogram	SOC				X	SOC	SOC		SOC	
Clinical course										
Fluid balance					X	X	X	X		
Diuretic requirement					X	X	X	X		
Inotropic support					X	X	X	X		
Ventilator Hours					X	X	X	X		
ICU LOS									X	
Hospital LOS									X	

Safety Adverse Event Reporting			X		X	X	X	X	X	X
Complications									X	X
Mortality									X	X

SOC=standard of care, MUF = modified ultrafiltration

11) Data and Specimen Banking

The data will be collected and saved on a password-protected Excel data sheet which will be stored on the password-protected computer of principal investigator.

12) Data Management

Sample Size Estimation

Sample size estimation was calculated using PASS 12 (15) based on the number of subjects needed to compare the difference in fluid balance as measured by the total amount of fluid administered in the first 24 and 48 hours for the two groups. Power analysis was run based on published data that showed a difference 130 mL between similar group assignments. (14) A sample size of 12 achieves 83% power to detect a mean difference of 75 mL with an estimated standard deviation of 60 mL and with a significance level (alpha) of 0.05 using a two sample t-test.

Data Management

Standard of care lab values and clinical indicators as identified in protocol Section 9 will be recorded in the electronic medical record (EMR) per routine hospital procedure. Lab values of markers being collected for research only will be received in a report directly from the labs. Investigators will extract study data from the EMR or lab results onto a source document for data collection and record into an electronic spreadsheet.

Statistical Analysis Plan

Data analysis will include measures of central tendency for all demographic and outcome variables. The primary endpoint of clinical indicators, including fluid balance, inotropic support, ventilator hours, and LOS will be compared between groups using a Repeated Measures ANOVA with post hoc comparisons, Student's t-test or chi-square as appropriate. A p-value less than, or equal to, 0.05 will be regarded statistically significant.

Secondary outcomes to be compared between groups include the biochemical markers. Analysis will be done using Student's t-test and a p-value less than, or equal to, 0.05 will be regarded statistically significant.

The other indicators, number of adverse events (as determined by methemoglobin) will also be described and reported as indicated in protocol Section 13. Differences in adverse event rates will be evaluated using descriptive and inferential statistics as appropriate.

Data Storage

All information gathered during this study will be kept confidential. Subjects will be assigned case numbers and data collection forms that including the patient name will only be seen by the research team. All data collection forms will be placed in a confidential folder within the principal investigator's locked office. Tabulation of data will be stored in a password protected file within a password protected computer. The results of this study may be published in scientific journals or be presented at professional meetings, but no individuals will be identified. The collection and handling of data will be in complete accordance with the HIPPA regulations. The research-related records for this study may be inspected by a federal regulatory agency and the Institutional Review Board.

Data collected for this study will be maintained for three years after the investigation is complete and the FDA is notified.

13) Provisions to Monitor the Data to Ensure the Safety of Subjects

MONITORING PLAN

The individuals responsible for data safety and monitoring will be Dr. Elzein (PI). Quality control will include regular data verification and protocol compliance checks by Dr. Elzein.

COLLECTION AND REPORTING OF SAEs AND AEs

Throughout the study, Dr. Elzein and the study research coordinator will monitor study participants for adverse events. Dr. Elzein will review all adverse events (AEs) and serious adverse events (SAEs) individually and in aggregate on a biweekly basis. Dr. Elzein will report all AEs and SAEs to the Advocate Health Care IRB according to the AE reporting guidelines.

For this study, the following standard AE definitions will be used:

Adverse event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease, that did not exist at the time of the subject's entry into the study and that is temporally associated with the use of the study medication, regardless of whether it is considered related to the study medication.

Suspected adverse reaction: A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

Adverse Reaction: An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

Unexpected Adverse Event: An adverse event or suspected adverse reaction is considered "unexpected" if it is not consistent with the risk information described in this plan.

