Milrinone in Congenital Diaphragmatic Hernia

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Contents

SECTION 1. ABSTRACT

Introduction: Infants with congenital diaphragmatic hernia (CDH) usually have pulmonary hypoplasia and persistent pulmonary hypertension of the newborn (PPHN) leading to hypoxemic respiratory failure (HRF) that is often associated with cardiac dysfunction. Pulmonary hypertension associated with CDH is frequently resistant to conventional vasodilator therapy including inhaled nitric oxide (iNO). Increased pulmonary vascular resistance (PVR) can lead to right ventricular overload and dysfunction (Patel 2012, James, Corcoran et al. 2015). In patients with CDH, left ventricular dysfunction, either caused by right ventricular overload or a relative underdevelopment of the left ventricle, is associated with poor prognosis. Right ventricular dysfunction in CDH is also associated with poor prognosis (Moenkemeyer and Patel 2014). Milrinone is an intravenous inotrope and lusitrope (enhances cardiac systolic contraction and diastolic relaxation respectively) with pulmonary vasodilator properties and has been shown anecdotally to improve oxygenation in PPHN (Bassler, Choong et al. 2006, McNamara, Laique et al. 2006, Bassler, Kreutzer et al. 2010, McNamara, Shivananda et al. 2012). Milrinone is commonly used during the management of CDH although no randomized trials have been performed to test its efficacy. Thirty percent of infants with CDH in the Children's Hospital Neonatal Database (CHND) (Grover, Murthy et al. 2015) and 22% of late-preterm and term infants with CDH in the Pediatrix database (Malowitz, Hornik et al. 2015) received milrinone. In the recently published VICI trial, 84% of patients with CDH received a vasoactive medication. (Snoek, Capolupo et al. 2016)

Objective: This is a Phase II pilot trial to determine if milrinone infusion in neonates ≥ 36 weeks' postmenstrual age (PMA) at birth with CDH would lead to an increase in PaO_2 with a corresponding decrease in OI (or OSI) by itself or in conjunction with other pulmonary vasodilators such as iNO at 24 h post-infusion. (Note: oxygenation index (OI), = Mean airway pressure x FiO_2 x 100 ÷ PaO_2 and oxygen saturation index (OSI) = Mean airway pressure x FiO_2 x 100 ÷ preductal SpO_2).
As secondary objectives, we will determine if milrinone improves oxygenation at 48 h and 72 h post-infusion, reduces right ventricular pressures on echocardiogram, and alters the risk of systemic hypotension, arrhythmias, intracranial hemorrhage, need for extracorporeal membrane oxygenation (ECMO), and chronic lung disease (CLD, defined as oxygen need at 28 d of postnatal age). Finally, we will also evaluate the infant's medical (pulmonary and nutritional) status at discharge and conduct a phone questionnaire at 4, 8, and 12 months of age.

Study Design: Double-blind randomized controlled pilot trial with 1:1 (treatment: control) randomization.

Eligibility criteria:

Infants are eligible if they meet all of the following criteria:

(i) ≥ 36 0/7 weeks postmenstrual age (PMA) at birth by best obstetric estimate AND birth weight of ≥ 2000g

(ii) postnatal age ≤7 days (168 hours of age, including post-operative infants with HRF)

(iii) invasive mechanical ventilation (defined as ventilation with an endotracheal tube) and

(iv) one arterial blood gas with an OI ≥ 10 (after tracheal tube obstruction, pneumothorax and other easily resolvable mechanical causes for increased OI are ruled out) on the most recent arterial blood gas within 12 hours prior to the time of randomization.

(v) if an arterial blood gas is not available at the time of randomization, a preductal OSI of ≥ 5 can be used as an inclusion criterion instead of OI ≥ 10; (the OSI should be based on the most recent preductal pulse oximetry recording and must be within 12 hours of randomization)

(vi) postnatal blood gas with PCO_2 ≤ 80 mmHg (arterial, capillary or venous blood gas) on the most recent blood gas sample obtained within 12 hours prior to randomization

Note: Criteria (iv) to (vi) must be met at the most recent analysis within 12 hours prior to randomization.

Stratification: Patients will be stratified based on severity of HRF using the prediction formula (PaO_2/FiO_2 ratio – $PaCO_2$ value) at the time of randomization. (Park, Lee et al. 2013) Patients with prediction value ≥ 0 will be considered as "moderate" and infants with a value of < 0 as "severe" HRF. If no arterial blood gas is available prior to randomization, an oxygen saturation index (OSI – see below) of 5 to 10 is considered "moderate" HRF and > 10 is considered "severe" HRF.

In addition, the following markers of disease severity will be recorded if available in the medical chart (but not used for stratification):

(a) <u>Prenatal</u>

a. observed to expected lung to head ratio – O/E LHR, by antenatal ultrasound
b. position of the liver,
c. observed to expected fetal total lung volume (O/E FTLV) assessment by fetal MRI (Ruano, Lazar et al. 2014) (Kastenholz, Weis et al. 2016),

(b) <u>Postnatal</u>

a. oxygenation index (OI), = Mean airway pressure x FiO_2 x 100 ÷ PaO_2
b. oxygen saturation index (OSI) = Mean airway pressure x FiO_2 x 100 ÷ preductal SpO_2, (Rawat, Chandrasekharan et al. 2015)
c. evidence of pulmonary hypertension as determined by echocardiogram:
    i. velocity of the tricuspid regurgitation jet – if present,
    ii. the position of the inter-ventricular septum and

iii. direction of shunt at the patent foramen ovale – PFO and patent ductus arteriosus – PDA level

(c) Intraoperative defect size (Congenital Diaphragmatic Hernia Study, Lally et al. 2007).

Exclusion Criteria:

Infants are ineligible if they meet any of the following criteria:

- known hypertrophic cardiomyopathy
  - Note 1: infants of diabetic mothers with asymmetric septal hypertrophy can be included as long as there is no evidence of obstruction to left ventricular outflow tract on echocardiogram,
  - Note 2: infants with other acyanotic congenital heart disease (CHD) and CDH may be included in the study and will be a predetermined subgroup for analysis)
- cyanotic CHD – transposition of great arteries (TGA), total anomalous pulmonary venous return (TAPVR), partial anomalous pulmonary venous return (PAPVR), truncus arteriosus (TA), tetralogy of Fallot (TOF), single ventricle physiology – hypoplastic left heart syndrome (HLHS), tricuspid atresia, critical pulmonic stenosis or atresia etc.,
- enrolled in conflicting clinical trials (such as a randomized controlled blinded trial of another pulmonary vasodilator therapy); Note: mothers enrolled in fetal tracheal occlusion studies such as FETO may be enrolled if permitted by investigators of the fetal tracheal occlusion study; More information about FETO can be found at http://www.chop.edu/centers-programs/center-fetal-diagnosis-and-treatment/fetoscopic-endoluminal-tracheal-occlusion-feto or http://childrens.memorialhermann.org/FETO-trial/ [FETO refers to fetoscopic endoluminal tracheal occlusion and involves occlusion of fetal trachea with a balloon device at mid-gestation and subsequent removal in later gestation]
- infants with bilateral CDH
  - Note 3: infants with anterior and central defects are included in the study
- associated abnormalities of the trachea or esophagus (trachea-esophageal fistula, esophageal atresia, laryngeal web, tracheal agenesis)
- renal dysfunction (with serum creatinine > 2 mg/dL not due to maternal factors) or oligohydramnios associated with renal dysfunction at randomization; renal dysfunction may be secondary to renal anomalies or medical conditions such as acute tubular necrosis
- systemic hypotension (mean blood pressure < 35 mm Hg for at least 2 h with a vasoactive inotrope score of > 30)
- decision is made to provide comfort/ palliative care and not full treatment
- Known intracranial bleed (including the following findings on the cranial ultrasound)
  - Parenchymal hemorrhage – including blood in the cerebral parenchyma, cerebellar parenchyma or basal ganglia

  - Blood/echodensity in the ventricle with distension of the ventricle
  - Midline shift secondary to mass effect from an intracranial hemorrhage
- thrombocytopenia (platelet count < 80,000/mm^3) despite blood product administration on the most recent blood draw prior to randomization
- coagulopathy (PT INR > 1.7) despite blood product administration on the most recent blood draw (if checked – there is no reason to check PT for the purpose of this study)
- aneuploidy associated with short life span (such as trisomy 13 or 18) will not be included in the study (infants with trisomy 21 can be included in the study)
- use of milrinone infusion prior to randomization (the use of other inhaled pulmonary vasodilators such as iNO, inhaled epoprosternol, inhaled treprostinil, inhaled PGE1 and oral such as endothelin receptor antagonists is permitted – Note: it is unlikely to be on oral pulmonary vasodilators early in the course of CDH)
- ongoing therapy with parenteral (intravenous or subcutaneous) pulmonary vasodilators such as IV/SQ prostacyclin analogs (Epoprostenol – Flolan® or Treprostinil – Remodulin®) or IV phosphodiesterase 5 inhibitors (sildenafil – Revatio®) at the time of randomization. Administration of IPGE1 is not an exclusion criteria. In addition, initiation of therapy with these two classes of parenteral medications (except IPGE1) during the first 24 hours of study drug initiation is not permitted and will be considered a protocol deviation. The risk of systemic hypotension is high during the first 24 hours of study-drug (milrinone) infusion and hence parenteral administration of other pulmonary vasodilators is avoided to minimize risk of hypotension.
- Subjects already on ECMO or patients who are being actively considered for ECMO by the neonatal or surgical team
- Infants with hypoxic-ischemic encephalopathy (HIE) undergoing therapeutic hypothermia
- attending (neonatal, critical care or surgical) refusal for participation in the trial (including concern about presence of hemodynamic instability)

Study Intervention/Methods: An initial cranial ultrasound is obtained prior to initiation of study drug or within 4 hours of commencement of the study drug. A second cranial ultrasound is obtained preferably within 24 hours (maximum - within 96 hours) of completion of the study drug. Both these cranial ultrasounds are covered by the study budget. Additional head imaging may be performed based on clinical indications (but are not covered by the study budget).
An echocardiogram, if clinically indicated, ***will preferably*** be obtained prior to (or within 6 hours of) initiation of the study drug. Infants with CDH will be randomized to receive a milrinone infusion at 0.33µg/kg/min or equal volume of placebo (D5W) infusion. The dose of the study drug will be increased to 0.66 µg/kg/min 2 to 4 hours after initiation of study drug if there is no evidence of hypotension (as defined as mean blood pressure < 35 mmHg and a vasoactive

inotrope score > 30; Vasoactive Inotrope score = dose of dopamine in μg/kg/min + dose of dobutamine in μg/kg/min + 100 X epinephrine dose in μg/kg/min + 100 X norepinephrine dose in μg/kg/min + 100 X phenylephrine dose in μg/kg/min + 10000 x vasopressin dose in U/kg/min) two hours after initiation of study drug. Infusion will be continued until the OI decreases to < 7 (or OSI < 3.5 if no arterial blood gas is available). The study drug will be continued during surgery and if the patient requires either veno-arterial or veno-venous extracorporeal membrane oxygenation (VV-ECMO or VA-ECMO) if permitted by the clinical team. The maximum duration of study drug infusion is 72 h. After 70 h (if the patient is on 0.66 μg/kg/min or 72h if the patient is on 0.33 μg/kg/min) of study drug infusion (or earlier if two consecutive OIs at least one hour apart are < 7 or OSIs < 3.5 if no arterial blood gas is available), the infusion rate of the study drug is weaned and subsequently discontinued. Data from a maximum of 4 echocardiograms, **if performed for clinical indications will be recorded,** (a) first echocardiogram, preferably prior to onset of study drug or within 6 hours of initiation of study drug, (b) second echocardiogram, 6 – 36 h after initiation of study medication (c) third echocardiogram, 36 h after initiation of study medication but prior to completion or discontinuation of study-drug and (d) first echocardiogram after completion of the study drug.

Infusion of the study drug will be stopped if the mean blood pressure decreases < 35 mmHg and by > 20 mmHg (from pre-study drug mean blood pressure) after initiation of study drug and remains low for 2 hours despite treatment with > 40 ml/kg/24h of fluids and vasoactive medications (with a vasoactive inotrope score of > 30). Once the study drug is discontinued for improvement in oxygenation or a decrease in systemic blood pressure, it will not be restarted even if the condition leading to discontinuation changes (such as change in oxygenation or blood pressure). However, clinical providers can initiate open-label milrinone at their discretion. Study drug will also be stopped in the presence of renal dysfunction and/or development of a large intracranial (intraventricular with distension of the ventricle or parenchymal) bleed/echodensity.

Primary Outcome:

The primary outcome is the oxygenation response, as determined by change in OI (or OSI if no arterial blood gas is available) at 24h after initiation of study drug. In patients that require ECMO or die prior to completion of 24h from the initiation of the study drug, the last OI (or OSI if no arterial blood gas is available) prior to initiation of ECMO or death will be used for analysis. In patients without an arterial line (or if the line is lost), oxygen saturation index (OSI) will be calculated using a preductal oximeter. (Rawat, Chandrasekharan et al. 2015)

Secondary outcomes:

(i) Oxygenation response at 24 h after initiation of study drug assigning a minimum OI of 40 or OSI of 20 for subjects that need ECMO or die after initiation of study drug infusion but prior to 24h of study-drug infusion. In this analysis if the patient's OI is 30 (or OSI of 15) and is cannulated for ECMO due to hemodynamic instability, he/she will be assigned an OI of 40 (or OSI of 20). However, if the OI prior to ECMO is 47 (i.e., > 40), the higher number (47) will be used for analysis of this secondary outcome.

(ii) Oxygenation index at 48 and 72 h after initiation of study drug (or OI/OSI at the time of initiation of ECMO or immediately prior to death, for infants placed on ECMO or died before these time points),

(iii) Change in echocardiographic findings – velocity of tricuspid regurgitation, left ventricular ejection fraction, position of the interventricular septum and direction of shunt at the PDA and PFO level – prior to and 24-72h after starting the study medication. This outcome will be available only for those infants who have a pre-study drug echocardiogram and a second echocardiogram between 24-72 h after initiation of study drug. These data will be extracted from echocardiograms performed for clinical indications.

