# Inhaled Selective Pulmonary Vasodilators for Advanced Heart Failure Therapies and Lung Transplantation Outcomes (INSPIRE-FLO)

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**1. Protocol Title:** Inhaled Pulmonary Vasodilator Therapy in Left Ventricular Assist Device (LVAD) Implantation, Heart Transplantation, and Lung Transplantation: Prospective, Randomized, Double-Blinded Study

**2. Purpose of the Study:**

*1. Aim I – Clinical Trial Investigation.* In order to utilize Inhaled Epoprostrenol (iEPO, Veletri®, Actelion Pharmaceuticals, South San Francisco, CA, USA) as an acceptable alternative to Nitric Oxide (iNO, INOMAX®, Mallinkrodt Pharmaceuticals, St. Louis, MO, USA) in adult patients, we propose a randomized, prospective, double-blinded trial in the cardiothoracic surgical population, which will evaluate the primary hypothesis that these two medications will have similar efficacy in pulmonary vasodilation and a similar impact on clinical outcomes in end-stage lung disease patients undergoing lung transplantation and end-stage heart failure patients under durable LVAD implantation or heart transplantation (Table 1).

*2. Aim II – Cost-Capture Analysis.* There will be a parallel prospective cost-capture analysis designed to precisely acquire the expenses that each drug incurs per patient averaged across all patients randomized to that drug.

**Table 1. Study Summary**

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|---|---|
| §**Sample Size** | N = 424 (50/50 by randomization strata) |
| **Population** | 1. Lung transplantation (N = 200)<br>2. Heart Transplantation / LVAD implantation (N = 224) |
| **Rationale** | • Comparison of iNO and iEpo impact on outcomes – evaluate for equivalency<br>• PVD therapy indications:<br>1. Lung Transplant: Improvement of ventilaton and perfusion matching after lung allograft implantation by vasodilation of ventilated pulmonary capillaries<br>2. Heart Transplant/LVAD Implantation: Improvement of RV contractility after cardiac allograft implantation or LVAD implantation by PVR reduction |
| **Study Design** | Prospective, Randomized, Double-Blinded |
| **Primary Outcomes** | 1. Lung Transplant: Severe PGD (grade 3)<br>2a. Heart Transplant: RVAD insertion<br>b. LVAD: INTERMACS Moderate or Severe RVF |
| **Secondary Outcomes** | All populations:<br>• ICU LOS (days)<br>• Hospital LOS (days)<br>• Mechanical ventilator duration (hours)<br>• Postoperative AKI<br>• In-hospital mortality<br>• Mortality 30-day, 90-day, 1-year |
| **Study length*** | 24-36 months |

§Sample size powered for primary outcomes
*Study length is determined through sample size divided by annual operations at Duke Universtiy Hospital; AKI = Acute kidney injury; iEpo = inhaled epoprostenol; iNO = Inhaled nitric oxide; ICU = Intensive care unit; LOS = Length of stay; LVAD = Left ventricular assist device; PGD = Primary graft dysfunction; PVD = Pulmonary vasodilators; PVR = Pulmonary vascular resistance; RVF = Right ventricular failure

**3. Background & Significance:**

*Introduction.* Inhaled Nitric Oxide (iNO) is a selective pulmonary vasodilator (PVD) with FDA-approval in the neonatal population alone. In adult patients, iNO is used off-label to treat pulmonary hypertension, right ventricular (RV) failure, and ventilation-to-perfusion mismatch. Adult patients who undergo durable LVAD implantation (e.g. Heartware®, Heartmate 2®, or Heartmate 3®) or cardiac transplantation for end-stage heart failure or those that have endured lung transplantation as a result of end-stage lung disease, compose the largest subpopulation which receives PVD therapy at Duke University Hospital. Intravenous Epoprostenol is FDA approved for adult patients with pulmonary hypertension and is the only agent which has displayed mortality benefit in these patients. The inhaled formulation of Epoprostenol (iEPO) was developed in order to maintain efficacy and avoid the systemic side effects of vasodilation and thrombocytopenia. Inhaled iEPO is used off-label in our cardiothoracic surgical patients for new-onset perioperative pulmonary arterial hypertension (PAH), known preoperative PAH, RV dysfunction with LVEF > 35-40%, and promotion of ventilation to perfusion matching through alveolar deposition of the prostanoid compound and vasodilation of the intimately associated intra-acinar pulmonary arteries. This vasodilation can decrease pulmonary vascular resistance and can improve oxygenation while avoiding systemic effects commonly seen in the intravenous formulation. iEPO has been introduced in the cardiothoracic operating rooms (OR) and ICU as a cost-conscious alternative medication to iNO. iEPO may display an equivalent efficacy profile to iNO for pulmonary vasodilation and oxygenation and have a similar impact on clinical outcomes. For the purposes of this writing, thoracic transplantation will refer to both heart and lung transplantation.

