PROTOCOL

NASO

A randomised controlled trial of nitric oxide administration during cardiopulmonary bypass in infants undergoing Arterial Switch Operation for repair of transposition of the great arteries.

Protocol Version 4 and date: 10th August2018

Document history:

Date of Change	Summary of Change
V1	First version
V2 19/02/2017	Updated prior to ethics submission
V3 04/05/2017	Amended in response to ethics Age stratification changed to 42 days for ANZ Addition of Perth as a site
V3.1 29/06/2018	Perth & QLD removed as sites until ready to start, removal of AI's in these sites
V 4 14/08/2018	-Changed definition of age stratification for randomisation -Updated adverse event reporting -addition of ESC -Reformatting in line with current MCRI recommendations - Clarification of secondary objectives -information added on patient overlapping in nitric oxdie on bypass study

This clinical trial will be conducted in compliance with all stipulations of this protocol, the conditions of the ethics committee approval, standards of Good Clinical Practice (as defined by the International Conference on Harmonisation), ethical principles that have their origin in the Declaration of Helsinki and all applicable national and local regulations.

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CONTENTS

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	*TERM*
AE	Adverse Event
ASO	Arterial Switch Operation
CEBU	Clinical Epidemiology and Biostatistics Unit
CHD	Congenital Heart Disease
CO2	Carbon dioxide
CPB	Cardio Pulmonary Bypass
CPI	Coordinating Principal Investigator
CRF	Case Report Form
CUF	Conventional UltraFiltration
CVD	Cardiovascular Diseases
DSMB	Data Safety Monitoring Board
ECMO	Extracorporeal membranous oxygenation
ECLS	Extracorporeal life support
ESC	Executive Steering Committee
FiO2	Fraction of Inspired Oxygen
HREC	Human Research Ethics Committee
ICU	Intensive Care Unit
ITT	Intention To Treat
LCOS	Low Cardiac Output Syndrome
MAE	Major Adverse Event
MCRI	Murdoch Children's Research Institute
MUF	Modified Ultra Filtration
NHMRC	National Health and Medical Research Council
NICU	Neonatal Intensive Care Unit
NO	Nitric Oxide
NOCPB	Nitric Oxide on Cardio Pulmonary Bypass (study)
PaCO2	Arterial Blood Gas Analysis - partial pressures of oxygen
PaO2	Arterial Blood Gas Analysis - partial pressures of carbon dioxide
PAH	Pulmonary Arterial Hypertension
PICU	Paediatric Intensive Care Unit
RCH	Royal Children's Hospital
SAE	Serious Adverse Event
SCUF	Slow Concentrated UltraFiltration
SIRS	Sepsis-Induced Systemic Inflammatory Response Syndrome
SUSARs	Suspected Unexpected Serious Adverse Reaction
TGA	Transposition of Great Arteries
TSC	Trial Steering Committee

PROTOCOL SYNOPSIS

TITLE	A randomised controlled trial of nitric oxide (NO) administration during cardiopulmonary bypass in infants undergoing Arterial Switch Operation for repair of transposition of the great arteries.
AIM	To demonstrate that exposure to gaseous NO reduces the incidence of major postoperative adverse events in infants on cardiopulmonary bypass who are undergoing an arterial switch (ASO) for correction of Transposition of the Great Arteries (TGA).
OBJECTIVES – PRIMARY	To evaluate an effect on major adverse events (death, Extracorporeal membranous oxygenation (ECMO), cardiac arrest, emergency chest opening) postoperatively in infants exposed to gaseous NO while on cardiopulmonary bypass (CPB) for ASO for TGA.
OBJECTIVES – SECONDARY	To investigate whether the exposure to gaseous NO results in improved clinical outcomes at 28 days (which includes ICU length of stay, hospital stay, ECMO days, ventilator days, inotropic hours, dialysis days, inhaled NO hours, and open sternum days).
DESIGN	Multi-centre, double blind, randomised controlled trial of two parallel treatment arms. Participants will be randomised (stratified by centre and age at time of surgery) into one of two treatment arms: Intervention arm will receive NO 20 ppm into the oxygenator of a Cardio-Pulmonary Bypass (CPB) circuit. Control arm will not receive NO At the end of CPB, the patient will return to the Intensive Care Unit (ICU), where normal care will continue. Approximately 20-30 centres across four continents are expected to participate.
PRIMARY OUTCOMES	The primary outcome for this trial is: Major adverse event postoperatively (end point combining cardiac arrest, ECMO, death, emergency chest opening).
SECONDARY OUTCOMES	The secondary outcomes for this study is a composite free day score consisting of a combination of 8 scores including length of stay in ICU, length of stay in hospital, ventilator free days, inotrope free days, dialysis free days, inhaled NO free days, ECMO free days, Closed sternum days, all calculated at day 28.
STUDY DURATION	Two years
INTERVENTION	1. Intervention: Participants will receive NO blended into the fresh gas flow of the CPB oxygenator, which will be maintained at 20 ppm via an INO Max DSIR (or similar) (Mallinckrodt, NJ, USA). The NO and gas as per standard concentration will be measured via an access port just prior to the oxygenator. NO administration will

	commence upon initiation of CPB and stopped at the cessation of CPB. 2. Control: Participants assigned to the control arm will NOT receive NO. Instead, they will receive gas blended as per standard concentration into the oxygenator of the CPB using the same formula as for NO delivery.
NUMBER OF PARTICIPANTS	800 participants The rate of major adverse events in the control arm is assumed to be 15% (based on data observed over the last 5 years at RCH). The rate in the intervention arm is assumed to be 8% (the rate observed in the previous study[30]). Assuming alpha of 0.05, a 2-sided test and no dropout, 400 participants per treatment group are required to provide 87% power to show the two arms significantly different given these rates if major adverse events observed.
POPULATION	Infants undergoing CBP during ASO for TGA

INVESTIGATOR AGREEMENT

I have read the protocol entitled "A randomised controlled trial of nitric oxide administration during cardiopulmonary bypass in infants undergoing Arterial Switch Operation for repair of transposition of the great arteries. (NASO)"

By signing this protocol, I agree to conduct the clinical trial, after approval by a Human Research Ethics Committee or Institutional Review Board (as appropriate), in accordance with the protocol, the principles of the Declaration of Helsinki, the good clinical practice guidelines adopted by the relevant local regulatory body and all other applicable national and local regulations.

Changes to the protocol will only be implemented after written approval is received from the Human Research Ethics Committee or Institutional Review Board (as appropriate), with the exception of medical emergencies.

I will ensure that trial staff fully understand and follow the protocol and evidence of their training is documented on the trial training log.

_____

Signature of PI & Date

1. ADMINISTRATIVE INFORMATION

1.1. Trial registration

1.1.1. Registry

This trial is registered with the Australia New Zealand Clinical Trials Registry. This trial will be registered with ClinicalTrials.gov as it meets the definition of an Applicable Clinical Trial.

1.2. Sponsor

Study Sponsor	Murdoch Children's Research Institute
Contact name	Professor Warwick Butt
Address	50 Flemington Road Parkville VIC 3052 Australia

1.3. Expected duration of study

This study is expected to run for 2 years: from the initiation of participant screening until the last participant has finished the study. Recruitment is expected to take 2 years. Participants will be followed to hospital discharge.

1.4. Study locations

This study will be conducted in the operating theatres and intensive care units of major paediatric hospitals in Australia, New Zealand, Malaysia, Canada, China, Israel, Thailand, Indonesia, Saudi Arabia, India, European Union, United Kingdom and the United States.

Potential sites who perform more than 12 Arterial Switch Operations for Transposition of the Great Arteries annually will be invited to express interest in participating in the study. Each site participating needs to meet the following criteria;

1. Can administer NO 20ppm to the oxygenator during CPB (blinded to all but the perfusionist)

2. Develop and follow locally designed protocols for anaesthesia, CPB and post-operative care (in other words, each centre can have its own protocol but patient management pre, intra and post-operatively must be identical in both groups)

A list of study sites will be maintained within the study record on ClinicalTrials.gov and updated in this section with protocol amendments or bi-annually (whichever comes first).