(iv) Vasoactive Inotrope score and systemic blood pressure

(v) Area under the curve for inspired oxygen after initiation of the study drug over the first 72 hours of study-drug infusion (or prior to ECMO or death, whichever is earlier) (Note: inspired oxygen and ventilator data from 4 time points per day – every 6 hours will be recorded to calculate area under the curve),

(vi) If subsequent to the study drug, any additional inotrope or pulmonary vasodilator is used (such as iNO), we will evaluate the oxygenation response to these agents. If inotropes or vasodilators were used prior to the initiation of study drug, similar values will be recorded. The OI or OSI and PaO_2/ FiO_2 ratio prior to and after initiation of the inotrope / vasodilator are recorded (these data are recorded at 6 hour intervals; the date and time of initiation of individual inotropes and vasodilators is recorded). The change in OI or OSI and PaO_2/ FiO_2 ratio in response to these agents is evaluated as a continuous variable and arbitrarily classified into responders, partial responders and non-responders similar to prior trials (NINOs 1997, 1997).

   a. complete response is defined as an increase in PaO_2/ FiO_2 ratio > 20 mmHg from baseline,
   b. partial response is an increase in PaO_2/ FiO_2 ratio of 10 to 20 mmHg from baseline,
   c. no response is defined as an increase of < 10 mmHg (or a decrease) in PaO_2/ FiO_2 ratio from baseline,

d. the site of sampling (preductal vs. postductal) is also recorded. (Gien and Kinsella 2016); right radial and ulnar arterial lines/sticks will be considered preductal; Left radial and ulnar arterial samples are usually preductal but can occasionally be postductal; umbilical arterial lines and posterior tibial lines (both sides) are always postductal.
e. in patients without arterial access, an OSI (oxygen saturation index) will be used

(vii) Supplemental oxygen at 28 d, and 56 d (or discharge, whichever comes earlier) from the hospital – primary institution or secondary institution to which patient was transferred. The use of supplemental oxygen at 28 d will be used to calculate the incidence of chronic lung disease. Chronic lung disease severity will be classified similar to the BPD classification in preterm infants at > 32 week gestation (Jobe and Bancalari 2001). Supplemental oxygen use should be continuous. Intermittent supplemental oxygen given only during feedings or for spells administered for < 60 minutes and if the infant is not receiving oxygen at any other time during the day will not be considered as "supplemental oxygen".

a. Time point of assessment: 28 d, 56 d postnatal age or discharge to home whichever comes first.
b. Mild CLD: treatment with oxygen > 21% oxygen for at least 28 days but breathing room air by 56 days' postnatal age or discharge to home whichever comes first.
c. Moderate CLD: need for > 21% but < 30% oxygen at 56 days postnatal age or discharge to home whichever comes first.
d. Severe CLD: need for ≥ 30% oxygen and/or positive pressure (high-flow nasal cannula – defined for the purpose of this study as > 2 LPM, non-invasive ventilation or nasal intermittent positive pressure ventilation – NIV or NIPPV, CPAP, or positive pressure ventilation) at 56 days' postnatal age or discharge whichever comes first.

(viii) Survival to discharge (or day 120, whichever comes earlier) without ECMO and

(ix) Clinical status – pulmonary (use of supplemental oxygen or respiratory medications – pulmonary vasodilators (sildenafil, bosentan etc.,), diuretics, methylxanthines, steroids, inhaled or nebulized steroids or bronchodilators) and nutritional (weight, length, head circumference, use of anti-reflux and prokinetic medications) at discharge from the hospital. Subsequently, a phone survey will be conducted to record the pulmonary and nutritional status of the subjects at 4, 8, and 12 months of age.

(x) Feasibility to perform a definitive trial (primary outcome – improvement in survival without ECMO) and safety (incidence of systemic hypotension, intracranial bleeding and arrhythmias)

Conclusion: Congenital diaphragmatic hernia is an orphan disease with high mortality and few randomized, masked clinical trials evaluating postnatal management. Intravenous milrinone is a commonly used medication in neonatal and pediatric intensive care units and is currently used in 17% of patients with CDH within the NRN institutions, 22% of units in the Pediatrix network and 30% of units in the CHND network. This pilot will provide data and enable further studies evaluating the efficacy of pulmonary vasodilator therapy in CDH.

SECTION 2. STATEMENT OF PROBLEM

2.1. HYPOTHESIS

The overall goal is to determine whether intravenous administration of milrinone to newborn infants ≥ 36 weeks' gestational age at birth with congenital diaphragmatic hernia (CDH) with an oxygenation index (OI) of ≥ 10 or an oxygenation saturation index (OSI) ≥ 5 will increase survival without extracorporeal membrane oxygenation (ECMO) by day 120 or discharge home (whichever comes first). The goal of evaluating a decrease in the incidence of death or ECMO would need to be tested in a large (possibly international or NRN and non-NRN centers in the US), randomized, controlled clinical trial. However, before a large randomized trial is performed, a study to evaluate feasibility, short-term efficacy and safety is required.

Our primary hypothesis <u>for the current pilot</u> is that milrinone infusion will lead to an increase in PaO_2, with a corresponding decrease in OI (or OSI) and changes in echocardiographic markers of pulmonary hypertension, either by itself or in conjunction with other pulmonary vasodilators such as inhaled nitric oxide (iNO) at 24 h post-infusion (*efficacy outcome*). We also hypothesize that milrinone will not increase the risk of systemic hypotension, arrhythmias, intracranial hemorrhage, need for ECMO, chronic lung disease (CLD,

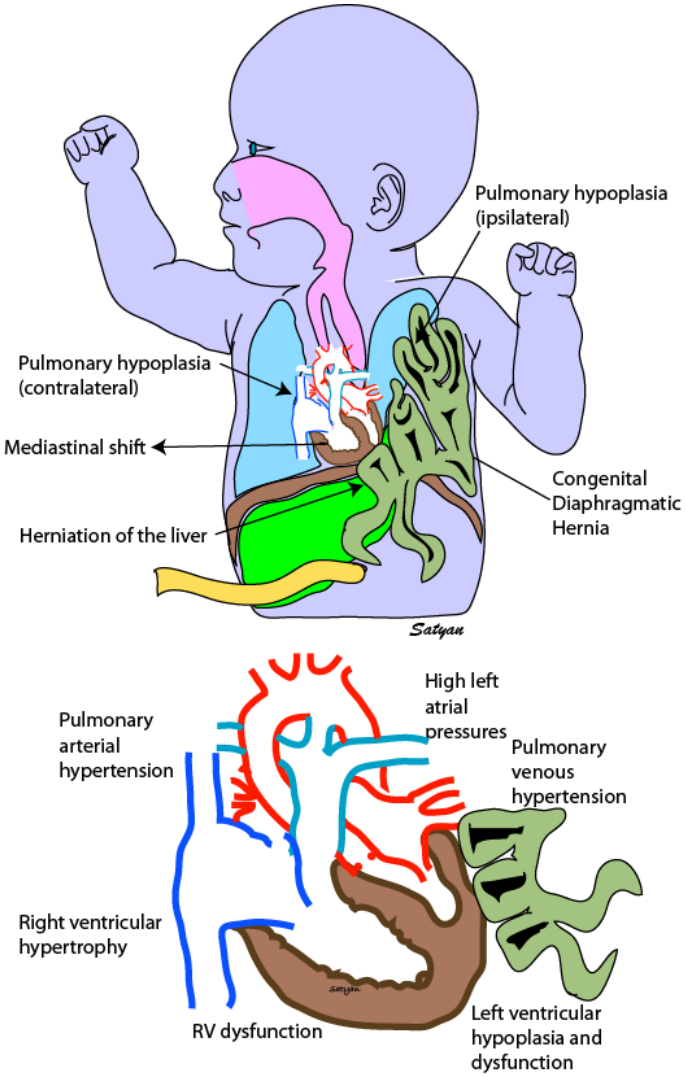

Figure 1. Pathophysiology of diaphragmatic hernia.
Herniation of abdominal contents into the thorax (commonly on the left side) results in ipsilateral pulmonary hypoplasia. Mediastinal shift leads to hypoplasia of the contralateral lung. Herniation of the liver into thoracic cavity is associated with poor prognosis. Pulmonary arterial hypertension leads to right ventricular hypertrophy and eventually right ventricular dysfunction. Left ventricular hypoplasia and dysfunction is common in fetuses with CDH and may lead to transient pulmonary venous hypertension in the immediate postnatal period.

defined as oxygen need at 28 d of postnatal age), or oxygen need at discharge from the hospital or at day 120, whichever is earlier (*safety outcomes*).

2.2. SPECIFIC AIMS

Specific aim #1 - Efficacy: To determine the acute changes in oxygenation and echocardiography during treatment with IV milrinone (improvement in OI/OSI and PaO_2 at 24 hours from baseline and changes in right ventricular pressure and function at 24 – 72 h from baseline)

Specific aim #2 - Safety: To determine the safety of IV milrinone treatment by comparing the incidence of serious adverse events (severe hypotension, bleeding - intracranial and extracranial hemorrhage and arrhythmias).

Specific aim #3 - Outcome: To determine the infant's status (pulmonary and nutritional) at discharge and conduct a series of telephonic surveys at 4, 8 and 12 months of postnatal age to assess the impact of baseline characteristics (gender, gestational age at birth, mode of delivery, birth weight, time of diagnosis – antenatal vs. postnatal), study intervention and other therapies and interventions on infant's status.

Specific aim #4 - Feasibility: To derive preliminary estimates of the incidence of survival without ECMO in the control and IV milrinone treatment groups and to demonstrate the feasibility of conducting a large randomized controlled clinical trial.

2.3. BACKGROUND

2.3.1. Congenital Diaphragmatic Hernia

Congenital diaphragmatic hernia (CDH) is one of the common serious congenital anomalies managed in the neonatal intensive care unit (NICU) (Yoder, Lally et al. 2012). Despite many technological advances over the past two decades, including high frequency ventilation, iNO, surfactant replacement therapy and ECMO, CDH presenting in the neonatal period continues to be associated with relatively high rates of morbidity and mortality (28-30%)(Keller 2012, Yoder, Lally et al. 2012) (Table 1). Survival of infants at tertiary care centers automatically selects for patients who survive birth, resuscitation, and transport from an outside facility, and therefore likely underestimates the true hidden mortality associated with CDH. The major underlying pathophysiology in such infants appears to be a combination of lung immaturity, hypoplasia, and persistent pulmonary hypertension of the newborn (PPHN), which may be further

aggravated by left ventricular underdevelopment (Schwartz, Vermilion et al. 1994, Irish, Karamanoukian et al. 1996, Aggarwal, Stockman et al. 2011, Menon, Tani et al. 2012) (figure 1). Between 7/2008 and 6/2013, CDH was the diagnosis associated with the highest mortality among patients requiring ECMO for neonatal respiratory indications (table 1 and 2). Infants with CDH have prolonged length of stay in the NICU compared to many other congenital anomalies including critical congenital heart defects (table 1).

Table 1. Survival and length of stay for babies with CDH and severe congenital heart disease cared in hospitals affiliated with Children's Hospital Neonatal Database (CHND) between June 2010 and March 2013

Condition	Discharge outcome	Number	Average length of stay	Median length of stay
Congenital diaphragmatic hernia (CDH)	Survival	341 (68%)	52 d	37 d
	Death	160 (32%)	22 d	16 d
Congenital heart disease (life threatening)	Survival	94 (73%)	24 d	16.5 d
	Death	35 (27%)	14 d	7 d

Table 2. Neonatal Respiratory ECMO Runs by Diagnosis (July 2008 – June 2013)
Data from ELSO registry – date of access July 01, 2013

Diagnosis	Total Runs (% total)	Survived	% Survived
Congenital diaphragmatic hernia (CDH)	1263 (29.8%)	625	49.5%
Meconium aspiration syndrome (MAS)	1069 (25.3%)	1002	93.7%
Persistent pulmonary hypertension of the newborn (PPHN/PFC)	896 (21.1%)	677	75.6%
Respiratory distress syndrome (RDS)	80 (1.9%)	65	81.3%
Sepsis	248 (5.9%)	141	56.9%
Pneumonia	53 (1.3%)	29	54.7%
Air-leak syndrome	11 (0.3%)	7	63.6%
Others	612 (14.4%)	329	53.8%
Total	4232	2875	67.9%

In spite of such high mortality, only two randomized controlled trials (NINOS CDH pilot) targeting treatment of PPHN associated with CDH with iNO has been published to date (1997). This study failed to demonstrate any benefit of iNO; in fact, the need for ECMO was significantly increased in iNO treated infants. A second trial (VICI) evaluating high frequency oscillation vs.

conventional ventilation in Europe was published in 2016 (table 3) (Snoek, Capolupo et al. 2014) (Snoek, Capolupo et al. 2016). The overall mortality by one year of postnatal age was 27% in this trial. This trial showed some benefits in the conventional ventilation arm compared to HFV but was stopped prior to completion of the expected sample size.

Table 3. Morbidity and mortality in CDH associated with initial mode of ventilation (VICI trial) (Snoek, Capolupo et al. 2014) (Snoek, Capolupo et al. 2016)

Variables: corrected for center (results are presented as n (%) or median (IQR)	**HFO (n = 80)**	**CMV (n = 91)**	**p-value**
Length of ventilation (days)	13 (8–23)	10 (6–18)	0.03
Severity CLD			
No CLD	37 (46.3%)	50 (54.9%)	
Mild CLD	7 (8.8%)	13 (14.3%)	
Moderate CLD	2 (2.5%)	1 (1.1%)	
Severe CLD	9 (11.3%)	6 (6.6%)	
Died	25 (31.3%)	21 (23.1%)	0.13
ECMO (in ECMO centers only)	24 (51.1%)	16 (26.2%)	0.007
Inhaled nitric oxide	45 (56.3%)	39 (42.9%)	0.045
Sildenafil	25 (31.3%)	11 (12.1%)	0.004
Inotropics	73 (91.3%)	73 (80.2%)	0.08
Duration inotropics (days) (in survivors only)	8 (4.25- 19)	6 (3.25- 11.75)	0.02
Presence of pulmonary hypertension	57 (71.3%)	59 (65%)	0.16
Severity of pulmonary hypertension			
None	20 (25%)	29 (31.9%)	
< 2/3 systemic	9 (11.3%)	10 (11%)	
2/3 systemic – systemic	26 (32.5%)	26 (28.6%)	
> systemic	22 (27.5%)	21 (23%)	
Missing	3	5	
Switch to CMV	24		
Switch to HFV		14	
Treatment failures (hypoxemia or hypercarbia or hypotension or OI>40 or increase in inspiratory pressure	27 (33.3%)	20 (22%)	0.01

2.3.2. Etiology of Pulmonary Hypertension Associated with CDH

The histological findings of CDH show pulmonary vascular remodeling superimposed on hypoplasia or pruning of the vascular bed associated with structural and functional changes in the heart, airways and lung parenchyma (Gien and Kinsella 2016). Pulmonary hypertension associated with CDH is often severe and resistant to treatment with conventional pulmonary vasodilators such as iNO (1997) (Gien and Kinsella 2016). Poor oxygenation response to iNO and other conventional vasodilators may be secondary to:

Right ventricular dysfunction: Remodeled pulmonary vasculature in CDH results in severe PPHN and leads to RV dysfunction. Milrinone significantly improves RV diastolic function and marginally increases RV systolic velocity in CDH (Patel 2012) resulting in improved pulmonary blood flow and oxygenation. A recent case series from Royal Children's Hospital, Melbourne, Australia demonstrated that right ventricular diastolic dysfunction measured by tissue Doppler on day 1 and 2 of life correlated with length of stay and duration of respiratory support (Moenkemeyer and Patel 2013).

