Study Protocol Title:

Treatment of Delayed Pulmonary Transition in Extremely Preterm Infants & Bronchopulmonary Dysplasia

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Treatment of Delayed Pulmonary Transition in Extremely Preterm Infants & Bronchopulmonary Dysplasia

Study Sponsor:

AdventHealth

Principal Investigator:

Principal investigator: Hussnain Mirza, MD

List of Abbreviations:

BPD Broncho-pulmonary dysplasia

GBS Group B streptococcus

HBs Hepatitis B surface antigen

iNO Inhaled nitric oxide

IVH Intraventricular hemorrhage

NEC Necrotizing enterocolitis

PAP Pulmonary arterial pressure

PDA Patent ductus arteriosus

PH Pulmonary hypertension

PVL Periventricular leukomalacia

RCT Randomized Controlled Trials

ROP Retinopathy of Prematurity

RT Respiratory Therapist

sBP Systemic blood pressure

sPO2 Pulse oximeter

VP Ventriculoperitoneal

Introduction

This document is a protocol for a human research study. This study is to be conducted in accordance with applicable Federal regulations and institutional research policies and procedures.

Background Information and Scientific Rationale

Bronchopulmonary Dysplasia (BPD) is the most common morbidity in extremely preterm infants affecting approximately 15,000 infants annually in the United States. Up to 70% of extremely preterm infants who require mechanical ventilation beyond 7 days of life can develop BPD.[1] It is considered a major contributor to the $22 billion annual cost of prematurity.[2] BPD increases the risk of mortality especially after respiratory syncytial viral infection. Although clinical symptoms may improve with time, pulmonary reserve are always restricted. Up to 50% infants with BPD may die within 2 years if the disease is complicated by late pulmonary hypertension.[3] There is no cure for BPD; however, reducing its incidence and ameliorating its severity is possible by minimizing the underlying contributing risk factors.

In an animal model, West et al. showed significant lung injury secondary to the elevated pulmonary capillary pressure up to 40 mmHg for as little as 4 minutes.[4] Multiple prospective clinical studies have shown a significant association between early PH and BPD.[5-7] Incidence of early PH in preterm infants has been reported in 6-42% range. However, time for early PH screening was variable (1-6 weeks of life). As the natural history of postnatal cardiopulmonary adaptation in extremely preterm infants was never studied before, the optimal time for early PH screening was unknown.[8]

iNO is an effective treatment for pulmonary hypertension in term and near-term infants. There is no randomized controlled trial to determine the efficacy of iNO to treat early PH in extremely preterm infants[9, 10] but the treatment can be effective when used in appropriate subjects.[11, 12] Safety of iNO treatment in preterm infants is well established in multiple RCTs.[2, 13-16] American Academy of Pediatrics (AAP)[17] and National Institute of Child Health (NICHD)[18] do not recommend routine iNO treatment to prevent BPD/death in preterm infants. However, these organizations recognize the potential benefit of iNO treatment for preterm infants with PH and encourages future research. Similarly, American Heart Association and American Thoracic Society also favor the iNO treatment for preterm infants with documented PH.[19] Using a case control study design Seth et al. have recently reported higher risk for BPD and intraventricular hemorrhage (IVH) among preterm infants with early PH. iNO treatment of early PH was associated with improved clinical outcomes and survival compared to the infants in the untreated group.[20]

Prophylactic iNO treatment of preterm infants did not decrease the risk of BPD in several randomized clinical trials.[21, 22 23] However, these trials were done without echocardiographic screening for PH[24] to selectively enroll high risk infants who may benefit from iNO intervention. Thus, a likely explanation for the negative outcome of efficacy is the enrollment of generic nature of study subjects that may have diluted the beneficial effect of iNO intervention. For these infants, iNO treatment continued for 7-14 days without monitoring hemodynamic changes. Prolonged iNO treatment may cause the formation of unmeasured toxic radicals such as peroxynitrite that can induce surfactant dysfunction, cause membrane damage by lipid peroxidation and increase the risk for BPD.[25] It is very important to identify PH in preterm infants and monitor pulmonary hemodynamic changes following the initiation of iNO treatment to prevent unnecessary and prolonged exposure to iNO.[26] In the presence of hemodynamically significant PDA, iNO treatment can increase left to right shunting by causing pulmonary vasodilatation.[26]

Based on the current literature and our preliminary data [27](presented below), we hypothesized that "iNO treatment of extremely preterm infants with delayed postnatal cardiopulmonary transition defined as presence of early pulmonary hypertension (PH) at - 96 hours of age will decrease the incidence of death or BPD".

If our hypothesis is correct, this study will provide evidence for beneficial effects of early diagnosis and treatment of pulmonary hypertension in preterm infants to minimize the risk of BPD. Our proposed RCT may lead to change in clinical practice.

Study Objectives

Primary Objective/Aim/Goal/Hypothesis:

Primary objective of this study is to show that iNO treatment of extremely preterm infants with early Pulmonary Hypertension (PH) defined as echocardiographic evidence of PH at 48-96 hours of age will decrease the incidence of death or BPD.