2. INTRODUCTION AND BACKGROUND

2.1. Trial rationale and aim

The aim of this trial is to determine if adding NO to CPB is superior to CPB without NO in the incidence of post-operative major adverse events in infants with TGA undergoing an ASO. Major adverse events include cardiac arrest, emergency chest opening, use of ECMO, and death.

2.2. Background

The incidence of congenital heart disease (CHD) is approximately 1/100 live born children, of which up to 50% require cardiac surgery to correct the underlying abnormality at some stage during their life. Over 80% of cardiac surgical procedures require CPB. In the USA, the cost for CHD was U$1.9 billion in 2011, with an average cost per patient of U$25,000 (Centre for Disease Control and Prevention, USA). In Australia, more than 2000 children are born each year with CHD, with over 30,000 children currently living with CHD. Transposition of the great arteries (TGA) presents in 5-7% of all patients with congenital heart disease. The overall annual incidence is 20-30 per 100,000 live births. Transposition of the great arteries is isolated in 90% of patients and is rarely associated with syndromes or extra-cardiac malformations.

Cardiac surgical and intensive care mortality in children following cardiac surgery is low, with a 2-5% peri operative death rate depending on the complexity of the procedure. However, postoperative morbidity is common after cardiac surgery, which translates to an increased rate of long term mortality, morbidity, and disability [1]. Despite major improvements in CPB devices, the exposure of host blood to large artificial organ surfaces, combined with myocardial injury during planned myocardial ischemia, results in a significant systemic inflammatory response. CPB-triggered systemic inflammatory syndrome is responsible for the most serious and potentially life-threatening side effects associated with cardiac surgery. CPB, hypoxic-ischemic injury, and the release of damage-associated molecular patterns trigger an inflammatory cascade closely related to sepsis-induced systemic inflammatory response syndrome (SIRS) [2]. It is characterized by endotoxin release, leukocyte and complement activation, and widespread activation of inflammatory mediators, resulting in endothelial leak, increased oxygen consumption, and organ dysfunction [3].

Cardioplegia during CPB allows the surgeon to operate on a still heart. However, while the heart is arrested, myocardial blood flow stops, resulting in ischemia and myocardial injury. Upon restoration of blood circulation during release of cross-clamping, CPB provides full flow with high oxygen content, but can also cause reperfusion injury. Central to the pathophysiology of reperfusion injury is a robust local inflammatory response, with children appearing to be particularly susceptible to developing multisystem organ failure as a result of these processes [4,5]. As a result of the combined effects of direct CPB-related inflammation, myocardial ischemia, and reperfusion, the heart is unable to meet the metabolic demands of the body, resulting in organ hypoperfusion. This situation is called Low Cardiac Output Syndrome (LCOS), and is commonly defined according to the following criteria: increased need for inotropes, increased arterial-venous oxygen extraction, increased blood lactate levels (metabolic acidosis), decreased urine output (oliguria), and need for extracorporeal life support (ECLS) [6].

Children post-bypass commonly develop a potentially life-threatening LCOS, a major determinant of postoperative outcomes. LCOS commonly presents as respiratory and renal failure, leading to organ hypoperfusion, cardiac arrest, and death. The severity of the LCOS is also influenced by the type of surgery performed, the pre surgical condition of the patient, as well as non-surgical injury to the heart muscle during CPB. Several studies have shown that LCOS is a powerful determinant of postoperative

morbidity and mortality (Table 1), which is evidenced in the 25-40% of children who present with LCOS in the hours following CPB during cardiac surgery [7]. LCOS may lead to transient or permanent organ damage, brain ischemia, cardiac arrest and death. Long-term outcomes may also be affected by acute injury to the developing brain caused by LCOS-related ischemia [8].

CPB-related injuries are more pronounced in infants and young children for a number of reasons, including: higher metabolic rate, stronger inflammatory responses, higher bypass circuit to patient blood volume, and altered homeostasis. At the same time, they also represent the cardiac surgical group with the highest mortality, and highest risk of long-term neurological sequelae due to the vulnerability of the developing brain. In addition, bypass-induced modulation of inflammatory cytokines can lead to subsequent immunoparalysis, thus enhancing the risk of postoperative invasive infections [9]. The presentation of LCOS symptoms should be treated with an increased amount of fluid boluses and inotropes. Organ replacement, such as renal dialysis and prolonged mechanical ventilation may also be required. In the most severe cases, the heart is supported mechanically with ECLS. Considering the severe impact of LCOS on patient-centred outcomes after surgery in children with congenital heart disease, improved strategies that target LCOS are urgently needed [8]. Interventions leading to reduced LCOS are likely to reduce the incidence of organ failure, reduce severe major events, reduce the need for ECLS, reduce the time on postoperative ventilation, and reduce the ICU length of stay.

Commonly used approach to reduce LCOS:

Steroids.

Preoperative administration of steroids, such as methylprednisolone, is the most common, but controversial approach for reducing the inflammatory response associated with LCOS. Only a few prospective randomised controlled trials of corticosteroid administration in children undergoing cardiac surgery have been performed, and they present with conflicting results [10]. Patients randomised to dexamethasone in a relatively small study had significantly less fever, required less supplemental fluid, had greater preservation of renal function, less impairment of oxygenation, experienced a significantly shortened duration of mechanical ventilation, and reduced length of stay in the intensive care unit. Another trial investigating the effect of methylprednisolone administration four hours prior to CPB showed that patients who received two doses of methylprednisolone had significantly less fever, required less fluid, had a significantly reduced oxygen extraction ratio, and experienced a trend towards a reduced length of stay in the intensive care unit ($p = 0.07$) [11]. A recent multicentre study on infants undergoing Norwood surgery reported increased mortality in patients receiving intraoperative steroids, confirming previous concerns about the risks associated with steroids [12]. The latest adult data suggests that steroids could cause more harm than benefit [13].

Modified Ultra Filtration (MUF).

MUF is another prophylactic approach being trialled in paediatric cardiac centres [14]. Ultrafiltration removes water, reverses haemodilution, and eliminates low-molecular-weight substances, including inflammatory mediators [15]. Ultrafiltration may be used during CPB (i.e., conventional ultrafiltration, CUF), or once CPB is complete (i.e., MUF), with the composition of filtrates being identical and the assertion being that a greater amount of fluid and therefore solute may be removed following CPB than can be removed with CUF alone. While some studies have reported significantly beneficial effects from using MUF post-operatively, others have failed to do so [16].

The proposed physiological effect of Nitric Oxide (NO)

NO is an endogenous anti-inflammatory mediator that helps to protect endothelial beds and immunologically active cells. NO has a myocardial protective effect by reducing reperfusion injury [17]. NO generation is essential for regulation of endothelial function and microvascular inflammation. However, dysregulation of endogenous NO during CPB may aggravate the subsequent inflammatory response [18]. Several animal and human studies have demonstrated that exogenous NO can reduce myocardial damage in clinical and experimental settings of ischemia and arrest [19-22]. Reduced NO signalling is associated with several known risk factors for the most common cardiovascular diseases (CVDs) [23]. Organic nitrates, such as nitro-glycerine (also known as glyceryl trinitrate), have been used clinically in the treatment of CVDs for more than 150 years. However it wasn't until the late 1970s that it was demonstrated that their beneficial effects were due to the release of NO [24,25]. NO has since been found to be produced endogenously and to play a key role in the regulation of many physiological processes, including cardiovascular function [3]. Together, these findings have triggered substantial interest in the identifying therapeutic ways of modulating NO signalling.

Only a few drug candidates designed to directly activate NO signalling have reached the clinic, the most notable of these being inhaled NO for the treatment of newborns with pulmonary arterial hypertension (PAH) [26], and phosphodiesterase inhibitors to treat erectile dysfunction [27]. Therapeutic modulation of NO signalling is challenging as the effect must occur at the correct location, time, and dose. Inhaled NO, which operates at the surface of the pulmonary vascular bed, has minimal side effects owing to its pharmacokinetic characteristics [26].