The following list of medically important unexpected events is intended to serve as a guideline for determining which condition may be considered reportable. The list is not intended to be exhaustive:

- Renal failure
- Delay of chest closure (i.e. > 7 days)
- Surgical wound from study related surgery necessitating debridement
- Sepsis (i.e. confirmed by positive laboratory culture)
- Delayed extubation (i.e. intubation period lasting > 3 weeks)

Serious Adverse Event: A significant hazard or side effect, regardless of the Investigator's opinion on the relationship to treatment group. SAEs will be recorded and reported using the INO Therapeutics/Ikaria SAE form (see Attachment A). A serious adverse event is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (defined in this case as an event in which the subject was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe)
- Requiring prolongation of existing hospitalization (i.e., > 4 weeks which is a typical hospitalization time for this population)
- Results in persistent or significant disability/incapacity (e.g., stroke, seizure)
- Required intervention to prevent permanent impairment/damage (e.g., re-intubation, ECMO, additional surgical intervention)

AEs will be graded by the investigator according to the following scale:

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities. This includes transient laboratory test alterations.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities. This includes laboratory test alterations indicating injury, but without long-term risk.

Severe: An event that prevents normal everyday activities. If prolongation of hospitalization is greater than 4 weeks, required for treatment it becomes an SAE. (An AE that is severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as mild, moderate or severe.)

However, relating the intensity of clinical AEs to functional daily life and everyday activities may not be assessable in children in the PSHU. Therefore, if not possible to assess, the intensity of clinical AEs will be assessed by the investigator as per his/her best judgment.

The study will use the following **AE attribution scale**:

Unrelated: The AE is clearly related to other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject (i.e., another cause of the event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event).

Unlikely related: The event was most likely produced by other factors such as the subject's clinical state, therapeutic interventions or concomitant drugs administered to the subject, and does not follow a known response pattern to the study drug.

Possibly related: The event follows a reasonable temporal sequence from the time of drug administration and/or follows a known response pattern to the study drug; but it could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Probably related: The event follows a reasonable temporal sequence from the time of drug administration and follows a known response pattern to the study drug; and it cannot be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

AEs will be monitored throughout the time that a subject is in the trial (hospital length of stay). AEs will be assessed through a review of the hospital chart on a weekly basis and with a physical examination of the subject, if indicated.

REPORTING OF ADVERSE EVENTS

Events determined by the PI to involve injury or to be unexpected problems involving risk and probably related to the study drug will be reported by the PI to the IRB within 5 days per HRP 214. Adverse events that are determined by the PI not to

involve injury or not a related risk of the use of NO during the Norwood surgery, will be reported per IRB policy at the time of continuing review.

An IND safety report will be reported to the FDA by the PI when an event meet all three of the definitions for *suspected adverse reaction, serious* and *unexpected*. All FDA reporting will be consistent with 21 CFR 312.32 (c). For each reportable event, the information to be recorded in the source document includes the nature, date, intensity, duration, attribution (relationship to study drug), action taken and outcome of the event.

- Unexpected serious suspected adverse reactions will be reported to FDA as soon as possible but no later than within 15 calendar days following the PI initial receipt of the information.
- Unexpected fatal or life-threatening suspected adverse reactions will be reported to FDA as soon as possible but no later than 7 calendar days following the PI initial receipt of the information.

DATA ANALYSIS PLANS AND INTERRIM REPORTS

An independent Data Safety Monitoring Board (DSMB) will perform a statistical review of the study data and will have access to fully unblinded* patient data if necessary, to ensure patient safety. The DSMB will be comprised of 3 individuals who are not involved in the conduct of the study, representing pediatric cardiology or pediatric intensive care, perfusion and research. The DSMB will be empowered to recommend, based on safety considerations, modifications to the protocol or early termination of the study. The board will function independently of the investigators. AEs and SAEs will be monitored by the DSMB in an unblinded* manner during the study after half of the subjects (N=12, 6 in each group) are enrolled. Subsequent safety reports will be completed after each set of 6 subjects.

*A the randomization code will be kept strictly confidential. It will be provided to the DSMB by the perfusionists (Vincenzo Rizzo, Cindy Urbas) to ensure patient safety.

The interim analyses will be performed to evaluate differences in AE and SAE rates between the two treatment groups, and to evaluate the primary endpoint (fluid balance, morbidity and mortality). The DSMB will prepare a report after each review which will be given to the PI, and, in turn, this report will be submitted by Dr. Elzein to the IRB.