*Pharmacology.* There are 3 major pathways that affect pulmonary vascular tone: 1) Nitric oxide (vasodilatory), 2) Prostaglandin (vasodilatory), and 3) Endothelin (vasoconstrictive) pathways. During cardiothoracic operations, particularly transplantation and LVAD surgery, there is an appreciable imbalance in these pathways, which favors vasoconstriction. iNO administration, exerts its mechanism of pulmonary vasodilation and ventilation-to-perfusion matching through exogenous NO delivery and iEPO applies a similar mechanism via exogenous prostacyclin delivery. Both agents are delivered through mechanical ventilation to ventilated alveoli in order to promote gas exchange at the capillary bed. Both inhaled medications are desirable in this population due to pulmonary selectivity, absence of systemic vasodilation, as well as fast onset (5-10 seconds for iNO and 30-60 seconds for iEPO) and quick titration owing to short-half lives (10-20 seconds for iNO and 1-2 minutes for iEPO). There is no decision tree involved in the use of iNO vs iEPO except for that patient's known drug allergies which may preclude use of one inhaled agent in favor of the other. Of note, endothelin antagonists (e.g. bosentan), which are not part of our perioperative standard practice, are PO medications

which require reliable gastrointestinal absorption that may not be present during high-dose inotropic support, and are not readily titrated to effect as are the inhaled PVD, iNO and iEPO.

*Contraindications and Adverse Effects.* Absolute indications for iNO in favor of iEPO are due to prostaglandin allergy leading to anaphylaxis (extremely rare) or if the patient is pregnant due to risk for labor induction as a result of prostacyclin agonism. Routine pregnancy testing is performed in the preoperative setting in line with established preoperative anesthesia testing criteria. Parturients rarely present for thoracic transplantation or LVAD implantation. There are no absolute contraindications to iNO therapy in adult patients but the iNO delivery device system routinely measures the toxic metabolite of iNO, nitrogen dioxide ($NO_2$), which can lead to hypoxemia during metabolite accumulation. Additionally, methemoglobinemia (MetHb) is another rare adverse occurrence of prolonged iNO administration and MetHb levels are measured during arterial blood gas analysis.

*Preliminary retrospective study supporting noninferiority hypothesis.* In a retrospective study of 51 adult cardiothoracic surgical patients (all-comers, including thoracic transplantation, durable LVAD implantation, and non-transplant and non-LVAD cardiac surgical patients), requiring pulmonary vasodilation, our group illustrated similar efficacy between the use of iEPO and iNO with respect to optimizing RV hemodynamic variables, including pulmonary vasodilation and mixed venous oxygenation (Table 2). During this investigation, iNO was initiated in the operating room (OR) and continued during transport and into the ICU. While in the ICU, postoperative hemodynamic stability was achieved within 2 hours and iNO was transitioned to iEPO over 30 minutes in order to provide continuous inhaled pulmonary vasodilation and allow the patient to self-control during medication cross-over between iNO and iEPO. Clinical variables were followed at 5-minute intervals for 1 hour after transition to iEPO. No statistically significant differences were seen in hemodynamic variables during this transition (Table 2). The small sample size and retrospective design, however, incorporated several confounding variables that could not be controlled and *prospective data was deemed necessary to achieve reliable conclusions by evaluating clinical outcomes in order to change clinician practice patterns*. Other investigations have demonstrated equivalence in hemodynamic variables, mixed venous oxygenation, and ventilation-to-perfusion matching when delivery of iNO was compared with iEPO. These studies were, however, also retrospective or inadequately powered to rely on conclusions related to outcome measures.

