

A Dose Escalation Study to Evaluate the Effect of Inhaled Nitrite (AIR001) on  
Cardiopulmonary Hemodynamics in Subjects with Pulmonary Hypertension

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Version 3.0  
October 12, 2017



## TABLE OF CONTENTS

<b>PROTOCOL SYNOPSIS.....</b>	<b>3</b>
<b>1. STUDY OBJECTIVE, SPECIFIC AIMS, BACKGROUND AND SIGNIFICANCE.....</b>	<b>10</b>
1.1 OBJECTIVE.....	10
1.2 SPECIFIC AIMS .....	10
1.3 BACKGROUND.....	10
1.4 SIGNIFICANCE .....	15
<b>2. RESEARCH DESIGN AND METHODS.....</b>	<b>16</b>
2.1 CLASSIFICATION AND METHODOLOGICAL DESIGN.....	16
2.2 DETAILED DESCRIPTION OF RESEARCH ACTIVITIES .....	16
2.3 STUDY TREATMENT .....	21
2.4 STUDY DESIGN SCHEMATIC .....	22
2.5 DURATION OF FOLLOW-UP .....	22
2.6 SAFETY MONITORING.....	22
2.7 STUDY INVESTIGATIONAL THERAPY SUPPLIES.....	23
2.8 STUDY INVESTIGATIONAL THERAPY STORAGE .....	23
2.9 STUDY INVESTIGATIONAL THERAPY ACCOUNTABILITY .....	24
2.10 STUDY PROCEDURES .....	24
2.11 SCHEDULE OF ACTIVITIES.....	27
2.12 DESCRIPTION OF STUDY PROCEDURES .....	28
2.13 ENDPOINTS.....	30
2.14 WITHDRAWAL CRITERIA.....	31
2.15 STATISTICAL ANALYSIS.....	32
<b>3. HUMAN SUBJECTS .....</b>	<b>33</b>
3.1 SUBJECT POPULATION.....	33
3.2 INCLUSION CRITERIA.....	33
3.3 EXCLUSION CRITERIA.....	34
<b>4. RECRUITMENT AND INFORMED CONSENT PROCEDURES .....</b>	<b>35</b>
4.1 RECRUITMENT METHODS .....	35
4.2 INFORMED CONSENT PROCEDURES.....	35
<b>5. POTENTIAL RISKS AND BENEFITS .....</b>	<b>36</b>
5.1 POTENTIAL RISKS .....	36
5.2 ALTERNATIVE TREATMENTS .....	39
5.3 BENEFITS .....	40



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5.4	RISK MANAGEMENT .....	40
5.5	DATA SAFETY MONITORING PLAN.....	40
6.	<b>STUDY ADMINISTRATION.....</b>	<b>45</b>
6.1	QUALITY CONTROL AND QUALITY ASSURANCE .....	45
6.2	DATA HANDLING AND RECORD-KEEPING.....	46
6.3	ETHICS .....	47
7.	<b>COSTS AND PAYMENTS.....</b>	<b>48</b>
7.1	COSTS.....	48
7.2	PAYMENTS.....	48
8.	<b>QUALIFICATIONS AND SOURCE OF SUPPORT.....</b>	<b>49</b>
8.1	QUALIFICATIONS OF INVESTIGATORS.....	49
8.2	SOURCE OF SUPPORT.....	49
9.	<b>REFERENCES.....</b>	<b>50</b>



## PROTOCOL SYNOPSIS

Protocol Title:	A Dose Escalation Study to Evaluate the Effect of Inhaled Nitrite on Cardiopulmonary Hemodynamics in Subjects with Pulmonary Hypertension	
Protocol Number:	PRO 11080686	
NCT Number:	NCT01431313	
Version # and Date:	Version 3.0/October 12, 2017	
Clinical Phase:	Phase II clinical investigation	
Investigational Drug:	Sodium Nitrite Inhalation Solution (AIR001 Inhalation Solution)	
Trial Site:	Single-Center Trial	
Sponsor:	Mark Schmidhofer, MD Associate Professor of Medicine University of Pittsburgh Heart and Vascular Institute	
Investigator:	Marc Simon, MD, MS, FACC Associate Professor of Medicine University of Pittsburgh UPMC Heart and Vascular Institute	
Sub-Investigators:	Mark T. Gladwin, MD Michael Risbano, MD	Allison Morris, MD Frank Sciruba, MD Dennis McNamara, MD
Study Monitor:	Mark Schmidhofer, MD Associate Professor of Medicine University of Pittsburgh Heart and Vascular Institute	
Research Facilities:	University of Pittsburgh Medical Center 200 Lothrop Street Pittsburgh, PA. 15213	



Clinical Laboratories:	University of Pittsburgh Medical Center 200 Lothrop Street Pittsburgh, PA. 15213
Manufacturer:	Aires Pharmaceuticals, Inc.
Study Rationale:	<p>Inhaled nitric oxide has been demonstrated to improve pulmonary hemodynamics in subjects with pulmonary arterial hypertension (PAH).</p> <p>We hypothesize that inhaled nitrite provides a therapeutic approach for patients with PAH for the following reasons:</p> <ol style="list-style-type: none"> <li>1. Inhaled nitrite therapy provides a potent, effective treatment that will vasodilate the pulmonary circulation (reduce pulmonary vascular resistance), reduce mean pulmonary artery pressure, and increase cardiac output.</li> <li>2. This study will provide valuable efficacy and safety insights into the interactions between NO and nitrite, both direct NO generators, with or without background PH therapy, particularly phosphodiesterase type-5 inhibitors (PDE-5I), on pulmonary hemodynamics and right heart function.</li> <li>3. This study will also provide novel assessments of right ventricular global myocardial function.</li> <li>4. The results obtained from this study will also lay the groundwork for future large-scale clinical trials that are needed to investigate the efficacy of inhaled nitrite and/or its long-term effects on pulmonary hemodynamics in patients with pulmonary hypertension.</li> </ol>
Study Objectives:	The main objective of this study is to investigate the effect of inhaled nitrite delivered in a dose escalation manner in patients that may be on background pulmonary hypertension therapy; endothelin receptor antagonist (ETRA), PDE-5I, or prostacyclin analog on cardiopulmonary hemodynamics in subjects with pulmonary hypertension.
Study Hypothesis:	<ul style="list-style-type: none"> <li>• We hypothesize that inhaled nitrite treatment will improve clinical parameters of pulmonary vascular and right heart function. Our primary endpoint will be the change in pulmonary vascular resistance (PVR) measured by right heart catheterization.</li> <li>• We hypothesize that inhaled nitrite treatment will improve global right ventricular function.</li> </ul>



	<ul style="list-style-type: none"> <li>We hypothesize that inhaled nitrite may be delivered safely, based on stable mean arterial blood pressure with minimal dose limiting symptoms, in patients on background PAH therapy, particularly PDE-5I (sildenafil, tadalafil).</li> </ul>
Study Aims:	<p><u>Specific Aims:</u></p> <ol style="list-style-type: none"> <li>To determine the efficacy of inhaled nitrite, on the change in pulmonary vascular resistance measured by right heart catheterization.</li> <li>To explore the effect of inhaled nitrite on echocardiographic and hemodynamic parameters measured: SVR, RV systolic (dP/dtmax/IP, PWRmax/EDV, RV EF, TAPSE), RV diastolic function (dP/dtmin, Tau), Pulmonary vascular impedance / Wave Intensity.</li> <li>To evaluate the safety of inhaled nitrite on systemic blood pressure in patients on background therapy for PAH.</li> <li>To identify the pharmacokinetic relationships of nitrite measures of drug exposure and plasma cGMP, using blood sampling.</li> <li>To identify the changes in mitochondrial oxygen consumption, using analysis of platelets.</li> <li>To compare effects of inhaled nitrite on pulmonary vascular resistance with inhaled NO gas at 40 ppm.</li> </ol>
	<p>This is a single-center, open label phase II study to evaluate the effect of inhaled nitrite delivered in a dose escalation manner on the change in pulmonary vascular resistance (PVR) in subjects with pulmonary arterial hypertension undergoing right heart catheterization.</p> <p>A total of 50 subjects with a confirmed diagnosis of pulmonary hypertension and meeting all inclusion/exclusion criteria will be enrolled in the study which will entail a single right heart catheterization. The study population consisted of subjects with WHO group I PAH (n=20), WHO group II PH (n=20) and subjects with WHO group III PH (n=10) will receive the dose escalation paradigm:</p> <ul style="list-style-type: none"> <li>Each subject will receive a starting dose of 45 mg inhaled nitrite (AIR001 Inhalation Solution), followed by a second dose of 90 mg inhaled nitrite, based on safety and tolerability.</li> </ul>



	<p>The study visit will occur on the same day subjects are scheduled for their clinically indicated right heart catheterization or who volunteer for a research right heart catheterization for this specific study. Subjects on oral background PAH therapy (ETRA or PDE-5I) will be instructed to hold their regular PAH regimen on the day of the study visit.</p> <p>Baseline pulmonary artery hemodynamic assessment, echocardiogram, micromanometry measured simultaneous pressure and flow velocity signals will be performed prior to inhaled nitrite treatment. Responses to 40 ppm inhaled NO will be measured before nitrite nebulization via a non-rebreather face mask to assess baseline, pre-AIR001 vasodilator responsiveness.</p> <p>Subjects will receive a starting dose of 45 mg (placed in the nebulizer cup) of inhaled nitrite (AIR001), with one subsequent planned dose of 90 mg of inhaled nitrite, based on safety and tolerability, approximately 60 minutes after the first dose. During the study, right heart/pulmonary artery hemodynamics will be measured continuously, and cardiac output measured at 15 minute intervals, as well as noninvasive systemic blood pressure monitoring. Subjects will be tested for the changes in hemodynamics and calculated pulmonary vascular resistance (PVR) with inhaled nitrite.</p> <p>Subjects will be monitored closely for changes in blood pressure during the study. .The presence of severe systemic hypotension defined as systolic blood pressure &gt;30 mm Hg drop from pre-dose baseline AND symptomatic or requiring fluid replacement or other therapy will lead to a discontinuation of the study treatment, and the next higher dose of inhaled nitrite will not be administered. Subjects will continue to be monitored closely.</p> <p>Oxygen saturation measured by pulse oximeter and methemoglobin levels monitored by co-oximetry prior to dose administration and through 2 hours post-dose will be monitored closely.</p> <p>Blood samples may be collected for Cyclic Guanosine Monophosphate (cGMP) concentration determination, pharmacokinetics (PK) and platelet mitochondrial analysis at pre dose and the end of the each peak dose of nitrite (approx.. 15 minutes post inhaled dose). PK and cGMP measurements will be made on mixed venous blood using PCW pullback samples and venous blood will be collected for platelet mitochondrial analysis.</p> <p>Subjects will be monitored carefully for occurrences of adverse events, laboratory test abnormalities, and changes in vital signs.</p>
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	Following the study treatment, patients will be followed as an outpatient on Day 30 (+/- 5 day window). An additional follow-up assessment by telephone will occur on Day 3.
Planned Sample Size:	50 subjects
Duration of Treatment:	One day
Inclusion Criteria:	<p><u>WHO Group I PAH (n = 20)</u>          Diagnosis of RHC confirmed WHO Group I PAH in any of the following 3 categories:</p> <ul style="list-style-type: none"> <li>Idiopathic, primary or familial pulmonary arterial hypertension (IPAH, PPH, or FPAH)</li> </ul> <p style="text-align: center;"><b>OR</b></p> <ul style="list-style-type: none"> <li>PAH associated with one of the following connective tissue diseases (CTD):             <ol style="list-style-type: none"> <li>Systemic sclerosis (scleroderma)</li> <li>Limited scleroderma</li> <li>Mixed connective tissue disease</li> <li>Systemic lupus erythematosus</li> <li>Overlap syndrome;</li> </ol> </li> </ul> <p style="text-align: center;"><b>OR</b></p> <ul style="list-style-type: none"> <li>PAH associated with:             <ol style="list-style-type: none"> <li>Human immunodeficiency virus (HIV) infection;</li> <li>Simple, congenital systemic-to-pulmonary shunts at least one year post-surgical repair.</li> <li>Exposure to legal drugs, chemicals and toxins, such as fenfluramine, derivatives, other anorexigens, toxic rapeseed oil or L-tryptophan. Subjects with PAH associated with illegal drug use, such as methamphetamine, are excluded.</li> </ol> </li> </ul> <p style="text-align: center;">Stable PH for at least 3 months if on disease specific therapy.</p> <p><u>WHO Group II PH (=20)</u>  <u>Diagnosis of RHC confirmed WHO Group II PH by:</u></p> <ul style="list-style-type: none"> <li>Pulmonary capillary wedge pressure (PWCP) &gt; 15</li> </ul> <p style="text-align: center;">AND</p> <ul style="list-style-type: none"> <li>Transpulmonary Gradient (TPG) &gt; 12</li> </ul> <p><u>WHO Group III PH (n = 10)</u>          Has WHO functional class II-IV symptoms          Had the diagnosis of PH confirmed by a cardiac catheterization with the following values:</p> <ul style="list-style-type: none"> <li>Mean pulmonary artery pressure (mPAP) ≥ 25 mm Hg (at rest)</li> <li>PCWP or left ventricular-end diastolic pressure ≤15 mm Hg (if diagnosed with PAH)</li> </ul>