Left ventricular dysfunction: Abnormalities of the left ventricle have been reported in infants with CDH (Siebert, Haas et al. 1984, Schwartz, Vermilion et al. 1994). When compared to neonates with other causes of PPHN, infants with left sided CDH had significantly lower left ventricular mass assessed by echocardiography. Moreover, infants with CDH who required ECMO had a lower left ventricular mass

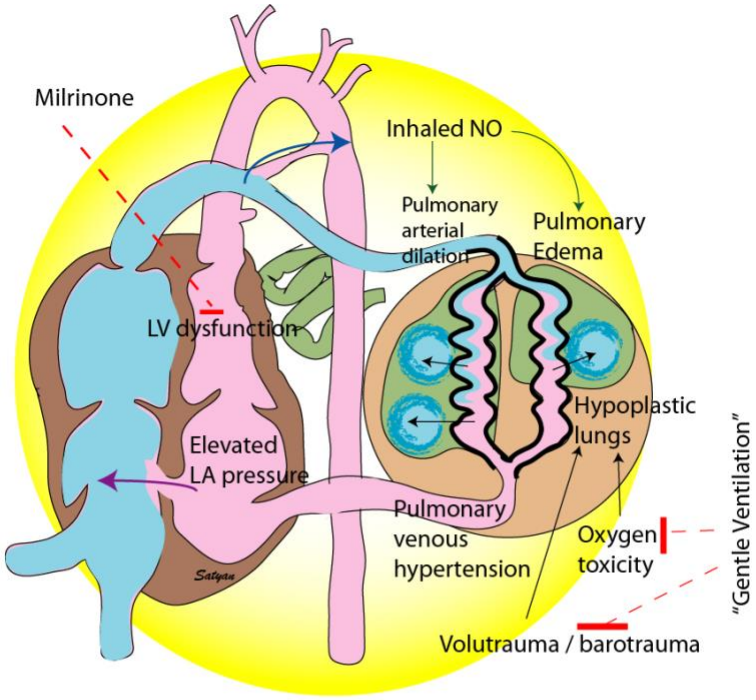

Figure 2. Patients with left sided CDH may have left ventricular hypoplasia and dysfunction. Such dysfunction may be associated with elevated left atrial pressure and pulmonary venous hypertension. Inhaled NO may result in pulmonary arterial dilation and exacerbate pulmonary edema in the presence of pulmonary venous hypertension. Milrinone, by improving left ventricular diastolic and systolic function reduces left atrial pressure and also dilate pulmonary vasculature resulting in improved oxygenation in CDH. The presence of hypoplastic lungs with remodeled pulmonary vasculature and volutrauma, barotrauma and oxygen toxicity contribute to poor response to pulmonary vasodilator therapy. We hypothesize that a combination of milrinone with "gentle" ventilation will improve oxygenation and response to pulmonary vasodilator therapy in CDH.

either by echocardiogram or at post-mortem, than those patients who did not require ECMO (Schwartz, Vermilion et al. 1994),(Siebert, Haas et al. 1984). Reduced left ventricular output has been documented in left sided and right sided CDH (Byrne, Keller et al. 2015). It has been proposed that reduced left ventricular mass contributes to functional LV hypoplasia and results in increased left atrial pressure and pulmonary venous hypertension (Kinsella, Ivy et al. 2005). This helps to explain why iNO and other pulmonary arterial dilators are perhaps less effective in diaphragmatic hernia. These agents can often worsen oxygenation in the presence of pulmonary venous hypertension and/or cardiac dysfunction (figure 2) (Lakshminrusimha 2012) in both children (Baird, Havalad et al. 2012) and adults (Bocchi, Bacal et al. 1994). Administration of an inodilator (an agent with both cardiac inotropic function and vasodilator effect) such as milrinone may improve LV dysfunction (figure 1) (Gien and Kinsella 2016) and promote pulmonary vasodilation and oxygenation (Cai, Su et al. 2008, Cai, Su et al. 2008).

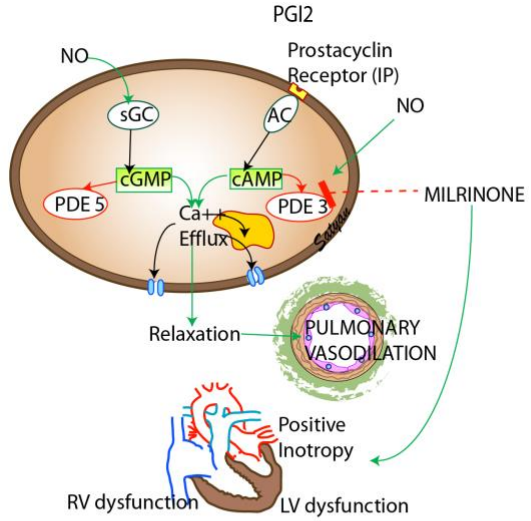

Figure 3. "Inodilator" (inotropy + vasodilator) function of milrinone: By inhibiting breakdown of cAMP in cardiac muscle, milrinone acts as an inotropic agent. Similar effect in arterial smooth muscle results in pulmonary (and systemic) vasodilation. Inhaled NO is shown to increase PDE 3 activity in animal studies. By acting through cGMP and cAMP, NO and milrinone demonstrate biochemical synergy and enhance pulmonary vasodilation.

Induction of cAMP / cGMP degrading PDE by hyperoxia and iNO: Cyclic GMP and cAMP are important second messengers that promote pulmonary arterial smooth muscle cell relaxation (Figure 3). Their concentration is regulated by specific phosphodiesterases such as the cGMP specific PDE5 and cAMP-specific PDE3. Animal models of PPHN demonstrate increased PDE3 expression, which could promote vascular dysfunction (Murray, MacLean et al. 2002). Recent laboratory studies also indicate that hyperoxic ventilation with iNO dramatically increases PDE3 activity (Chen, Lakshminrusimha et al. 2009), suggesting that these therapies may reduce cAMP and paradoxically contribute to vascular dysfunction (Thelitz, Oishi et al. 2004). Inhibiting PDE3 by milrinone enhances cAMP levels and promotes vasodilation of pulmonary arterial rings in animal models of PPHN (Lakshminrusimha, Porta et al. 2009) and following hyperoxic ventilation and/or iNO (Chen, Lakshminrusimha et al. 2009) similar to that encountered by many babies with CDH.

2.3.3. Milrinone

Milrinone is a phosphodiesterase 3 (PDE3) inhibitor with inotropic and lusitropic effects that was approved in the 1980s for intravenous use in decompensated congestive heart failure.

Vascular PDE3 breaks down cAMP in arterial smooth muscle cells and myocardium. By inhibiting PDE3, milrinone also functions as a vasodilator independent of β1-adrenergic receptor stimulation and has been called an "inodilator" because of these dual effects (figure 2 and 3) (Opie 1986, Lakshminrusimha and Steinhorn 2009, Lakshminrusimha and Steinhorn 2013). Milrinone increases cAMP levels in cardiac muscle and vascular cells, improving ventricular function both directly and by reducing afterload (Lakshminrusimha, Konduri et al. 2016). Intravenous or enteral sildenafil (a PDE5 inhibitor) is another pulmonary vasodilator with potential for use in CDH and is currently being investigated for chronic use in severe diaphragmatic hernia (NCT00133679). (Abman, Kinsella et al.)

Animal models of PPHN: In an ovine model of PPHN induced by antenatal ductal ligation, milrinone relaxed pulmonary arterial rings in a dose dependent manner (Lakshminrusimha, Porta et al. 2009). This model of PPHN is associated with significant remodeling of the pulmonary artery with smooth muscle hypertrophy and muscularization of small, distal pulmonary arteries (Wild, Nickerson et al. 1989) similar to that described in infants with PPHN and CDH and was used in preclinical studies for iNO (Zayek, Cleveland et al. 1993). In the same model, an intravenous loading dose of 100μg/kg followed by 1μg/kg/min for an hour reduced pulmonary vascular resistance from 0.503 ± 0.06 mmHg/mL/kg/min to 0.383 ± 0.03 mmHg/mL/kg/min ($p<0.05$) and was not associated with a significant decrease in systemic mean blood pressure (54 ± 3 to 51 ± 4mmHg) (Rashid, Morin et al. 2006). In lungs isolated from lambs with PPHN, protein levels of adenylate cyclase and PDE3A (the predominant PDE3 isoform in vascular tissue) were similar to control lambs without any change in PDE3A activity suggesting that the target enzymes for milrinone were unchanged by vascular remodeling in PPHN (Lakshminrusimha, Porta et al. 2009).

Milrinone improves left ventricular output following cardiac surgery: The benefits of milrinone in children following surgery for congenital heart disease have been well established in several studies (including the randomized, double-blind PRIMACORP study, n=238)(Chang, Atz et al. 1995, Bailey, Miller et al. 1999, Hoffman, Wernovsky et al. 2002, Hoffman, Wernovsky et al. 2003). The PRIMACOR study was a double-masked, placebo-controlled trial conducted in neonates and young children following cardiac surgery (Hoffman, Wernovsky et al. 2003). Three parallel groups (low dose, 25 μg/kg bolus over 60 minutes followed by a 0.25 μg/kg per min infusion for 35 hours; high dose, 75 μg/kg bolus followed by a 0.75 μg/kg per min infusion for 35 hours; or placebo) were studied. The composite end point of death or the development of low cardiac output syndrome (LCOS) was evaluated at 36 hours and up to 30 days after randomization. Among 238 treated patients, 25.9%, 17.5%, and 11.7% in the placebo, low-dose milrinone, and high-dose milrinone groups, respectively, developed LCOS in the first 36 hours after surgery. High-dose milrinone significantly reduced the risk the development of LCOS compared with placebo, with a relative risk reduction of 55% ($P = 0.023$) in 238 treated patients

and 64% (*P* = 0.007) in 227 patients without major protocol violations. There were 2 deaths, both after infusion of study drug. The use of high-dose milrinone reduced the risk of the LCOS through the final visit by 48% (*P* = 0.049). This study established that the role of high-dose milrinone after pediatric congenital heart surgery to reduce the risk of LCOS.

Case reports of milrinone use in PPHN not due to CDH: Anecdotal reports have shown that milrinone can be an effective therapeutic option in PPHN. (McNamara, Laique et al. 2006, Bassler, Kreutzer et al. 2010) These retrospective case reports from two different hospitals in Ontario, Canada looked at 24 critically ill late preterm/term infants (except one infant at 26 weeks postmenstrual age PMA) with HRF or PPHN unresponsive to iNO. Bassler et al reported 4 infants with PPHN. Some of the infants were "primed" with normal saline (15ml/kg) and administered a loading dose of 50 μg/kg over 30 min followed by 0.33 μg/kg/min. None of the infants developed systemic hypotension, and all of them showed consistent improvement in oxygenation. One of the infants was born at 26 weeks PMA and developed bilateral intraventricular hemorrhage (IVH) with moderate dilation of all ventricles. Another term infant developed an IVH. The third infant (39 weeks PMA) showed a small left subependymal hemorrhage. In a subsequent study, McNamara et al reported 9 term infants with PPHN with poor response to iNO who received IV milrinone. Because of the potential risk of systemic hypotension, a loading dose was avoided in these patients with PPHN. The infusion was started at 0.33 μg/kg/min and increased in increments of 0.33 according to clinical response to a maximum of 0.99 μg/kg/min. There was a significant improvement in oxygenation after commencement of milrinone, particularly in the first 24 h of infusion. Tachycardia improved and there was no hypotension. The same authors performed pharmacokinetic studies in 11 late preterm and term infants with PPHN resistant to iNO with a loading dose of 50 μg/kg over 60 min followed by an infusion of 0.33 to 0.99 μg/kg/min and demonstrated an improvement in oxygenation (figure 4) and RV / LV output and a reduction in pulmonary arterial pressure by echocardiography without any IVH (McNamara, Shivananda et al. 2012).

Recently, James et al have described 17 term infants with PPHN resistant to iNO and treated with milrinone infusion (James, Corcoran et al. 2015). An echocardiogram was obtained one hour (median) prior to milrinone infusion. Milrinone infusion increased left ventricular output, right ventricular output, right ventricle strain and strain rate. These echocardiographic changes were associated with a reduction in iNO dose and oxygen requirement over the subsequent 72 hours (James, Corcoran et al. 2015). There was a significant decrease in OI at 24 h post milrinone infusion and this effect persisted at 72 h [OI at baseline – 12 (6-21); 24 h – 5 (2-10) and 72 h – 3 (1-7)]. There was a reduction in systolic, diastolic and mean systemic blood pressures at 6 h after infusion with an increase in use of vasopressor medications. However, blood pressure began to increase after 12 h of milrinone administration with a peak at 72 h.

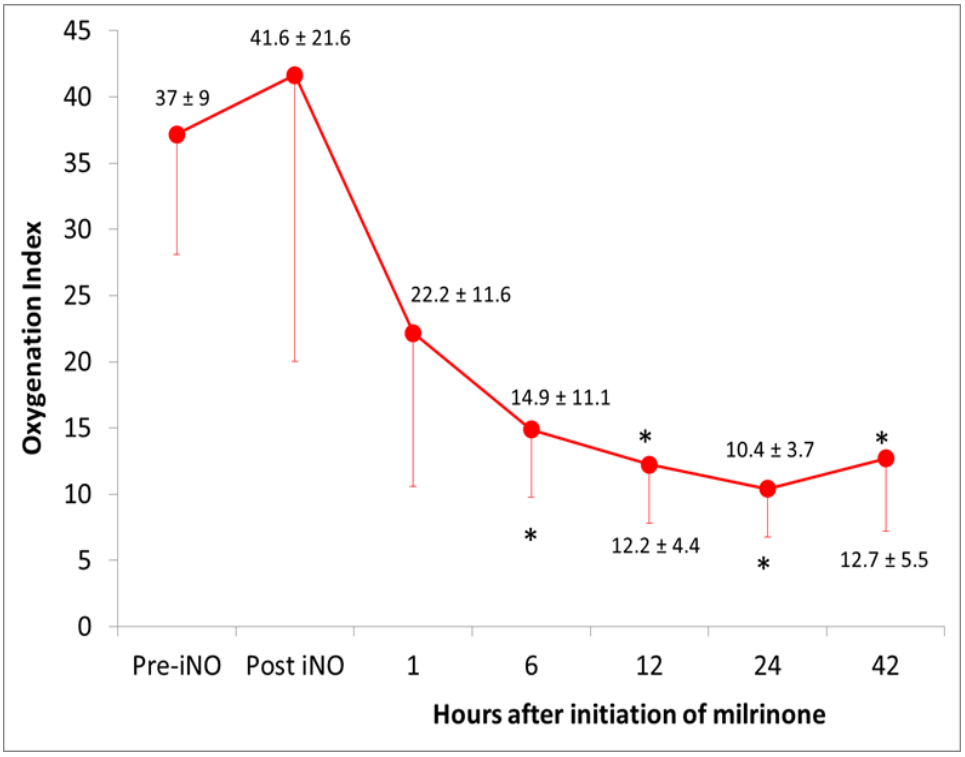

Figure 4. Change in oxygenation index (OI) in patients with iNO resistant PPHN (does not include patients with CDH) treated with IV milrinone. * p < 0.05 compared to post iNO OI (McNamara, Shivananda et al. 2012).