NO, when administered as an inhaled gas to adults on CPB undergoing cardiac surgery, blunts the release of myocardial injury markers, and ameliorates left ventricular subclinical dysfunction [28]. Therefore, direct delivery of NO to CPB may have the capacity to reduce the CPB-induced systemic inflammatory response with minimal systemic side effects due to the short half-life and localized delivery of the gaseous drug, thus mitigating the detrimental consequences of CPB to the patient. A small, single centre U.S. study tested this hypothesis and reported a reduction in bypass-induced inflammation following the delivery of gaseous NO to bypass circuits [29]. Children receiving gaseous NO into CPB had a significantly shortened duration of mechanical ventilation (8.4 versus 16.3 hours; $P < .05$) and reduced intensive care unit length of stay (53.8 versus 79.4 hours; $P < .05$), compared to the placebo group. These patients also reported significantly lower troponin and B-type natriuretic peptide levels postoperatively. In addition, NO reduced the positive fluid balance, resulting in

significantly less diuretic usage, and higher haemoglobin levels postoperatively. However, this study had a small number of participants (n = 16) with the congenital heart condition Tetralogy of Fallot.

A separate, randomised controlled study was conducted by the Royal Children's Hospital in Melbourne on 198 children [30]. This pilot study confirmed the positive effects of gaseous NO reported in the U.S. trial, as well as a reduction in the incidence of LCOS. Other improved patient outcomes included a reduced need for ECLS, trends towards a reduced length of stay, and shorter duration of ventilation. In light of these promising preliminary results from these two separate studies, a large multicentre trial to test these findings in children requiring cardiac surgery is needed.

Currently there is a multicentre study ongoing in Australia and New Zealand evaluating the effect of NO on LCOS in all infants undergoing any type of bypass surgery (Nitric Oxide on Cardio pulmonary bypass (NOCPB)). The Royal Children's Hospital is participating in the NOCPB trial. The NASO study will restrict recruitment to infants with the same diagnosis, TGA, and undergoing the same operation, ASO. Isolated TGA is managed in a similar manner all over the world, and hence this single operation and diagnosis allows a universally applicable definitive answer, on the efficacy of NO in the CPB circuit, to be obtained.

Why is this study important?

Postoperative paediatric cardiac surgery patients consume a large fraction of intensive care resources, and are at a very high risk of major complications; including cardiac arrest, long term neurological impairment, and death. Approximately 10-20% of children with CHD present with major neurological sequelae postoperatively, which creates a lifelong burden for patients, families, healthcare systems, and society as a whole [8]. Minimising perioperative morbidity would not only reduce intensive care resource utilisation, but may also impact positively on long-term neurological outcomes.

Why multi-centre?

Multi-centre trials reduce the bias inherent in single site approaches, and provide a true indication of the efficacy and applicability of potential therapies in real-world conditions. Inter-centre differences that may affect patient outcomes include: the heart defect specific surgical approach, operator experience and expertise, the use of other techniques such as priming of the bypass, management on bypass, and MUF post-bypass. The post-operative intensive care management strategies also vary between intensive care units. These include: respiratory strategies, fluid and inotrope management, as well as the threshold at which ECLS is administered. Therefore, only an appropriately designed multi-centre study would be capable of demonstrating the generalizable impact of NO on outcome.

Feasibility

The two completed studies from the US and Australia have demonstrated the feasibility of conducting a study with a similar population and design [29, 30].

The pilot trial conducted at the Royal Children's Hospital Melbourne was undertaken to investigate the feasibility and safety of NO delivery in infants and children undergoing CPB [30]. The Royal Children's Hospital, Melbourne, has the largest paediatric cardiac surgical service in Australia and New

Zealand. Over a period of 12 months, a total of 198 children with CHD requiring surgical correction were randomised (101 to the NO group; 97 to the control group) and then stratified into three groups: children < 6 weeks of age; > 6 weeks – 2 years of age; >2 years of age. All staff were blinded, except for the perfusionist, who was responsible for monitoring the concentration of NO, thus ensuring that it did not exceed the target concentration of 20 ppm. The surgeons, anaesthetists and ICU staff were all blinded to gas delivery. All clinically relevant data was recorded as per standard practice and was accessed through registry data and medical records. The primary outcome was to assess the feasibility and safety of NO delivery, as well as the reduction in LCOS in children undergoing cardiac surgery with CPB.

The pilot study reported a reduction in LCOS, and a trend towards shortened length of mechanical ventilation in patients allocated to the NO intervention. This was particularly significant in the younger children and those having high risk surgeries RACHS 4-6 (Risk adjustment for congenital heart surgery). This pilot study did not show an NO treatment effect in children > 2 years of age. However, postoperative LCOS is much less common in the older age group compared to the < 2 year group, which may be related to different surgical procedures, the proportional surface of the bypass circuit, or disparate immune responses. A total of 170 eligible patients were identified who were less than 2 years of age. Of these, 133 (88%) were randomised to receive NO delivered into the oxygentator of the bypass machine (n=65) or no NO (n=68). Of the 37 patients not randomised, 9 patients needed ECLS, 8 died, and 20 refused consent.

Table 1 and 2 show further unpublished analyses of the subgroup of participants from the pilot study with TGA who had ASO (n=24). This analyses supports the findings of a reduction in LCOS in the full cohort NO treatment group compared to the control group, In particular, there was significantly less ECMO and cardiac arrest in the group receiving Nitric Oxide.

Table 1.

Variable	All patients (N=24)	Nitric (N=12)	No Nitric (N=12)	P Value
Age at surgery (d), median (IQR)	9(6.5-22.5)	9.5(7-20.5)	9(5.5-23)	0.73
Male sex, n/N (%)	18/24 (75)	9/12(75)	9/12(75)	1.0
<36 weeks gestation, n/N (%)	2/24 (8)	1/12 (8)	1/12 (8)	1.0
Comorbidities, n/N (%)	6/24(25)	4/12(33)	2/12(17)	0.32
ECMO preoperatively, n/N (%)	1/24	0/12(0)	1/12(8)	1.0
RACHs-4 score, n/N (%)	10/24 (42)	6/12 (50)	4/12 (33)	1.0
Aristotle	10(8-11)	10(7.5-11)	10(9-11)	0.609
Duration CPB (min),median (IQR)	179(168-217)	179(169-193)	192(165-314)	0.488
Duration XC (min),median (IQR)	109(95-134)	111(105-127)	102(88-200)	0.644

Table 1. Demographic, clinical, and operative characteristics of subgroup ASO for TGA in the Melbourne pilot study.

Table 2

Variable	Nitric (N=12)	No Nitric (N=12)	P Value
ECMO postoperatively, n/N (%)	0/12 (0)	4/12 (33)	0.047
Any LCOS, n/N (%)	2/12 (17)	7/12 (58)	0.089
Delayed chest closure, n/N (%)	1/12 (8)	0/12 (0)	0.3
Cardiac arrest postop, n/N (%)	0/12 (0)	3/12 (25)	0.64
Renal replacement therapy, n/N (%)	7/12(58)	9/12(75)	0.667
Inhaled Nitric Oxide during ICU, n/N (%)	0/12(0)	2/12(17)	0.239
Duration Ventilation (hr),median (IQR)	70(40-88)	75(49-116)	0.2039
PICU LOS (hr),median (IQR)	95 (59-125)	139 (86-160)	0.046
Hospital LOS days (d),median (IQR)	10.5(9.5-12)	13.5(11-17)	0.029

Survival discharge home, n/N (%)	12/12 (100)	10/12(83)	0.478

Table 2. Clinical outcomes in Subgroup ASO for TGA in Pilot Study, Melbourne

Expected enrolment opportunity

Arterial Switch Operation (ASO) for TGA is generally done in the second week of life as an elective procedure in a baby with few other congenital abnormalities. By allowing each centre to have their own protocols of care (pre, intra and postoperatively) and the only data is 'routine clinical data" we anticipate each centre having high rates of screening and gaining consent. Also with randomisation by centre and the only cost that of the NO (and with multiple companies now providing the gas at cheaper price) many centres will participate. It is anticipated recruitment will be completed within 2 years.

This study will run concurrently with the NOCPB trial. However, both studies will run independently and reach completion as per individual study protocol. Participants who meet criteria for this study and have been previously consented for NOCPB will be consented retrospectively for use of their data only. They will not require further intervention but if they consent relevant data will be obtained from their patient electronic medical record.

