

Clinical Protocol

Sodium Nitrite Administration at the Time of Lung Organ Procurement and Transplantation to Minimize the Risk of Pulmonary Graft Dysfunction

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PROTOCOL TITLE: Sodium nitrite administration at the time of lung organ procurement and transplantation to minimize the risk of pulmonary graft dysfunction.

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Synopsis

Protocol Title:

Sodium nitrite administration at the time of lung organ procurement and transplantation to minimize the risk of pulmonary graft dysfunction

Study Site: University of Pittsburgh Medical Center

Phase of Development: Phase II A

Objectives:Primary Objective(s):

- To evaluate the safety of Sodium Nitrite infusion into the procured lungs and lung transplant recipient as reflected in blood mean arterial pressure and methemoglobin levels.

Secondary Objectives:

- To evaluate the efficacy of Sodium Nitrite infusion into the procured lungs and the lung transplant recipient in the prevention of grade 2-3 Primary Graft Dysfunction.
- To evaluate the efficacy of Sodium Nitrite infusion into the procured lungs and the lung transplant recipient in the prevention of delayed allograft complications including the incidence of acute rejection and BOS
- To identify the pharmacokinetic relationship of Sodium Nitrite and

metabolism of sodium nitrite in lung transplant recipients using blood sampling measures before and after drug exposure (will measure levels of plasma and whole blood nitrite, plasma nitrate, red cell iron-nitrosyl-hemoglobin, S-nitroso-hemoglobin and methemoglobin).
Statement of Primary Efficacy Hypothesis and Primary Analysis: The administration of Sodium Nitrite at the time of organ procurement and transplantation will be safe and significantly reduce the rate of Primary Graft Dysfunction in patients undergoing lung transplantation.
Planned Sample Size: A total of 8 subjects will be enrolled into the protocol. Recruitment is expected to continue for six months.
Study Design: Phase IIA observational non-randomized cohort.
Diagnosis and Key Patient Selection Criteria: Males or females greater than 18 years of age or older undergoing lung transplantation.
Treatments: Sodium Nitrite infusion to the donor lungs and the lung transplant recipient.

1 Abstract

While increasing numbers of patients with advanced lung disease are candidates for lung transplantation, the short- and long-term outcomes are severely compromised by graft dysfunction, primarily in the form of organ rejection. The earliest manifestation of lung allograft dysfunction, termed primary graft dysfunction (PGD), represents a form of ischemia-reperfusion acute lung injury, and occurs in its severest form (Grade 3) in from 10 to 35% of lung transplant recipients¹⁻⁶. PGD is the primary cause of early morbidity and mortality after transplantation and is strongly associated with the late development of chronic lung rejection or Bronchiolitis Obliterans Syndrome (BOS)⁷⁻⁹. Early graft dysfunction contributes significantly to the suboptimal outcomes of lung transplantation and to the failure of lung transplant recipients to achieve five-year survival rates comparable to patients who receive other solid organs such as the heart and liver. The risk of PGD further limits the time that lungs can be stored ex-vivo, therefore restricting the pool of available donors. A critical advance in the prevention of both early and late lung allograft dysfunction will occur if PGD can be successfully prevented or minimized.

In this study, we propose to test the hypothesis that administration of Sodium Nitrite to donor lungs and lung transplant recipients at the time of transplantation will be safe and will reduce the incidence of grades 2 and 3 PGD, thereby improving clinical outcomes with minimal toxicity.

Sodium Nitrite will be obtained from a commercial preparation (Hope Pharmaceuticals, Sodium Nitrite Injection USP (30mg/mL) NDC Number 60267-079-02) and the UPMC Pharmacy will prepare the formulations, which will be infused at three time points. First it will be infused into the preservation solution bag at the time of organ procurement from the donor, then to the allograft at the time of transplantation, and finally as a direct infusion into the organ recipient.

We plan to enroll total of 8 subjects undergoing lung transplantation for this Phase IIA observational non-randomized pilot investigation to evaluate the safety, efficacy, and pharmacokinetics of Sodium Nitrite administration when administered to the procured lung and lung transplant recipient, for the prevention of Primary Graft Dysfunction (PGD). It is anticipated that positive results from this trial lead to a larger clinical investigation of Sodium Nitrite administration directed at producing a reduction in PGD and perhaps secondary obliterative bronchiolitis; and will potentially allow for extended organ storage, extended use of more marginal organs, and more effective use of Donation after Cardiac Death (DCD) organs which undergo combination of warm and cold ischemia for organ procurement.

2 Objective, Specific Aims and Endpoints

2.1 Objective

The objective of this Phase IIA observational non-randomized pilot investigation is to evaluate the safety, efficacy, and pharmacokinetics of Sodium Nitrite administration when administered to the procured lung and lung transplant recipient, for the prevention of Primary Graft Dysfunction (PGD).

2.2 Hypothesis

Administration of Sodium Nitrite to donor lungs and lung transplant recipients at the time of transplantation will be safe and will reduce the incidence of grades 2 and 3 PGD, thereby improving clinical outcomes with minimal toxicity.

2.3 Specific Aims

The specific aims of this study are:

Primary Objective(s):

- To evaluate the safety of Sodium Nitrite infusion into the procured lungs and the lung transplant recipient as reflected in blood mean arterial pressure and methemoglobin levels.

Secondary Objectives:

- To evaluate the efficacy of Sodium Nitrite infusion into the procured lungs and the lung transplant recipient in the prevention of grade 2-3 primary graft dysfunction.
- To evaluate the efficacy of Sodium Nitrite infusion into the procured lungs and the lung transplant recipient in the prevention of delayed allograft complications including the incidence of acute rejection and BOS.
- To identify the pharmacokinetic relationship of Sodium Nitrite and metabolism of sodium nitrite in lung transplant recipients using blood sampling measures **before and after** drug exposure (levels of plasma and whole blood nitrite, plasma nitrate, red cell iron-nitrosyl-hemoglobin, S-nitroso-hemoglobin and methemoglobin will be measured).

2.4 Endpoints

Primary Endpoints Analysis

- The primary endpoint for the safety of Sodium Nitrite administration will be assessed by measurement of methemoglobin levels and mean arterial pressure during the nitrite infusion in the transplant recipient.
- The primary endpoint for the efficacy of Sodium Nitrite infusion will be the incidence of grades 2 and 3 PGD using the International Society for Heart and lung transplant (ISHLT) criteria for PGD based upon the worst value obtained over the initial 72 hours post lung reperfusion (T_{0-72}).

Secondary Endpoint Analysis

- Secondary endpoints for efficacy will include AUC curves for the oxygenation index during the initial 72 hours post transplantation, ventilator free days, intensive care free days and hospital days.
- The secondary endpoints for the prevention of Bronchiolitis Obliterans(BO) will be captured for up to 12-months following lung transplantation and include clinically

indicated spirometric and lung volume assessments of lung function performed as an indicator of Bronchiolitis Obliterans Syndrome (BOS), and evidence of pathological rejection by surveillance transbronchial lung biopsies.