	<ul style="list-style-type: none"> <li>○ Pulmonary Vascular Resistance (PVR) <math>\geq 3</math> mm Hg/L/min or <math>\geq 240</math> dynes*sec/cm<sup>5</sup></li> </ul> <p><u>Both WHO Group I PAH, WHO Group II and III PH</u></p> <ol style="list-style-type: none"> <li>1) Age 18 and older;</li> <li>2) Able to participate in right heart catheterization</li> <li>3) Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the study</li> <li>4) Subjects who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures</li> </ol>
Exclusion Criteria:	<ul style="list-style-type: none"> <li>• Age less than 18 years;</li> <li>• Baseline systemic hypotension, defined as MAP less than 50 mmHg;</li> <li>• Required intravenous inotropes within 30 days prior to study participation;</li> <li>• Has uncontrolled systemic hypertension as evidenced by sitting systolic blood pressure <math>&gt;160</math> mm Hg or sitting diastolic blood pressure <math>&gt;100</math> mm Hg at screening;</li> <li>• Has a history of portal hypertension or chronic liver disease, including hepatitis B and/or hepatitis C (with evidence of recent infection and/or active virus replication) defined as moderate to severe hepatic impairment (Child-Pugh Class B-C);</li> <li>• Has chronic renal insufficiency as defined by serum creatinine <math>&gt;2.5</math> mg/dL at screening or requires dialysis support;</li> <li>• Has a hemoglobin concentration <math>&lt;9</math> g/dL at Screening;</li> <li>• History of atrial septostomy within 6 months prior to Day 1 visit</li> <li>• Repaired or unrepaired congenital heart disease (CHD);</li> <li>• Pericardial constriction;</li> <li>• Confirmed diagnosis of restrictive or constrictive cardiomyopathy;</li> <li>• Left ventricular ejection fraction (EF) <math>&lt;40\%</math> by multiple gated acquisition scan (MUGA), angiography or echocardiography;</li> <li>• Symptomatic coronary disease with demonstrable ischemia;</li> <li>• Other severe acute or chronic medical or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the subject inappropriate for entry into this study;</li> <li>• Has a psychiatric, addictive or other disorder that compromises the ability to give informed consent for participating in this study. This includes subjects with a</li> </ul>



	<p>recent history of abusing alcohol or illicit drugs 30 days prior to study screening Day 0 and for the duration of the study;</p> <ul style="list-style-type: none"> <li>• Poorly controlled asthma defined by active wheezing and/or cough with FEV1 &lt; 70% predicted, responsive to inhaled BD (&gt;15% increase in FEV1 with BD);</li> <li>• Investigators, study staff or their immediate families;</li> <li>• Clinically significant intercurrent illness (including lower respiratory tract infection) or clinically significant surgery within 4 weeks before the administration of study drug;</li> <li>• Personal or family history of congenital or acquired methemoglobinemia;</li> <li>• Personal history of RBC CYP B5 reductase deficiency;</li> <li>• Known or suspected hypersensitivity or allergic reaction to sodium nitrite;</li> <li>• Personal history of glucose-6-phosphate dehydrogenase (G6PD) deficiency or any contraindication to receiving methylene blue;</li> <li>• If female, is pregnant or breast feeding, or has a positive urine or blood pregnancy test result predose;</li> <li>• Receipt of an investigational product or device, or participation in a drug research study within a period of 15 days (or 5 half-lives of the drug, whichever is longer) before the first dose of study drug;</li> <li>• Blood loss or blood donation &gt;550 mL within 90 days or plasma donation &gt;500 mL within 14 days before administration of study drug;</li> <li>• RHC &lt; 2 weeks from treatment visit unless clinically indicated</li> </ul>
Study Endpoints:	<p><u>Primary endpoint:</u>          The primary outcome measures for this study is change in pulmonary vascular resistance measured by right heart catheterization from time zero compared with peak effect within 60 minutes post completion of nebulized dose of nitrite.</p> <p><u>Secondary endpoints:</u>          The secondary endpoints measure:</p> <ul style="list-style-type: none"> <li>• Time to maximum PVR decrease;</li> <li>• Repeated measures ANOVA (RM-ANOVA) for change in PVR calculated from the start of inhalation and at times 15, 30, 45 and 60 minutes post end of nebulization.</li> <li>• Change in mean pulmonary artery pressure, transpulmonary gradient and cardiac output (CO)/cardiac index (CI),</li> <li>• Change in systemic blood pressure,</li> <li>• Change in SVR, RV systolic (dP/dtmax/IP, PWRmax/EDV, RV EF, TAPSE), RV diastolic function (dP/dtmin, Tau),</li> </ul>



	<ul style="list-style-type: none"> <li>• Change in pulmonary vascular impedance / Wave Intensity,</li> <li>• Change in plasma nitrite concentrations in mixed venous blood at specified time points,</li> <li>• Change in pulmonary artery occlusion (capillary) pullback nitrite and plasma cGMP at baseline compared with peak nitrite concentration.</li> <li>• Change in mitochondrial oxygen consumption compared to baseline after each dose of nitrite.</li> </ul>
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## 1. OBJECTIVE, SPECIFIC AIMS, BACKGROUND, AND SIGNIFICANCE

### 1.1 OBJECTIVE

The main objective of this study is to investigate the effect of inhaled nitrite delivered in a dose escalation manner in patients on background pulmonary hypertension therapy (ETRA, PDE5I, or prostacyclin analog) on cardiopulmonary hemodynamics in subjects with pulmonary hypertension.

### 1.2 SPECIFIC AIMS

#### Hypothesis:

- We hypothesize that inhaled nitrite treatment will reduce pulmonary vascular resistance. Our primary endpoint will be the change in pulmonary vascular resistance (PVR) measured by right heart catheterization.
- We hypothesize that inhaled nitrite treatment will improve cellular and global right ventricular signaling and function.
- We hypothesize that nitrite will be safe and well-tolerated in patients on background PAH therapy (PDE-5I and ETRA)

#### Specific Aims:

- 1) To determine the efficacy of inhaled nitrite, on the change in pulmonary vascular resistance measured by right heart catheterization.
- 2) To explore the effect of inhaled nitrite on echocardiographic and hemodynamic parameters measured: SVR, RV systolic (dP/dtmax/IP, PWRmax/EDV, RV EF, TAPSE), RV diastolic function (dP/dtmin, Tau), Pulmonary vascular impedance / Wave Intensity
- 3) To evaluate the safety of inhaled nitrite on systemic blood pressure in patients on background therapy for PAH.
- 4) To identify the pharmacokinetic relationships of nitrite measures of drug exposure and plasma cGMP, using blood sampling.
- 5) To identify the changes in mitochondrial oxygen consumption, using analysis of platelets



- 6) To compare effects of inhaled nitrite on pulmonary vascular resistance with inhaled NO gas at 40 ppm.

## 1.3 BACKGROUND

### 1.3.1. Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a relatively rare and devastating illness characterized by high morbidity and mortality rates.<sup>1</sup> The 1993 American College of Chest Physicians consensus statement reports that the incidence ranges from one to two cases per million.<sup>2</sup> PAH shows a female bias with F:M ratio of 2.3:1. The median age at diagnosis is 36. In the NIH registry, the diagnosis of pulmonary hypertension required a mean pulmonary artery pressure greater than 25 mm Hg at rest or greater than 30 mm Hg with exercise. PAH may occur in either sporadic or familial forms. Six percent of subjects in the NIH registry had a first-order relative with PAH. Familial PPH is inherited as an autosomal dominant disorder with incomplete penetrance, and has been mapped to a locus designated PPH1 on chromosome 2q33 that produces loss of function mutations in BMPR2.<sup>3</sup> Bone morphogenetic proteins, members of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily of signaling molecules, regulate cell differentiation and proliferation, apoptosis, morphogenesis and cell fate determination. Identified defects of this receptor are predicted to disrupt ligand binding, kinase activity, and heteromeric dimer formation – cellular processes that may ultimately be important in the maintenance of blood vessel integrity.

Familial and sporadic PAH appear to be phenotypically identical. The median survival for all subjects in the NIH registry was 2.8 years; the one, three, and five year survivals were 68%, 48%, and 34%, respectively.<sup>1</sup> With the advent of epoprostenol therapy, the one, three, and five year survivals for PAH subjects have improved to 87%, 63%, and 54%, respectively.<sup>4</sup> Despite these improvements in outcome, mortality remains unacceptably high. The lack of a routine screening test for PAH, and the fact that early symptoms are nonspecific ensures that subjects typically present with advanced disease.

Pulmonary hypertension is characterized by high pulmonary artery pressures and a high resistance to blood flow. The development of clinical PPA may be contingent on a genetic susceptibility (“hit” one) combined with an inciting injurious event(s) to the pulmonary artery EC (“hit” two) that renders it dysfunctional. The inciting event(s) probably cause a state of recurrent or chronic pulmonary vascular injury and repair, resulting in an excessive and adverse vascular remodeling process with associated alterations in gene expression. Adverse vascular remodeling and EC dysfunction results in excessive growth factor expression<sup>5</sup>, decreased eNOS expression and NO production<sup>6</sup>, vascular smooth muscle dysfunction, *in situ* thrombosis<sup>7</sup> and a deficiency in endogenous prostacyclin synthesis<sup>8, 9</sup>

### 1.3.2. Investigational Drug

The active ingredient in AIR001 Inhalation Solution is sodium nitrite in a phosphate-buffered, pH adjusted solution for nebulization. Pre-clinical data suggest that under the hypoxic, acidemic conditions present in the pulmonary hypertensive lung, inhaled nitrite results in the sustained release of NO which will act as an acute pulmonary vasodilator. In addition, because decreased levels of NO have been shown to stimulate vascular remodeling, the increased NO resulting from nitrite inhalation is postulated to attenuate or reverse the remodeling process, resulting in sustained pulmonary hemodynamic improvement in subjects with PAH.



### **1.3.3. Inhaled NO for Pulmonary Hypertension**

Nitric oxide released from the endothelium as a gas or attached to transport molecules activates soluble guanylyl cyclase in smooth muscle after binding to its heme group, resulting in increased cyclic GMP. Cyclic GMP activates GMP-dependent kinases that decrease intracellular calcium concentration in smooth muscle, producing relaxation. Nitric oxide also inhibits synthesis of a potent constrictor peptide, endothelin-1, an action that may contribute to vasodilation. Inhaled NO may prove efficacious in patients with sickle cell disease and secondary pulmonary hypertension due to its ability to selectively dilate the pulmonary vasculature.<sup>10, 11</sup> Nitric oxide diffuses to ventilated alveoli and dilates the blood vessels subserving these gas exchange units but is then inactivated by hemoglobin, preventing systemic hypotension. This results in a selective reduction in pulmonary artery pressures.

Inhaled nitric oxide (NO) is a potent acute vasodilator and results in improved hemodynamics in the 15% of PAH subjects who demonstrate vasoreactivity. However, the usefulness of NO for chronic therapy is limited by the need for continuous inhalation. In subjects who demonstrate vasoreactivity during an acute challenge with inhaled NO or a prostanoid challenge, therapy with calcium channel blockers has been demonstrated to result in improved symptoms and survival. In subjects who do not demonstrate an acute vasodilator response, available therapies for PAH include prostanoids, ETRA, and PDE-5I. Inhaled NO is now one of the currently used drugs for vasodilator challenges in the cardiac catheterization laboratory and therefore will be used as a comparator for nitrite responses in the current study.

Inhaled NO will be delivered using FDA approved doses of 40 ppm for approximately 5-15 minutes as used routinely in the catheterization laboratory for evaluation of pulmonary vascular responses in patients with PAH. At doses up to 80 ppm (40 ppm will be used in this study) inhaled nitric oxide rarely produces adverse effects. Buildup of NO-O<sub>2</sub> reaction products in tubing such as NO<sub>2</sub>, a molecule that can precipitate bronchospasm and pulmonary edema, can occur if the NO delivery system is not purged daily. The system will be purged prior to use, and inhaled levels of NO<sub>2</sub> maintained well below 1 ppm. The INOvent NO delivery system that will be used in this study continuously monitors inhaled NO and NO<sub>2</sub>. NO will only be used as clinically indicated using standard methodologies to evaluate pulmonary arterial vasodilatory capacity.

### **1.3.4 Effects of nitrate and nitrite on mitochondria and metabolism.**

Mitochondria are critical for oxidizing fuels (i.e., glucose, fatty acids, amino acids) for cellular energy production. Accumulating evidence suggests that mitochondrial dysfunction, characterized by decreased mitochondrial number, altered mitochondrial ROS generation or decreased substrate metabolism is associated with insulin resistance within skeletal muscle. A human *in vivo* study by Larsen et al recently reported that skeletal muscle mitochondria harvested after dietary nitrate supplementation displayed improved mitochondrial efficiency. These findings suggest that dietary nitrate enhances the efficiency of energy generation through direct effects on muscle mitochondria and may have implications in the metabolic syndrome where mitochondrial dysfunction may play a central role.

During ischemia nitrite is reduced to NO and N2O3 by different nitrite reductase enzyme



systems<sup>12, 13</sup>. Mitochondrial NO and S-nitrosothiols formed from nitrite dynamically and reversibly inhibit complex I during reperfusion, which limits ROS formation from complex I and III<sup>14, 15</sup>. This ultimately prevents the opening of the mitochondrial permeability transition pore and the release of cytochrome c. It has recently been shown that the site of nitrosation is on Cys 39 of the ND3 subunit of complex I<sup>15</sup>. A number of enzymes are required to convert nitrite into NO during organ ischemia. For example, in the heart, deoxygenated myoglobin acts as a functional nitrite reductase<sup>16</sup>. Nitrite-dependent NO formation is significantly decreased in myoglobin deficient hearts<sup>17</sup> and nitrite administration reduces myocardial infarction with abrogated effects in the myoglobin knockout mice<sup>18</sup>. These studies by the Shiva and Gladwin laboratories have demonstrated that nitrite is a potent cytoprotective agent to mitochondria after ischemia and reoxygenation by inhibiting mitochondrial complex I activity, thereby blocking electron transfer and blocking the formation of superoxide during reperfusion. Further, nitrite has been shown to induce the expression of PGC1 $\alpha$  as well as the mitochondrial biogenesis pathway in other cell types. These data suggest that nitrite inhibits mitochondrial respiration and attenuates mitochondrial ROS generation. Preliminary data from our research team suggest that nitrite specifically inhibits platelet mitochondrial basal respiration and enhances maximal respiratory capacity *in vivo*, effects which potentially decrease mitochondrial ROS generation and enhance substrate utilization in the platelet thereby attenuating platelet activation<sup>19</sup>.

Measurement of platelet mitochondrial respiration and dysfunction from a standard blood draw. We have now developed new methodologies to measure mitochondrial function and respiration in isolated platelets<sup>19</sup>. Considering emerging data on the role of nitrite and NO in regulating mitochondrial function, we plan to obtain fresh platelets to utilize with extracellular flux analysis (XF analysis (XF24, Seahorse Biosciences, Billerica, MA), a high throughput methodology to evaluate mitochondrial bioenergetics. We aim to measure platelet cell mitochondrial function before and after nitrite inhalation studies. Further, platelet mitochondrial bioenergetics will be measured along with thrombotic activation in patients with pulmonary hypertension.