Pharmacokinetics of milrinone in late-preterm/term neonates: The half-life, total body clearance, volume of distribution and steady state concentration of milrinone in neonates with PPHN are shown in table 4. Similar values were obtained from 48 neonatal, post-op cardiac patients in the PRIMACORP study (table 4) which also showed that the clearance in neonates is only 25% of that in children (Bailey, Miller et al. 1999). With a constant-rate infusion, neonates take a much longer time to achieve steady-state concentration. Without a loading dose, neonates reached 50% of steady-state concentration by 2h (compared to 45min in older children). However, for the same infusion rate, the steady-state concentration will be higher in neonates than older patients. These findings underscore the necessity of a loading dose for rapid achievement of a therapeutic blood concentration in neonates, but lower infusion rate to avoid higher levels secondary to poor clearance (Bailey, Miller et al. 1999). Milrinone pharmacokinetics have also been evaluated in preterm infants (Paradisis, Jiang et al. 2007, Paradisis, Evans et al. 2009), infants following cardiac surgery (Lindsay, Barton et al. 1998, Hoffman, Wernovsky et al. 2003) and more recently in neonates with iNO resistant PPHN (McNamara, Shivananda et al. 2012) (table 4). It appears to be a safe and effective medication and has been used in 209 CDH patients without associated heart defects within the CDH

registry (Menon, Tani et al. 2012). Seventeen percent of patients with CDH in the Neonatal Research Network (NRN) are treated with milrinone.

Table 4. Pharmacokinetic data on milrinone in adults, children, term and preterm neonates

Study	Adult (Young and Ward 1988)	Child (Bailey, Hoffman et al. 2004)	Neonate (post-op CHD) (Bailey, Miller et al. 1999)	Neonate (PPHN) (McNamara, Shivananda et al. 2012)	Preterm neonate (Paradisis, Jiang et al. 2007)
Gestational age/age	Healthy adult volunteers	Pediatric patients	Neonates	39.2 ± 1.3 weeks 14h (10-30h)	26 weeks (23-28)
Weight (kg)		5.9 ± 4*		3.481 ± 0.603	0.85 (0.52-1.26)
Half-life (h)	0.8 ± 0.22	3.7		4.1 ± 1.1	10.3
Total body clearance (mL/kg/min)	6.1 ± 1.3	2.5 to 10.6 (increases with age)	1.64 ± 0.37	1.83 ± 0.17	0.64
Volume of distribution (L/kg)	0.32 ± 0.08	0.7 – 0.9	0.523 ± 0.028	0.56 ± 0.19	0.576
Steady-state concentration (ng/mL)				290.9 ± 77.7	195 (78-257) at 21h after infusion
Dose regimen	12.5 to 75 µg/kg load followed by 0.5 µg/kg/min	25 to 75 µg /kg load followed by 0.25 to 0.75 µg /kg/min infusion	25 µg /kg over 60 min followed by a 0.25 µg/kg/min (low dose) or 75 mcg/kg load and 0.75 µg/kg/min (high dose)	50 mcg/kg load over 1h followed by 0.33 (to 0.99) µg /kg/min	0.75 mcg/kg/min for 3h followed by 0.2 µg /kg/min

* This weight is an approximate value - based on high dose arm in PRIMACORP study

Milrinone in CDH: Patel recently reported improved right ventricular diastolic function and oxygenation in 6 neonates with CDH following milrinone infusion (table 5) (Patel 2012). All of them were treated with iNO or IV sildenafil or both. Four of these patients had undergone surgery prior to initiation of milrinone. Oxygenation index decreased from 10.6 ± 5.6 to 7.9 ± 6.2 by 12-24h and to 5.1 ± 2.6 by 48-72h after commencement of milrinone infusion. The editorial accompanying this case report urged the need for randomized controlled trials of milrinone in CDH (Giaccone and Kirpalani 2012). Milrinone is commonly used during the

management of CDH without any randomized trials conducted to show benefit. Thirty percent of infants with CDH in the Children's Hospital Neonatal Database (CHND) (Grover, Murthy et al. 2015) and 22% of late-preterm and term infants with CDH in the Pediatrix database (Malowitz, Hornik et al. 2015) received milrinone.

Table 5. Change in oxygenation index (OI) before and 12-24h and 48-72h after initiation of milrinone in CDH

Patient #	PRE-Milrinone	12-24 h	48-72h
1	18.9	16.5	9.1
2	13.1	11.9	5.3
3*			
4	8	6.4	3.3
5	4.25	1.5	2.5
6	8.9	3.4	5.2
mean	**10.6**	**7.95**	**5.1**
SD	**5.6**	**6.2**	**2.6**

*** Missing mean airway pressure values prevented accurate calculation of OI (Data courtesy Neil Patel MD, FRACP)**

Oxygenation index (OI) on the first blood gas after admission in CDH: Preliminary data obtained from the Medical College of Wisconsin, Milwaukee* (Khmour, Konduri et al. 2014), University of Iowa† (Mann, Morriss et al. 2012) and Women and Children's Hospital of Buffalo¶ on the OI obtained from the first gas after admission is shown below. Data at 6 h after admission are shown in table 6 below.

Table 6. Comparison between CDH patients who survived without ECMO and patients that needed ECMO and/or died prior to NICU discharge. Data shown are mean ± SD. The number in parenthesis is the number of patients in that category.

Characteristic	Survived without ECMO	ECMO and/or death	Death
OI at admission (n=225)	**12 ± 10 (n=123)**	**33 ± 16 (n=102)**	**33 ± 18 (n=73)**
OI at 6 h after admission (n=127)	**9 ± 8 (n=81)**	**29 ± 20 (n=46)**	**26 ± 23 (n=27)**
Patients with OI ≥ 10, 6 h after admission (n=57)	**20 ± 8 (n=21)**	**35 ± 19 (n=36)**	**35 ± 22 (n=18)**

*** Data courtesy Dr. Mir Basir, MD – Medical College of Wisconsin; † data courtesy Dr. Jonathan Klein, MD – University of Iowa and ¶Ms. Ashley Williams, University at Buffalo. Please note that the number of patients with arterial blood gas data available at each time point is variable as this table does not represent a single cohort.**

Based on table 6, among patients with an OI ≥ 10 at 6 hours after admission, the need for ECMO and/or risk of death before discharge is 36/57 = 63% and represents moderate to severe PPHN.

Use of (PaO_2 / FiO_2) – $PaCO_2$ index for stratifying severity of PPHN and lung hypoplasia in patients with CDH: The two main determinants of outcome in CDH are lung hypoplasia and PPHN. Arterial CO_2 is an important predictor of outcome in infants with pulmonary hypoplasia due to CDH (Salas, Bhat et al. 2014). Oxygenation index is also a good predictor of PPHN (Ruttenstock, Wright et al. 2015) but does not take $PaCO_2$ levels into account. An index that includes both oxygenation and ventilation parameters (such as (**PaO_2 / FiO_2) – $PaCO_2$ index)** may be a better indicator of severity of PPHN and lung hypoplasia. A cut-off value of zero for this index distributes patients into moderate and high risk of ECMO/death (table 7).

Table 7. Preliminary data: Outcomes – survival without ECMO, ECMO/death among infants with CDH with an OI of ≥ 10 6 hours after admission and stratified into severe and moderate hypoxemic respiratory failure (HRF) based on the (P/F ratio – $PaCO_2$) index

Index @ 6h after admission & OI ≥ 10	Survived without ECMO (n=21)	ECMO and/or Died (n=37)	Died (n=18)
Severe HRF (P/F ratio – $PaCO_2$) index ≤ 0 (n=21)	3 (14%)	18 (86%)	8 (38%)
Moderate HRF (P/F ratio – $PaCO_2$) index > 0 (n=37)	18 (49%)	19 (51%)	10 (27%)

Milrinone lactate injection is currently indicated for the short-term (~48h) intravenous use in adults with decompensated cardiac failure. Being an "inodilator" and lusitrope, milrinone is likely to improve cardiac function and promote pulmonary vasodilation and be effective in managing PPHN associated with CDH.

This investigation is not intended to support a new indication for use or any significant change in labeling for milrinone. This study does not involve change in route of administration. ***Only FDA approved milrinone products will be used in this study. FDA has exempted this study from requiring an IND.***

2.4. JUSTIFICATION FOR THE STUDY

Significance:

Approximately 1600 newborn infants with CDH are born in the US every year (http://www.cherubs-cdh.org/) and CDH is an orphan disease that is excluded from many trials involving PPHN. The overall survival based on the CDH registry is 71% (75% for isolated CDH) (http://utsurg.uth.tmc.edu/pedisurgery/cdhsg/CDHSG.pdf) and has not significantly improved in the last 10-15 years. The main causes of mortality are pulmonary hypoplasia and therapy-resistant pulmonary hypertension (figure 5). Survivors with CDH have long-term problems including pulmonary hypertension and chronic lung disease. In spite of high mortality, to date, only one randomized controlled study has been published evaluating the postnatal management of CDH. This study involving iNO was conducted through the Neonatal Research Network and the Canadian Institute of Health Research over 15 years ago. In the past decade, significant changes in mechanical ventilation, 'gentle' approach to ventilation of newborn infants, since the NINOS-CDH study was completed, recognition of oxygen toxicity, tolerance to lower SpO_2 and higher $PaCO_2$ (permissive hypercapnia) and delayed surgical repair have changed the management of CDH.

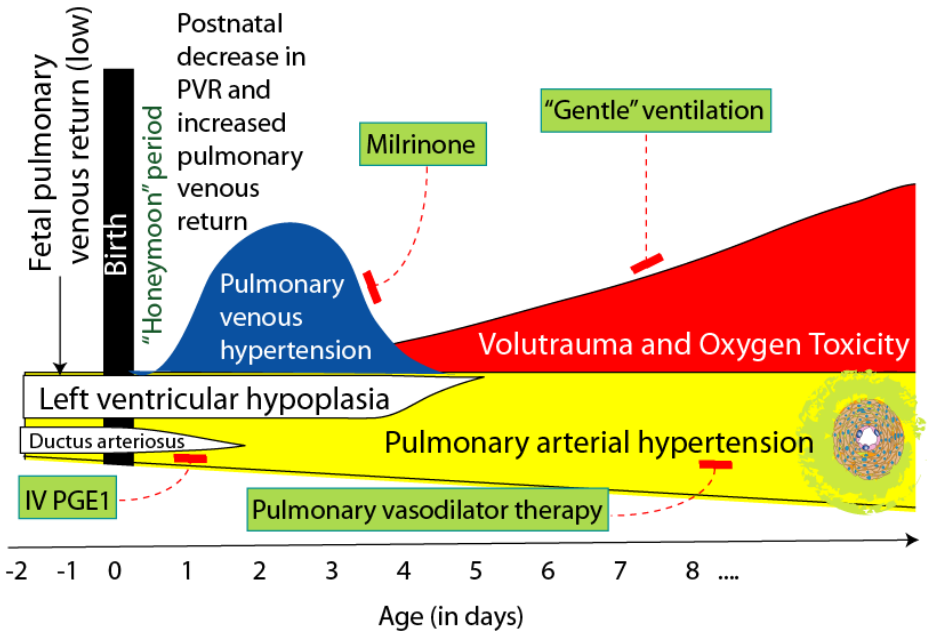

Figure 5. Pathophysiology of hemodynamic abnormalities in congenital diaphragmatic hernia. During fetal life, reduced pulmonary venous return contributes to left sided cardiac hypoplasia. In the immediate postnatal period, a short period of better oxygenation is referred to as "honeymoon" period. Subsequently, a period of left ventricular dysfunction results in pulmonary venous hypertension. Progressive volutrauma and oxygen toxicity leads to chronic lung disease and contributes to pulmonary hypertension. Maintenance of ductal patency with intravenous PGE1 and enhanced left ventricular function with milrinone may improve oxygenation in CDH.

Seventeen percent of patients with CDH in the NRN currently receive milrinone (same as sildenafil) (table 9, page 33). Based on case reports, compelling low-level evidence exists that

milrinone may be beneficial in PPHN and possibly CDH. There is a potential for confounding and co-intervention bias secondary to the lack of a control group in these case series (Giaccone and Kirpalani 2012). Even in a small pilot study, randomization can strengthen results and make them more interpretable (Giaccone and Kirpalani 2012).

Investigators: Congenital diaphragmatic hernia is a relatively rare condition and most patients are often referred to tertiary care centers. Approximately 10% of all cases in the US are cared for within the NRN. Many NRN investigators have experience in conducting trials on postnatal management of CDH (1997). The NRN is the ideal venue for conducting this pilot trial in the US. A successful pilot within the NRN may provide data for a multicenter definitive trial. With the change in the composition of NRN in 2016, the number of CDH patients has increased to approximately 227 / year.

Innovation: We speculate that poor response to conventional therapy in CDH is related to left ventricular dysfunction in the immediate postnatal period followed by ventilation induced oxygen toxicity and volutrauma (figure 5). (Gien and Kinsella 2016, Snoek, Reiss et al. 2016) Improving cardiac dysfunction with milrinone in combination with "gentle" ventilation may potentially improve oxygenation and reduce the risk of chronic lung disease and the need for ECMO. To our knowledge, this is the first randomized trial using an inodilator in CDH.

Approach: A prenatal consult by maternal-fetal medicine, surgery and/or neonatology is a common practice in many centers for mothers diagnosed with fetal CDH. Explaining the study to the parents and consenting during the antenatal period will allow us to enroll adequate number of patients. The intervention proposed in this protocol is intravenous infusion of milrinone, a medication that is commonly used in many NICUs. Based on the survey of NRN centers, 17% of babies with CDH already receive this medication (table 9). It is not clear if this medication is used early in the course of the disease or after documenting a poor response to iNO or secondary to the presence of ventricular dysfunction.

A potential problem with this protocol is slow recruitment. Based on the number of patients with CDH in the NRN, we anticipate that enrollment will be completed over a period of 24 to 36 months. However, if enrollment is slow over the first 6 months, it will be crucial to increase consenting during the antenatal period and to enhance involvement of pediatric surgeons at these centers.

Infants from all races and ethnicities and both genders will be included in this study. A Spanish consent form will be required by all sites with > 5% of subjects speaking Spanish only.

Environment: The Neonatal Research Network is ideally suited to conduct this pilot trial for the following reasons:

(i) Vast experience with neonatal clinical trials
(ii) Availability of an adequate number of patients to conduct this pilot trial
(iii) Recent experience and training and lessons learnt from trials involving management of PPHN
(iv) Many investigators in the NRN have experience in one of the two postnatal randomized controlled trials published in CDH population (NINOS 1997).
(v) Excellent collaboration between neonatologists and pediatric surgeons in previous and ongoing trials (e.g., NEST – Laparotomy vs. drainage in necrotizing enterocolitis NCT01029353).