3 Background and Rationale

While increasing numbers of patients with advanced lung disease are candidates for lung transplantation, the short- and long-term outcomes are severely compromised by graft dysfunction, primarily in the form of organ rejection. The earliest manifestation of lung allograft dysfunction, termed primary graft dysfunction (PGD), represents a form of ischemia-reperfusion acute lung injury, and occurs in its severest form (Grade 3) ranging from 10 to 35% of lung transplant recipients 1-6. PGD is the primary cause of early morbidity and mortality after transplantation and is strongly associated with the late development of chronic lung rejection or bronchiolitis obliterans syndrome (BOS) 7-9. Early graft dysfunction contributes significantly to the suboptimal outcomes of lung transplantation and to the failure of lung transplant recipients to achieve five-year survival rates comparable to patients who receive other solid organs such as the heart and liver. The risk of PGD further limits the time that lungs can be stored ex-vivo, therefore restricting the pool of available donors. A critical advance in the prevention of both early and late lung allograft dysfunction will occur if PGD can be successfully prevented or minimized.

The International Society for Heart and Lung Transplant (ISHLT) guidelines have provided a standardized definition for PGD based on the PaO₂/FiO₂ (P/F) ratio and the presence of chest radiograph infiltrates assessed within the first 72 hours following transplantation ¹⁰. The frequency of PGD has varied in published reports within the range of 70-80% as outlined in Table 1.

Table 1: Frequency of Reported PGD by Grade and Definition Using a Component of ISHLT Criteria						
Reference	Time (N)	Definition	PGD 0 (%)	PGD 1 (%)	PGD 2 (%)	PGD 3 (%)
Prekker 2005 (5)	1992-2004 (402)	T (0-48)		38%	28%	34%
Bobadilla 2008 (1)	1999-2008 (33)	T72				30%
Daud * 2007 (7)	1998-2004 (334)	T0	19%	39%	21%	21%

Table 1: Frequency of Reported PGD by Grade and Definition Using a Component of ISHLT Criteria						
Reference	Time (N)	Definition	PGD 0 (%)	PGD 1 (%)	PGD 2 (%)	PGD 3 (%)
Huang * 2008 (3)	1998-2004 (334)	T48	17%	65%	8%	9%
Christie 2009 (11)	2003-2007 (317)	T24				20%
Pittsburgh, unpublished	2003-2004 (76)	T (0-72)	21%	34%	20%	25%

+ Grouped 0-1 into Group 1. Did not include radiographic scoring component

* Identical cohort analyzed at different time points

PGD grade 3 has been associated with a 90-day mortality of 16.2% in comparison to a mortality of 9.5% for subjects with PGD Grades 0-1. However, the prognostic significance of PGD extends well beyond the immediate post-operative period. Grade 3 PGD has been associated with a more rapid decline in post-operative pulmonary function and the late development of bronchiolitis obliterans syndrome (BOS) ⁷. These data suggest that new approaches to reduce PGD are fundamental to improving the morbidity and mortality in lung transplant recipients.

4.3.1 Complex Interaction of Donor and Recipient Risk Factors Contributing to Primary Graft Dysfunction

Using the standardized definition for PGD, numerous donor, recipient, and treatment variables have been identified as potential clinical risk factors for the development of PGD ¹¹. These reported associations remain limited by the lack of multicenter, prospective, observational studies using consistent procurement techniques. Despite this limitation, both single center and registry reviews have suggested that variables such as donor age ⁶, prolonged ischemia time ¹², recipient pulmonary hypertension ^{6,13,14}, recipient diagnosis ¹⁵, and donor requirement for blood transfusion ¹⁶, may all be independent risk factors for PGD.

4.3.2 Rationale for the Investigation of Nitrite Therapy in PGD

Sodium Nitrite has been shown to limit ischemia-reperfusion injury and myocardial infarction. Nitrite is a potent cytoprotective agent, with its optimal dose (48 nmol - which raises murine plasma nitrite concentration to 10 μ M) decreasing infarct size by 50-70% in the heart, liver and

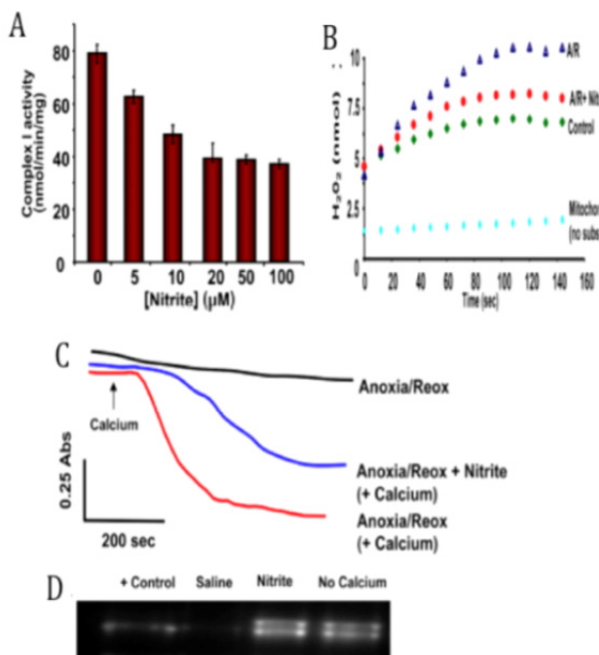


Figure 1: Inhibition of mitochondrial ROS and cytochrome C release by nitrite during anoxia and reoxygenation. A) nitrite inhibits complex I activity; B) decreases ROS generation; C) decreases permeability pore opening (measured as mitochondrial swelling; and D) Western blot for cytochrome c remaining in the mitochondria. Shiva et al. J. Ex. Med 2008.

brain of respective animal models¹⁷⁻²⁰. This cytoprotective effect of nitrite after ischemia/reperfusion (IR) has now been observed by a number of groups and in a number of organs/models. Although this cytoprotection has been shown to be dependent on the formation of nitric oxide (NO), it is independent of endothelial NO synthase. Recent data have also demonstrated that dietary nitrite supplementation can increase plasma and tissue levels of nitrite, and these increased levels can protect against IR injury in the heart and liver²¹. The mechanism of bioactivation of nitrite in the ischemic organ is the subject of intense current study, with groups proposing a central role for xanthine oxidoreductase²², mitochondrial enzymes^{23,24}, NO synthase²⁷, as well as non-enzymatic acidic disproportionation²⁶⁻²⁸.

The mechanism of nitrite cytoprotective effect involves the inhibition of mitochondrial respiration and decreased ROS at reperfusion. Nitrite appears to be mitochondrially-targeted and able to modulate mitochondrial reactive oxygen species (ROS) generation^{29,30}. We have demonstrated that nitrite dose dependently S-nitrosates and inhibits mitochondrial complex I in vivo models of IR as well as in vitro models of anoxia/reoxygenation (Figure 1).