Research Team Performance

This trial will be conducted in major tertiary paediatric centres. The members of the steering group have experience in conducting multi-centre RCTs within paediatric intensive care units.

3. STUDY OBJECTIVES

3.1 Primary objective

To investigate whether the exposure to gaseous NO reduces the incidence of postoperative major adverse events in infants on CPB. Major adverse events include cardiac arrest, emergency chest opening, use of ECMO, and death.

3.2 Secondary objectives

To investigate whether the exposure to gaseous NO results in improved clinical outcomes of infants on CPB undergoing an ASO for correction of TGA after the exposure to NO.

4 OUTCOMES

4.1 Primary outcome

The primary outcome is the number of participants with major adverse events (MAEs) within 28 days post-operatively. MAEs include cardiac arrest, emergency chest opening, use of ECMO, and death.

4.2 Secondary outcomes

The secondary outcomes for this study to be measured at Day 28 post surgery are:

1) length of stay in ICU (hours)
2) length of stay in hospital (days)
3) ventilator free days
4) inotrope hours
5) dialysis free days
6) inhaled NO hours
7) ECMO free days
8) Closed sternum days

All data related to secondary outcomes will be obtained from data in Redcap.

1. Length of stay in ICU (hours) will be calculated from date and time of admission to ICU date and time of discharge to ICU.
2. Length of stay in hospital (days) will be calculated from date and time of admission to hospital to date and time of discharge to hospital.
3. Ventilator free days will be calculated from date and time of intubation to date and time of extubation. Each day (or part of a day) will be counted as a day.
4. Inotrope hours will be calculated from data input into REDCAP.
5. Dialysis free days will be calculated from date and time of start of dialysis to date and time of stopping dialysis. Each day (or part of a day) will be counted as a day.
6. Inhaled NO free days will be calculated from data input into REDCAP.
7. ECMO free days will be calculated from date and time of start of ECMO to date and time of stopping ECMO. Each day (or part of a day) will be counted as a day.
8. Closed sternum days will be calculated from date and time of start of chest opening (or return to ICU time if delayed chest closure) to date and time of chest closure. Each day (or part of a day) will be counted as a day.

These scores will be combined to create a composite free day score consisting of a combination of the individual scores for outcomes 1 to 8.

5 STUDY DESIGN

This is a multi-centre, double blind, randomised, controlled superiority trial of two parallel arms.

Participants will be randomised 1:1 into one of two arms:

- Intervention arm will receive NO 20 parts per million (ppm) into the oxygenator of a cardio-pulmonary bypass circuit
- Control arm will not receive NO

Patients will be stratified by centre and by age, 0-21 days and > 21 days, at time of surgery.

At the end of CPB, the participants will return to the Intensive Care Unit where standard care will continue. Participants will be followed up until Day 28 post surgery.

Approximately 20 centres across Australia/Oceania, Asia, Europe and North America are expected to participate.

6 PARTICIPANTS AND RECRUITMENT

6.1 Number of Participants

A total of 800 participants will be enrolled in the study.

6.2 Eligibility Criteria

6.2.1 Inclusion criteria

Each participant must meet all of the following criteria to be enrolled in this study:

- Infant aged greater than or equal to 36 weeks gestation
- Infants less than 2 years
- Diagnosed with TGA and requiring Arterial Switch Operation
- Consent of parents/guardian.

6.2.2 Exclusion criteria

Potential participants will be excluded if they meet any of the following criteria:

- They have multiple major congenital anomalies (anomalies which affect the infant's life expectancy or health status as outlined in appendix 2: RACHS-1 Major non-cardiac structural anomalies)
- They have multiple other cardiac abnormalities (with the exception of ASD, VSD or PDA)
- They weigh less than 2.2kgs.
- Prior surgical exposure to cardio-pulmonary bypass

6.3 Identification and recruitment of potential participants

Infants with TGA will be identified through review of medical records and admission diagnosis by the research coordinators, the on call cardiac surgical team and/or Intensive Care Unit (ICU) staff.

Consent will be sought from the parent(s)/guardian of every eligible infant scheduled for the Arterial Switch Operation over the period of recruitment. Parents will be approached after the medical team have discussed surgery with parents but prior to time of surgery. For those consented retrospectively who are already recruited in the NOCPB study, they will be contacted by a phone call prior to sending out written information.

For Australian centres, the consent for this study will be done concurrently with the consent for NOCPB. It is not anticipated that children will be recruited solely for this study within the Australian centres while both studies are running, however, parents may opt to participate in only one study. Should recruitment for the NOCPB be completed prior to the completion of this project participants will be recruited solely to this project. International sites not involved in NOCPB will solely recruit for this study.

A pre-screening log will be maintained to document patients for whom the parent/guardian was not approached (i.e. ineligible or missed), and to document patients for whom consent was refused.

6.4 Consent

The study will be explained to the parent(s)/guardian by the nominated person at each centre (research coordinator, perfusionist, intensivist, surgeon etc.) who will emphasise that they are free to refuse consent at any time, and that if they do so their child's medical care will not be affected in any way. Consultants involved in the surgical procedure or clinical care will not be involved in the consent

process. In addition, the parent/guardian(s) of the child will be informed that the data collected about themselves and their child will be de-identified for use in any publications that may arise from the trial. The parent(s)/guardian will be given time to consider study participation and will be given the study Information Statement to review. Further explanation will be offered by a member of the research team, who will be available to respond to questions from the parents prior to the consent form being signed. Written consent will be obtained if the family wishes to take part in the study.

It is important that the investigator obtaining consent ensures that the parent(s)/guardian has a good understanding of what the study means for the child prior to consent. If the parent/guardian(s) is unable to give informed consent, their child should not be enrolled into the study.

7 INTERVENTION

7.1 Treatment arms

Participants will be randomly assigned to the experimental treatment arm or control arm to receive nitric oxide during CPB or CPB without NO (standard bypass), respectively. Both groups treatment will be blinded to all except the perfusion team by draping the regulator and facing it away from all other staff.

7.2 Trial Interventions

Experimental treatment: *Participants* will receive NO blended into the fresh gas flow of the CPB oxygenator and maintained at 20 ppm via an Ikaria INO Max DSIR (or following standard procedure in each hospital). NO will be started when the patient is on CPB, and stopped when the patient comes off CPB. The unit will face away from the anaesthetist or remain draped to maintain blinding.

Each Coordinating Principal Investigator is responsible for ensuring the participating sites in their country fulfil their regulatory requirements for using nitric oxide in this trial. Individual sites are responsible for obtaining nitric oxide for use in this study.

Use of nitric oxide in this study in Australian sites is within the conditions of it's marketing approval and therefore a CTX/CTN is not required for lodgement with the TGA.

Control: Participants will receive standard gas concentration blended into the oxygenator of the CPB. The Ikaria (or alternative NO delivery device) will remain attached and turned on with NO at zero ppm and the unit will face away from the anaesthetist or remain draped to maintain blinding.
PERIOPERATIVE CARE

Centres will adhere to their own methods for anaesthesia, surgical techniques, and CPB perfusion. *Rationale:* to allow centre specific individual practice, and to avoid interfering with centre specific cardiac surgical loads and outcomes.

All participants will receive a minimum sweep gas flow of 2.0 L/min. Carbon dioxide (CO_2) will be maintained at $PaCO_2$ 35–40 mmHg to ensure NO mixing and delivery to the experimental treatment group. Sweep gas FiO_2 will be set at 0.9–0.6, depending on temperature, aiming for PaO_2 of 200+mmHg.

7.3 Post-operative Care

Post-operative care will be performed according to the standard protocol for each site. Inotrope delivery, fluid management, renal replacement therapy, and ECLS will be administered as per standard care. *Rationale*: site specific intensive care practice will continue during the trial.

8 RANDOMISATION AND BLINDING

8.1 Randomisation

Participants will be randomly allocated to the NO or control group in a 1:1 ratio, stratified by centre and age at time of surgery, using a computer generated, blocked randomisation schedule. Randomisation will be web-based (REDCap), hosted by the Clinical Epidemiology and Biostatistics Unit (CEBU) at the Trial Coordinating Centre, MCRI. Participants will only be randomised once eligibility has been confirmed, immediately prior to surgery.