#### **1.3.4. Effects of Inhaled Nitrite and Interaction between Inhaled Nitrite and Background Therapies for PAH**

##### Nitric oxide and nitrite.

NO produced from eNOS is subject to rapid inactivation reactions with hemoglobin that greatly limit its lifetime in blood. Recent studies also indicate that NO formed from eNOS is can be oxidized to nitrite by oxygen or plasma ceruloplasmin.<sup>12</sup> Nitrite transport in blood provides an endocrine form of NO that can be shuttled from the lungs to the periphery, while limiting the exposure of authentic NO to the scavenging red cell environment. Then during the rapid hemoglobin deoxygenation from artery-to-vein, nitrite is reduced back to NO via reactions with hemoglobin, myoglobin, and other heme containing enzymes, such as xanthine oxidoreductase. Once formed, nitrite is a potent vasodilator, potentially regulating basal blood flow and hypoxic vasodilation<sup>14, 20-25</sup>. Nitrite limits ischemia/reperfusion-induced apoptosis and cytotoxicity in heart, liver and brain<sup>26-28</sup>. Additionally, nitrite limits hypoxic pulmonary vasodilation in a deoxyhemoglobin- and pH-dependent fashion<sup>29</sup>. Given the central role of NO in vasoregulation, the association of decreased NO bioavailability in PAH and recently emerging signaling actions of nitrite, a strong foundation of knowledge supports that inhaled nitrite can reduce pulmonary pressures in patients with PAH.



### Inhaled nitrite for hypoxia-induced pulmonary hypertension in a new born sheep model.

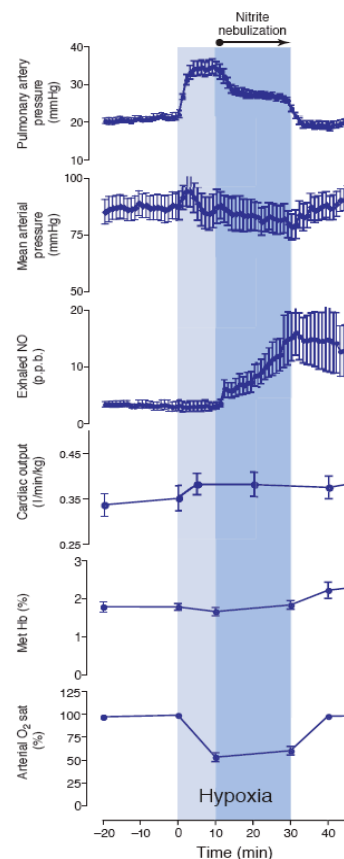
The above supports that inhaled nitrite could potentially exert the same actions as inhaled NO, by locally generating NO and promoting pulmonary vasodilation. Nitrite therapy has advantages over inhaled NO: ease of administration (intermittent use with a standard nebulizer), better availability, and lower cost. Pre-clinical data from a new-born sheep model of hypoxemia-induced fetal pulmonary hypertension indicate that a single nebulized dose of sodium nitrite produced the same vasodilatory effect as 20 ppm inhaled NO (Figure 1). From this, we hypothesize that (a) low-dose, nebulized nitrite can reduce pulmonary artery pressures and pulmonary vascular resistance in patients with PAH.

### Nitrite reverses experimental mouse hypoxia-induced PAH.

Chronic exposure of mice to hypoxia (10% O<sub>2</sub>) results in pulmonary vascular remodeling, characterized by increased muscularization, increased right ventricular pressures (RVP) and right ventricular hypertrophy. Treatment with low concentrations of nebulized nitrite (dosing and delivery as described in experimental plan), either once or three times per week, significantly prevented the development of PAH, as defined by all outcome measurements (Figure 2, A-C). Additionally, nitrite treatment 2 weeks into the hypoxic exposure, after the establishment of PAH, halted the progression of PAH and reversed increases in right ventricular pressures. Thus, inhaled nebulized nitrite inhibited both the development of hypoxia-induced PAH and reversed established PAH.

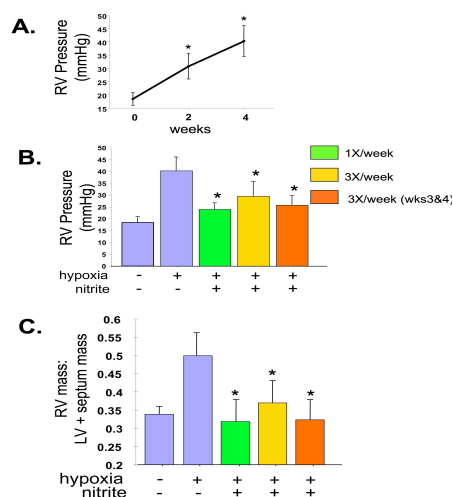
### Nitrite reverses monocrotaline-induced PAH.

To determine whether the effects of nitrite therapy on PAH were specific to chronic hypoxia, we examined an additional model of experimental PAH, sodium monocrotaline (MCT)-induced PAH. Similar to its effects in the hypoxia model of PAH, nitrite reduced monocrotaline-induced PAH (Figure 3 A-D). Nitrite treatment significantly reversed monocrotaline-induced right ventricular hypertrophy and protected against increases in right ventricular pressures.

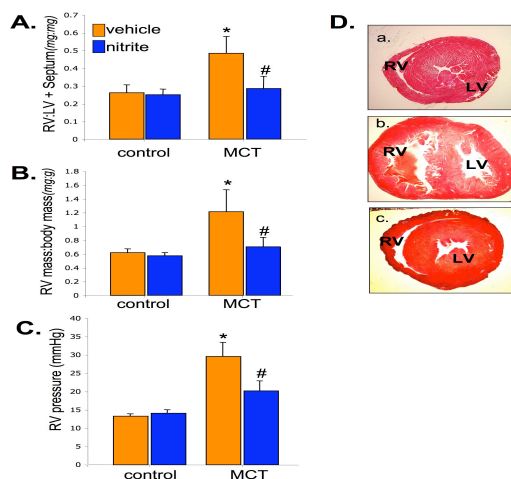


**Figure 1.** Vasodilatory Effects of Nitrite During Hypoxia-Induced Pulmonary Hypertension





**Fig. 2. Nitrite prevents and reverses hypoxia (10% O<sub>2</sub>) induced pulmonary hypertension.** Nitrite treatments were 1X per week, 3X per week, and when administered after PAH development in week 3 and 4.



**Fig. 3. Nitrite reverses monocrotaline-induced pulmonary hypertension in rats with treatment 3X per week.** A. Reduction in RV mass. B. Reduction in RV mass to body mass. C. Reduction in RV pressure. D. a) control, b) vehicle treated rat with PAH showing hypertrophied right ventricle (RV), c) nitrite treated with normal RV.

### 1.3.5 Rationale for Dose of Nitrite

The low doses of nitrite used in these treatment regimens – less than 45 mg or 13% of the dose 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. Aires Pharmaceuticals, who licensed intellectual property pertaining to inhaled nitrite from the NIH, has completed a Phase 1a single dose escalation study designed to define the maximum tolerated dose of inhaled sodium nitrite delivered to normal volunteers following electronic nebulization. These data, presented in abstract form at the 2009 ATS meetings, show that at the highest dose (176 mg) a significant decrease in systemic blood pressure and increase in heart rate was found. At the maximal tolerated dose (MTD); (125 mg) there was an increase in HR (+24 +/- 5 bpm) without significant changes in systolic (-3 +/- 2 mmHg) or diastolic (-3 +/- 2 mmHg) blood pressure. Bioconversion of nitrite was demonstrated by an increase in exhaled NO. Methemoglobin levels increased at the highest doses administered, but remained less than 3.5% in all subjects. Pharmacokinetic modeling demonstrated dose-proportional increases in peak and AUC plasma nitrite.

Note that in 80 volunteers in phase I studies at the NIH methemoglobin levels never rose higher than 3%. 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.

The effects of the interaction between FDA approved oral therapies for PAH (ETRA and PDE-5I) and inhaled nitrite are unknown. It is possible that the combination of the drugs



could lower systemic blood pressure to potentially clinically significant levels. This is of particular concern for the PD5 inhibitors which will increase the levels of cGMP for any given dose of NO or nitrite. For this reason the doses have been reduced significantly below the MTD of 125 mg and a dose escalation plan will be followed with clear stopping rules. The patient will be carefully monitored with a pulmonary artery catheter during drug administration and intravenous fluids and vasopressors will be immediately available for the treatment of symptomatic hypotension. In addition we propose a dose escalation regimen to ensure careful drug dose titration.

#### **1.4 SIGNIFICANCE**

Inhaled nitric oxide has been demonstrated to improve pulmonary hemodynamics acutely in subjects with pulmonary hypertension. It has been demonstrated to lower pulmonary arterial pressure acutely in preclinical models of pulmonary hypertension, putatively through a mechanism of sustained NO release. Repeat dosing of inhaled sodium nitrite has also been demonstrated to result in sustained improvement in pulmonary hemodynamics and in pulmonary vasculopathy in animal models of pulmonary hypertension.

We hypothesize that inhaled nitrite provides an innovative therapeutic approach for patients with PAH for the following reasons:

- 1) Inhaled nitrite therapy provides a potent, effective treatment that will vasodilate the pulmonary circulation (reduce pulmonary vascular resistance), reduce mean pulmonary artery pressure, and increase cardiac output.
- 2) This study will provide valuable efficacy and safety insights into the interactions between NO and nitrite, both direct NO generators, with or without background PH therapy, particularly PDE5I, on pulmonary hemodynamics and right heart function.
- 3) This study will also provide novel assessments of right ventricular global myocardial function.
- 4) The results obtained from this study will also lay the groundwork for future large-scale clinical trials that are needed to investigate the efficacy of inhaled nitrite and/or its long-term effects on pulmonary hemodynamics in patients with pulmonary hypertension.

## **2. STUDY DESIGN AND METHODS**

### **2.1 CLASSIFICATION AND METHODOLOGICAL DESIGN**

This is a single-center, open label phase II study to evaluate the effect of inhaled nitrite delivered in a dose escalation manner on the change in pulmonary vascular resistance (PVR) in subjects with pulmonary arterial hypertension undergoing right heart catheterization.

### **2.2 STUDY DESIGN AND METHODS**

#### **2.2.1. Study Design**

A total of 50 subjects with a confirmed diagnosis of pulmonary hypertension and meeting all inclusion/exclusion criteria will be enrolled in the study which will entail a single right heart catheterization. The study population consisted of subjects with WHO group I PAH (n=20), WHO group II (n=20) and subjects with WHO group III PH (n=10) will receive the following dose escalation paradigm: Each subject will receive a starting dose of 45 mg inhaled nitrite



(AIR001 Inhalation Solution), with one subsequent planned dose escalation of 90 mg inhaled nitrite, based on pulmonary vascular resistance response and safety and tolerability.

Screening (Day 0):

The potential study subjects are followed on a routine basis in the UPMC Heart and Vascular Institute (HVI), Comprehensive Lung Center (CLC) or an inpatient at UPMC Presbyterian, are well known or referred to the study investigator. Initial screening evaluations including physical examination, medical history, and clinical laboratory assessments will be conducted to determine study eligibilities during a routine clinic visit at the UPMC HVI, CLC or inpatient at UPMC Presbyterian. Subjects who meet the inclusion criteria and none of the exclusion criteria will be entered into the study.

Experimental Procedures (Day 1):

The study visit will occur on the same day subjects are scheduled for their clinically indicated right heart catheterization or who volunteer for a research right heart catheterization for this specific study. Subjects on oral background PAH therapy (ETRA or PDE5I) will be instructed to hold their regimen on the day of the study visit.

Subjects will be evaluated for additional medical history, physical examination, vital signs and pulse oximetry since screening. Laboratory testing will be repeated if baseline laboratory values are clinically significant and warrant repeat testing or inadvertently not collected at the screen visit. An echocardiogram with documentation of TRV for estimation of right ventricular systolic pressure and assessment of LV systolic and diastolic function will be performed pre and post nitrite inhalation.

Right heart catheterization is performed routinely as a clinical standard of care procedure for diagnostic purposes in this disease population. The standard right heart catheterization is performed with a balloon tipped, flow-directed pulmonary artery catheter (Swan-Ganz catheter), inserted through a sheath in the internal jugular vein. As per standard clinical protocol, hemodynamic recordings of right atrial, right ventricular, and pulmonary artery pressures, in addition to cardiac output, are made at baseline, and then repeated following pulmonary vasodilator challenge with inhaled nitric oxide (iNO, which is part of the standard clinical right heart catheterization when assessing pulmonary hypertension). Responses to 40 ppm iNO will be measured before nitrite nebulization to assess vasodilator responsiveness. As a part of this protocol the subjects may undergo an additional hemodynamic measurement using a micromanometer pressure catheter that may be inserted via the Swann-Ganz catheter distal port. Measurements with micromanometer and/or Swann-Ganz catheter will be made with simultaneous flow velocity from transthoracic echocardiographic doppler ultrasound at baseline just prior to nebulized AIR001 administration and at the time of the final hemodynamic assessment (60 minutes after second dose). This portion of the study that involves the micromanometer instrumentation is expected to add an additional 5-10 minutes onto the procedure.

Subjects will receive nebulized AIR001 doses escalated based upon safety and tolerability. The dose of inhaled nitrite will be delivered via electronic nebulizer. During the study right heart/pulmonary artery hemodynamics will be measured as well as noninvasive systemic blood pressure monitoring. Subjects will be tested for the changes in pulmonary vascular resistance (PVR) using standard clinical protocol hemodynamic recordings of right atrial, right ventricular, and pulmonary artery pressures, in addition to cardiac output at time zero, and approximate timelines 15, 30, 45 and 60 minutes after completion of each nebulization dose. For details, see sections 2.10.2 and 2.12 below.



Subjects will be monitored closely for changes in blood pressure during the study. A decrease in systolic blood > 30 mm Hg drop from pre-dose baseline AND symptomatic or requiring fluid replacement or other therapy will lead to a discontinuation of the study treatment, and the next higher dose of inhaled nitrite will not be administered. Subjects will continue to be monitored closely and treated as needed.

Oxygen saturation measured by pulse oximeter and methemoglobin levels monitored by co-oximetry prior to dose administration and through 2 hours post-last dose will be monitored closely. Venous methemoglobin levels may be assessed periodically at investigator discretion. Due to circumstances unknown, co-oximetry may not read MetHgb level, in the event, venous metHgb may be collected at approx. time intervals 30 and 45 minutes for each inhaled dose.