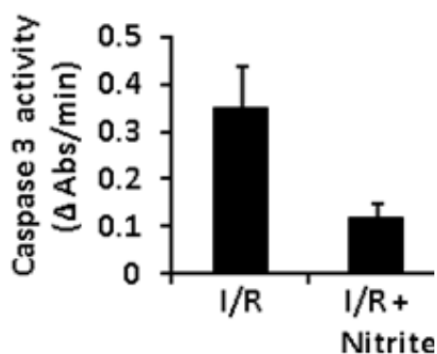
This post-translational modification decreases electron transfer through complex I, which effectively decreases ROS generation upon reperfusion and prevents protein oxidation and loss of function³⁰. Similar to other complex I inhibitors, the nitrite dependent S-nitrosation of complex I reduces reperfusion reactive oxygen species formation and oxidative damage to the mitochondria (indicated by a reduction in aconitase oxidation). In addition, nitrite prevents

opening of the mitochondrial permeability transition pore and the release of cytochrome c from the mitochondria in response to calcium, an effect similar to that of cyclosporine on mitochondrial function

4.3.3 NO And Nitrite Regulate Cellular Apoptosis

The mitochondrial apoptotic pathway is initiated by opening of the mitochondrial permeability transition pore and cytochrome c of mitochondrial ROS and cytochrome C release in a process inhibited by nitrite (Figure 1) and regulated by the pro- and anti-apoptotic proteins bax and bcl-2. This pathway drives the activation of caspase 9 and assembly of the apoptosome, which leads to the eventual activation of caspase 3³¹. This pathway plays a central role in the cytotoxicity of ischemia and reperfusion, as demonstrated by studies in which inhibiting caspase 9³² or overexpressing the anti-apoptotic protein bcl-2³³, significantly decreases infarct size in myocardial infarction models.

At low concentrations, NO regulates several steps in the apoptotic pathway including inhibiting the release of cytochrome C³⁴ and the activation of caspases 9 and 3³⁵, and maintaining levels of the anti-apoptotic protein bcl-2³⁶.



Consistent with these data, nitrite inhibits caspase 3 activation (Figure 2).

Figure 2: Caspase 3 activity in wild type mice after I/R with or without nitrite treatment.

4.3.4 Sodium Nitrite Therapy as the Most Promising Pilot Trial to Model in The Treatment Of PGD

Accumulating evidence suggests that the ubiquitous anion, nitrite (NO₂⁻), is a physiological signaling molecule, with roles in intravascular endocrine nitric oxide (NO) transport, hypoxic vasodilatation, and cellular cytoprotection³⁷⁻⁴⁰.

Numerous pre-clinical studies in mice, rats, dogs, sheep and primates have shown that nitrite has both vasodilatory and tissue protective effects in models of ischemia-reperfusion injury to the central nervous system, liver, kidney, heart and lung⁴¹⁻⁴⁷. Additionally, recent pre-clinical studies, as outlined below, now indicate that nitrite has potent effects at reducing primary graft dysfunction and late rejection after lung and heart transplantation^{39,40,47}. Most importantly, phase I-II studies of nitrite infusion, at doses much higher than the 40 mg dose planned for this trial, have been completed in more than 80 human volunteers and patients with sickle cell disease with no observed toxicity^{37,38,40}. In collaborative studies at the University of Pittsburgh with the Department of Surgery and the Starzl Transplantation Institute, we have evaluated Sodium Nitrite in rat models of lung transplantation and heart transplantation. These studies show that nitrite not only potently inhibits early ischemia and reperfusion injury but also prevents down-stream allograft rejection.

Heart Transplantation Models: In these studies, fully allogeneic heterotopic heart transplantation was performed in Lewis (LEW) to Brown Norway (BN) rats. Animals were all given tacrolimus therapy, and either a regular diet or a low nitrite/nitrate chow validated in Dr. Gladwin's laboratory or the low chow but with water containing nitrite (50 mg/L) for 120 days, beginning 7 days before transplant. Serum nitrite/nitrate levels were significantly higher in animals given nitrite water for 30 days, and lower in the animals fed with low nitrite/nitrate diet than those in animals who received standard diet. Supplementation of drinking water with nitrite enhanced heart graft survival to a median of >120 days from 49.5 days in animals fed a standard diet. In contrast, the low nitrite/nitrate diet led to nitrite and nitrate insufficiency and resulted in significantly earlier rejection of allografts (30.5 days). These data demonstrate that nitrite supplementation is significantly effective in preventing development of heart allograft rejection in a relevant pre-clinical model⁴⁷.

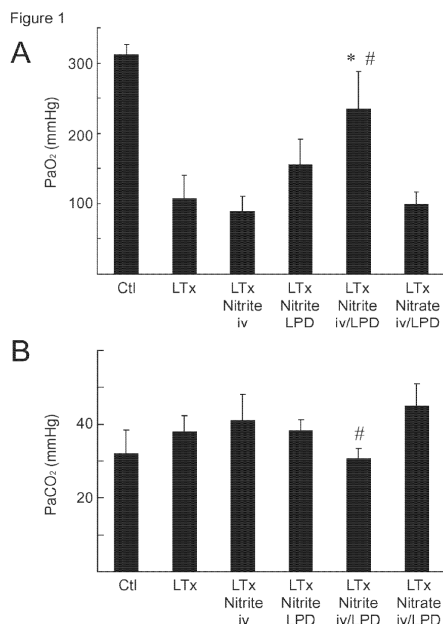


Figure 3: Increase in oxygenation after lung transplantation (LTx) in mice receiving nitrite in both the lung preservation solution (LPD) and IV at the time of reperfusion.

Lung transplantation models: In these studies, orthotopic left lung transplantation (LTx) was performed syngeneic rats. Grafts were preserved with or without Sodium Nitrite (10 μ M) in the cold storage

solution at 4°C for 6 hours.

Groups of recipients also received Sodium Nitrite at a

dose of 480 nmoles intravenously 5 minutes before graft reperfusion to target a plasma concentration of 10 μ M. Graft function, as measured by the pulmonary vein PaO₂ while breathing 100% oxygen and inflammatory lung injury were assessed 2 hours after transplantation.

We found that oxygenation was significantly higher in animals receiving Sodium Nitrite in both the preservation solution and given to the recipient compared to those in the control group (Figure 3; 234 \pm 53 vs 99 \pm 18 mm Hg; P<0.05). In contrast, nitrate did not alter gas exchange.

The increase of graft mRNA for IL-6, IL-1 β , TNF- α , iNOS, and COX-2 in the control group was markedly inhibited in nitrite group. Lung myeloperoxidase activity, lung wet to dry weights and histological evidence of edema and cellular inflammation were all significantly reduced with nitrite treatment.

4 Significance

It is anticipated that positive results in this trial will lead to a larger clinical investigation of Sodium Nitrite administration directed at producing a reduction in PGD, and perhaps secondary obliterative bronchiolitis; and will potentially allow for extended organ storage, extended use of

more marginal organs, and more effective use of Donation after Cardiac Death (DCD) organs which undergo combination warm and cold ischemia for organ procurement.

5 Study Design and Methods

5.1 Overview

This is a Phase IIA observational nonrandomized pilot investigation to evaluate the safety and efficacy of Sodium Nitrite administration for the reduction of PGD in patients undergoing lung transplant. The study will enroll 8 subjects, undergoing lung transplant at the University of Pittsburgh Medical Center (UPMC).