**Table 2. Hemodynamic values in CT surgical patients comparing inhaled Nitric Oxide and Epoprostenol**

| N= 51 | *HR | ¶MAP | ¶PAPs | ¶PAPd | ¶PAPm | ¶CVP | ¥CI | PI | §LVAD flow | SVO2 (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| [a]iNO | 98 | 78 | 37.9 | 18.6 | 25.3 | 12.5 | 2.61 | 5.36 | 4.66 | 71 |
| [a]iEpo | 100 | 80 | 39.1 | 19.0 | 26.8 | 12.2 | 2.67 | 4.93 | 4.82 | 70 |
| *P*-value | 0.41 | 0.40 | 0.48 | 0.58 | 0.24 | 0.74 | 0.63 | 0.52 | 0.65 | 0.52 |

a = reported as mean values; * units = beats per minute; ¶ units = mm Hg; ¥ units = L/min/$m^2$
CI = Cardiac Index, CVP = Central Venous Pressure, HR = Heart Rate, iNO = Inhaled nitric oxide, iEpo = Inhaled epoprostenol, LVAD = Left Ventricular Assist Device, MAP = Mean Arterial Pressure, PAPs = Systolic Pulmonary Artery Pressure, PAPm = Mean Pulmonary Artery Pressure, PAPd = Diastolic Pulmonary Artery Pressure, PI = Pulsatility Index, $SvO_2$ = Mixed Venous Oxygen Saturation

The large cost differential between these two agents remains an important concern for the health system: iNO is approximately 8-fold more expensive than iEPO, according to preliminary estimates based on PVD usage. Previous reports have estimated the cost of iNO administration to be between $95.00 – $115.00 per hour during medication delivery. The cost, however, has not precisely captured the time required to assemble the iNO delivery system as well as resources utilized to breakdown this setup into individual components following termination of delivery. The cost of iEPO delivery is captured at $14.83 per hour, which includes solution compounding by pharmacy as well as processing for delivery and nebulization by respiratory care services. Additionally, the iEPO delivery-system setup is a one-time, fixed cost for the duration of administration. Similar secondary resource utilization capture for iEPO is required for accurate cost comparison between these two agents.

## 4. Design & Procedures:

*Aim I – Development of a Definitive Clinical Trial Investigation.*

*1. Randomization and Double-Blinding.* The clinical research unit (CRU) will receive preoperative notification of lung and heart transplantation patients by reviewing the transplant waitlist. Preoperative notification of LVAD implantation will be done by the review of the cardiothoracic surgical schedule. Using a 50% randomization process utilized and established by the CRU at Duke University Hospital, each eligible patient will be randomized to receive either iNO or iEPO. The primary endpoint data will be collected and documented in an electronic data capture system during the period of time the patient, clinical care team, and study team are blinded. Primary endpoint data collection will be complete prior to the subjects' discharge from the ICU, at which point the unblinding will occur. Since primary endpoint data collection will occur during the blinded period, the potential for bias will be substantially minimized.

*2. Measured Outcomes.* The primary endpoint for the comparison of efficacy in the Lung Transplant population will be the incidence of Grade 3 Primary Graft Dysfunction (PGD). This is defined by the International Society of Heart and Lung Transplantation (ISHLT) as severe hypoxemia with a $PaO_2$-to-$FiO_2$ ratio < 200 or the presence of venovenous extracorporeal membrane oxygenation (VV ECMO) at an time-point within the first 72

hours after lung transplantation. The primary endpoint for the comparison of efficacy in LVAD patients will be incidence of moderate or severe RV failure according to Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) scoring. The primary endpoint for the comparison of efficacy in the heart transplant subset will be the incidence rate of RVAD insertion. Secondary endpoints related to clinical outcomes for all populations will be duration of postoperative mechanical ventilation, , ICU Length of Stay (LOS), hospital LOS, incidence of acute kidney injury, incidence of in-hospital mortality, as well as postoperative mortality at 30-days, 90-days, and 1-year after operation (Table 1).