Blood samples may be collected for Cyclic Guanosine Monophosphate (cGMP) concentration determination, pharmacokinetics (PK) and platelet mitochondrial analysis at pre dose and the end of the peak dose of nitrite may be collected. PK and cGMP measurements will be made on mixed venous blood using PCW pullback samples and venous blood will be collected for platelet mitochondrial analysis

Subjects will be monitored carefully for adverse events, laboratory test abnormalities, and changes in vital signs. Adverse experiences will be evaluated according to criteria outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Following the study treatment, subjects will be followed as an outpatient on Day 30 (+/- 5 day window). An additional follow-up assessment by telephone will occur on Day 3.

## **2.2 STUDY TREATMENT**

Following initial standard clinical measurements for right heart catheterization, 40 ppm inhaled NO gas will be administered for approximately 5-15 minutes through a facemask, continuously monitoring subject vasoreactivity. This is considered part of the clinical right heart catheterization procedure for pulmonary hypertension. Repeat hemodynamic measurements will be performed upon completion of inhaled NO. Pre-dose baseline PK and methemoglobin levels may be collected. The inhaled nitrite will be delivered per protocol below.

Following completion of all baseline assessments, each subject will receive a starting dose of inhaled nitrite, with one subsequent planned dose escalation of inhaled nitrite, based on safety and tolerability. Subjects will be monitored carefully during and after the study treatment.

The study will be conducted at UPMC Presbyterian Hospital, 3<sup>rd</sup> FL Catheterization Laboratory. Baseline vasoreactivity will be recorded after Inhaled NO at 40 ppm for approximately 5-15 minutes. Subjects will receive a starting dose of AIR001 (45 mg), with one subsequent planned dose escalation to 90 mg based upon safety and tolerability. The dose of AIR001 will be delivered by an electronic nebulizer system that is portable, highly efficient utilizing continuously vibrating mesh aerosol generation technology that allows a high percentage respirable dose delivery, minimal loss of drug to the environment between inhalations, and a reproducible droplet size distribution for optimal delivery of drugs to the distal pulmonary tree.

During, and at approximate timelines of 15, 30, 45 and 60 minutes post-inhalation for each dose, right heart/pulmonary artery hemodynamics will be measured as well as noninvasive systemic



blood pressure monitoring. Subjects will be monitored closely during the study. Dose limiting toxicity (DLT) is defined in Table 1.

A dose level will also be considered intolerable if, in the judgment of the Investigator, the type, frequency, or severity of AEs becomes unacceptable. If an intolerable dose is identified, no further doses at that or higher doses will be administered.

**Table 1 Dose Limiting Toxicity Criteria**

Sign or Symptom	Moderate	Severe <sup>1</sup>
Bronchospasm/ wheezing	Symptomatic but not requiring therapy	Symptomatic and requiring therapy
SBP <sup>2</sup>	Systolic > 20 mm Hg drop from pre-dose baseline AND symptomatic, or SBP <80 mm Hg AND symptomatic	Systolic > 30 mm Hg drop from pre-dose baseline AND symptomatic or requiring fluid replacement or other therapy
Dyspnea	Causes more discomfort (than a mild, easily tolerated AE) and interrupts the subject's usual daily activities	Incapacitating and causes considerable interference with the subject's usual daily activities
Cough	Causes more discomfort (than a mild, easily tolerated AE) and interrupts the subject's usual daily activities	Incapacitating and causes considerable interference with the subject's usual daily activities
Hypoxia <sup>3</sup>	SaO <sub>2</sub> < 5% from baseline AND symptomatic	SaO <sub>2</sub> ≤10% baseline AND symptomatic
Venous or percutaneous methemoglobin <sup>4,5</sup>	>5% and <7%	≥7%
Other drug-related signs or symptoms <sup>6</sup>	Causes more discomfort (than a mild, easily tolerated AE) and interrupts the subject's usual daily activities OR CTCAE v.4 <sup>6</sup> Grade 2 toxicity	Incapacitating and causes considerable interference with the subject's usual daily activities OR CTCAE v.4 <sup>6</sup> Grade 3 or 4 toxicity

AE = adverse event; CTCAE = Common Toxicity Criteria for Adverse Events; DLT = dose limiting toxicity;  
 SBP = systolic blood pressure; SaO<sub>2</sub> = oxygen saturation (hemoglobin);

- <sup>1</sup> Severe DLT seen in any subject which is deemed to be study drug-related will preclude further dosing with AIR001 for that subject.
- <sup>2</sup> Moderate or Severe SBP without symptoms will be continuously monitored with BP measurements every 5 minutes. Symptomatic refers to dizziness or fainting. Discontinuation of further dosing will be based upon follow up blood pressure measurements, subject symptoms and physician-investigator discretion (see Stopping Rules).
- <sup>3</sup> Moderate or Severe SaO<sub>2</sub> without symptoms will be continuously monitored and recorded every 5 minutes. As most clinically feasible; in patients with difficulty measuring SaO<sub>2</sub> at baseline, most usually those with scleroderma but not limited to this condition, the follow up will not be based upon repeated SaO<sub>2</sub> measurements but on clinical decision of the operating physician that will be documented as such.
- <sup>4</sup> Elevated percutaneous methemoglobin levels measured by pulse co-oximeter will be continuously monitored and confirmed by venous methemoglobin level. Follow up will be based upon the clinical decision of the operating physician and will be documented as such.
- <sup>5</sup> A single occurrence of venous methemoglobin levels >5% in an individual subject will result in no further dosing for that individual subject for the remainder of the study period
- <sup>6</sup> Common Terminology Criteria for Adverse Events v. 4.0 ([CTCAE](#)). CTCAE criteria will be applied if considered related to study drug and confirmed on repeat testing.



Baseline for the calculation of supine blood pressure changes is defined as the supine measurement taken prior to AIR001 dosing.

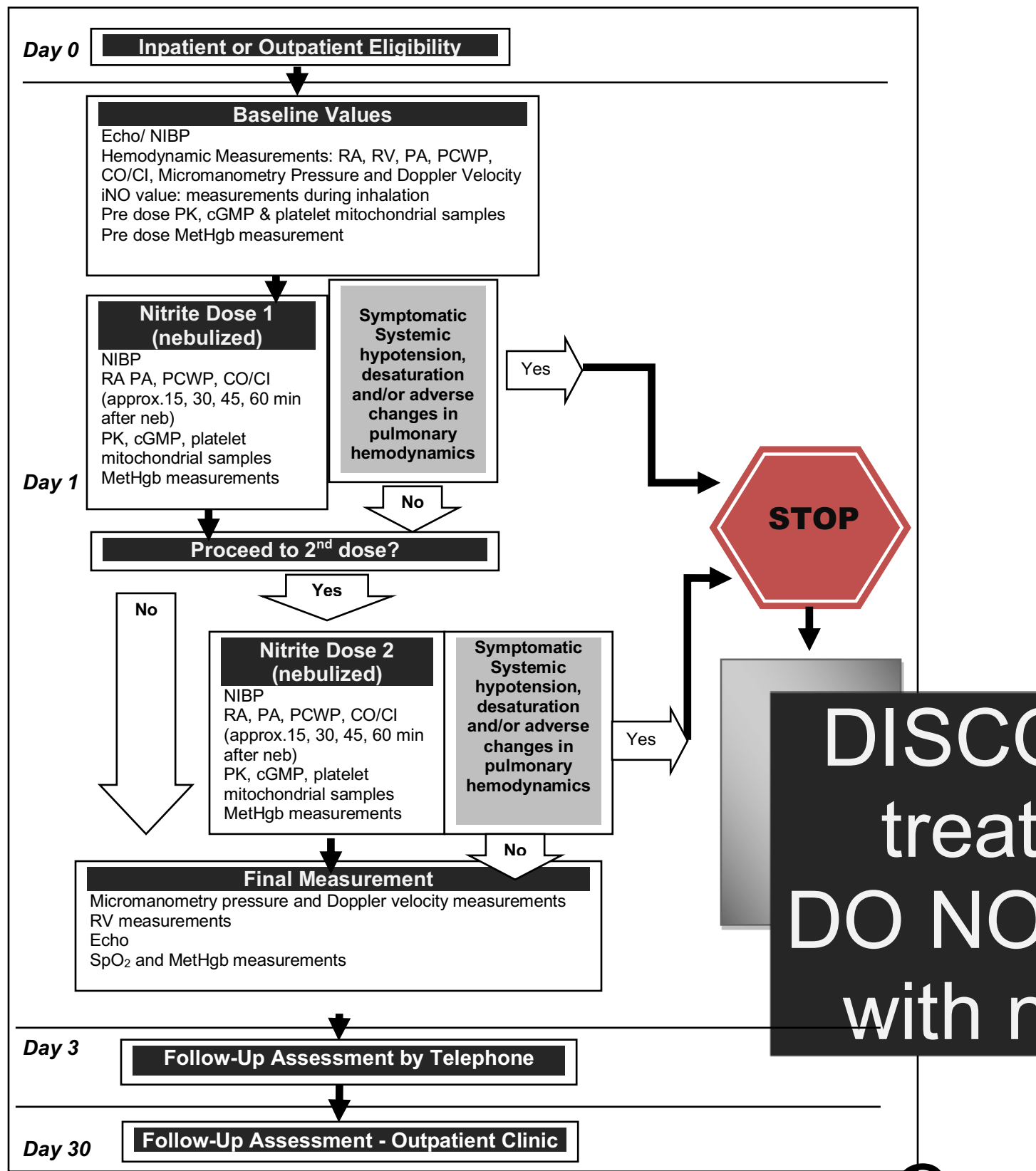
The presence of severe hypotension will be defined by a decrease in systolic blood pressure >30 mm Hg from pre-dose baseline AND symptomatic or requiring fluid replacement or other therapy will lead to a discontinuation of the study treatment, and the next higher dose of inhaled nitrite will not be administered to that subject. Subjects will continue to be closely monitored. Oxygen saturation will be measured by pulse oximeter and methemoglobin levels monitored by continuous co-oximetry prior to dose administration and 2 hours post last-dose will be monitored closely.

Blood samples for PK analysis, Cyclic Guanosine Monophosphate (cGMP) concentration determination, and platelet mitochondrial analysis may be collected at pre-dose and at the end of nebulization at the presumed peak nitrite concentration for each tolerated dosage time points; approximately 15 minutes post inhalation. Blood samples collected for measurements will be made on mixed venous blood using PCW pullback samples at baseline (pre-dose) and after each nitrite dose. Platelets will be isolated by differential centrifugation. Measurements of platelet mitochondrial activity will be determined at baseline (pre-dose) and after each nitrite dose.

Subjects will be monitored carefully for adverse events, laboratory test abnormalities, and changes in vital signs. Adverse experiences will be evaluated according to criteria outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.



## 2.3. STUDY DESIGN SCHEMATIC





All subjects enrolled in the study will be followed for 30 days (+/- 5 day window) after completion of the study treatment. Subjects removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

## **2.5. SAFETY MONITORING**

Prior studies suggested that hypotension and methemoglobinemia may occur with the use of sodium nitrite. The study treatment of inhaled nitrite will be used safely with careful monitoring and prompt discontinuation of the study treatment in response to systemic hypotension with definitions of dose limiting toxicity as outlined in Table 1.

All subjects will be studied in UPMC Presbyterian Hospital Catheterization Laboratory with an investigator and nurse present at all times, and with continuous monitoring of vital signs and oxygen saturation. Subjects will also be monitored closely for methemoglobin levels during the study treatment.

Following study drug treatment, subjects will be evaluated as an outpatient at the clinic on Day 30 (+/- 5 day window). In addition, an interval telephone assessment will occur on Day 3. Subjects will be followed for evidence of acute or delayed adverse effects from the study treatment and to assess their clinical status. All adverse events experienced by subjects will be collected from the time of dosing, through the study and until the final study visit. Subjects continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible. If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used.

In addition to these formal evaluations, subjects will be encouraged to immediately contact the study investigator and/or the study coordinator with questions, concerns, or to report new symptoms that occur during their study participation. If appropriate, based upon the evaluation, medical treatments will be provided to subjects, including appropriate referral to physicians or other services at the UPMC.

## **2.6. STOPPING RULES**

In addition to the dose limiting toxicity as outlined in Table 1 above, study treatment will be discontinued if any of the following occurs:

- A decrease in systolic BP > 40 mm Hg drop from pre-dose baseline;
- Desaturation > 10% from baseline;
- A single occurrence of venous methemoglobin level >5%;
- Severe DLT criteria met and symptomatic or requiring fluids or treatment;
- Any serious adverse event thought to be possibly related to the study treatment.

## **2.7. DRUG SUPPLIES**

### **2.7.1. Formulation and Packaging**

Formulation:



The active ingredient of the Sodium Nitrite Inhalation Solution (AIR001 Inhalation Solution) is sodium nitrite in a phosphate-buffered, pH adjusted solution for nebulization. The Sodium Nitrite Inhalation Solution is intended to be prepared prior to inhalational delivery to patients via electronic nebulization.

**Packaging:**

All vial formulations are labeled, packaged and distributed by Aires Pharmaceuticals, Inc. Aires Pharmaceuticals, Inc. will provide drug supplies to the investigational site.

**2.7.2. Availability**

AIR001 is an investigational drug and will be obtained from Aires Pharmaceuticals, Inc, San Diego, California. AIR001 will be supplied free-of-charge to investigators under a Collaborative Agreement between Aires Pharmaceuticals, Inc. and the University of Pittsburgh.

**2.7.3. Preparing and Dispensing**

The study drug will only be diluted and dispensed by the research pharmacist to the required dose concentrations.

The drug product will be delivered to subjects utilizing a nebulizer system supplied by Aires that is portable, highly efficient and that utilizes continuously vibrating mesh aerosol generation technology that allows a high percentage respirable dose delivery, minimal loss of drug to the environment between inhalations, and a reproducible droplet size distribution for optimal delivery of drugs to the distal pulmonary tree.

**2.7.4. Drug Administration**

The route of administration is by nebulization. It is anticipated that each nebulization will take approximately 10 – 15 minutes to deliver the dose. All doses specified in the protocol are the amounts of AIR001 placed into the nebulizer cup.