SECTION 3. STUDY DESIGN

3.1. PRIMARY OUTCOME

The primary outcome is the oxygenation response, as determined by change in OI (or OSI if no arterial blood gas is available) at 24h after initiation of study drug. In patients that require ECMO or die prior to completion of 24h from the initiation of the study drug, the last OI (or OSI, if no arterial blood gas is available) prior to initiation of ECMO or death will be used for analysis. A difference in OI of 4 points in mean change from baseline OI at 24 h between experimental and control groups will be considered significant (OI increased by 4 ± 14.8 in the control group and decreased by 2.7 ± 23.4 following iNO in the CDH NINOS study (1997). In the Patel study, following milrinone infusion, OI decreased by 2.7 ± 1.7 from baseline by 12-24 h and 5.5 ± 3.2 by 48-72 h; the mean change in OI of both these time points is a decrease by 4.1 ± 1.5 (Patel 2012)). This outcome will be used for sample size calculation. Although a decrease in OI by 4 points may not be clinically significant, this number is based on the only published case series showing the use of milrinone in CDH. In patients without an arterial line (or if the line is lost), oxygen saturation index (OSI) will be calculated using a preductal oximeter. (Rawat, Chandrasekharan et al. 2015)

Oxygenation index (OI) = Mean airway pressure (cm H_2O) x FIO_2 x 100 ÷ PaO_2 (mm Hg)
Oxygen saturation index (OSI) = Mean airway pressure (cm H_2O) x FIO_2 x 100 ÷ SpO_2 (%)

3.2. SECONDARY OUTCOMES

(i) Oxygenation response at 24 h after initiation of study drug assigning a minimum OI of 40 or OSI of 20 (if no arterial blood gas is available) for subjects that need ECMO or die after initiation of study drug infusion but prior to 24h of study-drug infusion. In this analysis if the patient's OI is 30 (or OSI of 15) and is cannulated for ECMO due to hemodynamic instability, he/she will be assigned an OI of 40 (or OSI of 20). However, if the OI prior to ECMO is 47 (i.e., > 40), the higher number (47) will be used for analysis of this secondary outcome.

(ii) Oxygenation index at 48 and 72 h after initiation of study drug (or OI/OSI at the time of initiation of ECMO or immediately prior to death, for infants placed on ECMO or died before these time points),

(iii) Change in echocardiographic findings – velocity of tricuspid regurgitation, left ventricular ejection fraction, position of the interventricular septum and direction of shunt at the PDA and PFO level – prior to and 24-72h after starting the study

medication. This outcome will be available only for those infants who have a pre-study drug echocardiogram and a second echocardiogram between 24-72 h after initiation of study drug. These data will be extracted from echocardiograms performed for clinical indications.

(iv) Vasoactive Inotrope score and systemic blood pressure

(v) Area under the curve for inspired oxygen after initiation of the study drug over the first 72 hours of study-drug infusion (or prior to ECMO or death, whichever is earlier) (Note: inspired oxygen and ventilator data from 4 time points per day – every 6 hours will be recorded to calculate area under the curve),

(vi) If subsequent to the study drug, any additional inotrope or pulmonary vasodilator is used (such as iNO), we will evaluate the oxygenation response to these agents. If inotropes or vasodilators were used prior to the initiation of study drug, similar values will be recorded. The OI (or OSI) and PaO_2/ FiO_2 ratio prior to and after initiation of the inotrope / vasodilator are recorded (these data are recorded at 6 hour intervals; the date and time of initiation of individual inotropes and vasodilators is recorded). The change in OI (or OSI) and PaO_2/ FiO_2 ratio in response to these agents is evaluated as a continuous variable and arbitrarily classified into responders, partial responders and non-responders similar to prior trials (1997, 1997).

   a. complete response is defined as an increase in PaO_2/ FiO_2 ratio > 20 mmHg from baseline,
   b. partial response is an increase in PaO_2/ FiO_2 ratio of 10 to 20 mmHg from baseline,
   c. no response is defined as an increase of < 10 mmHg (or a decrease) in PaO_2/ FiO_2 ratio from baseline,
   d. the site of sampling (preductal vs. postductal) is also recorded. (Gien and Kinsella 2016); right radial and ulnar arterial lines/sticks will be considered preductal; Left radial and ulnar arterial samples are usually preductal but can occasionally be postductal; umbilical arterial lines and posterior tibial lines (both sides) are always postductal.
   e. in patients without arterial access, an OSI (oxygen saturation index) will be used

(vii) Supplemental oxygen at 28 d, and 56 d (or discharge, whichever comes earlier) from the hospital – primary institution or secondary institution to which patient was transferred. The use of supplemental oxygen at 28 d will be used to calculate the incidence of chronic lung disease. Chronic lung disease severity will be classified similar to the BPD classification in preterm infants at > 32 week gestation (Jobe and Bancalari 2001). Intermittent supplemental oxygen given only during feedings or for spells administered for < 60 minutes and if the infant is not receiving oxygen at any other time during the day will not be considered as “supplemental oxygen”.

a. Time point of assessment: 28 d, 56 d postnatal age or discharge to home whichever comes first.
b. Mild CLD: treatment with oxygen > 21% oxygen for at least 28 days but breathing room air by 56 days' postnatal age or discharge to home whichever comes first.
c. Moderate CLD: need for > 21% but < 30% oxygen at 56 days postnatal age or discharge to home whichever comes first.
d. Severe CLD: need for ≥ 30% oxygen and/or positive pressure (high-flow nasal cannula – defined for the purpose of this study as > 2 LPM, non-invasive ventilation or nasal intermittent positive pressure ventilation – NIV or NIPPV, CPAP, or positive pressure ventilation) at 56 days' postnatal age or discharge whichever comes first.

(viii) Survival to discharge (or day 120, whichever comes earlier) without ECMO and

(ix) Clinical status – pulmonary (use of supplemental oxygen or respiratory medications – pulmonary vasodilators (sildenafil, bosentan etc.,), diuretics, methylxanthines, steroids, inhaled or nebulized steroids or bronchodilators) and nutritional (weight, length, head circumference, use of anti-reflux and prokinetic medications) at discharge from the hospital and at 12 months of age. In addition, a review of clinical status will also be performed at 4 and 8 months of postnatal age.

(x) Feasibility to perform a definitive trial (primary outcome – improvement in survival without ECMO) and safety (incidence of systemic hypotension, intracranial bleeding and arrhythmias)

3.3. STUDY INTERVENTION

The study intervention is an intravenous infusion of milrinone (***only FDA approved milrinone products will be used in this study***) or placebo (**D5W**).

3.4. BLINDING/MASKING

The intervention will be blinded by the study pharmacist at each center. Infants, parents, care-givers will not be aware of the treatment assignment.

Accidental unmasking: if the identity of the study drug is accidentally revealed to the research or the clinical team, data collection and analysis will continue as previously planned and a protocol violation will be documented.

Intentional unmasking: if the neonatal/surgical attending requests unmasking based on the presence of a serious adverse event, he/she will be informed that the study drug may be

immediately stopped. The request for unmasking will be forwarded to the site PI, study PI and discussed with the NICHD-NRN director. If approved, the study pharmacist will be directed to reveal the identity of the study drug to the attending physician only and not the research team. If the neonatal/surgical attending requests unmasking based on a positive effect of the study drug, he/she will be informed that after the completion of study drug therapy, the clinical team may initiate open-label milrinone.

3.5. POTENTIAL RISKS AND BENEFITS TO SUBJECTS

Infants with CDH represent a high-risk population with mortality ranging from 30 to 40%. Additional risks from the use of IV milrinone include systemic hypotension, intracranial hemorrhage (reported in critically ill neonates with PPHN) and arrhythmias (reported in adults). The anticipated benefit is improvement in oxygenation and reduced need for ECMO.

Systemic hypotension is commonly seen in patients with CDH. In recent studies 80-90% of patients with CDH required treatment with vasoactive medications. (Snoek, Capolupo et al. 2016) Patients will be closely monitored for systemic hypotension due to its association with increased right –to-left shunting and hypoxemia. Study drug will be discontinued in the presence of 'severe' or 'life-threatening' systemic hypotension as outlined in the MOP that cannot be explained by other causes (such as sepsis or pneumothorax) and described below.

a. Severe hypotension:
    - decrement in mean BP > 20 to 30 mmHg compared to the most recent value during the period prior to study drug or at baseline OR
    - receiving extensive volume therapy (40 to 80 ml/kg during a 24-hour period of the study monitoring period: onset of study drug to 24 hours after discontinuation of study drug) OR
    - escalation of vasoactive inotrope score by 20 to 30 compared to the score at baseline
b. Life-threatening hypotension:
    - Decrease in mean BP > 30 mmHg compared to the most recent value during the period prior to study drug or at baseline OR
    - receiving volume therapy (> 80 ml/kg during a 24-hour period of the study monitoring period: onset of study drug to 24 hours after discontinuation of study drug) OR
    - escalation of vasoactive inotrope score by > 30 compared to the score at baseline)

(i) Vasoactive inotrope score calculation (vasoactive inotrope score = dopamine dose (μg/kg/min) + dobutamine dose (μg/kg/min) + epinephrine dose (μg/kg/min x 100) + norepinephrine dose (μg/kg/min x 100) + phenylephrine dose (μg/kg/min x 100) + vasopressin dose (U/kg/min x 10000) (Gaies, Gurney et al. 2010)

Intracranial hemorrhage has been reported in 2 patients with milrinone use in one case series. Subjects in the study will be closely monitored for intracranial hemorrhage with a first head ultrasound and a repeat ultrasound after completion of the study drug infusion. Study drug is discontinued if a 'severe' or 'life-threatening' intracranial bleed/echodensity as defined in the MOP and as described below.

a. Severe intracranial bleed: unilateral or bilateral blood/echodensity filling the ventricle(s) resulting in distension without parenchymal bleeding and without a midline shift.
b. Life-threatening intracranial bleed: Parenchymal hemorrhage/echodensity (including cerebellar hemorrhage) and / or any bleed/ echodensity associated with a midline shift.

Extracranial hemorrhage has not been reported in neonates with PPHN receiving milrinone. However, the study-drug should be discontinued in the presence of 'severe' or 'life-threatening' extracranial bleed (that cannot be attributed to other factors such as trauma, surgery etc,) as outlined in the MOP and described below:

a. Severe category:
   - pulmonary or GI or other forms of extracranial bleed requiring >15-30 ml/kg PRBC transfusion during the study monitoring period: (onset of study drug to 24 hours after discontinuation of study drug) OR
   - associated with a 5 to 10 mm Hg decrease in mean blood pressure from the time prior to the onset of bleeding
b. Life-threatening category: pulmonary or GI or other forms of extracranial bleed requiring PRBC transfusions (total volume of PRBC > 30 ml/kg during the study monitoring period: onset of study drug to 24 hours after discontinuation of study drug) OR associated with > 10 mm Hg decrease in mean blood pressure from the time prior to onset of bleeding.

Milrinone does not directly induce renal dysfunction. However, renal dysfunction can prolong the half-life of milrinone. New-onset renal dysfunction of 'severe' or 'life-threatening' category as outlined in the MOP) are indications for discontinuation of study-drug.

a. Severe renal dysfunction: Serum creatinine > 2 mg/dL but ≤ 3 mg/dL OR increase by 0.5 to 1 mg/dL from the most recent value during the period prior to study drug or at baseline
b. Life-threatening renal dysfunction: Serum creatinine > 3 mg/dL OR increase by > 1 mg/dL compared to the most recent value during the period prior to study drug or at baseline

Milrinone use has been associated with arrhythmias in adults. Arrhythmias have not been reported in the neonatal population in the absence of cardiac disease or surgery. However, if 'severe' or 'life-threatening' arrhythmias (as described in the MOP) are noted after the use of milrinone and other causes (such as electrolyte disturbances or presence of a central line in the right atrium) are ruled out, the study drug should be discontinued.

a. Severe arrhythmia:
    i. rhythm abnormality including bradycardia (heart rate ≤ 70/min) or tachycardia (heart rate ≥ 220 but ≤ 300/min) OR
    ii. arrhythmia associated with a 5 to 10 mm Hg decrease in mean blood pressure from the time prior to the onset of arrhythmia OR
    iii. therapy with anti-arrhythmic medications, AND
    iv. No use of pacing, cardioversion or defibrillation.
b. Life-threatening arrythmia:
    i. rhythm abnormality – tachyarrhythmia with heart rate > 300/min OR
    ii. treated with pacing, cardioversion or defibrillation OR
    iii. arrhythmia associated with a > 10 mm Hg decrease in mean blood pressure from the time prior to the onset of arrhythmia

Note: Respiratory deterioration (as reported on the toxicity table in the MOP) is common in the natural history of CDH and should not be a reason for discontinuing the study drug.

SECTION 4. METHODS

4.1. STUDY POPULATION

Infants with an antenatal or postnatal diagnosis of CDH will constitute the study population. Infants of all ethnicities and both sexes will be included. **Stratification** based on the severity of respiratory failure ($PaO_2/FiO_2 – PaCO_2$) will be performed. If the ($PaO_2/FiO_2 – PaCO_2$) is ≥ 0, subjects are stratified as moderate hypoxemic respiratory failure (HRF) and if < 0, as severe HRF. If no arterial blood gas is available prior to randomization, an OSI of 5 to 10 is considered "moderate" HRF and > 10 is considered "severe" HRF.

4.1.1. Inclusion Criteria

Infants are eligible if they meet all of the following criteria:

(i) ≥ 36 0/7 weeks PMA by best obstetric estimate AND birth weight of ≥ 2000g
(ii) postnatal age ≤7 days (168 hours of age), including post-operative infants following CDH repair with HRF within this postnatal age,
(iii) invasive mechanical ventilation (defined as ventilation with an endotracheal tube) and
(iv) one arterial blood gas with an OI ≥ 10 (after tracheal tube obstruction, pneumothorax and other easily resolvable mechanical causes for increased OI are ruled out) on the most recent arterial blood gas within 12 hours prior to the time of randomization.
(v) if an arterial blood gas is not available at the time of randomization, a preductal OSI of ≥ 5 can be used as an inclusion criterion instead of OI ≥ 10; (the OSI should be based on the most recent preductal pulse oximetry recording and must be within 12 hours of randomization)
(vi) postnatal blood gas with PCO_2 ≤ 80 mmHg (arterial, capillary or venous blood gas) on the most recent blood gas sample obtained within 12 hours prior to randomization

Note: Criteria (iv) to (vi) must be met at the most recent analysis within 12 hours prior to randomization.