*Aim II –Cost-Capture Analysis.*
In parallel with the design & procedures of *Aim I*, the cost capture analysis component will be essential in order to better gauge the cost due to duration of administration (variable cost) according to each inhaled PVD. Established clinical criteria specific to each group (lung transplantation vs. heart transplantation/LVAD implantation) have been developed to determine the inception of protocolized PVD weaning. Weaning medications according to established protocols will allow for accurate interpretation of the comparative length of therapy between iNO and iEPO and help prevent erroneous PVD usage without criteria for discontinuation. Secondary resource utilization will be documented by respiratory care services and itemized cost sheets will be developed.

Subject Groups
Inhaled PVD therapy is administered to every patient undergoing thoracic transplantation and LVAD implantation at our institution and each patient is eligible for enrollment. Over a 3-year period (1 year for follow-up) we will prospectively enroll 200 lung transplant subjects and 224 heart transplant or LVAD implantation patients who will be informed and consented prior to their scheduled procedure. Potential subjects will be under the care of 1 or more investigators in this study. Consented subjects will be randomly assigned to 1 of 2 groups, iNO vs iEPO, to be initiated in the OR on the day of the operation based on accepted standard of practice and study protocol. Medication administration will be double-blinded, such that neither the surgical nor anesthesiology teams will be notified of the inhaled agent to which the patient has been randomized. Ability to unblind the delivery system will be made available to both teams if required to preserve optimal patient care. As per our standard practice, respiratory care services will manage the initiation and maintenance of inhaled PVDs in the OR and ICU, and these personnel will be the only practitioners notified of the actual delivered medication during study blinding.

Exclusion Criteria
- Combined Organ Transplantation (Heart-Lung, Heart-Liver, Heart-Kidney)
- Age < 18 years old
- Pregnancy (females of child bearing potential will receive pregnancy testing prior to cardiothoracic surgery as a standard of care)
- Known allergy to prostaglandin (rare)
- Refusal of blood products due to personal or religious preference.
- Subject is enrolled in another study protocol, which does not allow randomization of PVD therapy
- Heart transplant or durable LVAD recipients with adult congenital heart disease (CHD)
- Caveat: Does NOT meet exclusion criteria if the scheduled heart transplant or LVAD implantation is due to heart failure from *a previous heart transplantation* related to CHD, performed more than 90 days previous to the date of trial enrollment
- Patient is scheduled to undergo lung transplantation but has undergone heart transplantation in the previous 90 days
- Patient is scheduled to undergo durable LVAD implantation but has undergone heart transplantation in the previous 90 days
- Patient is scheduled to undergo heart transplantation but has undergone lung transplantation in the previous 90 days
- Patients with preoperative VV ECMO as a bridge to lung transplantation
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Stopping Criteria – In the event the following criteria are met and the clinical team is in agreement, subjects will be weaned off of their iPVD per instutional standard iPVD weaning practice. If adverse events are encountered, the drug will be immediately stopped without weaning.
- Venoarterial (VA) ECMO insertion remains at end of operation
- VA ECMO insertion is performed postoperatively in the ICU
- LVEF < 30% on echocardiogram at the end of the operation for heart and lung transplant subjects

- LVEF < 30% for heart and lung transplant subjects on echocardiogram noted postoperative in the ICU
- Inhaled pulmonary vasodilation is halted for reasons other than standard weaning ordered by the clinical care team
- Adverse events related to the INO or EPO that affect the subject's welfare