**2.8 DRUG STORAGE**

The investigational drug will be kept in its original packaging and stored in a locked, secure area at controlled room temperature (20 – 25° C) with the investigation drug services (IDS). The temperature of the storage area must be monitored to ensure compliance with required temperatures. A temperature log will be maintained to make certain that the drug supplies are stored at the correct temperature at all times maintained by IDS. Access to and administration of the investigational drug will be limited to the study investigators and authorized research staff. Study drugs may only be dispensed to subjects enrolled in this study.

**2.9. DRUG ACCOUNTABILITY**

The study investigators or the study coordinator will document the amount of study drugs dispensed and/or administered to subjects. The study drugs accountability records will be maintained throughout the course of the clinical trial by IDS. Any discrepancies in drug supplies will be noted and explained.

**2.10 STUDY PROCEDURES**

**2.10.1. Screening**



Screening evaluations to determine eligibility will take place prior to receiving study treatment. The potential study subjects are followed on a routine basis in the UPMC Heart and Vascular (HVI), Comprehensive Lung Center (CLC) or an inpatient at UPMC Presbyterian and are well known or referred to the study investigators. During routine clinical visit, patients who express interest in participation will undergo the screening assessment to determine that all inclusion/exclusion criteria are met prior to receiving the study treatment.

If the patient has had any of the tests done within the 6 months prior to the screening period, they will not be repeated for study purposes. The results collected from these tests and procedure will serve as a baseline for comparison of the subject's overall condition following study treatment. This visit may take up to 2 hours to complete.

### Screening

- Medical history review and demographics
- Complete history and physical examination to include vital signs, blood pressure, body weight, and height.
- Assessment of oxygen saturation
- Baseline clinical laboratory evaluations including complete blood count with differential, platelets, electrolytes, glucose, BUN, serum creatinine, liver function tests, PT/PTT, nt-ProBNP..
- Urine pregnancy test for female of childbearing potential.
- Informed, written consent may be obtained after subject meets eligibility criteria or on day of Study visit (Day 1)

### **2.10.2. Experimental procedures (Day 1)**

Subjects who meet the inclusion criteria and none of the exclusion criteria will receive the study treatment of inhaled nitrite. The study will take place at UPMC Presbyterian Hospital, 3<sup>rd</sup> FL Catheterization Laboratory, and may take up to 6 hours to complete.

#### Pre-Dose: Baseline

- Physical examination to include vital signs, blood pressure, weight and oxygen saturation
- Laboratory testing that clinically warrants repeating testing or inadvertently not collected for screening may be obtained prior to research procedures
- Echocardiogram
- Non-invasive blood pressure measurement
- Right Heart Catheterization (RHC)-RA, RV, PA, pulmonary capillary wedge pressure (PCWP), CO/CI
- Inhaled NO testing at 40 ppm if PCWP < 25 with hemodynamic response recorded (RA, PA, PCWP, CO/CI)
- Micromanometry to record high fidelity pressure signals with simultaneous flow velocity from transthoracic echocardiographic ultrasound for the measurement of pulmonary arterial input impedance. Micromanometer pressure catheter is inserted via the Swann-Ganz catheter distal port.
- Repeat 2<sup>nd</sup> baseline hemodynamic measurement: RA, RV, PA, PCWP. CO/CI
- Pre-dose baseline PK, cGMP and platelet mitochondrial samples
- Pre-dose methemoglobin measurement

#### AIR001 Dose 1 45 mg Nebulized



- Non-invasive blood pressure measurement
- PK, cGMP and platelet mitochondrial samples (approx. 15 minutes after nebulization)
- RA, PA, PCWP, CO/CI (approx. 15, 30, 45, 60 minutes after nebulization)
- AE assessment
- Methemoglobin (MetHgb) measurements (approx. 5, 15, 30, 60 minutes after nebulization)

#### AIR001 Dose 2 90mg Nebulized

- Non-invasive blood pressure measurement
- PK, cGMP and platelet mitochondrial samples (approx. 15 minutes after nebulization)
- RA, PA, PCWP, CO/CI (approx. 15, 30, 45, 60 minutes after nebulization)
- AE assessment
- MetHgb measurements (approx. 5, 15, 30, 60 minutes after nebulization)

#### Final Measurements:

- Includes 60 minute hemodynamic measurements (RA, PA, PCWP, CO/CI) from last tolerated AIR001 dose
- RV measurement
- AE assessment
- Micromanometry to record high fidelity pressure signals with simultaneous flow velocity from transthoracic echocardiographic ultrasound for the measurement of pulmonary arterial input impedance. Micromanometer pressure catheter is inserted via the Swann-Ganz catheter distal port.
- Echocardiogram
- SpO2 and MetHgb measurements at approx. 120 minutes post last tolerable dose

#### PK Sampling for Nitrite/Nitrate:

- Blood samples for PK analysis may be collected at pre-dose and at the end of nebulization at the presumed peak nitrite concentration for each tolerated dosage time points; approximately 15 minutes post inhalation.

#### Methemoglobin (metHgb) levels:

- MetHgb levels will be recorded via co-oximetry at the following approximate time points: pre-dose, 5, 15, 30, 60 minutes after each dose of AIR001 and at 120 minutes post last dose of AIR001. Venous methemoglobin levels will be assessed as clinically indicated at the discretion of the investigator. Due to circumstances unknown, co-oximetry may not pick up methemoglobin readings. In those instances, venous methemoglobin levels will be collected at approximate 30 and 45 minute post inhalation time points.

#### **2.10.3. Follow-up monitoring (Day 3)- Telephone Assessment**

The interval telephone assessment should be completed as close to the scheduled visit date as possible. However, a variance of 2 days will be allowed for the follow-up monitoring to facilitate scheduling or to account for weekends or holidays.

Subjects will be contacted by the study investigator and/or the study coordinator for a follow-up assessment by telephone to assess for symptoms potentially related to adverse effects of



study drug, review of medication changes, and interval medical or surgical histories including emergency or physician office visits. This phone assessment may last about 30 minutes.

- Interval history assessment
- AE assessment

#### **2.10.4. Follow-up monitoring (Day 30 +/- 5 day window)- Outpatient Clinic Assessment**

Outpatient visits should be completed as close to the scheduled visit dates as possible. However, a variance of 5 days will be allowed for the follow-up monitoring to facilitate scheduling or to account for weekends or holidays. This visit will take place at the UPMC HVI and CLC outpatient clinic, and may take up to 2 hours to complete.

- Physical examination to include vital signs, blood pressure and oxygen saturation
- Clinical laboratory evaluations
- AE assessment

In addition to these formal evaluations, subjects will be encouraged to immediately contact the study coordinator and/or the investigators with questions, concerns, or to report new symptoms that occur during their study participation. If appropriate, based upon the evaluation, medical treatments will be provided to subjects, including appropriate referral to physicians or other services at the UPMC.

Any clinically significant adverse event, laboratory test or physical examination observed during final assessments will be followed as medically appropriate until resolved or explained.



## 2.11. SCHEDULE OF ACTIVITIES (STUDY ASSESSMENT TABLE)

The table below summarizes the protocol procedures that will be performed at screening, during the study treatment, and at follow-up.

Study Phase	Screening	Pre-Treatment	Treatment				Post-Treatment Follow-Up Monitoring	
Visit Type	Outpatient Clinic	Outpatient Cath Lab				Phone Assessment	Outpatient Clinic	
Days on Intervention	Day 0	Day 1				Day 3	Day 30	
Study Procedures		Baseline	Dose 1	Dose 2	Final Measurement			
Informed Consent	X							
Medical History and Demographics	X							
I/E Criteria	X							
Physical Exam	X	X					X	
Vital Signs	X	X					X	
Oxygen Saturation	X	X	X	X	X		X	
Laboratory Tests***	X	X					X	
Urine Pregnancy Test Δ	X	X						
Non-invasive blood pressure measurement		X	X	X				
Right Heart Catheterization (RHC): RA, RV, PA, PCWP, CO/CI (RV measurements at baseline, pre-dose and 60minute interval post last dose AIR001)		X	X	X	X			
Micromanometry		X			X			
Echocardiogram		X			X			
Inhaled NO at 40 ppm if PCWP < 25		X						
PK Sampling*		X	X	X				
metHgb Level§		X	X	X	X			
Plasma cGMP		X	X	X				
Platelet Mitochondrial Analysis		X	X	X				
AE Assessment	X	X	X	X	X	X	X	
Interval History Assessment						X		

Δ Urine pregnancy test will be performed only on female of childbearing potential.

\*\*\* Clinical laboratory tests may be repeated on study visit 1 if screen lab results deemed clinically significant and warrant repeat testing or inadvertently not collected at screen visit.

\* PK Sampling for Nitrite: Blood samples will be obtained at pre-dose and at the end of nebulization at the presumed peak nitrite concentration for each tolerated dosage time points; 15 minutes post inhalation.

§ Methemoglobin (metHgb) levels: will be measured at the following time points: pre-dose, 5, 15, 30 and 60 minutes each dose of AIR001 and at 120 minutes post last dose of AIR001.



## **2.12. DESCRIPTION OF STUDY PROCEDURES**

### **2.12.1. Right Heart Catheterization and Simultaneous Pressure and Doppler Flow Velocity Measurement**

The right heart catheterization is performed as a clinical standard of care procedure, routinely performed for diagnostic purposes in this disease population. The standard right heart catheterization is performed with a balloon tipped, flow-directed pulmonary artery catheter (Swan-Ganz catheter), inserted through a sheath in the internal jugular vein. As per standard clinical protocol, hemodynamic recordings of right atrial, right ventricular, and pulmonary artery pressures, in addition to cardiac output, are made at baseline, and then repeated following pulmonary vasodilator challenge with inhaled nitric oxide (iNO, which is part of the standard clinical right heart catheterization when assessing pulmonary hypertension).

As a part of this protocol the subjects may undergo an additional hemodynamic measurement using a micromanometer pressure catheter that will be inserted via the Swann-Ganz catheter distal port. The micromanometer catheter will record high fidelity pressure signals for the measurement of pulmonary arterial input impedance. Measurements with micromanometer will be made with simultaneous flow velocity from transthoracic echocardiographic ultrasound at baseline just prior to nebulized AIR001 administration and at the time of the final hemodynamic assessment (60 minutes after second dose). This portion of the study that involves the micromanometer instrumentation is expected to add an additional 5-10 minutes onto the approximate 4 hour procedure.

#### **Cardiac Output (CO)**

Cardiac output is the amount of blood pumped through the left ventricle of the heart in a time interval of one minute. There are a number of ways to calculate CO. Thermodilution and/or Fick cardiac index are 2 means which will be used for measuring CO for this protocol. The CO will be the average of the recorded CO collected.

#### **Cardiac Index (CI)**

Cardiac index is a measurement that relates the CO from the left ventricle in one minute to body surface area (BSA) therefore determining cardiac function to size of the individual. CI is calculated by  $CO/BSA$ .

### **2.12.2. Pulmonary Capillary Wedge Pressure**

Pulmonary capillary wedge pressure (PCWP) provides an indirect estimate of left atrial pressure (LAP). PCWP is measured by the balloon-tipped, multi-lumen catheter (Swan-Ganz catheter) while it is in a branch of the pulmonary artery. Just behind the tip of the catheter is a small balloon that can be inflated with air (~1 cc). The catheter has one opening (port) at the tip (distal to the balloon) and a second port several centimeters proximal to the balloon. These ports are connected to pressure transducers. When properly positioned in a branch of the pulmonary artery, the distal port measures pulmonary artery pressure and the proximal port measures right atrial pressure. The balloon is then inflated, which occludes the branch of the pulmonary artery. When this occurs, the pressure in the distal port rapidly falls, and after several seconds, reaches a stable lower value that is very similar to left atrial pressure (normally about 8-10 mmHg). The balloon is then deflated and pulmonary artery pressure can again be recorded. The same catheter can be used to measure cardiac output. Blood samples will be withdrawn from the tip of the wedged catheter at baseline (pre-dose), and at 15 minutes after each dose of inhaled nitrite (45 mg dose and 90mg).

### **2.12.3 Echocardiography**



Transthoracic echocardiograms will be completed to assess RV function by measures such as TAPSE as well as to assess the Doppler waveform of the pulmonary artery. The echoes will be completed pre-dose and after final dose. This is a noninvasive test.

#### **2.12.4. Percutaneous Oxygen Saturation and Methemoglobin**

The device (Masimo Rainbow SET® CO-Oximeter) selected for use in this clinical trial has been validated to provide accurate determinations of both SaO<sub>2</sub> and methemoglobin levels under conditions of hypoxia and methemoglobinemia. Continuous SaO<sub>2</sub> and methemoglobin monitoring will occur prior to study drug administration until 2 hours post-dose. Measurements will be recorded pre-dose, approx. 5, 15, 30 and 60 minutes after each dose of AIR001 and approximately 2 hours post last dose of AIR001.

#### **2.12.5. Pharmacokinetic (PK) Studies**

Pharmacokinetic studies will be performed on all subjects enrolled to measure the concentration of inhaled nitrite and its metabolites in blood. The evaluation of the relationship between dosage and blood concentrations, and the relationship between blood concentrations and pharmacologic effects will provide valuable mechanistic information about the effects of inhaled nitrite on cardiopulmonary hemodynamics in Subjects with Pulmonary Hypertension.

To determine the pharmacokinetic profiles of the inhaled nitrite, blood samples will be collected at pre-nitrite dosing and at the end of nebulization at the presumed peak nitrite concentration for each tolerated dosage time points; approximately 15 minutes post inhalation. Vital signs, blood pressure and pulse oximetry will be measured and monitored closely at each time points. Samples will be immediately centrifuged and frozen at -20°C until the time of analysis.

PK samples will be accurately recorded for the collection time. Covariate information, e.g., age, body weight, height, disease type, concomitant medications, laboratory values, etc., will be obtained at the study visit. This is expected to yield an information-rich data set through dense sampling, enabling better characterization of the PK relationship of inhaled nitrite in subjects with PAH.

#### **2.12.6. Laboratory Testing**

Peripheral blood samples will be evaluated for baseline and follow-up comparison purposes which include complete blood count with differential, platelets, electrolytes, glucose, BUN, serum creatinine, liver function tests, PT/PTT, and nt-ProBNP. Urine pregnancy test may be performed only on female of child-bearing potential on Day 0 and Day 1.

Screening lab samples may be collected and analyzed at UPMC laboratories or at outlying facility of subject's choice. Lab results will be added to research chart for comparison. All 30-day samples will be analyzed at UPMC Presbyterian laboratory with the exception of the ntPro-BNP which may be processed and analyzed through the Pulmonary, Allergy and Critical Care Medicine (PACCM) research lab.