Secondary Objective/Aim/Goal/Hypothesis:

Secondary objective is to show that iNO treatment of extremely preterm infants with early PH will decrease the pulmonary artery pressure and improve the oxygenation within 72 hours of intervention. The change in pulmonary artery pressure and oxygenation from

initiation of treatment () to 3 days later (approximately 72 hours later) will be compared for the two treatment arms.

Study Design

Research Design

This research will be carried out as a masked randomized controlled trial. Randomization will be designed by our study statistician (Dr. Julie Pepe). Study population will be extremely preterm infants with early pulmonary hypertension diagnosed by echocardiography at 48- 96 hours of age. They will be randomized to either iNO or placebo.

Study Agent, Device, and/or Intervention Description

Inhaled nitric oxide (iNO) will be used as the intervention agent. This therapy is currently used in infants with late pulmonary hypertension that developed as a complication of BPD. Mallinckrodt Corporation, the manufacturer and supplier of iNO has agreed to provide the iNO as well as the placebo gas tanks at no cost for the study.

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	*X*	*X*	
21 CFR 54	*X*	*X*	
21 CFR 210	*X*		
21 CFR 211	*X*		
21 CFR 312	*X*		
21 CFR 812		*X*	*X*
21 CFR 820		*X*	

An IND for this study has been approved by the FDA. The PI, Hussnain Mirza, MD, is the holder of the IND, #139794. This study will comply with all FDA requirements, IRB requirements, and ICH-GCP guidance.

iNO and placebo devices will be stored separate from our clinical equipment in NICU. The clinical team will not have access to the study devices. Trained, unblinded respiratory therapists will obtain the study devices after study consent is signed and randomization has occurred. The study will be conducted per GCP and FDA requirements.

Study Site(s)/Location(s) and Number of Subjects

This is a single site clinical study to be conducted at the Neonatal Intensive Care Unit of AdventHealth for Children. A total of 136 infants will be enrolled in the trial, and 68 of those infants will be randomized (34 in each arm). Screen failures, which are defined as infants who were consented but the echocardiogram showed no PH, will be enrolled into the comparison group and no further intervention will occur for them. To compare the incidence of death or BPD, outcome data will continue to be collected on the comparison group for the duration of their hospital stay.

Subject Selection

Vulnerable Populations

This research involves Neonates of Uncertain Viability. If this population was not included, the validity of the study would be compromised because the hypothesis is based on the uniquely high incidence of early pulmonary hypertension (PH) in this group which in turn is associated with a very high risk for bronco-pulmonary dysplasia (BPD), a very serious morbidity with extremely high probability of death and neurodevelopmental impairment if survived. We aim to provide intervention in the form of inhaled nitric oxide to alleviate PH and reduce the incidence of BPD. A successful trial will change practice and improve the clinical outcome of this population

We would like to point out that the survival rate of this vulnerable population (extremely preterm infants, gestational age between 23-29 weeks) has improved remarkably in the U.S. during the past decade (based on National Institute of Child Health, Neonatal Research Network statistics) because of extensive clinical trials and intervention conducted by clinical investigators in the U.S. and globally. These studies were all approved by the local IRBs with the notion that research such as our proposed trial will provide evidence to guide our practice in enhancing the rate of intact survivors among this vulnerable population.

Our neonatologists do not determine the viability of any newborn in the delivery room or during the code. We make all decisions to offer limited life support or comfort care well before the delivery if any fetus has known congenital anomaly that will not be compatible with life. We also offer limited or comfort care only for infants born before 24 weeks gestation if the family make that decision during perinatal consultation with the neonatologist. For infants that code in the delivery room or in the unit, Neonatal Resuscitation Program (NRP) Guidelines are followed. The entire team (MD, ARNP, RN, RT) is involved when resuscitation is decided to be ineffective and is stopped.

Withdrawal of life support is an extremely rare occurrence in our NICU that requires two neonatologists to independently assess the clinical status of an infant. Dr. Mirza, will not participate in determining the withdrawal of life support or DNR for any infant enrolled in this study.

Inclusion Criteria

There will be two steps of inclusion and exclusion criteria:

Step 1:

Inclusion Criteria:

These infants are eligible for echocardiographic screening of early pulmonary hypertension:

1. Extremely preterm infants born at $23^{0/7}$-$29^{6/7}$ weeks gestation admitted to our neonatal ICU
2. Positive pressure ventilation at 48-96 hours of age, irrespective of oxygen concentration via ETT or CPAP

Exclusion Criteria:

1.Death prior to the 48 hours of age

2.Chromosomal or major congenital anomalies

3. Myocardial Dysfunction seen on screening echocardiogram

4. Infants dependent on right to left shunting of blood or any complex cardiac defect

5. Mother is COVID-19 positive

Step 2:

Inclusion criteria

- Infants with early pulmonary hypertension will be randomized to iNO treatment or placebo at 48 -96 hours of age.