4.1.2. Exclusion Criteria

Infants are ineligible if they meet any of the following criteria:

- known hypertrophic cardiomyopathy
    - Note 1: infants of diabetic mothers with asymmetric septal hypertrophy can be included as long as there is no evidence of obstruction to left ventricular outflow tract on echocardiogram,

    - Note 2: infants with other acyanotic congenital heart disease (CHD) and CDH may be included in the study and will be a predetermined subgroup for analysis)
- cyanotic CHD – transposition of great arteries (TGA), total anomalous pulmonary venous return (TAPVR), partial anomalous pulmonary venous return (PAPVR), truncus arteriosus (TA), tetralogy of Fallot (TOF), single ventricle physiology – hypoplastic left heart syndrome (HLHS), tricuspid atresia, critical pulmonic stenosis or atresia etc.,
- enrolled in conflicting clinical trials (such as a randomized controlled blinded trial of another pulmonary vasodilator therapy); Note: mothers enrolled in fetal tracheal occlusion studies such as FETO may be enrolled if permitted by investigators of the fetal tracheal occlusion study; More information about FETO can be found at http://www.chop.edu/centers-programs/center-fetal-diagnosis-and-treatment/fetoscopic-endoluminal-tracheal-occlusion-feto or http://childrens.memorialhermann.org/FETO-trial/ [FETO refers to fetoscopic endoluminal tracheal occlusion and involves occlusion of fetal trachea with a balloon device at mid-gestation and subsequent removal in later gestation]
- infants with bilateral CDH
    - Note 3: infants with anterior and central defects are included in the study
- associated abnormalities of the trachea or esophagus (trachea-esophageal fistula, esophageal atresia, laryngeal web, tracheal agenesis)
- renal dysfunction (with serum creatinine > 2 mg/dL not due to maternal factors) or oligohydramnios associated with renal dysfunction at randomization; renal dysfunction may be secondary to renal anomalies or medical conditions such as acute tubular necrosis
- systemic hypotension (mean blood pressure < 35 mm Hg for at least 2 h with a vasoactive inotrope score of > 30)
- decision is made to provide comfort/ palliative care and not full treatment
- Known intracranial bleed (including the following descriptions on the cranial ultrasound)
    - Parenchymal hemorrhage – including blood in the cerebral parenchyma, cerebellar parenchyma or basal ganglia
    - Blood/echodensity in the ventricle with distension of the ventricle
    - Midline shift secondary to mass effect from an intracranial hemorrhage
- thrombocytopenia (platelet count < 80,000/mm^3) despite blood product administration on the most recent blood draw prior to randomization
- coagulopathy (PT INR > 1.7) despite blood product administration on the most recent blood draw (if checked – there is no reason to check PT for the purpose of this study)
- aneuploidy associated with short life span (such as trisomy 13 or 18) will not be included in the study (infants with trisomy 21 can be included in the study)

- use of milrinone infusion prior to randomization (the use of other inhaled pulmonary vasodilators such as iNO, inhaled epoprosternol, inhaled Treprostinil, inhaled PGE1 and oral such as endothelin receptor antagonists is permitted – Note: it is unlikely to be on oral pulmonary vasodilators early in the course of CDH)
- ongoing therapy with parenteral (intravenous or subcutaneous) pulmonary vasodilators such as IV/SQ prostacyclin analogs (Epoprostenol – Flolan® or Treprostinil – Remodulin®) or IV phosphodiesterase 5 inhibitors (sildenafil – Revatio®) at the time of randomization. Administration of IPGE1 is not an exclusion criteria. In addition, initiation of therapy with these two classes of parenteral medications (except IPGE1) during the first 24 hours of study drug initiation is not permitted and will be considered a protocol deviation. The risk of systemic hypotension is high during the first 24 hours of study-drug (milrinone) infusion and hence parenteral administration of other pulmonary vasodilators is avoided to minimize risk of hypotension.
- Subjects already on ECMO or patients who are being actively considered for ECMO by the neonatal or surgical team
- Infants with hypoxic-ischemic encephalopathy (HIE) undergoing therapeutic hypothermia
- attending (neonatal, critical care or surgical) refusal for participation in the trial (including concern about presence of hemodynamic instability)

Note:

1. A clotting profile evaluation is not required prior to enrollment. However, a cranial ultrasound (covered by the study budget) must be obtained either prior to initiation of study drug or within 4 hours of initiation of study drug. Not obtaining a cranial ultrasound within this time period will be considered a protocol deviation.
2. One complete blood count (CBC) with a platelet count of > 80,000/ mm^3 on the most recent evaluation prior to enrollment is required for randomization. This CBC may be drawn after one or multiple platelet transfusions.
3. The use of therapies such as sedation, analgesia, paralysis, surfactant, high frequency ventilation, iNO, pressors, inhaled prostaglandins (including PGE_1 and prostacyclin) and steroids are permitted at the discretion of the clinical team (and/or based on written center guidelines – a copy of each center's guidelines, if available, will be collected prior to enrollment).
4. Infants with CDH as part of a congenital syndrome that does not cause cardiopulmonary or renal compromise (e.g., rib cage anomalies, renal dysplasia) can be included in the study. Infants with trisomy 21 and CDH without cyanotic heart disease or kidney disease affecting renal function may be included in this trial.

5. A baseline echocardiogram (if ordered for clinical indications) prior to initiation of study drug or within 6 hours of initiation of study-drug is preferred as a baseline study. However, consenting, randomization and preparation of study drug by the pharmacy may proceed without an echocardiogram. It is not necessary to wait for the "official" results of the echocardiogram to commence study drug (unless there is a high suspicion for cyanotic congenital heart disease).

4.2. DETAILED STUDY PROCEDURES

For the purpose of the study, time period prior to study drug is defined as 12 hours prior to initiation to 30 minutes prior to initiation of study drug. Baseline is defined as the period 30 min prior to initiation of study drug. The monitoring period is defined as the period of study drug infusion and the first 24-hours following study drug cessation.

4.2.1. Screening

All mothers and infants with an antenatal or postnatal diagnosis of CDH will be included in screening.
The screening log will include – confirmation of gestational age, birth weight and antenatal or postnatal diagnosis of CDH.

4.2.2. Consent Procedures

Mothers with an antenatal diagnosis of fetal CDH and infants admitted with a postnatal diagnosis of CDH will be screened and approached for consent.

(i) In many institutions, mothers are referred by the Obstetrician and/or Maternal-Fetal Medicine (MFM) specialist for a consult with Pediatric Surgery and Neonatology. This would be an ideal time to obtain consent (brochures for distribution and consenting will be provided to all centers).
(ii) Mothers admitted to labor and delivery with an antenatal diagnosis may be approached for consent (if not in active labor or on narcotic analgesia).
(iii) Infants that are diagnosed postnatally, or born to mothers that could not be consented during the prenatal period or transferred from outside hospitals may be approached in the first 168 hours of life (7 days).
(iv) Permission to access maternal records to record antenatal glucocorticoid therapy, ultrasound and MRI based assessment of severity of CDH will be requested at the time of consent (included in the consent form).

(v) Permission to access infant's records from providers (subsequent hospitalizations, pediatric primary care providers and subspecialty consultants to obtain follow-up information until the baby's first birthday will be requested at the time of consent (included in the consent form).

4.2.3. Randomization Procedures

The following information must be checked and/or confirmed prior to randomization. (a) chest X-ray to confirm the presence of unilateral CDH, (b) complete blood count – CBC to check platelet count and (c) a blood gas – arterial, venous or capillary to document a $PCO_2 \leq 80$ mm Hg. These labs are routinely obtained to evaluate babies with suspected CDH in most institutions. Data from a baseline echocardiogram prior to initiation of study drug (if obtained for clinical purposes) is recorded but is not necessary for randomization (see 4.1.2 (5) above).

(i) The diagnosis of CDH and the side of the defect must be confirmed with a chestX-ray.

(ii) Adequate platelet count ($\geq 80{,}000/mm^3$) on the most recent CBC (after a platelet transfusion if necessary).

(iii) Optional - An indwelling arterial line is placed (if not available oxygen saturation index or OSI is collected).

(iv) Concurrent, ongoing therapy with a parenteral (IV/SQ) pulmonary vasodilator (milrinone, epoprostenol, treprostinil or sildenafil) is a contraindication to the study.

(v) An arterial blood gas (preductal or postductal) with an oxygenation index of ≥ 10 (or OSI ≥5 in the absence of an arterial blood gas) and an arterial, venous or capillary blood gas with $PCO_2 \leq 80$ mmHg on the most recent blood gas (or pulse oximeter recording) should be recorded within 12 hours prior to randomization. An acute reversible mechanical factor such as endotracheal tube obstruction as a cause of elevated OI should be ruled out prior to randomization. If both preductal and postductal gases are available (this is rare), a preference is given to preductal – right radial or ulnar arterial draws, for calculation of OI. (Gien and Kinsella 2016) The same site (preductal or postductal) must be used at various time points for consistent comparison.

(vi) Infants with CDH are eligible for enrollment if there are no exclusions and the most recent blood gas drawn within 12 hours prior to randomization has an OI ≥ 10.

a. In patients without arterial access and/or an arterial blood gas, oxygen saturation index (OSI) can be calculated and if ≥ 5, randomization can be performed.

(vii) Once the infant meets these eligibility criteria and is randomized, the study drug infusion should start within 5 hours of randomization (if not, a protocol deviation form should be filled out). Once the infant is randomized, study drug is initiated even if a subsequent blood gas obtained for clinical purposes has an OI < 10 (or OSI is < 5 if there is no arterial blood gas).

Eligible infants will be randomized to either milrinone arm or placebo arm using a phone or web system in a 1:1 ratio.

4.2.4. Stratification:

Patients will be stratified based on the severity of HRF using the prediction formula (PaO_2/FiO_2 ratio – $PaCO_2$ value) at the time of randomization. Patients with prediction value ≥ 0 will be considered as "moderate" and infants with a value of < 0 as "severe" HRF. If arterial line is not present prior to randomization, preductal OSI value of 5 to 10 (moderate) and > 10 (severe) will be used to determine severity of respiratory failure.

In addition, side of the defect, center, prenatal (observed to expected lung to head ratio – LHR, position of the liver, lung volume assessment by MRI) (Ruano, Lazar et al. 2014), postnatal (OI (Ruttenstock, Wright et al. 2015), oxygen saturation index = mean airway pressure x FiO_2 x 100 ÷ preductal SpO_2) (Rawat, Chandrasekharan et al. 2015) (Park, Lee et al. 2013), evidence of pulmonary hypertension (velocity of the tricuspid regurgitation jet – if present, the position of the inter-ventricular septum and direction of shunt at the patent foramen ovale – PFO and patent ductus arteriosus – PDA level) and intraoperative (defect size) (Congenital Diaphragmatic Hernia Study, Lally et al. 2007) markers of disease severity will be recorded (but not used for stratification).

4.2.5 In-hospital Procedures

Pharmacy: Once the randomization code is received, the study pharmacist at the center will prepare an infusion of milrinone (pre-mixed bags contain 200 µg/mL of milrinone are preferred and do not need any further reconstitution in the pharmacy; Milrinone is also available in 1 mg/mL solution for injection as 10-, 20-, and 50-mL single dose vials). ***Only FDA approved milrinone products will be used in this study.*** Infusion is initiated at 0.33 µg/kg/min (20 µg/kg/h; about 0.1 ml/kg/h) after priming the infusion circuit. An equivalent volume of 5% dextrose (D5W) will be used for infants randomized to the placebo arm. The premix solution in D5W has a pH of 3.2 to 4.0. Milrinone solution prepared from a single-dose vial is stable for 24

hours. The ready-to-use 200 µg/ml pre-mix solution is stable for a longer period of time if no further dilution/alterations are done.

Although 0.9% saline, 0.45% saline and dextrose 5% can be used as diluents, only dilution with dextrose 5% solution is permitted. This is necessary to maintain similarity to premix bags and placebo (also dextrose 5% solution).

Approximate time for compounding at the pharmacy using premixed solution on weekdays during daytime is approximately 30 min and during weekends and nights is 60-75 min based on staffing (data from Women and Children's Hospital of Buffalo). A maximum duration of 5 hours is permitted between randomization and initiation of study drug. Failure to initiate study drug within this time frame is a protocol deviation.

Dose: Milrinone/placebo will be initiated an infusion of 0.33 µg/kg/min (or 20 µg/kg/h or 0.1 ml/kg/h of pre-mixed 200 µg/ml solution). It is recommended that infants receive a fluid bolus (Lactated Ringers solution - preferred or 0.9% normal saline 10 mL/kg) *before* initiating study drug to avoid systemic hypotension, unless there is a contraindication. If after 120 min of infusion of this dose of study drug (and within 240 min of study drug initiation), no evidence of hypotension (defined as mean blood pressure < 35 mmHg for at least 30 minutes) and a vasoactive inotrope score ≤ 30 (see below for calculation of vasoactive inotrope score) , the rate of the study drug infusion is increased to 0.66 µg/kg/min (40 µg/kg/h or 0.2 ml/kg/h of pre-mixed solution).

Compatibility: Milrinone is compatible with D5W, normal saline and Lactated Ringers solution. Milrinone is incompatible with furosemide, imipenem/cilastatin and procainamide.

Vasoactive Inotrope score:

A vasoactive inotrope score is a quantitative assessment of the degree of therapeutic support required by the patient to maintain adequate perfusion and/or blood pressure. It is calculated by the following equation (Rosenzweig, Starc et al. 1999, Kumar, Sharma et al. 2014, Maeda, Toda et al. 2015) (Gaies, Gurney et al. 2010):

Inotrope score = dose of dopamine in µg/kg/min + dose of dobutamine in µg/kg/min + 100 X epinephrine dose in µg/kg/min + 100 X norepinephrine dose in µg/kg/min + 100 X phenylephrine dose in µg/kg/min) + vasopressin dose (U/kg/min x 10000)
Note – the original formula includes 10 x milrinone dose (µg/kg/min). This has been excluded as patients will not be on open-label milrinone while on study drug infusion.