Data Collection

*Secondary measures will be hemodynamic variables* (similar to those measured in Table 2) such as transesophageal echocardiographic (TEE) evaluation of RV function based on stand-of-practice protocol, intravenous administration of inotropes, serial measures of postoperative serum creatinine and GFR, resolution of elevated liver function tests (heart failure patients, illustrates improvement in RV function), incidence of thrombocytopenia (platelet count < 150 x $10^9$/L) and trajectory of resolution, as well as ventilation-to-perfusion matching (arterial oxygen tension, $PaO_2$; arterial carbon dioxide tension, $PaCO_2$; and fraction of inspired oxygen, $FiO_2$). Variables will be recorded at designated time points during the entire duration of administration – from initiation in the operating room to cessation in the ICU. These time points include: Intraoperative before surgical incision, time = 0 (initiation of PVD), 30 minutes, 2 hours, 6 hours, 12 hours, 18 hours, 24 hours, and every 6 hours up through 72 hours after initiation. These secondary measures will be obtained up through 72 hours after initiation regardless of cessation or continuation of the inhaled PVD. After 72 hours, increments of every 12 hours thereafter will be assessed if PVD administration continues. Ventilation and perfusion nuclear scans will be obtained and recorded per standard clinical practice for each group of lung transplant recipients. Established protocols with criteria for initiation of medication weaning have been created according to each medication based on individual pharmacokinetic properties. Once established criteria are met, weaning of each inhaled PVD will begin and continue until the medication is terminated according to standardized weaning protocols established for lung transplant patients and heart transplant/LVAD patients.

*Subject follow up.* Subject will be contacted by phone by a member of the research team and be asked a short series of questions to assess their current medical condition and any changes since surgery at 30-days (± 3 days), 90-days (± 5 days), and 1-year (± 7 days) after surgery completion date. The phone follow-up should take approximately 5 minutes of the subject's time. If subjects have been admitted to a hospital outside of Duke Health after surgery they will be asked to sign an authorization of release to provide us permission to obtain medical information related to their hospitalization.

Blood Sampling

Blood samples will be drawn for analysis as a part of this study. One 9 ml sample of blood will be obtained from each patient prior to the initiation of PVD therapy and stored at 4°C prior to processing. This sample will be stored for Genomic DNA analysis at the completion of this study in order to assess patients who are responders to inhaled pulmonary vasodilaton through upregulation and down regulation of notable vasoactive substances (e.g. endothelin, thromboxane, nitric oxide, prostaglandin, etc.). In addition, each subject will also be asked to sign the Genomic and Proteomic Database Repository (IRB Pro00015651) consent form, thus allowing the banking of their plasma and DNA samples as well as data to be used for future research. Participation in IRB Pro00015651 is voluntary and optional to all subjects consented in this parent study. Blood samples (7 ml each) will be drawn at 3 separate time points: 1) directly after insertion of the invasive blood pressure monitoring (arterial) line, 2) POD 1 (8 to 24 hours after completion of surgery), and 3) POD 7 (6 days from POD 1). In each 7ml blood sample, 3.5ml will be collected in Sodium Citrate tubes for coagulation analysis and another 3.5ml will be collected in EDTA tubes for metabolomic and proteomic analysis. Plasma will be separated from these samples and banked at -80°C for analyses of proteomic and metabolomic signatures. Up to 30ml of blood will be collected during the 12 month study participation period.

**6. Subject Identification, Recruitment, & Compensation:** Subjects will be recruited either during the outpatient or inpatient evaluation phase, or contacted by phone. Recruitment may also occur on the day of the operation given the complexities of the transplant process, which may provide obstacles to earlier enrollment. After obtaining permission from the operating surgeon, surgical subjects will be screened by the study coordinator by reviewing the transplant pre-list. Prior to asking any patient for consent to participate, the patient or Legally Authorized Representative (LAR) will be approached first by the surgeon or one of the members of the surgical care team to determine if the patient or LAR is willing to consider enrollment in the study. If so, the subject or LAR will either be seen during an inpatient or outpatient visit, or be contacted by phone and informed about the study by a member of the research team. If the individual or LAR is willing to consider enrollment and does not meet exclusion criteria, then the research coordinator will present the research protocol in its entirety. During this time, the study coordinator will answer any and all questions as they arise. If

the subject or LAR agrees to participate, the coordinator will ask the them to sign and date the appropriate consent form. A copy of this consent form will be given to the subject and a copy of the consent form will be added to the subject's medical record. The subject or LAR will be given the option to sign a separate consent form to allow us to store portions of the collected blood specimens and any data collected under this research study and maintain these samples and data in a database/repository (PRO00015651) for possible use in future research studies relating to surgical outcomes. In the event a LAR provides consent at the time of enrollment, the subject will be approached once they regain the ability to provide an informed consent.