The DSMB will be comprised of the following individuals:

- Denise Angst, PhD, RN – Chair of DSMB, Director, Advocate Center for Pediatric Research

- Christine Steffensen, PharmD, MA - Clinical Coordinator, Pediatrics and Pediatric Critical Care, Advocate Children's Hospital-Oak Lawn

- Luis E. Torero, MD – Pediatric Pulmonologist, Torero Pulmonary Specialists, S.C.

14) Withdrawal of Subjects

The FISH test to test for chromosomal abnormality is done routinely on all neonates with cardiac disease. If the FISH test result is abnormal after the Norwood procedure is performed, indicating genetic syndromes, the subject will be withdrawn from the study.

It may be possible that RV wall and endocardial fibroelastosis may not be evident on the pre-operative echo but that it would be identified during the operation. If this condition is identified during the operation, the subject will be withdrawn at that time, randomization will be unblended and the subject will be treated as if they are not in the study (e.g. will not have extra study blood drawn).

At any time, the attending physician can withdraw a patient from this study if they feel it is warranted based on clinical condition. Parents will be notified by the attending physician. The parents have the right to withdraw their child from the study at any time. If the parent decides to withdraw their child from the study, they will be asked if medical record collection can continue until the end of hospitalization. Documentation of the conversation and their decision will be entered into the research chart and medical record.

15) Risks to Subjects

We have been using *inhaled* NO during and after surgery at our center for over 8 years. The direct risk of using NO in the CPB circuit during surgery is related to elevated methemoglobin serum level. An elevated methemoglobin level is usually treated effectively by decreasing the rate of NO administration. All surgical staff and team members are familiar with NO use.

NO administration via the cardiopulmonary bypass circuit will be monitored by checking frequent methemoglobin levels. Per our standard practice, this level is checked with every arterial blood gas which is usually performed every 30 minutes while the patient is on cardiopulmonary bypass. If the methemoglobin level increases to 3%, NO administration will be decreased until methemoglobin falls below this level. No other research-related risks are anticipated.

After the surgery, subjects will be monitored for any adverse events throughout the course of their hospitalization as outlined in safety monitoring plan protocol (Section 13). Appropriate medical care will be provided for any adverse events.

16) Potential Benefits to Subjects

There are no guaranteed benefits to any subjects in this study. The results of this study will be used to enhance our understanding of the effectiveness of NO to potentially decrease ischemia reperfusion injury that can complicate the surgical procedure and postoperative recovery and may benefit future patients. Potential benefits to the subject could include decreased ischemia/reperfusion injury and systemic inflammatory reaction that is commonly seen after neonatal cardiac surgery. This will be reflected by improved post-operative myocardial function and preserved end organ function (kidney, lung, liver), shorter intensive care unit and hospital stay and improved mortality and morbidity.

17) Vulnerable Populations

This research involves children as a vulnerable population. Informed consent/parental permission will be assured by offering parents multiple opportunities to ask and have their questions answered about the study before enrollment and throughout the study time period. Informed consent/parental permission documentation will be completed as discussed in Section 30. Subjects will be too young to provide assent as they will all be neonates.

18) Multi-Site Research

The study will be performed exclusively at ACH-OL.

19) Community-Based Participatory Research

Not applicable

20) Sharing of Results with Subjects

The results of some tests routinely performed as standard of care (including lab values) may be shared with the family by the physician involved in the care. No further sharing of research-only procedures or data will be provided to subjects or their families.

21) Setting

The research will be conducted at ACH-OL with the research interaction taking place in the operating room and post-surgical data collection occurring while subjects are cared for in the PSHU.

22) Resources Available

The Norwood procedure has been performed at our institution for over 20 years. Our operative mortality varies between 5 and 10%; the national operative mortality for the Norwood procedure is around 16%. In the past, we performed between 20 and 30 Norwood procedure per year. During 2013, we performed around 15 procedures with zero mortality. Our estimated period to recruit the 24 subjects for the study is 2 years (taking into consideration parents who decline participation).