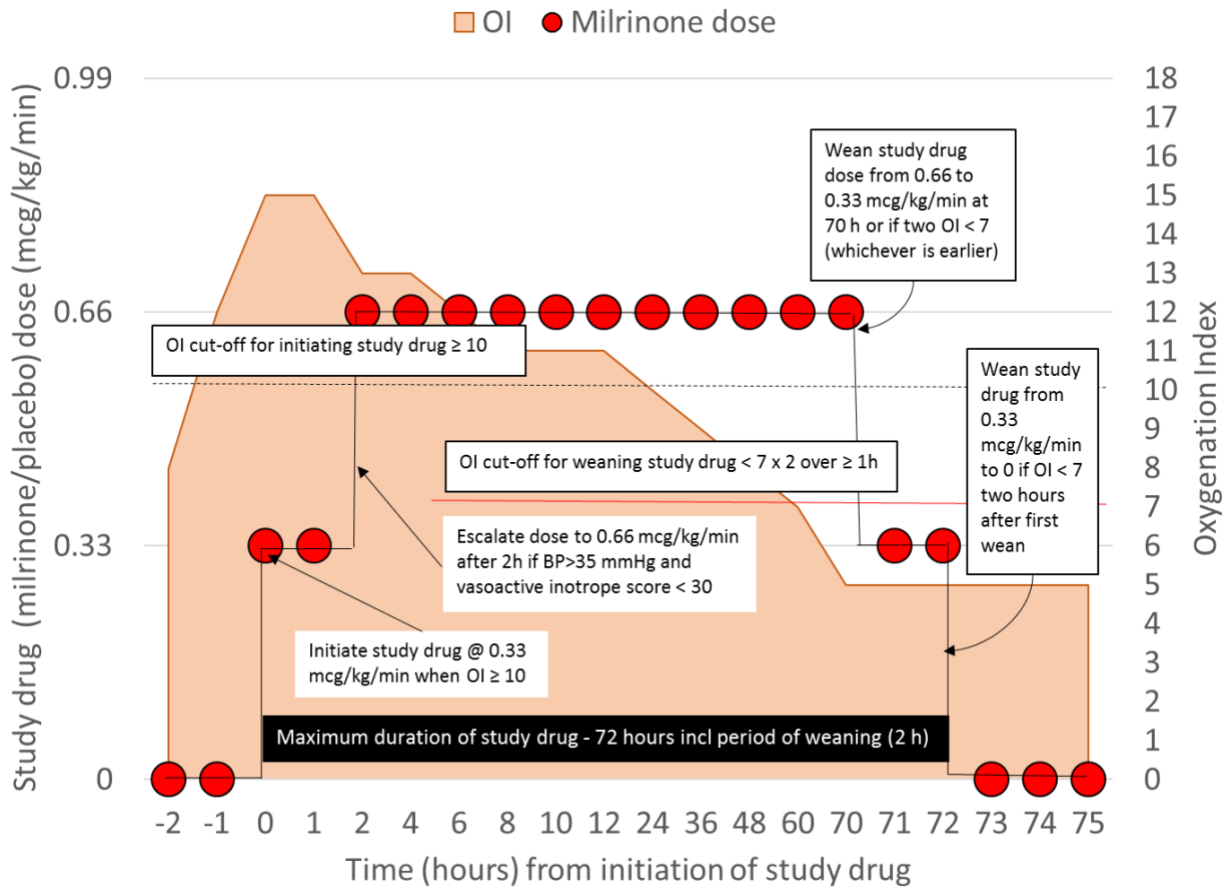

Figure 6. Initiation, maintenance and weaning of study drug (black line) in relation to oxygenation index (OI – orange shaded area). The study infusion is initiated at 0.33 µg/kg/min when OI ≥10. The dose is escalated to 0.66 µg/kg/min 2 to 4 h later if there are no adverse effects (mean BP < 35 mmHg and vasoactive inotrope score of > 30). The dose is maintained at 0.66 µg/kg/min for the duration of the study. The study drug is weaned to 0.33 µg/kg/min at 70h of infusion or if OI < 7 on two consecutive blood gases at least an hour apart (whichever time point is earlier). Two hours after the first wean, the study drug must be stopped. Study drug is also stopped if there is a severe or life-threatening adverse event (hypotension, hemorrhage, arrhythmia or renal dysfunction)

Management of the patient:

Management of the infant with CDH is completely based on center protocols or as per individual physician. The reference list includes publications on guidelines for CDH management

from the European consortium.(Reiss, Schaible et al. 2010) (Snoek, Capolupo et al. 2014, Snoek, Reiss et al. 2016)

If the patient is found to have any of the following criteria during the course of therapy, the study drug should be discontinued as described below.

(i) Diagnosed with severe or life-threatening intracranial bleed/echodensity as defined in the MOP

(ii) Diagnosed with severe or life-threatening renal dysfunction as defined in the MOP

(iii) Diagnosed with severe or life-threatening arrhythmia as defined in the MOP not attributed to other causes such as electrolyte disturbances or a central line in the right atrium

(iv) Diagnosed with severe or life-threatening intracranial bleed as defined in the MOP

(v) Confirmed trisomy 13 or 18 by chromosomal analysis

Note: Respiratory deterioration is common in the natural history of CDH and should not be a reason for discontinuing study drug.

Duration of study intervention

<u>In the absence of complications</u>:

(i) Milrinone/placebo is continued until oxygenation improves and two OIs obtained at least one hour apart are less than 7 (OSI < 3.5, if no arterial blood gas is available), then weaned according to the protocol below, or

(ii) Milrinone/placebo is weaned and discontinued after a maximum of 3 days of therapy (72 hours from the initiation).

<u>Surgery and ECMO</u>:

(i) Milrinone/placebo should preferably be continued if the patient requires ECMO irrespective of the mode of ECMO – VA vs. VV (Reason: although pharmacokinetics and volume of distribution are altered, milrinone may be beneficial with improved cardiac function especially during VV ECMO). (Note: ECMO use varies from center to center, but averages around 30%. This will mean that a fair number of the high risk patients are excluded unless they are enrolled soon after birth)

(ii) It is recommended that the study drug be continued through surgery. If the surgical team prefers to discontinue the study drug, this indication should be recorded and study drug weaned as per protocol.

(iii) Note: if the clinical team decides to wean the baby off the study drug prior to ECMO or surgery, these reasons are documented as indications for discontinuing the study drug. It is not considered a protocol deviation or violation to wean the medication prior to surgery or ECMO. <u>If serious, potentially drug-related complications occur, drug should be discontinued immediately (without weaning)</u>.

Study drug discontinuation criteria:

Milrinone/placebo is discontinued (without weaning) if the patient develops 'severe' or 'life-threatening' hypotension, intracranial or extracranial hemorrhage, renal dysfunction or arrhythmia that cannot be attributed to other causes.

<u>Note</u>:

The study drug can be discontinued (with or without weaning) by the clinical team at any time point if the team is concerned that the study drug poses a risk to the infant. An indication for discontinuation should be recorded. The reason for discontinuation of study drug i.e., (a) due to completion of 72 h of infusion, (b) due to achievement of improved oxygenation (OI < 7 or OSI < 3.5, if no arterial blood gas is available), (c) secondary to occurrence of a side effect (intractable hypotension, intracranial hemorrhage, arrhythmia or renal dysfunction) or (d) due to a decision of the clinical team is recorded.

Weaning of study drug (figure 6):

(i) Once discontinuation criteria are reached, milrinone/placebo is weaned by 0.33 µg/kg/min every two hours and discontinued (total duration of wean 2 h if the patient is on 0.66 µg/kg/min and abrupt cessation of the medication infusion if the patient is on 0.33 µg/kg/min). If the subject is on 0.66 µg/kg/min, weaning is initiated at 70 hours after initiation of study drug. If the subject is on 0.33 µg/kg/min, no weaning is required and the study drug is stopped at 72 hours after initiation. NOTE: Once discontinuation criteria are reached and weaning is initiated, the study drug is discontinued after the wean. If the study drug is not weaned within 4 hours of meeting discontinuation criteria, this is considered a protocol deviation. If the wean was initiated following two OI values of < 7 (or OSI of < 3.5) and oxygenation worsens after initiating the wean, the study drug should still be discontinued as the end-point was met. However, open-label milrinone can be started soon after discontinuation of the study drug.

(ii) If adverse events such as severe or life-threatening hypotension, intracranial or extracranial bleed,, arrhythmia or renal dysfunction are observed, study drug is abruptly stopped without gradual weaning.

Open-label milrinone use:

Immediately after completion of study intervention, open-label milrinone is permitted at the discretion of the clinical team. The study drug infusion will be 0.33 μg/kg/min at the time of cessation if gradual weaning protocol is adopted. It is recommended that the clinical team start open-label milrinone at this rate and adjust the dose based on clinical response. If the clinical team initiates open-label milrinone at 0.33 mcg/kg/min immediately after weaning the study drug, there is no discontinuity in therapy (for patients in the milrinone arm).

Criteria for re-initiation of study drug:

After the patient has met criteria for study drug discontinuation once (due to improvement in oxygenation and meeting OI criteria, or due to side effects such as severe hypotension, intracranial bleeding or renal dysfunction), study drug is discontinued and cannot be reinitiated even if the indication for initial discontinuation is resolved (for e.g., OI exceeds 10 after cessation of study drug or hypotension is resolved and blood pressure is normal etc.,). The clinical team may start open-label milrinone.

During the post-study drug infusion period, data regarding open-label milrinone use, pressor use, and vasodilator use will be recorded for 24 hours after cessation of study-drug.

Fluid intake:

(i) As systemic hypotension and fluid bolus administration are common in patients with CDH and PPHN, it is important to record daily fluid intake.
(ii) Total fluid intake (including maintenance fluids/TPN, flushes, medications, boluses and blood products) will be recorded for three 24-hour periods as entered in the nursing notes/EMR (please see MOP for details).

Clinical criteria for ECMO:

The decision to place an infant with CDH on ECMO is ***entirely up to the clinical team***. Many centers prefer to use ECMO for preoperative stabilization. The following general guidelines are provided as criteria for ECMO (Reiss, Schaible et al. 2010) (Snoek, Reiss et al. 2016):

(i) Oxygenation index is consistently ≥ 40 for at least 3 h and/or
(ii) Inability to maintain preductal saturations > 85% or postductal saturations > 70% and/or
(iii) Increased $PaCO_2$ and respiratory acidosis with pH < 7.15 despite optimization of ventilator management and/or
(iv) PIP > 28 cm H_2O or mean airway pressure > 17 cm H_2O is required to achieve saturations > 85% and $PaCO_2$ < 65 mmHg and/or
(v) Inadequate oxygen delivery with metabolic acidosis as measured by elevated lactate ≥ 5 mM/L and pH < 7.15 and/or
(vi) Systemic hypotension (mean blood pressure < 30 mmHg), resistant to fluid and inotropic support, resulting in hypoperfusion and/or oliguria (urine output < 0.5 mL/kg/h) for at least 12-24h

Subject specimens: Information about blood counts, imaging and blood gases will be obtained from blood draws conducted for the purpose of clinical care. No additional blood work will be performed for the purpose of research.

Echocardiography: Data from a maximum of 4 clinical echocardiograms will be recorded. No additional echocardiograms will be performed for the purpose of this study. To estimate the secondary outcome of improvement in cardiac function and pulmonary hypertension, data from at least two echocardiograms will be obtained if available – the first echocardiogram preferably prior to initiation of the study drug. If echocardiogram is not performed prior to study drug initiation, study drug may be initiated.

If clinical echocardiograms are obtained during the study drug infusion, data from the clinical echocardiogram closest to 24 hours after study drug infusion is used to calculate the secondary outcome of change in echocardiographic findings. If a third echocardiogram is obtained close to 72 hours after initiation of study drug, data from this study will be recorded. In addition, data from any other echocardiograms obtained for clinical purposes after completion of study drug will be recorded (maximum of 4 echocardiograms).

Cranial ultrasound: As mentioned previously, an initial cranial ultrasound is obtained prior to initiation of study drug or within 4 hours of commencement of the study drug. A second cranial ultrasound is obtained preferably within 24 hours (maximum within 96 hours) of completion of the study drug. Both these cranial ultrasounds are covered by the study budget. Additional head imaging may be performed based on clinical indications but are not covered by the study budget. If the patient has a screening ultrasound that demonstrates intracranial hemorrhage

(as outlined in the exclusion criteria) prior to starting the study-drug, the patient is not randomized. In this case, only one screening cranial ultrasound is covered by the study budget.

4.2.6 Post-hospital Procedures

No procedures are required after discharge from the hospital (except for a telephonic questionnaire conducted at 4, 8, and 12 months of age – see 4.2.7 below).

4.2.7 Additional Follow-up Examinations and/or Questionnaires

The subject's pulmonary and nutritional status will be initially recorded at discharge. Subsequently, a study questionnaire evaluating breathing outcomes and nutritional status will be administered at 4, 8, and 12 months of age by a telephonic interview. This questionnaire is a modification of the questionnaire used for the SUPPORT trial. (Stevens, Finer et al. 2014)

SECTION 5. ANALYTICAL PLAN

5.1 SAMPLE SIZE AND POWER ESTIMATES

Sample Size: In the case series describing the effect of milrinone among neonates with CDH, baseline OI (mean ± SD) was 10.6 ± 5.6, and improved to 7.9 ± 6.2 and 5.1 ± 2.6 at 12-24 h and at 48-72 h, respectively (Patel 2012). Assuming that children receiving placebo will have no additional change in OI (mean change in OI = 0 at 24 hours), that children receiving milrinone will improve to a mean OI of 6.0 (a mean change in OI of 4.6) at 24 hours, and that the standard deviation of the change from baseline OI at 24 hours in each group is 6.2, 33 subjects in the control group and 33 subjects in the milrinone group would have 84% power to detect this difference with a two-sided alpha of 0.05 (note that 6.2 is a conservative standard deviation estimate, as data from the Patel et al study indicate that the standard deviation for change from baseline in OI at 24 hours may be as low as 1.5). Phrased differently, if both the control and treatment OI's were to change, for any given difference in OI, the sample size would be sufficient to detect an effect size of 0.7 with a power of 84%. The sample size will be sufficient to provide estimates of adverse event rates in the treatment group. The following table provides the 95% confidence intervals for observed adverse event rates varying from 6% to 49% for a sample size of 33 subjects.

Table 8. Confidence Intervals for Observed adverse event rates.

Point estimate	0.06	0.09	0.21	0.30	0.39	0.49
95% CI	(0.007, 0.2)	(0.02, 0.24)	(0.09, 0.39)	(0.16, 0.49)	(0.23, 0.59)	(0.31, 0.66)

Within the NRN centers, approximately 36% of neonates with CDH who are ≥ 36 weeks PMA require ECMO. In a selective population (isolated CDH with OI ≥ 10), we estimate that the risk of ECMO or death prior to discharge (or by day 120) will be approximately 40%. A sample size of 33 in the placebo arm would provide a 95% confidence interval for this estimate of (26%, 58%). The precision of estimates for outcome rates in the treatment group can be seen in table 8.

Limitations:

(a) This study is not powered to compare rates of the outcome of death or ECMO between groups. To detect a reduction of 10% (from 40% incidence of death/ECMO in control arm to 30% incidence in the milrinone arm), with a 1:1 ratio of treatment and control subjects, a power of 0.8 and an alpha value of 0.05, we would require 376 subjects in each arm.

(b) The study is also not powered to compare a sicker cohort of CDH patients similar to that evaluated in the CDH NINOs study (1997). The patients in CDH NINOs study had an OI of approximately 45 with standard deviations of 16.3 and 14.5 in control and treatment groups respectively. Such a cohort will require a sample size of 207 patients in each arm to achieve the primary outcome outlines in the current protocol. We intend to enroll patients early at a lower OI (or OSI).

5.2 AVAILABLE POPULATION

Available population/duration of the study: There are currently no protocols involving term/late preterm infants with CDH in the NRN (except the observational study for early-onset sepsis which does not interfere with this protocol). The International CDH study group (not associated with NRN) comprises of approximately 75 centers. These centers enrolled 300 babies into the registry in 2009. The number of babies with CDH within the NRN per year was approximately 170/ year from 2011 to 2012. More recent data obtained from the NRN grant applications shows a higher number (approximately 227/ year). Information about the number of patients available within the NRN during 2011 and 2012 is given in table 9.