Exclusion criteria:

Infants whose echocardiogram done at 48-96 hours shows at least one of the following:

- PH exclusively due to excessive pulmonary blood flow (left to right shunting due to large PDA and normal septal position and no significant TR without bidirectional shunt)
- Pulmonary blood flow obstruction secondary to pulmonary vein stenosis, mitral valve stenosis, cor triata, and aortic valve atresia.

Resources Available

The PI will meet with the research team prior to commencement of the study to discuss the protocol and address any questions about the protocol or individual duties and functions. Team members will meet as needed for the duration of the study to address any questions or concerns that may arise. Research personnel in the study have completed all training requirements to participate in research as required by AdventHealth. The study will be conducted in accordance with the protocol and Institutional Review Board (IRB).

The PI and Co-Is have worked together in conducting the observational study that successfully obtained the preliminary data for the current randomized controlled trial.

The experience enhances the successful implementation of the current research application. In regard to the patient resource, in 2016, our NICU admitted 132 extremely preterm infants. Over a three-year proposed study period, we will have 362 available subjects for enrollment into our trial.

We will secure the collaboration of our Echocardiography department in conducting the echocardiograms as described in the protocol.

We have received three years of funding for this study from the Thrasher Foundation.

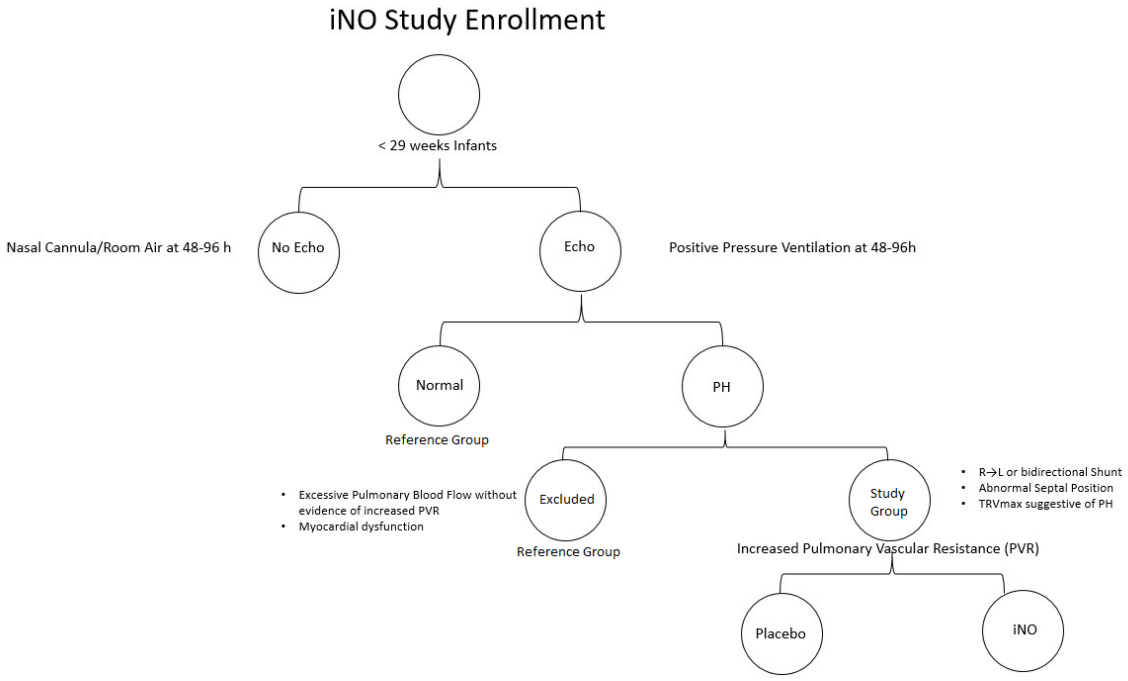
Study Procedures

Subject Recruitment and Screening

The study will commence after IRB approval and OSP clearance and full execution of agreements with Thrasher Foundation and Mallinckrodt. The research team members indicated on the

delegation log will screen the NICU census daily for infants who may be eligible for the study. If an infant is identified as potentially eligible through review of the medical records, permission will be sought from the attending neonatologist to approach the parents about study participation. After informed parental consent by a research team member, all eligible infants (per step 1 inclusion criteria above) will be screened by echocardiography at 48-96 hours of life to identify infants with early PH. If an echocardiogram is ordered by the clinical team at this time point, a study echo will not be performed and the data will be retrieved from the clinically indicated echo. If an infant is critically ill and unable to tolerate the procedure as determined by attending clinicians, echocardiogram can be delayed up to 96 hours of age. Any significant clinical finding, e.g. pericardial effusion, large PDA, ventricular dysfunction or any major structural anomaly will be disclosed to the clinical care team. All infants will be managed by the clinical teams in NICU. Eligible infants (per step 2 inclusion /exclusion criteria cited above) will be enrolled in the randomized controlled trial for treatment with iNO or placebo as shown in Figure 1. Infants who have no evidence of pulmonary hypertension on the screening echocardiogram will have no further research intervention activity, but their data will be collected (comparison(reference) group).