Dr. Michel Ilbawi and I (Chawki Elzein) are the surgeons who will be performing these surgeries. Dr. Ilbawi has over 20 years experience and Dr. Elzein has approximately 10 years' experience with the Norwood procedure and the postoperative care of patients. The PSHU is covered 24 hours a day, seven days a week, with a team of experienced pediatric cardiac intensivists and experienced nursing staff. Dr. Andrew VanBergen, chief of cardiac critical care, has approximately 7 years' experience with postoperative care of Norwood procedure patients. The perfusionists run the cardiopulmonary bypass machine during the Norwood procedure. They have more than 10 years experience with extracorporeal circulation during complex pediatric cardiac operations. There is a back-up ECMO (extracorporeal membrane oxygenation) team available in case of sudden postoperative cardiorespiratory decompensation. In addition, Vivian Cui and Robert Metzger, Pediatric Echo cardiographers will be performing the echocardiography on the subjects.

23) Prior Approvals

Not applicable

24) Recruitment Methods

See protocol Section 7.

25) Local Number of Subjects

See protocol Section 6.

26) Confidentiality

All information gathered during this study will be kept confidential. Data collection forms and the database that include the patient name and other PHI will be seen only by the principal investigator and research team. All information and data gathered in this study will be placed in a password protected computer with restricted access by

only the investigative team. The results of this study may be published in scientific journals or be presented at professional meetings, but no individuals will be identified. The research-related records for this study may be inspected by a federal regulatory agency and the Institutional Review Board.

Data will be stored on Advocate's secure computer network which will only be accessible by the study investigators.

27) Provisions to Protect the Privacy Interests of Subjects

See protocol Section 12 and 26.

28) Compensation for Research-Related Injury

No compensation will be provided for research-related injury. No funds have been set aside by Advocate Health Care as compensation for injury or any associated costs.

29) Economic Burden to Subjects

The patient is not responsible for any extra costs related to the research. The cost of NO used in CPB during surgery, and the cost of the serum ischemia/reperfusion and inflammatory markers, will be covered by IKARIA Inc.

30) Consent Process

Written parental permission/informed consent will be obtained from each child's parent or legal guardian prior to surgery (See Attachment B- Parental Permission form and Attachment C – HIPPA Authorization Form). We will follow procedures outlined in SOP HRP-090 to obtain informed consent/parental permission. The neonate remains in the hospital from birth until the Norwood procedure is performed. Parents will be informed about the study by our team of Pediatric Cardiac surgeons when they see the child for a clinical preoperative exam. This exam typically occurs after the child is born and the diagnosis is confirmed by echocardiography, and before the scheduled surgical procedure.

The informed consent/parental permission process will take place at the same time as obtaining consent for surgery. The consent for surgery is usually obtained 1-2 days before surgery date. Only the study investigators (Chawki Elzein, Andrew VanBergen) will be involved in obtaining the research consent. Approximately 15 to 30 minutes will be assigned for discussing the research study and potential related risks to the subject. The person obtaining the research consent will clearly distinguish its difference from the surgical

consent. If the parents want to think about the research study, they will have ample time to decide whether to enroll or not. All questions will be answered prior to obtaining consent.

Non-English Speaking Subjects

There is a potential that a neonate with Spanish speaking parents will be invited to participate in this study. In this case, the Spanish short form will be used for consent with the English consent presented orally in Spanish with the assistance of an interpreter (we have computer Skype based interpreters for over 10 languages are available at ACH-OL). In addition, one of the sub-investigators, Andrew VanBergen, MD, speaks fluent Spanish and may be available to present the study to any Spanish speaking patients. It is anticipated that this process may be used for 1-2 patients only.

Subjects who are not yet adults (infants, children, teenagers)

All patients are neonates with HLHS or its variants. The Norwood procedure is usually performed at 4 to 7 days old. The consent form will be obtained from at least one parent before any study procedures are performed, though both parents will be encouraged to discuss the child's participation. The mother will have to sign the consent form if the parents are not married. If there are social issues and DCFS is involved in consenting for the patient, the patient will not be enrolled.

31) Process to Document Consent in Writing

We will be following procedures outlined in SOP HRP-091. The consent for the research study will be documented together with the consent for surgery as a note in the medical record of the patient.