Baseline laboratory results within 6 months of screen visit may be used as baseline comparison and will not be repeated for research purposes. Blood samples may be collected on study visit 1 if baseline screening results are deemed clinically significant and warrant repeat testing or inadvertently not collected at screen visit.



### **2.12.7. Additional Laboratory Testing**

Blood samples may be collected for Cyclic Guanosine Monophosphate (cGMP) concentration determination and platelet mitochondrial will be collected at the end of nebulization at the at pre dose and the end of the peak dose of nitrite may be collected. cGMP measurements will be made on mixed venous blood using PCW pullback samples and venous blood will be collected for platelet mitochondrial analysis. These de-identified blood samples will be sent to the research lab on the 12th floor BST for processing.

### **2.12.8. Specimen collection and management**

#### Specimen Collection / Documentation

At each sampling time, one mL of blood will be withdrawn and discarded to assure that the solution used to maintain catheter patency does not dilute the sample. Blood samples will be accurately recorded for the actual sample collection time. Each research PK sample will be labeled with subject's unique identifier, sample date, scheduled sample collection time, and actual sample collection time. Covariate information, e.g., age, body weight, height, disease type, concomitant medications, laboratory values, etc., will be obtained at each time point. This is expected to yield an information-rich data set through dense sampling, enabling better characterization of the biomarkers in this patient population.

#### Specimen Handling and Labeling (De-Identification)

Specimens collected will be properly labeled. All research biological specimens and all records associated with the samples will be labeled only with a unique code that contains no personal identifiers. The information linking these code numbers to the corresponding subject's identity will be kept in a secure location in the investigator's office, and will not be available to staff managing samples at the research laboratories.

Immediately upon receipt of the biological specimens, all attempts will be made to process, isolate, collect, and store the specimens. The code number and date on which the specimen is frozen, all other information about the specimen, and subsequent processing will be entered on the specimen processing worksheet.

#### Specimen Management and Storage

Specimens in excess of immediate assay requirements may be stored indefinitely in a locked freezer under the control of the principal investigator.

The blood samples will be stored after appropriate coding to remove patient identifiers. The coding information linking patient identifiers to the stored samples will be maintained in a locked, secure area that will be accessible only to the study investigator. Subjects may request to have their samples destroyed at any time. These samples will be destroyed immediately upon receipt of the subjects' written request to do so. Identification of which samples to destroy will be available from the coding information linking patient identifiers to the stored samples as described earlier in this paragraph.

#### Restrictions to Direct Access of Specimens

Specimens will be kept in the responsible study investigators' laboratories indefinitely and will be under the control of the principal investigator. Investigators or other personnel not involved with the management or operations of the study are not permitted direct access to the specimens.



## **2.13. ENDPOINTS**

### **2.13.1. Primary Endpoints**

The primary outcome measures for this study is change in pulmonary vascular resistance (PVR) measured by right heart catheterization from time zero compared with peak effect within 60 minutes post completion of nebulized dose of nitrite.

### **2.13.2. Secondary Endpoints**

The secondary endpoints measure:

- Time to maximum PVR decrease;
- Repeated measures ANOVA (RM-ANOVA) for change in PVR calculated from the start of inhalation and at times 15, 30, 45 and 60 minutes post end of nebulization.
- Change in mean pulmonary artery pressure, transpulmonary gradient and cardiac output (CO)/cardiac index (CI),
- Change in systemic blood pressure,
- Change in SVR, RV systolic (dP/dtmax/IP, PWRmax/EDV, RV EF, TAPSE), RV diastolic function (dP/dtmin, Tau),
- Changes in pulmonary vascular impedance / Wave Intensity,
- Change in plasma nitrite concentrations, cGMP, and platelet mitochondrial activity in mixed venous blood at specified time-points,

Change in pulmonary artery occlusion (capillary) pullback nitrite and plasma cGMP at baseline compared with both nitrite doses.

## **2.14. SUBJECT WITHDRAWAL**

A subject may voluntarily discontinue participation in this study at any time. The investigator may also, at his or her discretion, discontinue a subject from this study at any time. Every effort should be made by the investigator to keep the subject in the study.

Subjects may be withdrawn from the study prior to completion if any of the following criteria are observed:

- Intercurrent illness or an unexpected fatal or life-threatening adverse event, which requires discontinuation of study treatment
- Pregnancy
- Subject reached protocol-defined stopping criteria
- Request by the subject to withdraw from the study
- Protocol violations
- Persistent non-compliance
- Lost to follow-up
- Investigator discretion
- Study closed/terminated

### **2.14.1. Dropouts and withdrawals**

To be considered complete, a subject must complete all study visits as specified in the protocol without violations of the protocol so significant as to obscure the response to study treatment.



Subjects who fail to complete all study required visits will not be considered complete and may not enroll at a later date and will not be replaced. A record will be kept of all subjects who fail to complete all study visits and their primary reasons for discontinuation.

In the event of subject withdrawal, subjects will be encouraged to continue all follow-up visits for safety monitoring or to continue follow up as directed by their personal primary physicians, unless the subject withdraws consent at any time (without having to justify the decision). All available data from subjects who discontinued during the study, for whatever reason, will be included in the safety analysis.

## 2.15 STATISTICAL ANALYSIS

### 2.15.1 Sample Size and Power

Limited data are available on expected vasodilatory responses to nitrite in patients with Group I PAH or Group III PH. We reviewed data from studies examining the PVR response to inhaled NO, fasudil, iloprost, and treprostinil<sup>30-32</sup>, which reported percent decreases in PVR after therapy ranging from 5-25%. Assuming a distribution of PVR among PAH patients similar to that reported by Voswinckel, et al (911 +/- 102 dyn·sec·cm<sup>-5</sup>), we estimate using the paired t-test that we will need 14 participants to achieve 80% power to detect a statistically significant 10% decrease in PVR at a significance level of 0.05. At a projected enrollment of approximately 20 patients with group I PAH for our primary outcome measure, we anticipate more than sufficient power to detect significant acute vasodilatory effects of nitrite.

### 2.15.2 Statistical Analysis

#### General Approach

The primary efficacy outcome variable is pulmonary vascular resistance (PVR), measured by right heart catheterization. T-test will be performed to determine difference of mean values for outcome variables at baseline and highest tolerated nitrite. The secondary endpoints will be analyzed with repeated measures ANOVA (RM-ANOVA) to compare the overall cardiopulmonary hemodynamic effects and the PK endpoints (eg: AUC) for all doses of nitrite.

#### Safety Analysis

Adverse events (AEs) will be grouped by body system. AEs occurring pre-treatment, during active treatment and post-treatment will be summarized separately. The number and percentage of subject experiencing at least one AE of any type, AEs within each body system and AEs within each preferred term will be tabulated and listed. Separate summaries will be provided for all AEs, AEs by maximum severity, drug related AEs, severe adverse events (SAEs), and for AEs leading to withdrawal.

#### Pharmacokinetic Analysis

PK endpoints will be summarized using descriptive statistics. The actual sampling times will be used in calculating PK parameters using non-compartmental methods. The PK endpoints namely, AUC, Incremental recovery,  $t_{1/2}$ , CI, AUC<sub>last</sub>, C<sub>max</sub> will be analyzed using a multiplicative linear mixed effect models.

#### Handling of missing data:

Every effort will be made to collect complete data on each study day. With respect to safety evaluation, it is not planned to impute missing data.

## 3. HUMAN SUBJECTS



### 3.1 SUBJECT POPULATION

The racial, gender and ethnic characteristics of the proposed subject population in this research protocol shall reflect the demographics of the population of Pittsburgh and the surrounding area. We shall attempt to recruit subjects in proportion to these demographics. No exclusion criteria shall be based on race, ethnicity or gender.

Every effort will be made to keep subjects in the study until they complete all study procedures.

#### 3.1.1 Inclusion of Women and Minority

Both men and women of all races and ethnic groups are eligible for this trial. Women who meet the inclusion criteria, and have none of the exclusion criteria, will be enrolled without restriction as dictated by the study protocols. Because of the use of a study medication, woman of child bearing potential must meet specialized inclusion/exclusion criteria to minimize this risk.

#### 3.1.2 Inclusion of Children

Children under the age of 18 will not be recruited for this protocol because of the need to explore potential adverse effects of the study treatment more fully in adults. .

### 3.2 INCLUSION CRITERIA

Potential study subjects must satisfy the following criteria to be enrolled in the study

#### WHO Group I PAH (n = 20)

Diagnosis of RHC confirmed WHO Group I PAH in any of the following categories:

- Idiopathic, primary or familial pulmonary arterial hypertension (IPAH, PPH, or FPAH)

**OR**

- PAH associated with one of the following connective tissue diseases:
  - i. Systemic sclerosis (scleroderma)
  - ii. Limited scleroderma
  - iii. Mixed connective tissue disease
  - iv. Systemic lupus erythematosus
  - v. Overlap syndrome;

**OR**

- PAH associated with:
  - i. Human immunodeficiency virus (HIV) infection;
  - ii. Simple, congenital systemic-to-pulmonary shunts at least one year post-surgical repair.
  - iii. Exposure to legal drugs, chemicals and toxins, such as fenfluramine, derivatives, other anorexigens, toxic rapeseed oil or L-tryptophan.Subjects with PAH associated with illegal drug use, such as methamphetamine excluded.

Stable PAH for at least 3 months if on therapy

#### WHO Group II (n=20)

- Pulmonary capillary wedge pressure (PWCP) > 15
- AND



- Transpulmonary Gradient (TPG) > 12

#### WHO Group III PH (n = 10)

Has WHO functional class II-IV symptoms

Had the diagnosis of PH confirmed by a cardiac catheterization with the following values:

- Mean pulmonary artery pressure (mPAP)  $\geq$  25 mm Hg (at rest);
- Pulmonary capillary wedge pressure (PCWP) or left ventricular-end diastolic pressure  $\leq$  15 mm Hg (if diagnosed with PAH); and
- Pulmonary vascular resistance (PVR)  $\geq$  3 mm Hg/L/min or  $\geq$  240 dynes\*sec/cm<sup>5</sup>;

#### WHO Group I, II and III

Age 18 and older;

Able and willing to participate in right heart catheterization;

Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the study;

Subjects who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures;

### **3.3 EXCLUSION CRITERIA**

Subjects meeting any of the exclusion criteria at baseline will be excluded from participating in study.

- Age less than 18 years;
- Baseline systemic hypotension, defined as MAP less than 50 mmHg;
- Required intravenous inotropes within 30 days prior to study participation;
- Has uncontrolled systemic hypertension as evidenced by sitting systolic blood pressure >160 mm Hg or sitting diastolic blood pressure >100 mm Hg at Screening;
- Has a history of portal hypertension or chronic liver disease, including hepatitis B and/or hepatitis C (with evidence of recent infection and/or active virus replication) defined as moderate to severe hepatic impairment (Child-Pugh Class B-C);
- Has chronic renal insufficiency as defined by serum creatinine >2.5 mg/dL at Screening or requires dialytic support;
- Has a hemoglobin concentration <9 g/dL at Screening;
- History of atrial septostomy within 6 months prior to Day 1 visit;
- Repaired or unrepaired congenital heart disease (CHD);
- Pericardial constriction;
- Confirmed diagnosis of restrictive or constrictive cardiomyopathy;
- Left ventricular ejection fraction <40% by multiple gated acquisition scan (MUGA), angiography or echocardiography;
- Symptomatic coronary disease with demonstrable ischemia;
- Other severe acute or chronic medical or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the subject inappropriate for entry into this study;
- Has a psychiatric, addictive or other disorder that compromises the ability to give informed consent for participating in this study. This includes subjects with a recent history of abusing alcohol or illicit drugs 30 days prior to study screening Day 0 and for the duration of the study;



- Poorly controlled asthma defined by active wheezing and/or cough with FEV1 < 70% predicted, responsive to inhaled BD (>15% increase in FEV1 with BD);
- Investigators, study staff or their immediate families;
- Clinically significant intercurrent illness (including lower respiratory tract infection) or clinically significant surgery within 4 weeks before the administration of study drug;
- Personal or family history of congenital or acquired methemoglobinemia;
- Personal or family history of RBC CYP B5 reductase deficiency;
- Known or suspected hypersensitivity or allergic reaction to sodium nitrite;
- Personal history of glucose-6-phosphate dehydrogenase (G6PD) deficiency or any contraindication to receiving methylene blue;
- If female, is pregnant or breast feeding, or has a positive pregnancy test result predose;
- Receipt of an investigational product or device, or participation in a drug research study within a period of 15 days (or 5 half-lives of the drug, whichever is longer) before the first dose of study drug;
- Blood loss or blood donation >550 mL within 90 days or plasma donation >500 mL within 14 days before administration of study drug;
- RHC < 2 weeks from treatment visit unless clinically indicated

## **4. RECRUITMENT AND INFORMED CONSENT PROCEDURES**

### **4.1 RECRUITMENT METHODS**

The potential study subjects will be recruited from the UPMC Heart and Vascular (HVI) and Comprehensive Lung Center (CLC) or inpatients at UPMC Presbyterian Hospital. Potential subjects will be first identified or referred to the investigator who may also be the primary care or treating physician. The investigator who may already have knowledge of and access to subjects' information may review the subjects' records to identify potential research subjects for the study. After identifying potentially eligible subjects, the investigator will then approach these subjects to discuss the research opportunity.

To minimize the possibility that subjects will feel obligated to participate, investigators will reinforce with their subjects that participation is voluntary, that they do not have to participate, and the decision not to participate will not affect their care, now or in the future. The investigator will also allow subjects to make further inquiries if they are interested.

### **4.2 INFORMED CONSENT PROCEDURES**

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects and their families. Consent forms describing in detail the study procedures and risks are given to the subject and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects



will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Prior to performing any of the research study procedures or interventions, subjects must provide informed consent. The investigator will verbally explain the study to the potential subject in a language understandable to subjects, providing all pertinent information (purpose, procedures, risks, benefits, alternatives to participation, etc.), and will allow potential subjects ample opportunity to ask questions to elicit a better understanding of the study. Following this verbal explanation, potential subjects will be provided with a local IRB approved consent form and will be asked to read and review the document. Upon reviewing the document, the investigator will provide adequate opportunity for the subject to consider all options, answer any additional questions the potential subject may have. Every effort will be made to ensure that subjects have comprehended the study information prior to obtaining subject's voluntary agreement to participate.