Figure 1: Study Enrollment Plan



Diagnosis of PH will be based on peak velocity of tricuspid regurgitation (TR_{max}), PDA gradient or end systolic interventricular septal position.[5] We will calculate right ventricular (RV) pressure gradient by modified Bernoulli equation (RV pressure = 4 x ($TR\ max^2$). Pulmonary artery pressure

(PAP) will be estimated by adding 5 to the RV gradient (to adjust for right atrial pressure). In the absence of measurable TR_{max}, PAP can be estimated by calculating the PDA gradient and measuring the concurrent systemic blood pressure (sBP) i.e. sBP- (4 x $PDAmax^2$) for left to right shunting across PDA or sBP+ (4 x $PDAmax^2$) for right to left or bidirectional shunting. In the absence of TR_{max} or PDA, PAP can be estimated by the end systolic interventricular septal configuration at the papillary muscle level in short axis view from multiple acoustic windows i.e. left parasternal, right high parasternal and subcostal. PH will be categorized as moderate if estimated pulmonary artery pressure (PAP) is more than half but less than systemic systolic blood pressure or if the septal position is flat. PH will be considered severe if PAP $\geq$ sBP or septal position is paradoxical.

Data Collection:

Demographic and clinical data will be prospectively collected from maternal and neonatal charts along with data from serial echocardiograms. Following variables will be included in the database

- **Maternal Data:** Age, ethnicity, educational level, smoking, alcohol or illicit use of drugs, medication history, any medical or surgical problem, antenatal screening lab results (GBS, HIV, HBs Ag, RPR) maternal UTI, chorioamnionitis, rupture of membrane, vaginal bleeding or discharge, antenatal antibiotics, steroids, indomethacin or magnesium.
- **Neonatal Data: BPD our primary outcome is determined based on the need for positive pressure ventilation or supplemental oxygen after room air challenge test** Gestation, Gender, mode of delivery, Apgar scores, IUGR, Birth Weight, Surfactant, Ventilation, pulmonary hypertension (early, intermittent or late), use of supplemental oxygen, culture proven infections, blood transfusions, Cardiotropic medication, bicarb infusion, saline boluses, use of indomethacin, PDA treatment/ ligation, use of diuretics, use of postnatal steroids, IVH, PVL, Post IVH hydrocephalus, NEC, ROP, ROP requiring treatment, VP shunt, Tracheostomy, Gastrostomy tube, home oxygen, home ventilation, length of hospital stay and death.

Consent Process

All consent form processes will adhere to HRP 802 and 803 guidance. This study will be explained to the parent/s and all questions will be answered. The study team member will ensure the parent/s understand the information and have sufficient time to review the consent. The signing of the consent will be as indicated on the signature page of the full consent, with a parent or LAR signing on the first line and the signature of a second parent signing unless one of the indications of why a second parent is not signing is checked, as is standard on the consent template for pediatric studies. The parent/s or LAR will be given a copy of the signed consent.

Non-English-Speaking Subjects

In the event a non-English speaking family is identified as having a child who is a potential candidate, the IRB Short Form procedures will be initiated. A certified interpreter will be used to discuss the study with the parents or legal guardian. The consent form has been translated into Spanish and Creole and will be used to enroll Spanish/Creole speaking subjects.

Documentation of Informed Consent Process

Documentation of the informed consent process is required to establish that the subject was accurately and adequately informed and that no study-related procedures were initiated prior to obtaining informed consent. A research team member will note in the source documentation the consent process, date consent was obtained, and that consent was obtained prior to initiating any research procedures.

