Title: **Nitric Oxide Administration During Pediatric Cardiopulmonary Bypass Surgery to Prevent Platelet Activation**

NCT03455218
Unique Protocol ID 1111115-1NO in CPB 001

Study Protocol and Statistical Analysis Plan

Document Date: 5/19/2020

Protocol

To complete the aims and objectives of this project we are planning a pilot randomized, double blinded, placebo controlled clinical trial of nitric oxide added to the sweep gas of the oxygenator during cardiac surgery requiring CPB in infants.

Inclusion Criteria: Infants less than one year of age undergoing cardiac surgery with the use of cardiopulmonary bypass.

Exclusion Criteria: Prior surgery requiring CPB within the same hospitalization, pre-operative need for ECMO or mechanical circulatory support, known hypersensitivity to nitric oxide, known hemostatic or thrombotic disorder that results in an altered transfusion/anticoagulation protocol

Equitable subject selection: All patients will be considered for eligibility that meet the inclusion/exclusion criteria regardless of race, gender, or ethnic background. Non-English speaking patients will be considered for inclusion if a suitable consent form can be approved prior to their surgical procedure. A Spanish language short form will be submitted along with the initial study as we anticipate some eligible subjects will be Spanish speaking only. Other languages encountered in eligible patients will be considered on an individual basis and if a suitable consent form can be drafted and approved prior to the patient's surgical date, they will be considered for enrolment.

Sample Size: 40 patients equally distributed amongst the treatment and placebo groups

Power Calculation: using 0.05 as type 1 error tolerance and previous data from neonatal CPB studies, a minimum sample size of 12 patients is necessary to detect a difference of a change in platelet count (Specific Aim 1) of 50 with a power of 91%.

Sample size justification: In order to maximize the ability to estimate a sample size for a larger multi-institutional trial (Specific Aim 2) we need to maximize the number of patients enrolled within this local pilot trial. Approximately 120 infants annually meet the inclusion/exclusion requirements annually at CHW. Given experience with prior interventional studies in this population, we anticipate a consent rate of approximately 33%. Therefore we anticipate being able to enroll 40 patients into this trial within one year.

Randomization: Stratified to insure the number of patients with Society of Thoracic Surgery (STS) Mortality Class 1-3 and 4-5 surgeries are equally distributed.

Justification for stratification of randomization: The complexity of congenital cardiac surgery is known to significantly impact morbidity and mortality (16). In order to insure an equal distribution of patients with this known confounder among groups we will stratify the randomization to insure an equal distribution to active and placebo groups.

Recruitment: The PI and co-investigators have access to the cardiac surgical scheduling coordinators. We will be in contact with them and browse the list of scheduled surgical patients at the weekly surgical conference. The PI and co-investigators are also active clinicians within the cardiac intensive care unit and will be looking for eligible patients amongst the census of the intensive care unit within the normal course of their clinical duties. No fliers or published materials will be used. If the patient has an identified primary cardiologist at the time they are deemed to be eligible, we will contact them prior to approaching the patient/family to answer any questions they would have.

Treatment: A minimum sweep gas flow of 2.5 L/min will be used in all patients with CO_2 added to maintain $PaCO_2$ 35-40 mmHg. All patients will have an INOmax DS_{IR} device in line with the sweep gas. Nitric oxide at a dose of 20 ppm will be added to the sweep gas of the treatment group from the initiation to the conclusion of CPB. The placebo group will not receive nitric oxide but will be connected to the INOmax DS_{IR} device.

The INOmax DS_{IR} device and nitric oxide will be supplied by Mallinckrodt Pharmaceuticals. A letter indicating this support from Mallinckrodt is included in the package.

Blinding: All care providers involved in the clinical care of the patient will be blinded to treatment group (and methemoglobin levels) for 30 days post-operatively or hospital discharge (whichever is later). A single study staff (perfusionist or respiratory care practitioner) will be responsible for nitric oxide delivery according to the randomization scheme outlined above.

Study Measurements (Primary objectives in **bold**):

Aim 1:

Baseline and at conclusion of cardiac repair/prior to transfusion: **Change in platelet count**, platelet response to TRAP, U46619, and CRP (to be done at the Blood Research Institute)

Aim 2:

30 day (all cause) mortality, length of hospital stay post-operatively, transfusion volumes of platelets and pRBCs, total number of transfused products, length of mechanical ventilation post-operatively, vasoactive infusion score (highest within the first 24 hours post-operatively), need for ECMO within 48 hours post-operatively, and total hospital cost.

Aim 3:

Baseline, at conclusion of CPB, and upon arrival to ICU: **Methemoglobin levels**

Statistical Analysis Plan

A redcap database will be created to collect all data fields and insure data integrity. Statistical support will be through a qualified statistician from the Clinical and Translational Science Institute of Southeast Wisconsin, Dr. Chiang-Ching Huang. We plan to compare continuous variables such as change in platelet count; platelet response to TRAP, U46619, and CRP; length of stay; length of mechanical ventilation; transfusion volumes; total hospital cost; and methemoglobin levels with either a Mann Whitney test or t-test. A chi-square analysis will be used for dichotomous variables such as mortality and need for ECMO. Multivariate or regression analysis will be done as needed to control for significant cofounders such as surgical complexity scoring, age, gender, race, ethnicity, weight, and presence of known genetic disorder.