5.2 Study Time Line

We propose to enroll eight patients over a 6-month interval.

5.3 Screening Procedures

Patients with end stage lung disease who are deemed eligible for placement on the lung transplant waiting list will be eligible for participation and written informed consent will be obtained at the time of their listing for lung transplantation. The medical records of the lung transplant patients will be reviewed for eligibility criteria by the PI and other Co- Investigators who by virtue of their role as a clinician already have the knowledge of and access to the patients' identifiable medical information. Subjects will be approached for consent after the decision has been reached to place the subject on the waiting list. After enrollment and prior to transplantation, blood will be drawn for baseline studies that include complete blood count (CBC), chemistry profile; and for storage to allow for subsequent exploratory PBMC gene expression profiling and proteomics which would include evaluation of IL6, IL β , TNF- α , iNOS and COX-2.

5.4 Study Drug Preparation

The Sodium Nitrite will be obtained from a commercially available, FDA-approved preparation (Hope Pharmaceuticals, Sodium Nitrite Injection USP (30mg/mL) NDC Number 60267-079-02) and the UPMC Pharmacy will prepare the formulations for this clinical study.

To minimize waste of study drug, the UPMC Pharmacy will prepare Sodium Nitrite as two stock solutions in syringes. The first stock solution will be a 12 mL (0.16mg/mL) stock solution to be used in the Perfadex preservation solution and the pneumoplegia solutions. The second stock solution will be 1.33 mL (30 mg/mL) stock solution to be used in the preparation of a 40 mg Sodium Nitrite infusion in normal saline. The stock solutions in syringe will be prepared from Hope Pharmaceuticals Sodium Nitrite Injection, USP. The stock solutions in syringe will be frozen for long term storage to be used as needed as enrollment progresses.

A specifically labeled frozen syringe of prepared stock Sodium Nitrite solution (12 mL, 0.16 mg/mL) will be available for the addition to the 2.8 L Perfadex bag. Once thawed, the addition of the 12 mL contents of the syringe will achieve a final concentration of 0.69 mg/L of solution (i.e., 10 uMolar or 1.93 mg for each 2.8 liter bag. Thawed syringes that are not used will be discarded.

An initial 1l bag of Perfadex administered to the donor organ **will not include the addition of Sodium Nitrite.**

Organ Implantation Prior To Reperfusion

A specifically labeled frozen syringe of prepared stock Sodium Nitrite solution (12 mL, 0.16 mg/mL) will be available to the OR at the time of lung transplantation. Once thawed, the Sodium Nitrite will be added to the pneumoplegia solution to achieve a final concentration of 0.69 mg/L (10 uMolar). Thawed syringes that are not used will be discarded. The pneumoplegia solution (see Appendix C) is prepared by the UPMC Pharmacy and the Sodium Nitrite will be added immediately prior to the infusion of the pneumoplegia solution into the donor lung.

Organ Reperfusion to Recipient

A specifically labeled frozen syringe of prepared stock Sodium Nitrite solution (1.33 mL, 30 mg/mL) will be used by the UPMC pharmacy to prepare a 40 mg Sodium Nitrite in a 250 mL infusion

bag of normal saline (i.e., 0.16 mg/mL) The entire volume of this bag will be used to administer to the patient at the time of Organ Perfusion.

5.5 Study Drug Intervention

Sodium Nitrite will be administered to the donated lung at the time of procurement and to the lung transplant recipient at the time of reperfusion.

Organ Procurement from the Donor

Consistent with current practice, the initial liter of antegrade flush of the donor lung with Perfadex includes the addition of prostaglandin and nitroglycerin to a 1L bag of the preservation solution. This initial liter of solution WILL NOT include the addition of Sodium Nitrite. Subsequent to this initial liter, any additional preservation solution (Perfadex) will be provided in 2.8 Liter bags only and will include the addition of Sodium Nitrite for injection to achieve a final concentration of 0.69 mg/mL (10 uMolar). Other additives to the preservation solution will include THAM and calcium chloride consistent with local practice. The Sodium Nitrite will be added prior to the infusion of the preservation solution. The actual volume of solution used for both antegrade (PA to PV) and retrograde (PV to PA) flush will be determined by the surgical team directing the procurement and recorded for the purpose of this study. A member of the study team will be present for monitoring of this procedure.

Organ Implantation prior to Reperfusion

At the time of transplant, the first donor lung will be flushed with a cold pneumoplegia solution after the bronchial (1st) anastomosis. The pneumoplegia solution used clinically at UPMC is a 50% Dextrose/autologous blood preparation, which includes Nitroglycerine, Verapamil and Heparin (refer to Appendix C for description of UPMC pneumoplegia solution); and the Sodium Nitrite will be added just prior to use. A second flush of pneumoplegia solution will occur after the PV (3rd, last) anastomosis. The identical pneumoplegia solution as outlined above will be used for the second flush, but at a temperature range of 34- 38 °C. The same process of flushing with pneumoplegia solution will be followed for the second donor lung.

To the recipient at the time of reperfusion:

Sodium Nitrite will be delivered intravenously to the recipient *immediately* prior to the first lung reperfusion as a single infusion of Infuse at 4 mL/min for the first 30 min, followed by 2.2 mL/min for the next 60 min.

5.6 Dosing Adjustments and/or Discontinuation due to Adverse Events

The Sodium Nitrite infusion will only be initiated if the patient's mean arterial pressure (MAP) > 50 mm Hg. The patient's blood pressure may be supported by vasoactive medications and/or volume resuscitation at the time of study drug infusion as directed by the patient's clinical involved anesthesiologist.

If the patient meets criteria for initiation of the study medication, the infusion will be started at the prescribed rate and continue as outlined above. The pre-infusion MAP will be established by the treating anesthesiologist. The study medication will continue unless the patient's MAP falls > 20% compared to the recorded baseline at the start of the study drug infusion. A fall in MAP > 20% will result in the study drug being stopped for 10 minutes and the patient's MAP will be continuously monitored.

If the MAP returns to within 10% of the MAP baseline determined prior to study drug infusion, the infusion may be restarted after a 10 minute interval. This transient fall in MAP with rapid reversal will be attributed to the study drug. If the MAP falls again to > 20% from the MAP baseline, the cycle may be repeated for a maximum of two intervals. The patient's MAP must return to within 10% of the baseline before any restart of the study medication.

If the MAP fails to return to within 10% of the MAP baseline determined prior to study drug infusion, the infusion will be suspended pending hemodynamic resuscitation as directed by the staff anesthesiologist. A more prolonged period of hypotension suggests the effects of organ reperfusion rather than study medication but the infusion will be held to allow stabilization of the patient. The study drug infusion will be restarted once the patient's MAP returns to within 10% of the baseline value.

The study medication may be infused up to 1 hour following the organ reperfusion if hemodynamic stability is delayed.

5.7 Study Procedures

5.7.1 Randomization and blinding

This study will be a non-randomized pilot observational study.

5.7.2 Study monitoring

1) Required end points during study drug infusion

- Mean Arterial Pressure:

The recipient's mean arterial pressure (MAP) will be monitored at 1 minute intervals during study drug infusion followed by 15 minute intervals for 1 hour.