In the event of REDcap failure a contact number will be provided for manual randomisation, this will be available 24 hours a day.

8.2 Blinding arrangements

The study perfusionist at each site will be the only staff member aware of the treatment assignment (NO delivery versus standard bypass) for each participant. The research coordinator will notify the perfusionist of the participant's inclusion to the study and provide the REDCAP participant number for patient that is sequentially allocated by REDCAP. The perfusionist will have access to REDCAP randomisation page on REDCAP which will be blinded to all other members of the research team.

Blinding in the operating theatre will be achieved by covering the NO delivery system with drapes. The surgeons, anaesthetists, ICU staff and parent/caregiver will not be aware which treatment arm a participant has been assigned to. Those analysing the data will remain blinded until final database lock, except for the independent statistician reporting to the Data Safety and Monitoring Board. The parent/caregiver can find out the treatment assignment as per local centre policy on completion of study.

The perfusionist will be instructed that all aspects of CPB, except for the provision of NO (or not), will be performed in a standard manner. The perfusionist will not attend the surgical handover in ICU.

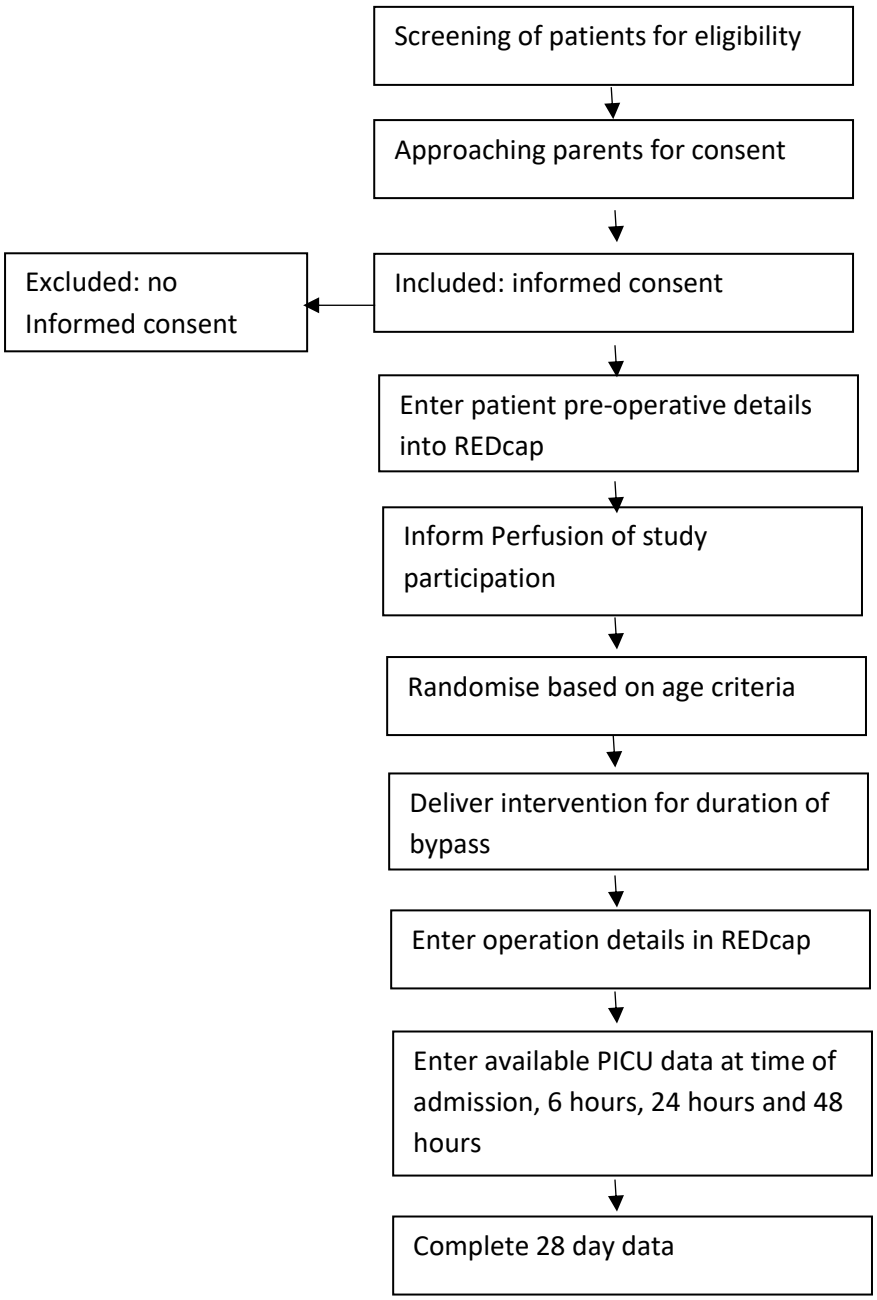
8.3 Breaking of the Study Blind

NO has been used in the inhaled form for over 20 years and has a sound safety record (18, 26). Since 2007, it has been registered with the Therapeutic Goods Administration in Australia. Given the very short half-life of NO (0.1-0.5 seconds), its administration via the CPB oxygenator, and the immediate cessation of effects following completion of CPB (and hence NO administration), we do not foresee any clinical situation in which the blind would need to be broken.

However, the randomisation code for an individual participant may be unblinded in emergency situations, where the investigator decides a participant cannot be adequately treated without knowing the identity of their treatment allocation. To break the randomisation code the Investigator must contact the randomisation personnel (the perfusionist) within their facility. If any unblinding occurs, the reason for unblinding must be documented in the protocol deviation section of REDcap and the CPI must be notified.

9 STUDY VISITS AND PROCEDURES

9.1 Trial timeline



9.2 Schedule of assessments

The Schedule of Assessments details the specific timing of procedures/evaluations. Enrolment can occur at anytime prior to surgery. Randomisation and intervention occurs in theatre, Patient assessment (vital signs and laboratory bloods results) occurring at time points; PICU admission (within an hour of admission), 6 hours post admission (4-8 hours post admission), 24 hours post PICU admission (20-28 hours post admission), 48 hours post PICU admission (40-56 hours post admission). Final data point occurs at 28 days post intervention for patient outcomes, this is a completion of data collection. Data must be entered initial surgical data within 28 days of date of surgery and completion within 56 days of surgery (4 weeks after 28days).

		TRIAL PERIOD						
	Enrolment	Randomisation to intervention	Admission to ICU	Post Admission (hours)				Post Admission (days)
TIME POINT	Before surgery	Within 24 hours of surgery	*Day 1*	*0*	*6*	*24*	*48*	*2-28 days*
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Allocation to intervention		X						
INTERVENTION: CPB with or without NO			x					
ASSESSMENTS:								
Baseline variables	X							
Vital signs and lab results				X	X	X	X	
Patient outcomes			X					X

Review of Adverse Events			X ← →

9.3 Screening

All patients undergoing either elective or emergency cardiac surgery for an ASO will be screened against the eligibility criteria. This screening will be performed by the on call research coordinators or cardiac surgical team or ICU team members. Screening logs will be maintained by local sites. When patients are deemed eligible they will be approached for consent. All eligible patients will be entered in to the patient eligibility page of the REDCAP database regardless of participation in the study including those who were approached but declined and those who were eligible but were missed.

9.4 Data Collection

Baseline variables, primary endpoints, secondary endpoints, parameters as defined in 9.6 will be prospectively recorded. All available data will be entered into REDCAP.