In addition, older potential participants whose competency to consent is in question will be tested for sufficient comprehension and recall of the information presented. Prospective subjects who do not remember the important facts about participation in the research study after repeated testing will not be included in the study. The investigators will also assess whether a participant understands experimental procedures over time, including assessment throughout the full duration of participation in the study.

The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

## **5. POTENTIAL RISKS AND BENEFITS**

### **5.1 POTENTIAL RISKS**

As with any experimental procedure, there may be adverse events or side effects that are currently unknown, and certain of these unknown risks could be permanent, severe or life threatening. Every attempt will be taken to minimize these risks.

#### Risks of Sodium Nitrite Inhalation Solution:

The first-in-man clinical study of AIR001 Inhalation Solution, Study AIR001-CS01, was a dose escalation study designed to identify the dose limiting toxicity, the maximum tolerated dose, and the pharmacokinetics of AIR001 administered. The most important safety finding was a decline in systolic and diastolic blood pressure at the highest dose tested, 176 mg. Dose-dependent asymptomatic increases in heart rate were noted at all doses, although at doses up to and including the maximum tolerated dose of 125 mg, the heart rate changes were well tolerated. Increase in heart rate was not always associated with a change in blood pressure. Significant levels of methemoglobin, or changes in pulmonary function, were not observed. Methemoglobin levels increased at the highest doses administered, but remained less than 3.5% in all subjects.

As with any drug product administered via inhalation, it is possible that susceptible individuals, such as those with unrecognized asthma, will develop bronchospasm upon exposure to inhaled sodium nitrite solution. There were no changes in pulmonary function noted in AIR001-CS01, which excluded subjects with asthma or abnormal baseline pulmonary function tests.



In Study AIR001-CS02, inhaled nitrite was generally well tolerated under hypoxic conditions at doses up to and including 113 mg. Additionally, a sustained reduction in hypoxia-induced pulmonary hypertension was demonstrated, suggesting that safely delivered doses of AIR001 Inhalation Solution B result in clinically significant vasodilatation, presumably through a mechanism of intrapulmonary reduction of nitrite to NO.

In this study, the significant treatment related adverse event was a decrease in blood pressure. The magnitude of the change in blood pressure from the hypoxic baseline was less than from the normoxic baseline, suggesting that hypoxia itself has an effect on BP.

#### Risks of Nitric Oxide Inhalation:

The inhalation of NO is usually well tolerated without side effects.

Infrequent risks: decrease pulmonary vascular tone, but because of its short biological half-life will be metabolized or inactivated before reaching the systemic circulation. Other risks: Massive overdoses of nitric oxide are fatal (from 500-1000ppm).

#### Risks of Sildenafil/Tadalafil (PDE-5 Inhibitor):

These medications are commonly used and FDA approved to treat Pulmonary Hypertension. These medications may lower blood pressure significantly if taken together with some other medications like nitroglycerin. AIR001 may cause similar effects in this study in combination with PDE-5I.

Subjects will be closely monitored throughout the entire procedure and should blood pressure drop significantly with the first inhaled dose of AIR001, study participant will not receive the next higher dose.

#### Risks of Blood Drawing:

The amount of blood to be drawn over the course of this research study could be a maximum of 5 tablespoons. To minimize the risks of blood tests, a licensed technician or registered nurse will draw your blood.

Common risks include temporary discomfort, bruising which may last for several days, redness, swelling, lower hemoglobin level. Infrequent risks include a subject may feel lightheaded or faint when blood is drawn. This is usually due to nervousness and is not usually serious. Rare risks include infection, and bleeding.

#### Risks of Echocardiography:

This is a noninvasive procedure. There is no known risk associated with this procedure.

#### Risks of Right heart Catheterization:

Common risks: pain at the needle entry site and slight risk of bleeding around the site. bruising at site, lightheadedness or dizziness during the needle stick.

Infrequent risks: puncturing the lung which would require a chest tube insertion, irregular heartbeats which usually stop when the long tube is removed from the heart.

Very rare complications include cardiac arrhythmias, cardiac tamponade, low blood pressure, infection, or embolism caused by blood clots at the tip of the catheter.

#### Risks of the Use of Micromanometer Catheter during Catheterization:

The micromanometer pressure and flow catheter are FDA approved, and have been used for clinical/investigative indications for over 25 years. Moreover, these catheters do not pose any inherent risk to patients. The catheter will be placed through the clinical Swann-Ganz catheter that is within the preexisting jugular venous sheath. This obviates the need for an additional



venipuncture. This catheter has successfully been used without complications by the study Principal Investigator for the past 5+ years.

#### Risks of Medications used during Catheterization:

The medications used for sedation are relatively brief in duration and should wear off within several hours. The side effects are listed below for each drug.

1 % Lidocaine: will be used to numb the area prior to the insertion of the cardiac catheter into either your neck or groin vein. A common side effect is slight burning at the site which dissipates quickly.

Fentanyl: Common side effects include temporary light-headedness, dizziness, nausea, vomiting or sweating.

Midazolam: Common side effect includes drowsiness. Infrequent side effects are nausea, vomiting. Breathing problems are rare.

Allergic reactions (e.g., hives, itching, etc.) to lidocaine, fentanyl, or midazolam are rare.

#### Risks of Chest X-Ray during the Catheterization

A chest x-ray may be done, if clinically indicated, on participants that a neck vein is used for the catheterization. A chest X-ray performed for the purpose of this research study involves exposure to radiation. Each chest X-ray will result in a radiation dose of approximately 0.03 rem to the chest with minimal exposure of other body areas. For comparison, radiation workers are permitted, by Federal regulation, to receive a maximum annual radiation dose of 20 rems to the most sensitive organs of their body. There is no minimum amount of radiation exposure that is recognized as being totally free of the risk of causing genetic mutations (abnormal cells) or cancer. However, the risk associated with the amount of radiation exposure received from participation in this study is considered to be low and comparable to everyday risks.

#### Reproductive Risks:

It is not known if the study drug can affect an unborn baby. Therefore, subjects should not become pregnant or father a baby while on this study. If subjects are physically able to father a baby, subjects must use an effective method of birth control while on this study. If subjects become aware that they or their sexual partner is pregnant during the course of their participation in this research study, subjects must contact, as soon as possible, the study investigator.

#### Risks of Breach of Confidentiality:

Participation in this research study does involve the potential risks of a breach of confidentiality of the medical record information and associated privacy of the participants. This research study will result in identifiable information that will be placed into the subject's medical records held at the University of Pittsburgh Medical Center. The nature of the identifiable information resulting from participation in this research study that will be recorded in the medical record includes laboratory test results. This potential for breach of confidentiality could impact future insurability, employability, or have other personal consequences for the subjects.

## **5.2. PROTECTIONS AGAINST RISK**

#### Protection Against Patient Risks Related to the Study Drug

The study has been designed with a focus on protecting subjects against risk from the medication



including:

- Specific exclusion criteria to provide a stable population of subjects and non-enrollment of subjects with significant co-morbidities that might place them at excess risk (see exclusion criteria above).
- Continuous monitoring by the DSMB.
- Involvement by trained staff / investigators with experience in the administration of inhaled nitrite.
- Specific holding criteria related to study adverse events.
- Frequent monitoring the cardiopulmonary hemodynamics for over the duration of the study.

#### Protection Against General Risks of Study Procedures

All research interventions/activities will be conducted in private patient care areas. The collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected.

All demographic and clinical information about the subject will be stored on an electronic password-guarded study database under the supervision of the investigator for this protocol. The electronic database has not been validated to be in full compliance with the FDA regulations at 21 CFR Part 11. Use of this electronic database is, however, felt to be acceptable due to the limited scope of this research study and the extent of data that will be collected. The data will be 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. Access to the database will be limited to the data manager and staff under the supervision of the PIs.

To prevent excessive blood sampling, a single withdrawal of blood for a combination of clinically indicated and this research study will not exceed 5% of the circulating blood volume, and the cumulative withdrawal over 1 month will not exceed 10% of the circulating blood volume. In addition, careful attention will be made to cardiovascular status and hemoglobin evaluation.

Specimens will be stripped of subject identifiers and stored securely according to a similar coding protocol as described above. These specimens will be stored safely in the custody of the Principal Investigator responsible for the individual assays. These Investigators will limit future access to any remaining sample to only those investigators with prior IRB approval for their studies.

All staff involved in this study are properly credentialed and instructed in the areas of testing, confidentiality, and safety. To minimize the risks associated with the study procedures and/or collection of specimens, trained staff or experienced investigators will perform the study procedures.

### **5.3 ALTERNATIVE TREATMENTS**

If subjects choose not to participate in this study, they are to continue their medical care under the direction of their primary physicians.

### **5.4 POTENTIAL BENEFITS**



There will be no direct benefit to the subjects participating in this study, but the society at large may benefit from the increased knowledge gained from this study that will lead to new treatment for individuals diagnosed with PAH in the future.

## 5.5 DATA SAFETY MONITORING PLAN

### 5.5.1 Data Safety Monitoring Board

A Data and Safety Monitoring Board (iDSMB) independent of the study investigators will monitor this clinical trial for additional measure of subject protection. The iDSMB consists of clinicians completely independent of the investigators who have no financial, scientific, or other conflict of interest with the trial.

The iDSMB will conduct interim monitoring 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.

### 5.5.2 Data Safety Monitoring Plan

Assuring patient safety is an essential component of this protocol. The study Principal Investigator has primary responsibility for the oversight of the data and safety monitoring. The study investigators will evaluate all adverse events. All subjects who have AEs, whether considered associated with the use of the study medication or not, must be monitored to determine the outcome. The clinical course of the AE will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the Principal Investigator considers it medically justifiable to terminate follow-up.

All untoward medical occurrences observed in subjects receiving the study drug will be recorded on the participants' adverse event worksheets by the study coordinator under the supervision of the principal investigator. The worksheets will then be reviewed for completeness and internal consistency. 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.

#### **\*Stopping Rule:**

For safety reasons, we propose to discontinue this study treatment if any of the following occurs:

- A decrease in systolic BP > 40 mm Hg drop from pre-dose baseline;
- Desaturation > 10% from baseline;
- A single occurrence of venous methemoglobin level >5%;
- Severe DLT criteria met and symptomatic or requiring fluids or treatment;
- Any serious adverse event thought to be possibly related to the study treatment.

The Sponsor and Investigator will prepare a detailed written summary of serious, unexpected, and treatment related adverse events, and will compare, and contrast the event with prior events. The detailed written summary will be provided to the DSMB and the IRB.



In addition, the DSMB Report addressed the following information will be submitted to the IRB at the time of continuing review annually or more often as required:

- A list of the research personnel who participated in the data and safety monitoring.
- The frequency of monitoring that took place during the renewal intervals and/or the dates that data and safety monitoring was conducted.
- A summary of cumulative data related to unanticipated problems (including adverse events) including a determination of causality and whether the risk to benefit assessment has changed.
- If appropriate, a summary of pertinent scientific literature reports, therapeutic developments, or results of related studies that may have an impact on the safety of study participants or the ethics of the research study.
- A summary of the outcome of reviews conducted to ensure subject privacy and research data confidentiality.
- Final conclusions regarding changes to the anticipated benefit-to-risk assessment of the study participation and final recommendations related to continuing, changing, or terminating the study.

#### **5.5.3 Parameters to be Monitored**

The following progress will be monitored throughout the course of the research to ensure the safety of subjects as well as the integrity and confidentiality of their data.

- An evaluation of the progress of the research study, including subject recruitment and retention, and an assessment of the timeliness and quality of the data.
- A review of collected data (including adverse events, unanticipated problems, and subject withdrawals) to determine whether there is a change to the anticipated benefit-to-risk assessment of study participation and whether the study should continue as originally designed, should be changed, or should be terminated.
- An assessment of external factors or relevant information (eg. Pertinent scientific literature reports or therapeutic development, results of related studies) that may have an impact on the safety and study participants or the ethics of the research study.
- A review of study procedures designed to protect the privacy of the research subjects and the confidentiality of their research data.

#### **5.5.4 Frequency of Monitoring**

The Investigator will review subject safety data as it generated. The Investigator and the research staff will meet on a quarterly interval to re-evaluate study goals, subject recruitment, data coding and retention, documentation and identification of adverse events, complaints and confidentiality of subjects. 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 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.

The iDSMB is expected to meet at least two times a year at the call of the Chairperson to review the progression of the study including patient enrollment, protocol compliance, and adverse event reports. An emergency meeting of the iDSMB may be called at any time by the Chair should participant safety questions or other unanticipated problems arise.

#### **5.5.5. Adverse Event Reporting:**

The study investigators will be responsible for detecting, documenting and reporting events that meet the following definition of an adverse event.

##### **5.5.5.1. Adverse event definitions**

Adverse event. Any untoward medical occurrence in a clinical study; regardless of the causal relationship of the event with the investigational drug or study treatment(s).

Associated with the use of the investigational drug or study treatment(s). There is a reasonable possibility that the adverse event may have been caused by the investigational drug or study treatment(s).

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse event. Any adverse event that places the patient or subject, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e., does not include an adverse event that, had it actually occurred in a more severe form, might have caused death).

Serious adverse event. Any adverse event occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

- Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse event; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event (e.g., for a preexisting condition not associated with a new adverse event or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse event.

Unexpected adverse event. Any adverse event, the frequency, specificity or severity of which is not consistent with the risk information described in the clinical protocol(s) or elsewhere in the current IND application, as amended.

##### **5.5.5.2. Recording/Reporting requirements**

###### Eliciting adverse event information

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

###### Recording requirements



All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of treatment group or 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 causal 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 study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Investigator.

#### 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 investigator-sponsor of the IND application

#### Causality and severity assessment

The investigator-sponsor of the IND application 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 investigator-sponsor'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. If the investigator-sponsor'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.

### **5.5.6. Reporting of adverse events**

#### **5.5.6.1. Reporting of adverse events to the FDA**

##### Written IND Safety Reports

The investigator-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 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 investigator-sponsor and Aires Pharmaceuticals, Inc. chief medical officer or designee 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-investigator'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.

#### Telephoned IND Safety Reports

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the investigator-sponsor 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 investigator-sponsor's initial receipt of the respective human adverse event information.

#### **5.5.7. 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'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 sponsor-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 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.