32) Drugs or Devices

Inhaled NO is already available and has been used at ACH-OL for over 7 years. This study will be the first time that NO will be administered via the cardiopulmonary bypass circuit at our institution but it has been used as part of the cardiopulmonary bypass circuit in children at another institution. (14) An investigator-initiated IND application was submitted to the FDA. Registration at clinicaltrials.gov has been submitted and is under review.

33) References

1- Society of Thoracic Surgery Database.

2- Cooper WA, Duarte IG, Thourami VH, Nakamura M, Wang NP, Brown WM III, et al. Hypothermic circulatory arrest causes multisystem vascular endothelial dysfunction and apoptosis. Ann Thorac Surg, 2000;69:696-703.

3- Chello M, Mastroroberto P, Perticone F, Celi V, Colonna A. Nitric oxide modulation if neutrophil-endothelium interaction: difference between arterial and venous coronary bypass graft. J Am Coll Cardiol. 1998;31:823-6.

4- Gianetti J, Del Sarto P, Bevilacqua S, Vassalle C, De Filippis R, Kacila M, et al. Supplemental nitric oxide and its effect on myocardial injury and function in patients undergoing cardiac surgery with extracorporeal circulation. J Thrac Cardiovasc Surg. 2004;127:44-50.

5- Uchiyama T, Otani H, Okada T, Ninomiya H, Kido M, Imamura H, et al. Nitric oxide indices caspase-dependent apoptosis and necrosis in neonatal rat cardiomyocytes. J Mol Cell Cardiol. 2002;34:1049-61.

6- Sawicki G, SalasE, Murat J, Miszta-Lane H, Radomski MW Release of gelatinase a during platelet activation mediates aggregation.. Nature. 1997;386:616-9.

7- Comini L, Bachetti T, Agnoletti L, Gaia G, Curello S, Milanesi B, et al. Induction of functional inducible nitric oxide synthase in monocytes of patients with congestive heart failure: links with tumor necrosis factor –alpha. Eur Heart J. 1999;20:1503-13.

8- Van Dervort AL, Yan L, Madara PJ, Cobb JP, Wesley RA, Corriveau CC, et al. Nitric oxide regulated endotoxin-induced TNF-alpha production by human neutrophils. J Immunol. 1994;152:4102-9.

9- Palatianos GM, Paziouros K, Vassili MI. Effects of exogenous nitric oxide during cardiopulmonary bypass on lung postperfusion histology. ASAIO J, 2005 Jul-Aug:51(4):398-403.

10- Zhou Jing, Wu Xiao-dong, Lin Ke. Blood Hibernation: a novel strategy to inhibit systemic inflammation and coagulation induced by cardiopulmonary bypass. Chinese Medical Journal 2010; 123(13):1741-1747.

11- Lowson SM, Hassan HM, Rich GF The effect of nitric oxide on platelets when delivered to the cardiopulmonary bypass circuit.. Anesthesia and Analgesia 1999 Dec; 89(6):1360-5.

12- Vinten-Johansen J, Sato H, Zhao ZQ. The role of nitric oxide and NO-donor agents in myocardial protection from surgical ischemic-reperfusion injury. Int Jour Cardiology 1995 July; 50(3): 273-81.

13- Lefer AM. Attenuation of myocardial ischemia-reperfusion injury with nitric oxide replacement therapy. Ann Thor Surg 1995 Sept; 60(3):847-51.

14- Checchia PA, Bronicki RA, Muenzer JT, Dixon D.Nitric oxide delivery during cardiopulmonary bypass reduces postoperative morbidity in children- a randomized trial. J Thorac cardiovasc Surg 2013 Sept;146(3): 530-6.

15- Hintze, J. *PASS 12*. Kaysville, Utah: NCSS, LLC; 2013.

Attachments

Attachment A – INO Therapeutics / Ikaria Serious Adverse Event Form

Attachment B- Parental Permission form

Attachment C – HIPPA Authorization Form

Attachment D – Eligibility Checklist

Attachment E – Data Collection Form

Attachment F – FDA letter of receipt