9.5 Measured Parameters

9.5.1 Baseline variables

Sex, age, weight, gestational age (at birth), primary cardiac diagnosis, secondary diagnoses (including comorbidities such as prematurity (less than 36 weeks) and diagnosis of a syndrome, hospital admission date, preoperative invasive ventilation, occurrence of atrial septectomy prior to their switch operation

9.5.2 Surgical intervention and bypass

- Length of cardiopulmonary bypass time and aortic cross clamp time
- Use of Modified ultrafiltration (MUF) or Slow concentrated ultrafiltration (SCUF) in theatre
- Steroids administered during theatre or within 48 hours prior to surgery

9.5.3 Postoperative status

- Date and time of return to PICU
- Data on postoperative cardiovascular status at each time point (on admission to ICU, 6 hours 24 hours and 48 hours post admission; (heart rate, blood pressure, central venous pressure, left atrium pressure (if available), respiratory status according to ventilator settings urine output (mL/kg/hr)
- Inotrope doses at each data point (on admission to ICU, 6 hours 24 hours and 48 hours post admission)
- Use of renal replacement therapy e.g. CVVF, Peritoneal Dialysis (hours)
- Use of inhaled Nitric Oxide (iNO). (hours)

- Arterial and mixed venous blood gas analysis (taken from jugular, subclavian, or femoral central venous catheter), including lactate measurements , blood Ph., arterial and venous saturations, pCO2, methemoglobin, bicarbonate/base excess
- Laboratory tests: platelets, white cell count, haemoglobin, creatinine, pO2, SataO2, and coagulation (APTT)
- Amount and type of blood products administered during first 24 hours
- Delayed chest closure and time of chest closure
- Occurrence of adverse events including emergency chest opening, extra corporal life support or death
- Arrhythmia and use of pacing post operatively
- Steroids administered during 48 hours postoperatively
- Patient PICU and hospital discharge date and time

10 ADVERSE EVENTS AND RISKS

10.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a patient or clinical trial participant administered a medicinal product and does not necessarily have a causal relationship with this treatment.

Adverse Reaction (AR): Any untoward and unintended response to an investigational medicinal product related to any dose administered.

Comment: All adverse events judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an investigational medicinal product would qualify as adverse reactions. The expression 'reasonable causal relationship' means to convey, in general, that there is evidence or argument to suggest a causal relationship.

Serious Adverse Event (SAE) / Serious Adverse Reaction (SAR): Any adverse event/adverse reaction that results in death, is life threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity or is a congenital anomaly or birth defect.

Note: Life-threatening refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

Medical and scientific judgement should be exercised in deciding whether an adverse event/reaction should be classified as serious in other situations. Important medical events that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in this definition should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): An adverse reaction that is both serious and unexpected.
A SUSAR is any SAE that is both suspected to be related to the trial treatment and is unexpected (i.e. not consistent with the available safety information in the approved Product Information.

Note that SUSARs require expedited reporting to stakeholders including the Sponsor, Investigators, HREC /IRB/IEC, local governance office and applicable regulatory body.

Safety issues (requiring expedited reporting)

The following definitions describe additional safety events that require expedited reporting to stakeholders including the Sponsor, Investigators, HREC, local governance office and TGA.

Significant Safety Issue (SSI): A safety issue that could adversely affect the safety of participants or materially impact on the continued ethical acceptability or conduct of the trial.
Comment: A SSI is a new safety issue or validated signal considered by the Sponsor in relation to the investigational medicinal product that requires urgent attention of stakeholders. This may be because of the seriousness and potential impact on the benefit-risk balance of the investigational medicinal product, which could prompt regulatory action and/or changes to the overall conduct of the clinical trial, including the monitoring of safety and/or the administration of the investigational medicinal product.

Urgent Safety Measure (USM): A measure required to be taken in order to eliminate an immediate hazard to a participant's health or safety. Note: This is a type of SSI that can be instigated by either the investigator or sponsor and can be implemented before seeking approval from HRECs or institutions.

10.2 Classification of Adverse Events

Monitoring AEs requires that they be classified as to seriousness, expectedness, and potential relationship to the study intervention (causality).

a. Seriousness

Adverse events are classified as serious or non-serious.
A serious adverse event (SAE) is defined as any AE that:

- results in death;
- is immediately life threatening;
- requires inpatient hospitalisation;
- requires prolongation of existing hospitalisation;
- results in persistent or significant disability/incapacity;

Important medical events will be considered an SAE if they jeopardise the patient, and/or require medical or surgical intervention to prevent one of the aforementioned outcomes.
The specific SAE related to NO delivery during CBP is increased MetHb (MetHb is >3%).

b. Expectedness

The investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected.

Expected: An event is considered expected if it is known to be associated with the study drug and/or the disease state. This study will be performed in infants undergoing high risk cardiac surgical

procedures. As such death, high risk organ support (ECLS), or renal replacement therapy are expected adverse events.

Unexpected: An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the trial intervention.

c. Causality

All adverse events must have their relationship to trial intervention assessed by the investigator who evaluates the adverse event based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the trial product should always be suspected.

The relationship of the event to the trial intervention will be assessed as follows:

- Unrelated: There is no association between the trial intervention and the reported event. AEs in this category do not have a reasonable temporal relationship to exposure to the test product, or can be explained by a commonly occurring alternative aetiology.
- Possible: The event could have cause or contributed to the AE. AEs in this category follow a reasonable temporal sequence from the time of exposure to the intervention and/or follow a known response pattern to the test article, but could also have been produced by other factors.
- Probable: The association of the event with the trial intervention seems likely. AEs in this category follow a reasonable temporal sequence from the time of exposure to the test product and are consistent with the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgement based on the investigators clinical experience.
- Definite: The AE is a consequence of administration of the trial intervention. AEs in the category cannot be explained by concurrent illness, progression of disease state or concurrent medication reaction. Such events may be widely documented as having an association with the test product or that they occur after rechallenge

10.3 Capturing and eliciting adverse event/reaction information

Adverse events and adverse reactions (non-serious and serious) will be captured from the time of intervention until 28 days post-surgery and will be followed until resolution or stabilisation.

10.4 IDENTFICATION AND DOCUMENTATION OF ADVERSE EVENTS

The site investigator will be responsible for reviewing all AEs by obtaining information from the source documents on all AEs, for the period from randomisation until completion of each individual child's participation in the study. The Site Principal Investigator/delegate is responsible for recording all safety events in the source document. The severity of an AE will be assessed by an investigator according to the definition in section 10.2. Changes in the severity of an AE will be reported. AEs characterised as intermittent will be documented for each episode. All AEs will be followed to an adequate resolution.

The AE will be described in the source documents (e.g. medical record) and captured on the CRF and will include:

- A description of the AE
- The onset date, duration, date of resolution
- Severity (mild, moderate or severe – what is the impact on the participant's daily life?)
- Seriousness (i.e. is it an SAE?)
- Any action taken, (e.g. treatment, follow-up tests)
- The outcome (recovery, death, continuing, worsening)
- The likelihood of the relationship of the AE to the trial treatment (Unrelated, Possible, Probable, Definite)

A condition that presents at screening, but that does not worsen, will not be classified as an AE. Abnormal laboratory values will not be considered as AEs, unless deemed clinically significant by the investigator and documented as such. This study will be performed in infants undergoing high risk cardiac surgical procedures. As such death, high risk organ support (ECLS), or renal replacement therapy are expected adverse events.

The mortality rate of participating units in Australia and New Zealand is monitored in three month intervals by the ANZPIC Registry as a standard safety measure. The registry has well defined rules around investigating excessive deaths and these will be applied to this study. In the event of increased mortality, the Data Safety Monitoring Board will be informed, and where required this information will be reported to HREC. Local sites are responsible for reviewing deaths as an AE and reporting deaths that are deemed related to the study.

10.5 Reporting of safety events

Any SAE occurring in a study participant from the time of randomisation until 28 days posy operatively will be documented in the source documentation and the SAE will be assessed by an investigator according to the definition in section 10.2. SAE that are deemed to be related to the study will be reported. This study will be performed in infants undergoing high risk cardiac surgical procedures. As such death, high risk organ support (ECLS), or renal replacement therapy are expected adverse events.

<u>Site Principal Investigator Reporting Procedures</u>
The Site Principal Investigator/delegate is responsible for recording all safety events in the source document.

The Investigator is responsible for expedited reporting (within 24 hours of becoming aware of the event) to the Sponsor-Investigator the following local safety events:

1. USMs (urgent safety matters)
2. SUSARs Suspected unexpected serious adverse reactions
3. Increased MetHb (MetHb is >3%)

The Site Principal Investigator is responsible for reporting SAEs (including SUSARs) to the Sponsor-Investigator as soon as possible but within 24 hours of the first knowledge of the event. These reports should be submitted using the trial adverse event and SUASAR forms on REDCAP

The Site Principal Investigator is also responsible for reporting SSIs, local USMs and local SUSARs to their research governance office within 72 hours of becoming aware of the event and in accordance with their local governance authorisation.