- Nitrite and metabolite levels:

Blood samples will be obtained prior to the infusion; at 5, 30, and 60 minutes during the infusion; and at 5, 10, 30, 60, 90, 150 after the infusion to measure plasma and whole blood nitrite, plasma nitrate, red cell iron-nitrosyl-hemoglobin, S-nitroso-hemoglobin and methemoglobin concentrations. However, no sample will be sent for methemoglobin concentration prior to drug infusion. Blood samples will also be obtained every 24 hours for two days for future exploratory biomarker/proteomic studies which would include evaluation of IL6, IL β , TNF- α , iNOS and COX-2. Nitrite levels will be measured by established methodologies (ozone-based reductive chemiluminescence) routinely performed in Dr. Gladwin's NO Metabolomics Core facility.

2) Required end points post study drug infusion to hospital discharge

Following administration of the Sodium Nitrite, the study subjects will continue to receive usual care for their condition consistent with local transplant treatment protocols. The study will not introduce any modification to the normal care algorithms. Day 0 (D₀) will be defined as the day of transplantation and reperfusion. Subsequent study days will be numbered sequentially. The following parameters will be required for collection prospectively. They will be obtained specifically for the study protocol if not requested by the treating physician. If requested by the treating physician the parameters will be recorded from the medical record. Specific parameters required to be monitored following lung transplantation/reperfusion will include:

- Arterial blood gas measurements from D0 to D3

The $\text{PaO}_2/\text{FiO}_2$ ratio and oxygenation index will be calculated based upon an arterial blood gas at D0 (within 6 hours of reperfusion), and D1, D2, and D3. The initial measurement (D0) will be obtained on a FiO_2 of 1.0 and PEEP of 5 while on mechanical ventilation. Subsequent measurements will be obtained on the prescribed ventilator settings. Ventilator parameters will be obtained with each blood gas determination.

There will be no arterial blood gas measurement on days patient is extubated.

- Chest radiography from D0 to D3

Plain film portable chest radiography will be obtained on D0 (within 6 hours of reperfusion), and D1, D2, and D3. All chest radiography will be referred to a radiology core for independent review and classification independent of the review required for clinical documentation.

The primary end point of PGD will be scored using the following criteria:

Grade	$\text{PaO}_2/\text{FiO}_2$	Radiograph
0	>300	Absent
1	>300	Present
2	200-300	Present
3	<200	Present

The absence of pulmonary infiltrates on plain film radiograph will be assigned Grade 0 independent of the $\text{PaO}_2/\text{FiO}_2$ ratio. All extubated patients will be graded as 0 or 1 based upon the interpretation of the chest radiograph. The need for extracorporeal support and/or inhaled nitric oxide will be documented and automatically assigned a Grade III classification.

The diagnosis of Primary Graft Dysfunction (PGD) requires the exclusion of alternative conditions, which might produce a similar radiographic and physiologic pattern including hyperacute rejection, cardiogenic pulmonary edema, venous anastomotic obstruction, and allograft infection. Exclusion of these conditions will be made by a PGD grading committee composed of transplant physicians not involved in the patient's care at the time of transplant until hospital discharge. Medical records will be reviewed within six to eight weeks of transplantation by the committee to classify PGD.

- Sequential Organ Failure Assessment Score (SOFA):

*The SOFA score is a recognized ICU scoring system to define the extent of the patient's organ failure. The scoring system has an individual component score for each of 6 organ systems including **respiratory, cardiovascular, hepatic, coagulation, renal and neurological** systems⁵⁸. The SOFA Score will be obtained daily at D1, D2, D3, and D4.*

- Ventilator Free Days (VFD): Defined as the number of days off mechanical ventilation at 8am through D30 post transplantation.
- ICU Free Days (IFD): Defined as the number of days outside the ICU through D30 post transplantation.
- Hospital Days: Defined as the total number of acute and long term acute (LTAC) hospital days during the index transplant admission.
- Outcome at 90 days: Patient clinical status at D90 post transplantation including living or deceased, and location. To confirm clinical status of patient on Day 90 post transplant as living or deceased and if living current location, a follow up call will be made to the patient or the family on day 90 +/- 7 days.

5.7.3 Optional end points to be obtained from admission to ICU discharge based on standard clinical monitoring

(1) Arterial blood gas measurements from D4 to ICU discharge

The PaO₂/FiO₂ ratio and oxygenation index will be calculated and the worst value for each individual day recorded for analysis. Corresponding ventilator parameters will be recorded.

(2) Plain film portable chest radiography from D4 to ICU discharge

All chest radiography will be referred to a radiology core for independent review and classification independent of the review required for clinical documentation.

(3) Requirement for extracorporeal support and days

Any day beyond D₀ to ICU discharge in which the patient receives extracorporeal support will be counted.

(4) Requirement for inhaled NO support and days

Any day beyond D₀ to ICU discharge in which the patient receives inhaled NO support will be counted.

- Results of Echocardiography -specifically the left ventricular function and estimated pulmonary arterial pressure (PA systolic, PA diastolic, and mean) to evaluate cardiac and pulmonary arterial function.
- D₀ to ICU discharge
 - ventilator settings
 - vital signs
 - 24 hour fluid balance
 - hemodynamic indices including MAP, PA pressures, central venous pressures, cardiac index.

3) Additional end points to one year post hospital discharge obtained only if needed for patient's standard care

- Results of pulmonary function tests - specifically the FVC, FEV1, and FEV1/FVC ratio.
- Lung Biopsy Results – specifically the diagnosis of acute cellular rejection and grade, bronchiolitis obliterans (BO), and treatment.
- Complications – including major opportunistic infections and subsequent hospitalizations.

5.8 Safety Assessment

The primary safety assessment will be based upon measured methemoglobin levels and hemodynamic indices during the Sodium Nitrite infusion. Because of the exploratory nature of this trial, all potential Adverse Events will be recorded for review.

5.9 Efficacy Assessment

The primary efficacy assessment will be the incidence of PGD Grade 3 or greater in comparison to age and disease-matched historical controls for the UPMC Lung Transplantation program.

6 Statistical Consideration

Statistical analysis for the protocol will be primarily exploratory (i.e., hypothesis generating), and no firm conclusion will be drawn based upon a confirmation oriented statistical analysis. The results will be displayed as mean \pm standard deviation for continuous variables, or frequency (percentage), for discrete variables. For variables with skewed distributions, both nonparametric analysis, and parametric analysis using logarithmically transformed data will be performed. The continuous efficacy variables will be analyzed by analysis of variance (ANOVA) models, which will contain terms of treatment dose, disease type, and treatment-by-disease type interaction. The treatment-by-disease type interaction will be assessed at significance level 0.10. If the treatment-by-disease type interaction is not statistically significant, the interaction effect will be removed from the model. The treatment differences will be assessed by pair-wise comparison between the study population and historical controls, using least-square means from the ANOVA model. If baseline value of each continuous efficacy variable is to be adjusted, data will be analyzed by analysis of covariance (ANCOVA).