Recruitment will not routinely occur on the day of the operation and most patients will be enrolled at least 12 hours in advance and provided at least the allowable time to review the study consent form and discuss their options with the PI and study personnel. There will be no direct compensation to the patient for recruitment.

If a subject is enrolled and randomized in this study for their LVAD implantation procedure and is later planned to receive a heart transplant, that previously enrolled subject is eligible to be re-enrolled. The following caveats apply to this subpopulation of LVAD patients:

A)Durable LVAD implantation may occur as a bridge to heart transplantation.

B)If LVAD implantation is followed by heart transplantation WITHIN 1 year following LVAD implantation, then data collected up through the time of heart transplantation will be recorded and valid as a patient in the LVAD group.

C)Data collected on or after the date of LVAD explantation/heart transplantation for such a patient will be considered as part of the heart transplant group.

D)If LVAD implantation is followed by heart transplantation AFTER 1 year following LVAD implantation, then the 1year follow-up period is complete and the patient may re-enter the trial as a heart transplant patient.

If a subject is enrolled and randomized in this study for their durable LVAD implantation procedure and is scheduled to receive a new durable LVAD via an LVAD exchange operation, the subject is eligible to be re-enrolled.

**7. Subject's Capacity to Give Legally Effective Consent:** Explicit (written) consent will be obtained from the patient or the patient's legal decision maker.

**8. Study Interventions:** Using a 50% randomization process utilized and established by the CRU, each eligible patient will be randomized to receive either iNO or iEPO, to be initiated in the OR based on accepted standard of practice at Duke University Hospital, during the clinical care of these patients.

**9. Risk/Benefit Assessment:** There is no direct benefit of this study to the enrolled subjects. Data gathered from this study may benefit future patients. Up to 30 ml of blood will be drawn during the 12 month study participation period. Blood sampling will be obtained, in the majority of subjects, from indwelling arterial or central venous lines inserted at the beginning of the intraoperative period as part of standard practice for these operations and there will be no additional risk to the patient for obtaining such vascular access. On rare occasion, blood sampling may be obtained from additional venipuncture sites during the postoperative period. Risks of blood sampling if obtained through venipuncture are pain, swelling, possible infection at the site of venipuncture. While these risks are minimal, the additional blood volume is highly unlikely to contribute to the patient's need for blood transfusion. To minimize any potential risk to the patient from genetic data, investigators and patients will be blinded to the individual patient's genotype. This information will not be included in the patient chart, will remain absolutely confidential, and will not be given to the patient or their family. DNA samples will be identified only by a coded number whose relation to the patient's name and other identifiers is available only to the data manager. The identity of the patient will remain anonymous in any publications which may result from this investigation.

There will be no additional risks to the subjects as a result of this study. Prior to June of 2015, iNO was the sole option for inhaled pulmonary vasodilation in this patient population and therefore utilized in each operation for this indication. As of June 2015, iEPO was introduced for the same indications as iNO in order to serve as a cost-conscious alternative to iNO and to potentially explore a different, equally impactful pathway for clinically evident pulmonary vasodilation (as measured by Swan-Ganz catheter data and determined by transesophageal echocardiography). There are no additional risks to the patient aside from the rare adverse effects such as allergic reaction, as previously discussed. The most common side effect of iNO is hypotension. The side effects common to intravenous iEPO are nausea, vomiting, hypotension, flushing, chest pain, anxiety, dizziness, bradycardia, difficulty breathing, abdominal pain, musculoskeletal pain and tachycardia.