The following information will be extracted from the source documents and entered onto the case report form (CRF):

- A description of the AE;
- The onset date, duration, date of resolution;
- Severity (mild, moderate or severe);
- Seriousness (SAE or non SAE);
- Any action taken (treatment, follow-up tests);
- The outcome (recovery, death, continuing, worsening);
- The likelihood of the AE impacting the study treatment (unrelated, possible, probable, definite).
- Whether the AE meets the criteria for a MAE (combining cardiac arrest, extracorporeal membranous oxygenation, death or chest opening [delayed sternal closure]).

Coordinating Principal Investigator Reporting Procedures

The CPI must assess and categorise the Safety Reports received from Investigators and report these to all Site Principal Investigators, the approving HREC and TGA in accordance with the NHMRC's 'Safety monitoring and reporting in clinical trials involving therapeutic goods' (November 2016) and any additional requirements of the approving HREC. All safety reports must clarify the impact of the safety event on participant safety, trial conduct and trial documentation.

The Sponsor-Investigator is responsible for the following reporting to PIs, the HREC(s) and TGA:

1. All SSIs that meet the definition of a USM within 72 hours of becoming aware of the issue.
2. All other SSIs within 15 calendar days of instigating or becoming aware of the issue
3. For SSIs leading to an amendment of trial documentation:
    a. Submit details of the SSI without undue delay and no later than 15 calendar days of becoming aware of the issue.
    b. Submit amendment to the HREC without undue delay.
4. For SSIs leading to temporary halt or early termination of a trial for safety reasons:
    a. Communicate reasons, scope of halt, measures taken, further actions planned without undue delay and no later than 15 calendar days of decision to halt.
    b. For a temporary halt, notify the PIs, HREC and TGA when the trial restarts, including evidence that it is safe to do so.

The Sponsor will also report SUSARs to the TGA as follows:

1. Fatal or life-threatening SUSARs immediately, but no later than 7 calendar days after being made aware of the issue (follow up info within a further 8 calendar days)
2. All other SUSARs no later than 15 calendar days of being made aware of the issue

The Sponsor is responsible for providing the additional safety information to the approving HREC:

1. Provide an annual safety report, including a summary of the evolving safety profile of the trial

2. Provide any updated Product Information/Investigator's Brochure for the investigational products (if applicable)

All sites outside Australia will be responsible for ensuring that they satisfy their local ethics/IRB reporting requirements regarding adverse event reporting.

11 DATA MANAGEMENT

11.1 Source Data

Each centre participating in the trial will have a site-specific Source Document Plan that will document the source, i.e. original recording, for each data item collected for the trial. This list should be prepared by the site prior to recruitment of the first participant and using the template provided by the Central Coordinating Centre and signed and dated by the Principal Investigator. The list should be filed in the Investigator Site File. Source documents that include source data include, but are not limited to, medical records (electronic or paper), recorded data from automated instruments, laboratory reports and the signed participant information and consent form.

The Principal Investigator is responsible for maintaining adequate and accurate source documents that include all pertinent observations on all the participant's at their site. Source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to source data must be traceable, not obscure the original entry, and explained if necessary.

11.2 Data Capture Methods and Data Storage

The Principal Investigator/delegate is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Hard copy data will be stored by the investigating site in a locked cupboard at a secure location, the local PI is responsible for the storage of codes linking the participant ID with patient personal identifiers.

Electronic data will be securely stored on the RedCAP database. REDCap is hosted on MCRI infrastructure and is subject to the same security and backup regimen as other systems (e.g. the network file servers). Data is backed up nightly to a local backup server, with a monthly backup taken to tape and stored offsite. All data transmissions between users and the REDCap server are encrypted. Regular data quality checks, such as automatic range checks, will be performed to identify data that appear inconsistent, incomplete, or inaccurate.

Access to REDCap is via an MCRI user account or (for external collaborators) via a REDCap user account created by the system administrator. The permissions granted to each user within each REDCap project is controlled by and is the responsibility of the project team. REDCap has functionality that makes adding and removing users and managing user permissions straightforward. REDCap maintains an audit trail of data create/update/delete events that is accessible to project users that are granted permission to view it. Instructions for date entry to REDCap must be read and training log signed prior to commencing data entry on REDcap.

11.3 Record Retention

In accordance with the Victorian Records Act 2001,all study information and documentation at each site must be securely stored, at a minimum until the youngest participant turns 25 years. This period must be extended if required by local requirements outside the state of Victoria (Australia).

Records should not be destroyed without the written consent of the Coordinating Principal Investigator. Participant confidentiality must be protected throughout the record destruction process. Paper records should be shredded. Records stored on a computer hard drive must use a process that completely erases the data. USB drives storing trial data must be physically destroyed. Records must be maintained stating what data was destroyed, how and when.

12 STUDY OVERSIGHT

12.1 Overview

The study will be overseen by an Executive Steering Committee (ESC).

The executive steering committee is responsible for the coordination of the trial over all participating sites. This includes continuous review of participant recruitment, accrual, retention, and withdrawal. It further involves oversight of participant management, adherence to protocol-specified regimens, discussion of adverse events and interim analysis, DSMB recommendations, procedures for data management and quality control.

Specifically, the role of the ESC will be to:

- Review participant recruitment, accrual, retention, and withdrawal,
- Discuss adverse events and relevant safety issues,
- Communicate study progress with all sites,
- Some members of the ESC will also be in the writing group.

ESC FREQUENCY AND FORMAT OF MEETINGS

The ESC will send monthly reports outlining recruitment rates, adverse events and study updates

They will meet four times a year to discuss;

-recruitment to date

-data related to safety

-data related to trial conduct

Additional ad hoc meetings of the ESC may be scheduled if requested by either the PI or the DSMB.

Steering Committee

The steering committee will consist of the lead clinician at each site and all members of the executive steering committee. They will receive the monthly report from the ESC and meet bi-annually to discuss study.

Lead sites

Alder Hey Children's Hospital (Liverpool) and Texas Children's Hospital, USA

Lead sites role and responsibilities

-Ethics,

-Support other sites,

-Participate in executive steering committee.

12.2 Data Safety and Monitoring Board (DSMB)

Prior to the commencement of the study, an independent DSMB will be established, they will be responsible for agreeing on DSMB procedures. These will then be formalised in a signed Charter. The DSMB will consist of one statistician and two independent clinicians, one independent intensive care specialist (from a non-cardiac centre) and one cardiac surgeon. The DSMB will be responsible for safeguarding the interests of trial participants, and monitoring safety of the trial interventions at interim analyses of results. This responsibility will be exercised by providing recommendations about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol-specified regimens and retention of participants, and the procedures for data management and quality control.

The DSMB will be advisory to the principal investigator the ESC, who will have ultimate responsibility for decisions regarding the trial. The PI will be responsible for promptly reviewing the DSMB recommendations, and presenting recommendations to the ESC who shall decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in study conduct are required. If ESC does not agree with the DSMB recommendations then a memo justifying the reasons for not complying with the recommendations must be promptly forwarded to the DSMB.

12.3 Central Coordinating Centre

The central coordinating team will be based at Royal Children's Hospital, Melbourne.

12.4 Local Site

At each participating site, the study will be coordinated by an on-site Study Management Team. The team will typically consist of the site PI, a site study coordinator/research assistant and a perfusionist. The site PI will be responsible for local oversight of the study, including: safety monitoring; ensuring that the study is conducted according to the protocol; and ensuring data integrity. The site PI will review the data for safety concerns and data trends at regular intervals, and will promptly report any significant protocol deviations or significant event that might arise during the study to the approving

HREC, institutional research governance office, and the central coordinating team.

12.5 Quality Control and Quality Assurance

Standardisation of all aspects of the study by local investigator teams, training of the centre PI and research assistant will be undertaken by the central coordinating team. The site PI and site research assistant will train the remaining local investigator team, in particular the senior medical staff conducting consent and randomisation.