Waiver of Written Documentation of Consent or Waiver of Consent

N/A

Randomization

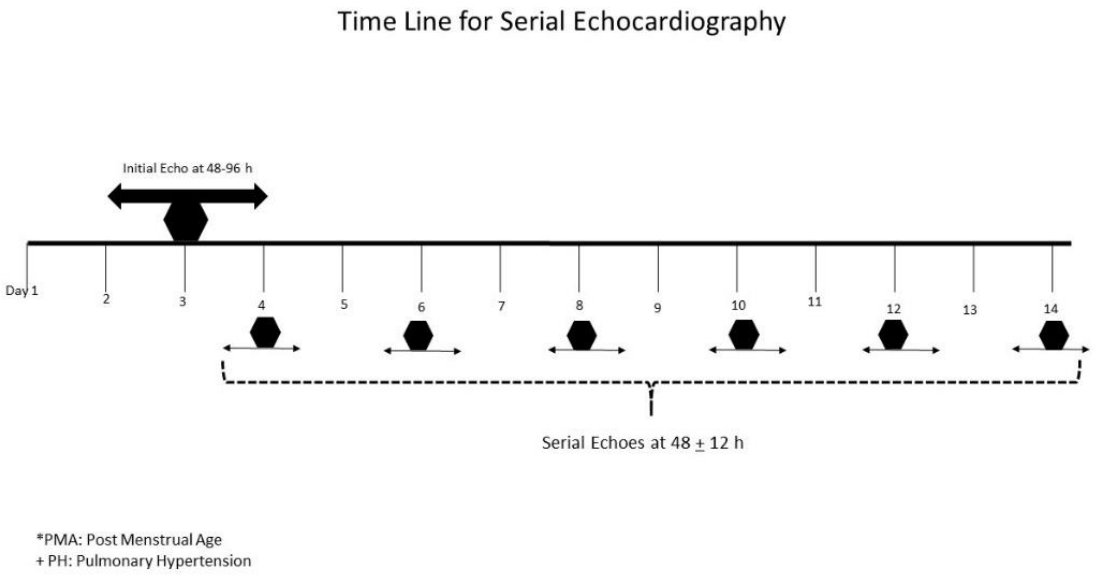
Randomization into 2 groups will be done using statistical software (SAS or Excel). To keep the balance between treatment and placebo groups, we will use stratified permuted block randomization. Randomization will be stratified by gestational age (23 $^{0/7}$-25 $^{6/7}$ weeks, 26 $^{0/1}$-29 $^{6/7}$ weeks) and severity of PH (moderate & severe). Because the number of enrolled infants per stratification group is unknown at the start of the study, additional envelopes will be generated with randomization sequences of various block sizes balanced between placebo and treatment. The envelopes will remain sealed until the infant has been enrolled in the study. The envelopes will be kept in a secured office in the RT department that will be accessible to only trained, unblinded RT personnel. Documents regarding the randomization process will be kept in the RT department. The randomization envelope will be pulled by one of the trained, unblinded RT personnel who will obtain the corresponding treatment/placebo tank prior to the initiation of intervention.

Study Visits:

Infants with early pulmonary hypertension will be randomized to the treatment group or the placebo group (see randomization plan). iNO or placebo treatment will start at 20 ppm. Treatment will continue for 2 weeks or until resolution of pulmonary hypertension, whichever comes first.

To ensure the safety and evaluate the clinical response to iNO treatment, we will perform serial echocardiograms as shown in figure 2.

Figure 2



Since the data of SpO_2 & FiO_2 are available in all infants, their ratios (SF ratio) will be calculated as markers of improved oxygenation. SF ratio has the advantage of avoiding invasive arterial blood sampling in situation when umbilical arterial line is unavailable. It is a reliable noninvasive surrogate for PaO_2: FiO_2 ratio to identify children with severe lung disease.[28, 29] Respiratory Severity Score (RSS= FiO_2 X Mean airway pressure (MAP)) will be monitored for infants on mechanical ventilation since the data of mean airway pressure will be available. Significant response to iNO treatment will be defined as 20% improvement in SF ratio or resolution of pulmonary hypertension (assessed by echo) within 72 hours. Serial echocardiograms will be performed every 24-48 ± 12hours until 14 days of life irrespective of the length of treatment.

If PH is resolved during the 2-week treatment window or after the completion of the 2-week treatment protocol, we will start weaning the iNO dose by decreasing to 15 ppm. Weaning will continue to 10, 5, 4, 3, 2 and 1 ppm every 4 hours. During the weaning process if SF ratio decreased by 20%, iNO /Placebo dose will be increased to the minimum dose where FiO_2 and PaO_2 were stable. Weaning can be resumed after 12 hours following the above guidelines. The iNO weaning process described above is the current standard of care in our NICU for infants with PH. If pulmonary hypertension recurs in the first 2 weeks of life after stopping the treatment, iNO/Placebo treatment will be resumed based upon the randomization at the time of enrollment.

About 1/3 of term and near-term infants with pulmonary hypertension may not respond to iNO. We will evaluate the effectiveness of iNO treatment by recording changes in serial echocardiogram and in mean airway pressure (MAP), FiO_2, SpO_2/FiO_2 ratio, and oxygenation index (OI) before and at 24 $\pm$ 6, 48 $\pm$ 6, and 72 $\pm$12 hours after beginning therapy. Non-responders will be defined as no improvement in pulmonary artery pressure or cardiac function on three serial echocardiograms and lack of 10% improvements from baseline for MAP, FiO_2, SpO_2/FiO_2 ratio and OI at 72 $\pm$ 12 hours after starting treatment. Once an infant is identified as a non-responder, we will start weaning treatment (iNO or placebo) as described in our iNO weaning plan given below. It is important because even in “non-responders,” one can still see significant rebound pulmonary hypertension with iNO discontinuation. If pulmonary hypertension is noted on subsequent echocardiograms, study intervention will not be resumed for non-responders. However, there is no restriction on clinical team to start iNO if needed based on clinical criteria.

During the course of hospitalization, all infants will receive standard of care and will be monitored as per NICU monitoring. Per AdventHealth NICU standardized guidelines, target oxygen saturations for ≤ 32 weeks PMA is 86-92%. We will follow these guidelines while the subject is receiving supplemental oxygen.

Study Duration

The study will commence as soon as we receive IRB approval and OSP clearance. The project enrollment period will last approximately three years with one year to clean the data and analyze results.