7 Human subjects

7.1 Subject Population

7.1.1 Human subjects involvement and characteristics

Subjects of both genders and all ethnic backgrounds will be considered for inclusion in this study. Patients with end stage lung disease who are deemed eligible for placement on the lung

transplant list will be enrolled. All volunteer subjects must be at least 18 years of age and have provided informed, written consent for participation in this study. Based on the population of patients referred for lung transplantation, we expect that approximately 50% of eligible subjects will be women and 10% will be non-Caucasian. Children under 18 years of age will not be considered for inclusion in this study because children are not eligible for lung transplant at the adult lung transplant programs involved in this protocol (children are referred to a pediatric lung transplant program). If the results of this study demonstrate beneficial effects of Sodium Nitrite administration for lung transplant recipients, future studies will attempt to incorporate children through collaboration with pediatric lung transplant centers.

7.1.2 Donor exclusion criteria

- Age > 55 years.
- Mechanical ventilation > 5 days prior to procurement
- Significant chest trauma or lung contusion
- Smoking history > 20 pack-year
- PaO₂/FiO₂ (O₂ challenge) < 300
- Donor radiograph with 2 quadrant infiltrates
- Donor that are determined single lung donors prior to transplant will be excluded

7.1.3 Recipient inclusion criteria

- Subjects undergoing lung transplantation.
- Subjects in the age range of 18-70 years Ability to understand and provide consent. Proxy consent will not be accepted.

7.1.4 Recipient exclusion criteria

- Recipient age > 70 years.
- Recipient history of pulmonary hypertension (idiopathic PAH, or secondary PAH with mean PA > 30 mm Hg)
- Recipient history of abnormal cardiac function defined as prior CABG or LVEF < 45 %)
- Recipient history of open thoracotomy/prior pleurodesis as exclusion criteria. However patients who have had limited VATS procedures for biopsies would NOT be excluded from the study. Recipient history of cirrhosis
- Recipient history of mechanical ventilation or extracorporeal support pre-operatively

- Recipient pre-operative hypotension defined by a systolic blood pressure less than 90 mm Hg not responsive to intravenous fluids or requirement for vasoactive medications
- Recipient preoperative history of renal insufficiency, dialysis or estimated glomerular filtration rate <30 ml/min/1.73 m² BSA
- Patients undergoing retransplantation
- Recipient history of significant coronary artery disease that is flow limiting and unable to be corrected by further percutaneous coronary artery interventions.

7.2 Recruitment and Informed Consent Procedures

Only patients with end stage lung disease deemed eligible for placement on the lung transplant list will be enrolled. All volunteer subjects must be at least 18 years of age and have provided informed, written consent for participation in this study at the time of listing for lung transplantation. The medical and surgical team will review the participation of the patient in this study at the time a decision is made for listing. Patients deemed eligible for participation will be approached by one of the study physicians for informed consent.

8 Potential Risks and Benefits

8.1 Risk of Experimental Intervention

Sodium Nitrite: Sodium nitrite has been used commercially as a food preservative, an anti-corrosive agent, a coloring agent, and an anti-anginal agent, with additional uses in laxatives, burn ointments, and liniments. Amyl nitrite has been inhaled or ingested as an euphoric stimulant. Nitrite has also been found as a contaminant in well water. Literature searches generated case reports of nausea, vomiting, abdominal pain, dizziness, headache, flushing, cyanosis, tachypnea, dyspnea, hypotension and death attributed to excess nitrite (high-dose) exposure from these sources as a consequence of methemoglobinemia due to oxidation of heme-iron in oxyhemoglobin. If levels of methemoglobin rise above approximately 30% of total hemoglobin, a subject may appear cyanotic and experience dyspnea, due to the reduced oxygen carrying capacity of hemoglobin (methemoglobin cannot bind oxygen). Levels above 50% can cause seizures, hypotension, coma and death. Note that methemoglobin levels have never risen higher than 3% at the currently used doses (30 mg) in 80 volunteers in phase I studies at the NIH.

Nitrite in the form of sodium nitrite for parenteral administration is currently available and approved by the FDA for use in the emergency treatment of cyanide poisoning. Sodium Nitrite administration for this clinical indication at the labeled dosage of 300 mg causes methemoglobinemia, a desirable effect in the treatment of cyanide poisoning, as methemoglobin binds to cyanide, thus protecting cellular mitochondria. Sodium nitrite (4mg/kg, approximately 300 mg) was infused intravenously over 10 minutes in eight healthy volunteer subjects. Methemoglobin levels in venous blood samples rose from 0.02% at baseline to 5.7% of total hemoglobin following infusion of sodium nitrite, with no subject experiencing adverse events. The sponsor of this IND application, Dr. Gladwin, has previously held an IND for sodium nitrite (IND # 70,411) for cardiovascular applications. The cardiovascular IND involved the administration of Sodium Nitrite to 69 normal volunteers in 4 phase I-II clinical trials without observed adverse effects. We have also treated 11 subjects with sickle cell disease on this IND without observed adverse effects. The low doses of nitrite used in these investigational treatment regimens – approximately 40 mg or 13% of the dose (300mg) used in the emergency treatment of cyanide poisoning – do not produce methemoglobin levels greater than 3% and have not been associated with clinically significant hypotension. There have been no adverse events noted in the 80 treated normal human volunteers and patients with sickle cell disease. While the safety of nitrite in lung transplant recipients has not been established, the short plasma half-life ($T_{1/2} = 42$ mins⁴⁹) and dose chosen for this study minimize the risk of subjects experiencing the known effects of nitrite, specifically methemoglobinemia and hypotension.

9 Protection against Patient Risk

9.1 Protection against General Patient Risk

All research interventions/activities will be conducted in private room/areas. The collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research.

All demographic and clinical information including the required and optional end points about the subject will be collected on hard copy case report forms used for data entry into a locked database. The hard copy casebooks will be maintained in the data center under the supervision

of Drs. Gladwin and Donahoe. The data will be stripped of individual identifiers and stored anonymously with a subject number. Information linking subject identifiers with the coded subject number will be stored under password protection on computers in locked areas, with access only to the database manager. Maintaining records in locked files in locked offices will protect confidentiality of subjects. Research Data are maintained on a password-protected computer that is accessible only to the data manager and his staff. All staff will sign confidentiality statements. Access to the database will be limited to the data manager and staff under the supervision of Drs. Gladwin and Donahoe. Blood specimens will be stripped of subject identifiers and stored according to a similar coding protocol as described above. These specimens will be stored safely in the custody of the sub-investigators responsible for the individual assays. These sub-investigators will limit future access to any remaining samples to only those investigators with prior IRB approval for their studies. For the PK assays these will be stored by Dr. Gladwin's laboratory.

All staff involved in this study are properly credentialed and instructed in the areas of testing, confidentiality, and safety.

The Investigator will retain the data for up to 2 years following termination of this IND application. The investigator may continue to use and disclose subjects' identifiable information for the purpose of this study for a minimum of seven years after final reporting or publication of the study. If the subject and/or legal representative decide to withdraw or be withdrawn from study participation, they may request that the study data and samples be destroyed.