Table 9. Infants with congenital diaphragmatic hernia in the Neonatal Research Network

	2011	**2012**	**Average**
Total CDH admissions	175	162	169
CDH ≥ 36 0/7 week PMA	142	133	138 (82%)
Inhaled NO in the preoperative period among CDH ≥ 36 0/7 weeks PMA	71	72	72 (43%)
Other medications used in the preoperative period among CDH ≥ 36 0/7 weeks PMA			
Milrinone	17	29	23 (17%)
Sildenafil	29	25	27 (20%)
Flolan ® (inhaled epoprostenol)	8	9	9 (6%)
Flolan ® (intravenous epoprostenol)	6	3	5 (3%)
Alprostadil (intravenous PGE1)	6	5	6 (4%)
Epinephrine	2	1	2 (1%)
ECMO among all CDH	59	56	58 (34%)
ECMO among CDH ≥ 36 0/7 weeks PMA	51	48	50 (36%)
Survival to discharge			
- all CDH	111	117	114 (68%)
- CDH ≥ 36 0/7 weeks PMA	94	101	98 (71%)
Concern regarding use of iNO in CDH	Yes – 8; No – 11		
Primary physician managing pulmonary vasodilator therapy Surgeons – 4; Neonatologists – 10; Joint (surgeons + neonatologists – 3); PICU – 1			

Duration of study (figure 7):

(i) There are approximately 138 babies with CDH ≥ 36 weeks PMA per year within the NRN.

(ii) Eighty percent of CDH patients have isolated CDH without other major anomalies (138 x 0.8 = 110 patients).

(iii) Approximately 35% of these patients do not meet inclusion criteria (OI < 10 mild disease or severe disease and/or hemodynamic instability): 110 x 0.65 = 71 patients (based on preliminary calculations using 11 patients in Buffalo and 116 patients from Milwaukee).

(iv) Refusal to participate in the study (parents and/ or physician not consenting to the study or are cared by another service that does not want participation in a trial) – 50%: 71 X 0.5 = 35 patients per year or 66 patients in approximately 2 years (figure 7).

5.3 PROJECTED RECRUITMENT TIME

We anticipate that it will take 24 to 36 months for enrollment and an additional year for administering the pulmonary/ nutritional status questionnaire. It will take approximately 48 months for the completion of this pilot study. Infants with CDH are a special population with high morbidity and mortality. We anticipate that enrollment might be slower than expected.

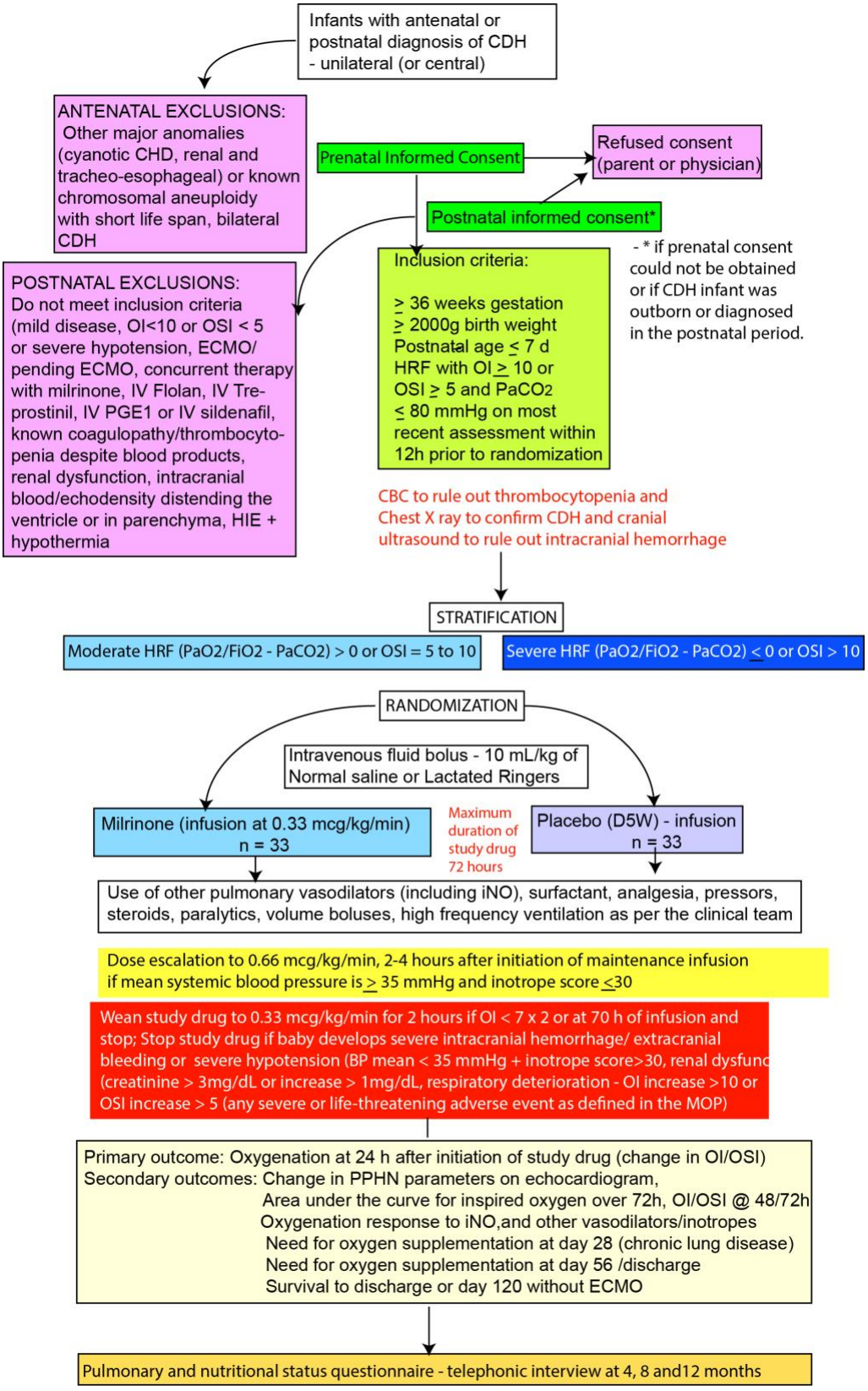

Figure 7. Outline of the study

5.4 STATISTICAL ANALYSIS PLAN

Baseline data will be collected prior to randomization including gestational age, side of the diaphragmatic hernia, birth weight, mode of delivery and arterial blood gas parameters (oxygenation index-OI) and/or pulse oximetry data (oxygen saturation index-OSI). Data from a baseline clinical echocardiogram is recorded (if performed prior to study drug initiation or within 2h of initiation of study drug). During the infusion of study drug, arterial blood gas parameters and hemodynamic data (blood pressure, heart rate and echocardiography results) will be recorded. After completing the study infusion, surgical details, and mortality information will be collected. Among survivors, respiratory and nutritional status at discharge and at 4, 8 and 12 months of age will be collected.

Data will be presented as mean ± SD for normally distributed variables and as median and ranges (with percentiles) for non-normally distributed data. All analysis will be by intention-to-treat. Baseline characteristics will be compared between treatment groups using chi-square and Fisher's exact test for discrete variables and t-test and ANOVA for continuous variables. The primary outcome, difference from baseline OI at 24 h, will be compared between treatment groups using a general linear model adjusted for the stratification factor of HRF severity. Other continuous outcomes will be analyzed similarly. Models for longitudinal outcomes such as oxygenation and vasoactive inotrope scores (short-term) and nutritional and pulmonary outcomes at 12 months (long-term) will include the effect of time, the interaction between time and treatment, and random effects to account for the correlation between measures taken on the same individual over time. Categorical outcomes will be analyzed using analogous generalized linear models. Cox regression models (with adjustment for HRF severity) will be used to plot proportion of responders who survive without ECMO against survival time in hours. Analyses may adjust for clinical site if treatment assignments are severely imbalanced for some sites provided that it is computationally feasible to do so. The potential influence of baseline measures such as time of diagnosis – antenatal vs. postnatal, side of the defect, and gender on pulmonary and nutritional outcome will be explored through the use of additional models similar to those described above.

5.5 DATA MONITORING PLAN

The DSMC will monitor the progress of the trial on a routine basis for monitoring enrollment progress and reviewing the accruing safety data. The DSMC will meet to look over the preliminary data after enrolling the first 10 subjects. Interim reviews are scheduled via e-mail or teleconference if DSMC identifies any emerging safety issues. A suggested schedule for further meetings based on subject enrollment is as follows:

25% enrollment (~ 17 subjects),
50% enrollment (33 subjects)
75% enrollment (~ 50 subjects)
Completion of enrollment (~ 66 subjects)

5.5.1 Reporting Adverse Events

There is high morbidity and mortality associated with the natural course of CDH. The following measures will be used to monitor safety of the trial based on a review of adverse events. Adverse event reporting will be handled as follows:

(i) **Potential Adverse Events** (as defined below) will be reported on the Adverse Events form.

(ii) Because of the nature of the study population, mild events will not be recorded as adverse events unless they are also unexpected.

(iii) Potential adverse events listed below that are severe, life-threatening or result in death (as per the MOP toxicity table) will be entered in the electronic data capture system (EDC) within 24 hours of discovery.

(iv) **Serious Adverse Events** will be reported on the Adverse Events form, entered in the electronic data capture system (EDC) and reported to NICHD and the NRN data center within 24 hours of discovery (via SAE Narrative) **when:**

a) they result in death **or**
b) they are possibly, probably or definitely related to the intervention **or**
c) they are unexpected. An unexpected event is defined as an adverse event not known to be associated with the natural course of CDH or a specified side-effect of interventions in the management of CDH.

(v) All serious adverse events that meet one of the above criteria will be reviewed by the NICHD NRN Program Scientist (as medical monitor).

(vi) All serious events that are marked as at least possibly related to the intervention will be forwarded to the Chair of the independent NRN DSMC for any further action.

The duration of reporting adverse events would include the period of administration of the study drug and 24 hours' post-infusion. This period will cover approximately 6 half-lives of milrinone and plasma levels are expected to be undetectable by 24 hours after completion of study-drug infusion.

5.5.2 Potential Adverse Events

The potential adverse events include:

1. Systemic hypotension
2. Abnormal cranial imaging consistent with intracranial bleed
3. Arrhythmias
4. Extracranial bleeding (especially pulmonary and gastrointestinal hemorrhage)
5. Renal dysfunction
6. Respiratory deterioration
7. Other events that are deemed severe (or worse in intensity), and either unexpected or at least possibly related to study or results in death

The data regarding serious adverse events will be reviewed after recruitment of the first 10 subjects. Subsequently, data regarding adverse events will be reviewed every 17 subjects (25% of the sample size). If DSMC is concerned about adverse events, the subjects can be unmasked for the DSMC to assess whether the event rates appear to differ between treatment arms. Should there be excess serious adverse events in one group, the committee may recommend breaking of the blinding and discontinuation of enrollment.

5.5.3 Interim Monitoring Plan and Stopping Rules

As mentioned previously, the DSMC will meet as per the following plan. A suggested schedule may include meeting after the first 10 subjects are enrolled and at 25% (~ 17 subjects), 50% (33 subjects), 75% (~ 50 subjects) of target sample size and after completion of enrollment.

In addition to monitoring trial progress, the DSMC will also review accumulating safety data for the trial. All safety data presented to the DSMC will be blinded to treatment group, though the report may be unmasked at the DSMC's request.

The three main adverse events that require close monitoring include severe hypotension , hemorrhage (intracranial and extracranial) and arrhythmia or death due to severe hypotension or intracranial hemorrhage or arrhythmia while on study drug infusion or 24 hours after cessation of study drug infusion.

The presence of the following adverse events *possibly, probably or definitely due to the study drug* in > 20% of subjects after enrollment of 33 patients with a statistically significant difference between the treatment arm and the placebo arm can lead to a discussion at DSMC regarding cessation of the trial.

(a) Severe hypotension is defined by a decrease in mean blood pressure > 20 to 30 mmHg compared to baseline OR receiving extensive volume therapy (>40 to 80 ml/kg during a 24 hour period of the study monitoring period OR escalation of vasoactive inotropic score by >20 to 30 compared to baseline) The study monitoring period is the time on study drug therapy and within 24 hours of completion of study drug therapy.
(b) Life-threatening hypotension - receiving volume therapy > 80 ml/kg during a 24 hour period of the study monitoring period OR escalation of vasoactive inotropic score by > 30 compared to baseline
(c) Severe intracranial hemorrhage (unilateral or bilateral blood/ echodensity filling the ventricle(s) resulting in distension without parenchymal bleeding and without a midline shift) while on study drug therapy and within 24 hours of completion of study drug therapy.
(d) Life-threatening intracranial hemorrhage - Parenchymal hemorrhage/ echodensity (including cerebellar hemorrhage) and / or any bleed/ echodensity associated with a midline shift.
(e) Severe arrhythmia – heart rate rhythm abnormality including bradycardia (heart rate ≤ 70/min) or tachycardia (heart rate 220 to 300/min) OR treated with anti-arrhythmic medications and no use of pacing, cardioversion or defibrillation OR associated with a 5 to 10 mm Hg decrease in MBP from the time prior to the onset of arrhythmia
(f) Life-threatening arrhythmia - tachyarrhythmia with heart rate > 300/min OR treated with pacing, cardioversion or defibrillation OR associated with a > 10 mm Hg decrease in MBP from the time prior to the onset of arrhythmia
(g) Death – resulting from severe hypotension, hemorrhage or arrhythmia.

5.5.3.1 Monitoring Enrollment

Because the focus of the study is to evaluate changes in oxygenation and determine the feasibility of conducting a larger randomized trial, the DSMC will routinely review enrollment rates on this study to assess whether the enrollment rates are likely to be insufficient to support a full randomized trial to determine the impact of milrinone on survival without ECMO. For the current pilot trial, we anticipate 3 patients to be enrolled per month. Given this accrual target, it is anticipated that at least 12 patients will be enrolled in the 6-month period after at least 75% of sites have begun randomization *and enrollment.*

This study will provide valuable data about CDH patients, response to various pulmonary vasodilators and pulmonary / nutritional outcomes at 12 months of age. Since this is a pilot trial to determine feasibility of a larger trial examining survival without ECMO as the primary

outcome, the interim efficacy of the Milrinone with respect to oxygenation will not be used as a criterion for stopping the study.

5.5.3.2 Future Plans:

Information obtained from this trial will be used for designing a definitive trial with a primary outcome of survival to discharge (or day 120, whichever is earlier) without ECMO. Preliminary sample size for the definitive trial is 752 (376 patients in each arm) and will require international collaboration (16 patients enrolled per month to complete the trial in 4 years).

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