Study Outcome Measures (Endpoints)

- Maternal demographics (listed under the data collection section of the protocol)
- Infant measures (listed under the data collection section of the protocol)

- Incidence of death or BPD measured at 36 weeks post menstrual age (yes or no)
    - BPD will be defined as need for supplemental oxygen for at least 28 days. Severity of BPD will be determined at 36 weeks postmenstrual age. Infants with no need for supplemental oxygen will be classified as mild BPD. Continued requirement for supplemental oxygen (<0.3 FiO2) will be considered moderate BPD. Infants requiring >0.3 FiO2 and or positive pressure respiratory support will be diagnosed with severe BPD. The need for continuing oxygen therapy will be determined by a room air challenge. We will prospectively collect data that will allow us to define BPD as recommended by the 2018 NICHD report
- Pulmonary arterial pressure based on serial echocardiograms)
- Oxygen saturation level (average for each 24-hour period after enrollment)
- Arterial pressure and oxygen saturation will be measured up to 2-weeks or until the treatment is discontinued, whichever is shorter.

Data Management and Quality Plan

Data de-identification

Each subject will be assigned a unique study identification number (001, 002, 003, etc.) for data collection. A master list will be created to link the subject's MRN with the study number. The master list will be saved electronically in a password protected file on an AdventHealth server accessible only to study team members.

Data Confidentiality, Storage, and Retention

Study documentation and paperwork will be stored in the locked Pediatric Research office and access will be restricted to the research team. The data file will be stored electronically on a password protected secure server at AdventHealth accessible only to the study team. Study documentation will be retained for 7 years after completion of the study. After that period, paper records will be placed in hospital approved shred bins. Electronic records will be placed in a computer electronic trash bin followed by immediate emptying of the electronic trash bin.

Data Quality

Quality control procedures for this research study include source data verification by randomly selecting 10% of subject records with comparison between the paper case report form (CRF) and the electronic database record of those same data. If errors are common, data will be completely checked prior to data analysis.

Data Sharing

N/A

Sample Size Determination

Sample size was calculated based on our pilot study showing that the incidence of death or BPD among extremely preterm infants with early pulmonary hypertension was 80%. Assuming a 30% relative decrease of the incidence we came up with a sample size of 34 infants in each arm with 80% power and alpha of 0.05.

Statistical Analysis Plan

Primary Objective Analysis

Data will be analyzed to define the demographic and clinical characteristics of the two study cohorts (iNO and placebo) and the infants without early PH (not enrolled for treatment, please see Fig. 1). Chi square test for categorical variables and student's t test or non-parametric equivalent test for continuous variables will be used. Relative Risk (with 95% CI) for death or BPD will be calculated for the placebo and iNO treatment groups. The outcomes of infants without early PH will also be compared with the two study cohorts (iNO and placebo). Logistic regression will be performed to adjust for significant co-variables if needed (unlikely because of randomization and stratification). Similarly, treatment and placebo groups will be compared to determine the short-term benefits of iNO treatment for PH in preterm infants.

Secondary Objective Analysis

We will analyze the change in pulmonary arterial pressure by serial echo within 72 hours of initiation of iNO. We will also compare the pulse oximeter to inspired oxygen ratio.

Potential Risks and Benefits

Potential Benefits

If our primary hypothesis is correct, the infant enrolled in the iNO arm may have the benefit of being a survivor without BPD. The infant in the placebo arm will have the same risk of surviving with BPD as in current standard of care.

Potential Risks:

A potential risk associated with iNO is methemoglobinemia. This risk is minimal as we are using low concentrations of iNO. Occasionally an extremely preterm infant is unstable and performing echocardiogram could cause oxygen desaturation and a temporary increase in FiO2 requirement. Heart failure has been described as a potential risk for iNO treatment. However, this complication is noted only among the patients with ischemic heart disease or myocardial dysfunction. No severe side effects of iNO have been reported at the starting dose of 20 ppm.[30] We will not include any infants with myocardial dysfunction noted on the initial echocardiogram. We will start weaning iNO treatment if worsening of hemodynamic status is noted on serial echocardiograms based upon the recommendations of our cardiologists. Very rarely, nitric oxide can chemically react with oxygen to make nitrogen dioxide (NO_2) that can cause inflammation of the airways if the level exceeds beyond 5 parts per million.

Mitigation of Risks:

We will measure levels of methemoglobin with blood gases as is current standard of care in the NICU. The first methemoglobin level will be checked within 4-8 hours of treatment initiation, and then repeated every 24-48 hours during the intervention. Methemoglobinemia can be reversed by stopping the iNO.

Our device has an alarm set at 0.7ppm for live monitoring of NO_2 that is set well below the threshold for any adverse effect.