9.2 Protection against Patient Risks Related to the Study Drug

The following components have been incorporated to protect the study subjects from risk related to the study drug:

- Small sample size consistent with the exploratory nature of the trial.
- Specific exclusion criteria to provide a stable population of patients listed for single or double lung transplant, and non-enrollment of patients listed for retransplantation with significant co-morbidities that might place them at excess risk (see exclusion criteria above).
- Continuous monitoring by an independent data and safety monitoring board.

- Involvement by expert staff with a broad range of expertise in the administration of Sodium Nitrite, and the pre and postoperative care of patients undergoing lung transplantation.
- Frequent monitoring for adverse effects of the study drug will be conducted during and following its administration.

10 Data and Safety Monitoring Plan

10.1 Data and Safety Monitoring Board

An Independent Data and Safety Monitoring Board (IDSMB) will be established; to be comprised of individuals who are not involved with this study protocol. The DSMB will oversee patient safety in this clinical trial. The DSMB will be comprised of members including senior experts in pulmonary medicine, clinical research, clinical trial design, biostatistics, psychosocial issues relating to chronic disease, and research ethics. The Data and Safety Monitoring Board will conduct interim mentoring of accumulating data from research activities to assure the continue safety of human subjects, relevance and appropriateness of the study, and the integrity of research data.

10.2 Data and Safety Monitoring Plan

Assuring patient safety is an essential component of this protocol. The study investigators have primary responsibility for subject safety monitoring. The investigators will evaluate all adverse events. The study coordinators must view patient records for possible adverse events until 72 hours after the last dose of study drug. All untoward medical occurrences observed in subjects receiving the study drug will be recorded on the participants' adverse event case report forms (CRF) by the study coordinator under the supervision of the clinical investigator. The CRF will then be reviewed for completeness and internal consistency. Subsequently, the CRFs will be recorded on an electronic password-guarded study database. In addition to internal safeguards built into a computerized system, external safeguards will be put in place to ensure that access to the computerized system and to the data is restricted to authorized personnel. Training conducted by qualified individuals on a continuing basis will be provided to individuals in the specific operations with regard to computerized systems that they are to perform during the course of the study.

The Clinical Investigator will work with the reporting sub-investigators to prepare a detailed written summary of serious, unexpected, and study drug-related adverse events, and will compare, and contrast the event with prior events. The detailed written summary will be provided to the IDSMB and also to the sponsor of the IND, Dr Gladwin. Adverse events will be reported. Adverse events will be reported to IRB as per guidelines.

10.3 Parameters to be Monitored

The Clinical Investigator will evaluate any changes in laboratory values and physical signs, and will determine if the change is clinically important, and different from what is expected in the clinical course of patients following a lung transplant. Expected events for lung transplant patients are untoward clinical occurrences that are perceived by the clinical investigator to occur with reasonable frequency in the day to day care of patients with a lung transplant, treated in the hospital setting. Examples of adverse events that are expected in the course of lung transplant care include infection, rejection, and organ failures. Such events will not be considered reportable adverse events unless the event is considered by the clinical investigator to be associated with the study drug or experimental procedures, or is unexpectedly severe or infrequent for a patient undergoing lung transplantation.

An event will be considered to be study drug- or experimental procedure-related if the event follows a reasonable, temporal sequence from the study drug/procedure, and could readily have been produced by the study drug/procedure. An event will be considered to be unexpected for the study drug if it is not identified as a potential adverse event in this clinical protocol, or is unexpectedly severe, or occurs more frequent than events described herein. The most common adverse events expected for this intervention would be hypotension and methemoglobinemia. These events will be reported to the Sponsor of the IND, Dr Gladwin within 5 days of the event.

10.4 Frequency of Monitoring

The Clinical Investigator, sub-investigators, and the research staff will meet every 4 months to re-evaluate study goals, subject recruitment, data coding and retention, documentation and identification of adverse events, complaints and confidentiality of subjects. However, the meeting may be held more frequently, if

necessary, for example depending on the subject enrollment. There will be an evaluation of the progress of the research study, including assessments of data quality, time lines, participant recruitment, accrual, and retention. The Clinical Investigator will also review the outcome and adverse event data to determine whether there is any change to the anticipated benefit-to-risk ratio of study participation and whether the study should continue as originally designed or should it be re-evaluated and changed. Summary of all adverse events and subject safety data will be prepared by the Clinical Investigator and submitted to the sponsor of the IND, Dr Gladwin. Dr Gladwin will review subject safety data as it generated to submit to the FDA, if needed.

The IDSMB will meet at least yearly and more often as necessary with trial. The IDSMB will also meet prior to initiation of the study.

The DSMP and IDSMB reports will be submitted to the IRB at the time of renewal.

10.5 Recording/Reporting Requirements

10.5.1 *Eliciting adverse event information*

Clinical study subjects will be routinely questioned about adverse events at study visits.

10.5.2 *Recording requirements*

All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of suspected causal relationship to the investigational drug or study treatment(s) will be recorded in the subjects' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a *serious adverse event*) and; 2) an assessment of the casual relationship between the adverse event and the investigational drug or study treatment(s).

Adverse events or abnormal test findings felt to be associated with the investigational drug or/and study treatment will be followed until the event (or its sequel), or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator-sponsor.

1) *Abnormal test findings*

An abnormal test finding will be classified as an *adverse event* if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study dosing or discontinuation of subject participation in the clinical study.
- The test finding is considered an adverse event by the sponsor of the IND application.

2) *Causality and severity assessment*

The clinical investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the investigational drug or study treatment(s); and 3) if the adverse event meets the criteria for a *serious adverse event*.

If the clinical investigator's final determination of causality is "unknown and of questionable relationship to the investigational drug or study treatment(s)", the adverse event will be classified as *associated with the use of the investigational drug or study treatment(s)* for reporting purposes. It will be promptly reported to the sponsor within 24 hours of notification of the event. If the investigator's final determination of causality is "unknown but not related to the investigational drug or study treatment(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

10.6 Reporting of Adverse Events

10.6.1 *Reporting of Adverse event to the Sponsor*

All adverse events that are unexpected, fatal or life threatening and associated with the use of the study drug will be reported by the Clinical investigator to the sponsor of the IND application

within 24 hours of notification of event either by telephone or secure email. Investigator will provide the sponsor of the IND with the detailed report upon complete investigation of the adverse event within 5 days of being notified of the event.

Any adverse event unexpected, serious but not life threatening and associated with the use of the drug will also be reported to the sponsor within 5-7 days of notification of the event.

10.6.2 Reporting of adverse events to the FDA

2) (1) Written IND Safety Reports

The sponsor will submit a written IND Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to the clinical investigator and all participating sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the sponsor's receipt of the respective adverse event information.

For each written IND Safety Report, the sponsor of this IND will identify all previously submitted IND Safety Reports that addressed a similar adverse event experience and will provide an analysis of the significance of newly reported adverse event in light of the previous, similar report(s).

Follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the relevant information is available. If the results of the sponsor's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the investigator-sponsor will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

3) *Telephoned IND safety reports*

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the sponsor of the IND will notify the responsible review division of the FDA by telephone or facsimile transmission of any observed or volunteered adverse event that is 1) *associated with the use of the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected*.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the sponsor's initial receipt of the respective human adverse event information.

10.7 Reporting adverse events to the responsible IRB

In accordance with applicable policies of the University of Pittsburgh Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator's receipt of the respective information. Adverse events which are 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected* will be reported to the IRB within 24 hours of the investigator-sponsor's receipt of the respective information.

Follow-up information to reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator-sponsor will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

11 Costs and Payments

At this point, the patients will not be compensated for participation in this study however **neither the patients nor their insurance provider be charged for the study drug, Sodium Nitrite and any procedures done specifically for the research purposes.**

Any procedures done as a part of their routine clinical care will be charged to either the patients or their insurance provider.

12 Potential Benefits of the Proposed Research to the Subjects and Others

There are potential direct benefits for the volunteer subjects participating in this study, as the pre-clinical data suggests that Sodium Nitrite administration at time of lung transplantation reduces the severity of Primary Graft Dysfunction (PGD). Studies in animal models of lung transplantation also suggest improved oxygenation, reduced inflammation and reduced chronic rejection. However, at the present time, it is not known whether these same benefits will also be observed in humans.

13 Importance of the Knowledge to be Gained

This study may help improve the outcomes of patients with end stage lung disease who undergo lung transplantation. In addition, the study may advance our understanding of the mechanisms involved in primary graft dysfunction and the effects of nitrite on lung function. The individuals and society stand to benefit from findings if nitrite is found to have beneficial effects.

14 Qualifications of the Investigators

Principal Investigator

Matt Morrell, MD: Dr. Morrell is Assistant Professor in the Division of Pulmonary, Allergy, and Critical Care Medicine. He has extensive clinical experience in the postoperative care of patients following lung transplantation.

Co-Investigators:

Norihisa Shigemura, MD: Dr. Shigemura is Assistant Professor in the Department of Surgery. He has extensive clinical experience in the pre, post, and intraoperative care of the lung transplant recipient.

Jonathan D'Cunha, MD, PhD: Dr D'Cunha is the Associate Professor of Cardiothoracic Surgery and Associate Director, Lung Transplantation. He has extensive clinical experience in the pre, post and intraoperative care of Lung transplant recipient.

Mark T. Gladwin MD: Dr Gladwin is the Chief of Pulmonary, Allergy, and Critical Care Medicine and Director of the Vascular Medicine Institute at the University of Pittsburgh. He is an internationally recognized authority in the field of sodium nitrite including both the basic science and a broad range of clinical applications in cardiovascular disease. He is a current IND holder for the investigation of sodium nitrite in cardiovascular disease.

Joseph Pilewski, MD: Dr. Pilewski is the Medical Chief of the Lung Transplantation Program at UPMC. He has extensive experience in the areas of management of patients both pre and post lung transplantation. He has also been an international leader in the development and implementation of complex clinical trials in cystic fibrosis.

Maria Crespo, MD: Dr. Crespo is Assistant Professors in the Division of Pulmonary, Allergy, and Critical Care Medicine and is involved in the daily care of the lung transplant patient population. Dr. Crespo has been actively involved in clinical research trials.

John McDyer, MD: Dr. McDyer is Associate Professor of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine (PACCM), Department of Medicine, University of Pittsburgh School of Medicine. Dr. McDyer also serves as the Director, Lung Transplantation Translational Research Program. Dr. McDyer is a transplant pulmonologist and immunologist, and has extensive experiences in human and mouse studies related to mechanisms of rejection and tolerance in lung transplant, and antiviral host defense.

Patricia George, MD: Dr. George is Assistant Professors in the Division of Pulmonary, Allergy, and Critical Care Medicine Dr. George's clinical and research interests centered on lung transplantation and lung disease in immunocompromised hosts.

Matthew Pipeling, MD: Dr. Pipeling is Assistant Professors in the Division of Pulmonary, Allergy, and Critical Care Medicine and is involved in the daily care of the lung transplant patient population.

Bruce Johnson, MD: Dr. Johnson is Assistant Professors in the Division of Pulmonary, Allergy, and Critical Care Medicine. Dr. Johnson's practice focuses on the pre and post-transplant care of lung transplant recipients, and he is the senior clinician in lung transplantation at UPMC.

Elizabeth Lendermon MD and Silpa Kilaru MD are both Assistant Professors in the Division of Pulmonary, Allergy, and Critical Medicine and are involved in the daily care of the lung transplant patient population.

Michael Donahoe, MD: Dr. Donahoe is Associate Professor in the Division of Pulmonary, Allergy, and Critical Care Medicine. Dr. Donahoe also serve as the Director of Medical Intensive Care Unit (MICU), and has extensive experience in the design and execution of numerous NIH-sponsored complex clinical trials, and co-investigator for studies in nutrition support in COPD, aging in critical care medicine, and the care of critically ill patients. In addition, he has been an active participant in industry sponsored clinical research trials in nutrition support in COPD, ARDS, ICU infections and sepsis investigational drug therapy.

Dr. Yingze Zhang is an Associate Research Professor at the Division of Pulmonary, Allergy and Critical Care Medicine. She is the Director of the Translational Research Core Laboratory (TRCL) at the PACCM division and has been conducting biomedical research for more than 20 years. The TRCL is dedicated to providing resources and technical support for clinical sample processing, sample banking, and high-throughput genetics and biomarker analysis associated with translational research of lung and vascular diseases. We have archived and are actively banking clinical research samples for multiple NIH-funded studies. The biological samples collected from this study will be banked in Translational Research Core Laboratory (TRCL) at the University of Pittsburgh under her oversight.

Diana Zaldonis MPH, BSN is a Clinical Research Coordinator of the Department of Cardiothoracic Surgery. Ms. Zaldonis is a registered nurse with 29 years of experience in cardio-thoracic surgery with the past 22 years in cardio-pulmonary transplantation as a clinical and research coordinator. She possesses a master's degree from the University Of Pittsburgh

Graduate School Of Public Health in Epidemiology. Ms. Zaldonis has extensive experience in managing and coordinating clinical trials. Her major role would be to provide guidance on regulatory submissions. She would not be involved with the consenting the patients.

Jeremiah Hayanga MD is an Assistant Professor of Surgery in the Department of Cardiothoracic Surgery. He is also the Director of Lung Transplant Outcomes Committee in the Thoracic Transplant Division. He is actively involved in clinical research and listed as Co-Investigator on several research projects within the Department of Cardiothoracic Surgery.

Ernest Chan MD is a Faculty Research Instructor at the University of Pittsburgh. He has previously worked on research projects in the Department of Cardiothoracic Surgery.

Nicholas Hess and Simeng Wang are both Medical Students at the University of Pittsburgh and involved in Lung Transplant Research in the Department of Cardiothoracic Surgery.

Collaborators

Susan Stuart, RN, MPM: Susan Stuart is the CEO for the Center for Organ Recovery and Education (CORE). She is experienced in the daily operations for organ procurement.

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