13 STATISTICAL METHODS

13.1 Sample Size Estimation

The rate of MAE in the control arm is assumed to be 15% based on the data observed over the last 5 years at RCH, while the rate in the intervention arm is assumed to be 8% according to a previous study[30]. A two-group chi-square test with a 0.05 2-sided significance level will have 87% power to detect a difference between the two proportions (0.15 and0.08) when the sample size in each arm is 400, assuming no drop out or loss to follow-up.

13.2 Statistical Analysis Plan

13.3 Population to be analysed

All participants randomised to either of the two treatment arms will be included in the intention to treat (ITT) analysis. Participants will be included in the treatment arm that they were randomised to, regardless of what treatment (if any), they received. The primary efficacy analysis will be performed on the ITT population.

All participants who received any study medication (NO or control) will be included in the safety analysis. Participants who were discontinued from the study prior to receiving any study medication, or who did not receive study medication due to any other reason, will be excluded from the safety population. The safety analyses, including analysis of AEs, but excluding analysis of the primary outcome variable (MAE), will be performed using the safety population.

13.4 Handling of missing data

Since this study will be performed with hospitalised patients, only limited missing data is expected. If more than 20% of participants do not have data on MAE, then the primary outcome variable will be analysed as time to MAE. All participants with missing 28 days data at the time of discharge or at last MAE will be censored. 28 day data will be obtained from routine follow up at local site or from the referring hospital, therefore limited missing data is expected.

13.5 Methods of analysis

Primary endpoint

The primary outcome will be MAE at post-operatively, which will encompass cardiac arrest, ECMO, emergency chest opening and death. The proportion of participants in each of the two treatment arms who experienced MAE will be reported, with 95% confidence intervals (CI) and a risk ratio or risk difference between the two treatment arms. The proportions in the two treatment groups will be compared using a Cochrane-Mantel-Haenszel test, stratified by site.

Secondary endpoint

Patient outcomes, including length of stay in ICU, length of stay in hospital, ventilator days, inotrope hours, dialysis days, inhaled NO hours, ECMO days, Open sternum days, all will be compared between the two treatment arms using fixed effects generalised linear models (with the stratification variables, age at surgery as a fixed effect and site, as a random effect). The difference between mean values for the two treatment arms will be reported along with a 95% CI and p-value. Patient outcomes will be combined to create a composite free day score consisting of a combination of the above scores.

Binary secondary outcome variables (for example mortality) will be analysed the same way as described for the primary efficacy outcome; by reporting a risk ratio or risk difference, and Cochrane-Mantel-Haenszel test, stratified by site.

Safety endpoint

Safety outcomes will be analysed using the safety population. Adverse events will be listed and summarised by treatment arm.

13.6 Interim Analyses

Interim analyses will occur when 400 participants have been recruited to the study to compare both arms of the study. In the event the interim results show that the addition of NO during cardiopulmonary bypass shows unequivocal benefit to patients the study may be completed early.

14 ETHICAL AND LEGAL REQUIREMENTS

14.1 Compliance

This study will be conducted in compliance with: all stipulations of this protocol; the conditions of the ethics committee approval; the International Conference on Harmonization-Good Clinical Practice (ICH-GCP), and any applicable regional or national regulatory requirements. In Australia, this study will be conducted in compliance with the NHMRC National Statement on Ethical Conduct in Human Research (2007), the Note for Guidance on Good Clinical Practice ICH E6 (R2), NHMRC Guideline Safety monitoring and reporting in clinical trials involving therapeutic goods (EHSQ, 2108) and the TGA guideline Pharmacovigilance responsibilities of medicine sponsors, Australian recommendations and requirements, version 2.1 June 2018.

All sites outside Australia will be responsible for obtaining ethics and ensuring that they satisfy their local ethics/IRB reporting requirements regarding adverse event reporting. They must also comply with all relevant regulatory requirements required by their regulatory body.

14.2 Research Ethics Approval

Local ethics (HREC/IRB) approval of this trial must be obtained by a site prior to enrolling participants. Sites must submit evidence of HREC/IRB approval to the Central Coordinating Centre and wait for approval before commencing recruitment.

14.3 Modifications to the protocol

This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, study design, patient safety, or the willingness of participants to continue participation in the study will be considered an amendment, and as such will be written and filed as an amendment to the protocol and/or informed consent form. All such amendments will be submitted to the approving HREC/IRB for approval prior to

implementation. Addition of sites will be submitted with the next protocol amendment or quarterly (whichever occurs first).

14.4 Protocol Deviations

All protocol deviations will be recorded in the patient record (source document) and on the CRF. In addition, deviations must be reported to the Principal Investigator, who will then assess the significance.

Those deviations deemed to affect to a significant degree rights of a trial participant or the reliability and robustness of the data generated in the clinical trial will be reported as serious breaches. Reporting will be done in a timely manner (Site Principal Investigator to report to the coordinating principal Investigator within 72 hours and to the Site research governance office within 7 day; CPI to review and submit to the approving ethics committee within 7 days).

Where non-compliance significantly affects human participant protection or reliability of results, a root cause analysis will be undertaken and a corrective and preventative action plan prepared.

Where protocol deviations or serious breaches identify protocol-related issues, the protocol will be reviewed and, where indicated, amended.

14.5 Confidentiality

Participant confidentiality will be strictly maintained by the participating investigators, research staff, and the sponsoring institution. This confidentiality extends to the testing of biological samples and genetic tests, as well as clinical information relating to participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence. All information will be stored using re-identifiable numbers for each participant.

No information concerning the study or the data will be released to any unauthorised third party, unless prior written approval has been granted from the sponsoring institution. Authorised representatives of the sponsoring institution may inspect all documents and records being maintained by the investigator, including participant medical (office, clinic or hospital) and pharmacy records. The clinical study site will permit access to such records. All laboratory specimens, evaluation forms, reports and other records that leave the site will be identified only by a participant identification number to maintain participant confidentiality. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring by HREC, research governance office, coordinating PI or regulatory agencies.

14.6 Participant Reimbursement

No reimbursements will be made for participation.

14.7 Dissemination and translation plan

It is highly likely that these findings will have impact across the participating countries given the role of the investigators in paediatric intensive medicine, their role in regional and state-wide guideline development processes, their involvement in professional colleges, and at key educational conferences.

Publication in high impact peer-reviewed journals will be pursued, and presentations at national and international conferences are anticipated. Novel and modern information dissemination strategies will also be used to generate discussion and to disseminate the outcomes of the study. These strategies include: social media, podcast presentations, and Free Open Access Medical education

(FOAM) resources. On completion of the study a plain language copy of results will be sent to all participants in the study.

Authorship Details

All publications on this study will be coordinated through a writing group consisting of some of the members of the executive steering committee and major centre contributors. Each participating centre will have two authors provided they have enrolled and completed data entry on 12 patients, sites will be allocated an additional author for each additional 12 patients enrolled and data entry completed.

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16 APPENDICES

Appendix 1 Causality and Assessment of Severity – Adverse Events

The severity of an Adverse Event will be assessed as follows:

- Mild: Events that require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate: Events that cause sufficient discomfort to interfere with daily activity and/or require a simple dose of medication.
- Severe: Events that prevent usual daily activity or require complex treatment.

The relationship of the event to the study drug will be assessed as follows:

- Unrelated: There is no association between the intervention and the reported event. AEs in this category do not have a reasonable temporal relationship to exposure to the intervention, or can be explained by a commonly occurring alternative aetiology.
- Possible: The event could have cause or contributed to the AE. AEs in this category follow a reasonable temporal sequence from the time of exposure to the intervention and/or follow a known response pattern to the intervention, but could also have been produced by other factors.
- Probable: The association of the event with the study seems likely. AEs in this category follow a reasonable temporal sequence from the time of exposure to the intervention and are consistent with the known adverse effects of the treatment, or judgement based on the investigators clinical experience.
- Definite: The AE is a consequence of administration of the intervention. AEs in the category cannot be explained by concurrent illness, progression of disease state or concurrent medication reaction. Such events may be widely documented as having an association with the intervention.