Study team will seek clearance from the clinical team before performing echocardiogram to ensure the safety of the infant. If an infant is clinically unstable, study echocardiogram can be delayed or cancelled

During the course of study, the attending clinicians may request unmasking of intervention (iNO or placebo) only if oxygenation index is higher than 40 for at least 4 hours or if there is risk of

death due to hypoxic respiratory failure without starting iNO. If the infant is on iNO, treatment will continue. If the infant is on placebo, iNO treatment may be allowed. The PI will be notified immediately. A protocol violation will be filed and intention to treat principle will apply.

Provisions to Protect the Privacy Interest of Subjects

Subjects will be assigned a study number for collecting data. All precautions will be taken to make sure that only authorized individuals will have access subject research records. The collection of sensitive information about subjects will be limited to the minimum necessary to achieve the aims of the research and no unnecessary sensitive information will be collected.

Early Withdrawal of Subjects

Investigator Withdrawal of Subjects

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A. The following stopping criteria will be followed: Patient experiences a serious adverse event (defined per regulation) related to study drug
B. Patient experiences any one of the following:
    I. Pulmonary edema due to worsening of left ventricular dysfunction
    II. Elevated met Hb level (>5%) on 2 samples 6 hours apart or > 7.5% on a single reading
    III. If nitrogen di-oxide (NO2) concentration is more than 3 ppm for 6 hours or more than 5 ppm on a single reading
C. Investigator judgement

Subject Request for Withdrawal from Study

If a parent would like to withdraw their infant from the study, the Withdraw from Study form will be given to the parent/s to complete and the infant will be removed from the study.

Criteria for Cessation of Enrollment Pending DSMB Review of Safety Data

1. Two or more infants experience an SAE related to iNO treatment
2. Two or more infants are discontinued from the study due to safety concerns

Data Collection and Follow-up for Withdrawn Subjects

Subjects who are withdrawn from the study will not be included in the final analysis. All AEs and SAEs will continue to be collected on withdrawn subjects throughout hospitalization, and that data will be reviewed by the DSMB when necessary.

Adverse Event Reporting

An adverse event is defined as “any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.”

A serious adverse event is defined as an adverse event “if, in the view of the investigator, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.”

Adverse events that meet criteria for reporting will be submitted to the FDA and/or IRB as applicable. Given this high-risk patient population, a number of adverse events are expected. The key monitoring function will be to assess whether evidence is accruing that suggests a differential adverse event rate associated with treatment. AE’s collected include elevated met hemoglobin or nitrogen dioxide, rebound pulmonary hypertension after discontinuing iNO, myocardial dysfunction/heart failure, hypotension, pulmonary hemorrhage, pneumothorax, PDA (if treated), NEC (stage 2b or 3), culture proven sepsis (treated with antibiotics ≥ 5 days), IVH (grade III or IV), ROP (severe), death, cardiac arrest or other life-threatening events. These adverse events will be collected from the time the subject starts intervention or placebo through the end of study participation (at 36 weeks corrected gestational age) and will be documented in the subject’s chart.

Serious Adverse Events (SAE) and Adverse Events related to iNO Treatment:

Severe adverse events of special interest include one or any combination of the following

1. Pulmonary edema due to worsening of left ventricular dysfunction (occur when infants with pre-existing LV dysfunction are treated with iNO)

2. Elevated met Hb level (>5%) on 2 samples 6 hours apart or > 7.5% on a single reading
3. If nitrogen di-oxide (NO2) concentration is more than 3 ppm for 6 hours or more than 5 ppm on a single reading

Methemoglobinemia and elevated NO2 levels are dose dependent adverse events seen among infant treated with higher dose (>20ppm)

Adverse Events of Special Interest related to iNO treatment will be defined as one or any combination of the following

1. Rebound Pulmonary Hypertension Syndrome: This is seen after the abrupt withdrawal of iNO. Signs and symptoms of Rebound Pulmonary Hypertension Syndrome include hypoxemia, systemic hypotension, bradycardia, and decreased cardiac output. If Rebound Pulmonary Hypertension occurs, reinstate iNO therapy immediately.
2. Other rare adverse events may include thrombocytopenia (Platelets <50,000), hypokalemia (< 2.5 mEq/dl), hyperbilirubinemia while on study intervention (at phototherapy threshold for age and gestation) in absence of other known risk factors, atelectasis while on study intervention (as noted on chest x ray with lung collapse) and hypotension (below 5th centile for age and gestation)

Severity Grading School for Adverse Events:

Adverse event severity will be graded as below

Classification	Examples
Mild (Grade 1) infants are asymptomatic or have mild symptoms associated with study intervention that require clinical or diagnostic observations only; no intervention indicated.	• Methemoglobinemia >2% • NO2 > 1.5 • Hypokalemia <3 • Left Ventricular Dysfunction: Ejection Fraction decrease by 20% • Thrombocytopenia: Platelets <100,000 • Hyperbilirubinemia in the absence of other known risk factors that requires single phototherapy • Mild Hypotension (mean BP < # gestation weeks) that requires no treatment
Moderate (Grade 2) if infants have moderate symptoms that require discontinuing study intervention, non-urgent treatment or intervention	• Methemoglobinemia >3% • NO2 > 2 • Hypokalemia <2.7 • Left Ventricular Dysfunction: Ejection Fraction decrease by 30% • Thrombocytopenia: Platelets <50,000