**10. Costs to the Subject:** There will be no additional costs to the subjects as a result of this study.

**11. Data Analysis & Statistical Considerations:** Summary statistics will be computed for demographic, clinical, and outcome variables in the form of frequencies (percentage) for categorical variables and mean (standard deviation) for continuous variables for each arm. Univariate analysis will be performed to compare the difference of each variable between treatment groups by chi-square or Fisher exact tests for categorical variables, and t-tests or Wilcoxon Rank-Sum tests for continuous variables depending on data normality. The univariate results for the outcome variables will provide information on iNO treatment effect in comparison to iEPO without taking into account other potential confounding factors. All non-outcome variables meeting $p< 0.15$ association with treatment group will be considered for variable selection to build a multivariable regression model. For each outcome of interest, we will start with a regression model (logistic regression for binary outcomes or generalized linear model for continuous outcomes) with all variables selected from univariate analysis described above. Based on stepwise variable selection, we will determine the final set of covariates to be included in the final multivariable model to test the treatment group effect. Based on the analysis results, we will be able to understand if iNO is equivalent to iEPO (no significant difference) or significantly better or worse than iEPO (significant treatment effect) to address the efficacy of iNO for Aim 1. Several of secondary measures will be obtained over time. We will apply generalized mixed model to take into account the repeated measures over time to test for treatment effect. In the case of patients have switched to the other arm due to clinical decision, we will conduct the primary analysis based on the intent to treat (ITT) without reclassifying treatment assignment. In addition, protocol analysis, where only patients follow the protocol assignment are included will also be conducted to verify ITT results. For Aim 2 to compare cost capture analysis, the comparison of cost measures between two groups will be tested by two sample t-test.

Based on recent *annual* operations, approximately 120 LVAD implantations, 60 heart transplantations, and 110 Lung transplantations were performed at Duke University Hospital during FY 2014 – 2015. This study has been individually powered to primary endpoints for each arm (Table 1) and the duration of study enrollment has been determined according to annual operations and sample-size calculations. We estimated sample size based on equivalence test of the incidence rates of a binary outcome (e.g. PGD grade 3 (PGD-3)) of two treatment groups as an illustration. Assuming the incidence rate of PGD-3 under iEPO treatment is 0.35 and acceptable margin of the equivalence is ± 0.19, we will need 224 patients to have 80% power to detect an actual difference at 0.05 between two treatment group under this margin. This implies that the acceptable range of incidence rate for iNO treatment is from 0.21 to 0.59. Based on this estimate, we propose to enroll 200 lung transplant patients and 224 LVAD and heart transplant patients (n = 424) over a period of 24 to 36 months; the exact time point for trial culmination between 24 and 36 months will be dependent on enrollment rate. There will be a 50% randomization rate for each inhaled agent such that 212 patients will receive iEPO and 212 patients will receive iNO.

**12. Data & Safety Monitoring:** The proposal is not introducing a new medication that has not been utilized by our group and safety has been established for this patient population through clinical practice and medication usage. Safety will, however, be determined by assessing reported, rare, adverse effects of iNO (systemic hypotension, methemoglobinemia, and rebound pulmonary hypertension after appropriate weaning) and iEPO (systemic hypotension, non-surgical bleeding related to thrombocytopenia, flushing, and rebound pulmonary hypertension after appropriate weaning) in order to accurately monitor adverse events (AE) during this study. The PI will review and sign off on AE's as they occur and perform a quarterly review and determine if AE's are related to the study or otherwise. AE's will be reported to the IRB per HRPP policies.

*Stopping Rule:* Subjects who meet the stopping criteria in section 4 continued to be enrolled and followed for primary outcome analysis. See section 4 for stopping criteria.

**13. Privacy, Data Storage & Confidentiality:** All data collected in the case report forms (CRF) will be collected by review of the subjects routine medical record documentation or during the intraoperative portion of the study. All subjects will be given a study ID in an order to maintain their identity and subject's identity will be protected and confidentially maintained. Barcodes will be affixed to each study sample collected according to the protocol. For future review, the study number and barcode will be the only identifying information associated with the subject. All paper data will be stored in a locked cabinet in the research teams office as outlined in the research data security plan. Any computerized data will be stored within the Duke University Medical Center's Database, which is password protected, and located behind Duke Computing firewalls. Only the PI and the statisticians will have access to the data obtained from these cases.