	• Hyperbilirubinemia in the absence of other known risk factors that requires double phototherapy • Moderate Hypotension (mean BP < # gestation weeks) that requires IV fluid boluses after starting intervention
Severe (Grade 3): Infants developed severe or medically significant symptoms that is not immediately life threatening but require treatment or intervention.	• Methemoglobinemia >5% • NO2 > 3 • Hypokalemia <2.5 • Left Ventricular Dysfunction: Ejection Fraction decrease by 40% • Thrombocytopenia: Platelets <30,000 • Hyperbilirubinemia in the absence of other known risk factors that requires triple phototherapy • Severe Hypotension (mean BP < # gestation weeks) that require single cardiotropic infusion after starting the study intervention
Life Threatening (Grade 4): A complication that is life threatening and requires urgent intervention	• Methemoglobinemia >7.5% • NO2 > 5 • Hypokalemia <2 • Left Ventricular Dysfunction: Ejection Fraction decrease by 50% • Thrombocytopenia: Platelets <30,000+ skin or mucosal bleeding • Hyperbilirubinemia in the absence of other known risk factors that requires exchange transfusion • Severe Hypotension (mean BP < # gestation weeks) that require multiple cardiotropic infusions after starting the study intervention
Death (Grade 5): Any death that is directly related to study intervention	• Any death that cannot be explained by the existing clinical status (Sepsis/ resistant respiratory failure etc.) and directly related to iNO toxicity (all points mentioned above)

Relatedness/Causality of an adverse event will be determined and classified below

Classification	Definition
Related	There is an association between the event and the administration of investigational study drug, plausible mechanisms for the event to be related to the investigational study drug and causes other than the investigational study drug have been ruled out, and/or the event reappeared on re-exposure to the investigational study drug.
Possibly related	There is an association between the event and the administration of the investigational study drug and there is a plausible mechanism for the event to be related to investigational study drug, but there may also be alternative

	etiology, such as characteristics of the subject's clinical status or underlying disease.
Unlikely related	The event is unlikely to be related to the investigational study drug and likely to be related to factors other than investigational study drug.
Not related	The event is related to an etiology other than the investigational study drug (the alternative etiology will be documented in the study subject's medical record).

All SAEs should be reported to the PI and study Coordinator within 48 hours. Deaths should be reported to the PI and study Coordinator within 24 hours. The medical monitor will then be notified and will review the event. After review, additional notifications will be made to the DSMB, IRB, and FDA as necessary. When deemed necessary by the medical monitor, the DSMB will be notified of any significant events immediately and a meeting will take place to determine appropriate actions.

Known Safety Profile of iNO:

Controlled studies have included 325 patients on iNO doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on iNO. In both the NINOS and CINRGI studies, the duration of hospitalization was similar in iNO and placebo-treated groups. From all controlled studies, at least 6 months of follow-up is available for 278 patients who received iNO and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae. In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage. In CINRGI, the only adverse reaction (>2% higher incidence on iNO than on placebo) was hypotension (14% vs. 11%).

Nitrogen dioxide (NO_2) forms in gas mixtures containing iNO and O_2. Nitrogen dioxide may cause airway inflammation and damage to lung tissues. If the concentration of NO_2 in the breathing circuit exceeds 5 ppm, decrease the dose of iNO. During iNO treatment, NO_2 shall be continuously monitored. Methemoglobinemia is a function of time and nitric oxide dose in neonates. The methemoglobin (MetHb) concentration and time profiles during the first 12 hours of exposure to 0, 5, 20, and 80 ppm of iNO are shown below.

Safety Monitoring Plan

Safety Monitoring

A head ultrasound will be completed prior to the initiation of the study product. As standard of care in our NICU for this population, head ultrasound will be performed for intraventricular hemorrhage (IVH) screening at approximately 7 days of life and at approximately 36 weeks corrected gestational age. or prior to discharge. Real time monitoring for NO_2 will be available. Methemoglobin level will be checked within 4-8 hours from initiation of intervention and then every 24-48 hours during the intervention. In addition to the primary and secondary study outcomes, we will monitor all infants for AE's as defined above.

Data and Safety Monitoring Board (DSMB) or Equivalent

A data safety monitoring committee (DSMC) will be established for this trial. Safety data will be monitored by this committee at 50% enrollment, and AEs will be reviewed by the DSMC during the meeting. The DSMC will review the event information and determine if any action is necessary. The DSMC may stop the study or put the study on hold pending further review.

Ethical Considerations N/A

Sharing of Results with Subjects N/A

Funding Source: We received a grant from Thrasher Foundation to support this study. We have also received iNO and placebo gas tanks for the study from Mallinckrodt Pharmaceuticals.

Subject Stipends or Payments N/A

Publication Plan

At the completion of the trial, we will prepare an abstract for presentation at a national pediatric meeting and then a manuscript will be prepared for submission to a peer review journal.

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