## **6 STUDY ADMINISTRATION**

### **6.1. QUALITY CONTROL AND QUALITY ASSURANCE**

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically (i.e., at a minimum of annually) by qualified staff of the Education and Compliance Office – Human Subject Research, Research Conduct and Compliance Office, University of Pittsburgh.

The investigator-sponsor and the University of Pittsburgh and University of Pittsburgh Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

### **6.2. DATA HANDLING AND RECORD-KEEPING**

#### **6.2.1. Data recording**

Study worksheets will be completed for each subject enrolled into the clinical study. The Investigator will review, sign and date completed worksheets; the Investigator's signature serving as attestation of the Investigator's responsibility for ensuring that all clinical and laboratory data are complete, accurate and authentic.

Appropriate coded identifications (i.e. Subject ID number) will be used. Every effort will be made to collect complete data for each study visit. Causes of missing data will be fully documented. With respect to safety evaluation, it is not planned to impute missing data.

#### **6.2.2. Record maintenance and retention**

The Sponsor and Investigator will maintain records in accordance with Good Clinical Practice guidelines; to include:

- FDA correspondence related to the IND and clinical protocol, including copies of submitted Safety Reports and Annual Reports
- IRB correspondence (including approval notifications) related to the clinical protocol; including copies of adverse event reports and annual or interim reports
- Current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent form(s) and, if applicable, subject recruitment advertisements
- Signed FDA Form 1572 Statements of Investigator (i.e., for the Sponsor and all identified sub-investigators)
- Financial disclosure information (Investigator-sponsor and clinical protocol sub-investigators)
- Curriculum vitae (Sponsor and clinical protocol sub-investigators)
- Certificates of required training (e.g., human subject protections, Good Clinical Practice, etc.) for Sponsor and listed sub-investigators
- Listing of printed names/signatures of Investigator-sponsor and listed sub-investigators



- Normal value(s)/range(s) for medical/laboratory/technical procedures or tests included in the clinical protocol
- Laboratory certification information
- Instructions for on-site preparation and handling of the investigational drug(s), study treatment(s), and other study-related materials (i.e., if not addressed in the clinical protocol)
- Decoding procedures for blinded trials (*incorporate only if applicable*)
- Master randomization list (*incorporate only if applicable*)
- Signed informed consent forms
- Completed worksheets; signed and dated by Investigator
- Source Documents or certified copies of Source Documents
- Monitoring visit reports
- Copies of Sponsor communications to the Investigator and copies of Investigator communications to sub-investigators
- Subject screening and enrollment logs
- Subject identification code list
- Investigational drug accountability records, including documentation of drug disposal.
- Retained biological specimen log
- Interim data analysis report(s)
- Final clinical study report

Subject-specific data and will be coded and the subject identification code list will be stored so as to protect the subjects' confidentiality. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

The Investigator-sponsor and Aires Pharmaceuticals, Inc. will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

### **6.3. ETHICS**

#### Institutional Review Board (IRB) Approval

The Investigator will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and brochures (i.e., directed at potential research subjects and clinical faculty/staff) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the Investigator will promptly notify the University of Pittsburgh IRB of the deviation.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).



In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of the Investigator's decision to modify the previously accepted clinical protocol:

The Investigator will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to this Phase 1 clinical protocol that significantly affects the safety of the subjects. For changes that do not affect critical safety assessments, the revisions to the clinical protocol will be addressed in the Annual Report to the IND.

#### Ethical and scientific conduct of the clinical study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on GCP; and relevant policies, requirements, and regulations of the University of Pittsburgh IRB, University of Pittsburgh and UPMC, Commonwealth of Pennsylvania, and applicable federal agencies.

The Investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the Investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

## **7. COSTS AND PAYMENTS**

### **7.1 COSTS**

The study is jointly supported by the NIH Program Project Grant (PPG; grant number: P01HL103455) and by Aires Pharmaceuticals, Inc. The current proposal is the human core component of this PPG. The NIH funding covers the majority of the study procedures as well as the study infrastructure. Study drugs and research testing will be supported by Aires Pharmaceuticals, Inc. Medication such as ETRA or PDE-5I, routine lab tests, and any procedures not related to this research study are considered routine medical care and will be billed to the subjects' health insurance company. Subjects will be responsible for paying any deductibles, co-payments or co-insurance that are a normal part of their health insurance plan. Subjects who do not have health insurance will be responsible for these costs.

### **7.2 PAYMENTS**

Subjects will be compensated \$250.00 after completion of right heart catheterization and study treatment at the end of Day 1 study visit, and \$50.00 after completion of the Day 30 outpatient clinic follow-up study visit. The total compensation for participation in all aspects of the clinical study will be \$ 300.00. Parking validations will be given for study visit 1 and Day 30.



## 8. QUALIFICATIONS AND SOURCES OF SUPPORT

### 8.1 QUALIFICATIONS OF THE INVESTIGATORS

#### Sponsor:

**Mark Schmidhofer, MD** is an Associate Professor of Medicine, at the University of Pittsburgh, Heart and Vascular Institute. Dr. Schmidhofer is the Director of the Coronary Intensive Care Unit and Director of Quality Improvement, Division of Cardiology. He has served as coauthor on several publications. Dr. Schmidhofer will work closely with Dr. Simon in maintaining the necessary regulatory documents for this established IND.

#### Investigator:

**Marc Simon, MD**, is an Associate Professor of Medicine in the Heart Failure and Cardiac Transplantation Section at the University of Pittsburgh. His clinical specialty is advance heart failure and pulmonary hypertension. His research has largely focused on adaptation and failure of the right ventricle in pulmonary hypertension. Dr. Simon has been involved in many research projects. He will play a large role in the accrual of patients who are seen at the UPMC Heart and Vascular Institute on a regular basis.

#### Sub-Investigators:

**Mark T. Gladwin, MD**, is a Professor of Medicine, Director of the Hemostasis & Vascular Biology Research Institute and Chief of the Division of Pulmonary, Allergy, and Critical Care Medicine at the University of Pittsburgh. Dr. Gladwin has established himself as one of leading international authorities on nitrite anion biology and its application to treat a variety of cardiopulmonary diseases. He is currently the PI on two, large multi-center clinical trials to test the efficacy of nitric oxide and sildenafil in subjects with sickle cell disease and pulmonary hypertension. He has served as a principal or associate investigator on more than 25 human subjects protocols and holds seven FDA INDs for the use of investigational therapeutic medications, including nitrite, carbon monoxide, L-NMMA, and sildenafil.

**Dennis McNamara, MD** is a Professor of Medicine at the University of Pittsburgh in the Heart and Vascular Institute. He is the Director of the Heart Failure/Transplantation Section at the University of Pittsburgh Medical Center. Dr. McNamara is recognized as a national leader in pharmacogenetic research in heart failure, and is the National Principal Investigator of an NHLBI multi-center genetics outcomes study.

**Alison Morris, MD**, is an Associate Professor of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine. She is the PI of the NIH R01 to study the pathogenesis of HIV-associated emphysema. Dr. Morris has also been the PI on a K23, a California Tobacco-disease Related Program grant, and PI or co-investigator on numerous research projects examining lung diseases in various populations.

**Michael Risbano, MD, MA** is an Assistant Professor in the Department of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine. Dr. Risbano's clinical interests are medications primarily focused on the diagnosis, management and treatment of pulmonary hypertension. He is an attending physician in the Comprehensive Pulmonary Hypertension Program. Dr. Risbano's past research has focused on the discovery and implementation of biomarkers for the early diagnosis of pulmonary hypertension in patients with scleroderma (SSc-PAH). He is



interested in the hemodynamic evaluation of subjects with pulmonary hypertension and correlation of hemodynamic values with biomarker levels. Dr. Risbano also has a research interest in the hemodynamic responses to vasoactive medications.

**Frank Sciruba, MD**, is an Associate Professor of Medicine at the University of Pittsburgh, Pulmonary, Allergy and Critical Care Medicine division and Director of The Emphysema Research Center and Pulmonary Function Exercise Physiology Laboratory. He is an active member of the American College of Chest Physicians, and serves on the Pulmonary Physiology, Function & Rehabilitation Network Steering Committee. He is a member of the American Thoracic Society and has served on the Ad Hoc Committee on Standards for Clinical Exercise Testing and currently serves on the Corporate Relations Committee and Clinical Problems Program committee and has chaired the ATS International Conference post graduate course in COPD for four consecutive years.

## 8.2 SOURCE OF SUPPORT

National Heart, Lung, and Blood Institute and Aires Pharmaceuticals, Inc.

## 9. REFERENCES

1. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Kernis JT, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Annals of internal medicine*. 1991;115:343-349
2. Rubin LJ. Primary pulmonary hypertension. *Chest*. 1993;104:236-250
3. Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA, 3rd, Loyd JE, Nichols WC, Trembath RC. Heterozygous germline mutations in *bmp2*, encoding a *tgf-beta* receptor, cause familial primary pulmonary hypertension. *Nature genetics*. 2000;26:81-84
4. Barst RJ, Rubin LJ, McGoon MD, Caldwell EJ, Long WA, Levy PS. Survival in primary pulmonary hypertension with long-term continuous intravenous prostacyclin. *Annals of internal medicine*. 1994;121:409-415
5. Farber HW, Loscalzo J. Prothrombotic mechanisms in primary pulmonary hypertension. *The Journal of laboratory and clinical medicine*. 1999;134:561-566
6. Fagan KA, Fouty BW, Tyler RC, Morris KG, Jr., Hepler LK, Sato K, LeCras TD, Abman SH, Weinberger HD, Huang PL, McMurtry IF, Rodman DM. The pulmonary circulation of homozygous or heterozygous *enos*-null mice is hyperresponsive to mild hypoxia. *The Journal of clinical investigation*. 1999;103:291-299
7. Gaine S. Pulmonary hypertension. *JAMA : the journal of the American Medical Association*. 2000;284:3160-3168
8. Badesch DB, Orton EC, Zapp LM, Westcott JY, Hester J, Voelkel NF, Stenmark KR. Decreased arterial wall prostaglandin production in neonatal calves with severe chronic pulmonary hypertension. *American journal of respiratory cell and molecular biology*. 1989;1:489-498
9. Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, Loyd JE. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *The New England journal of medicine*. 1992;327:70-75



10. Cockrill BA. The use of nitric oxide in primary pulmonary hypertension. *Respiratory care clinics of North America*. 1997;3:505-519
11. Gladwin MT, Schechter AN, Shelhamer JH, Ognibene FP. The acute chest syndrome in sickle cell disease. Possible role of nitric oxide in its pathophysiology and treatment. *American journal of respiratory and critical care medicine*. 1999;159:1368-1376
12. Sparacino-Watkins CE, Tejero J, Sun B, Gauthier MC, Thomas J, Ragireddy V, Merchant BA, Wang J, Azarov I, Basu P, Gladwin MT. Nitrite reductase and nitric-oxide synthase activity of the mitochondrial molybdopterin enzymes marc1 and marc2. *The Journal of biological chemistry*. 2014;289:10345-10358
13. Tejero J, Gladwin MT. The globin superfamily: Functions in nitric oxide formation and decay. *Biological chemistry*. 2014;395:631-639
14. Shiva S, Sack MN, Greer JJ, Duranski M, Ringwood LA, Burwell L, Wang X, MacArthur PH, Shoja A, Raghavachari N, Calvert JW, Brookes PS, Lefer DJ, Gladwin MT. Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *The Journal of experimental medicine*. 2007;204:2089-2102
15. Chouchani ET, Methner C, Nadtochiy SM, Logan A, Pell VR, Ding S, James AM, Cocheme HM, Reinhold J, Lilley KS, Partridge L, Fearnley IM, Robinson AJ, Hartley RC, Smith RA, Krieg T, Brookes PS, Murphy MP. Cardioprotection by s-nitrosation of a cysteine switch on mitochondrial complex i. *Nature medicine*. 2013;19:753-759
16. Shiva S, Huang Z, Grubina R, Sun J, Ringwood LA, MacArthur PH, Xu X, Murphy E, Darley-USmar VM, Gladwin MT. Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. *Circulation research*. 2007;100:654-661
17. Rassaf T, Flogel U, Drexhage C, Hendgen-Cotta U, Kelm M, Schrader J. Nitrite reductase function of deoxymyoglobin: Oxygen sensor and regulator of cardiac energetics and function. *Circulation research*. 2007;100:1749-1754
18. Hendgen-Cotta UB, Merx MW, Shiva S, Schmitz J, Becher S, Klare JP, Steinhoff HJ, Goedecke A, Schrader J, Gladwin MT, Kelm M, Rassaf T. Nitrite reductase activity of myoglobin regulates respiration and cellular viability in myocardial ischemia-reperfusion injury. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105:10256-10261
19. Cardenes N, Corey C, Geary L, Jain S, Zharikov S, Barge S, Novelli EM, Shiva S. Platelet bioenergetic screen in sickle cell patients reveals mitochondrial complex v inhibition, which contributes to platelet activation. *Blood*. 2014;123:2864-2872
20. Zhan J, Nakao A, Sugimoto R, Dhupar R, Wang Y, Wang Z, Billiar TR, McCurry KR. Orally administered nitrite attenuates cardiac allograft rejection in rats. *Surgery*. 2009;146:155-165
21. Gonzalez FM, Shiva S, Vincent PS, Ringwood LA, Hsu LY, Hon YY, Aletras AH, Cannon RO, 3rd, Gladwin MT, Arai AE. Nitrite anion provides potent cytoprotective and antiapoptotic effects as adjunctive therapy to reperfusion for acute myocardial infarction. *Circulation*. 2008;117:2986-2994
22. Duranski MR, Greer JJ, Dejam A, Jaganmohan S, Hogg N, Langston W, Patel RP, Yet SF, Wang X, Kevil CG, Gladwin MT, Lefer DJ. Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver. *The Journal of clinical investigation*. 2005;115:1232-1240



23. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nature reviews. Drug discovery*. 2008;7:156-167
24. Mack AK, McGowan Li VR, Tremonti CK, Ackah D, Barnett C, Machado RF, Gladwin MT, Kato GJ. Sodium nitrite promotes regional blood flow in patients with sickle cell disease: A phase i/ii study. *British journal of haematology*. 2008;142:971-978
25. Dezfulian C, Raat N, Shiva S, Gladwin MT. Role of the anion nitrite in ischemia-reperfusion cytoprotection and therapeutics. *Cardiovascular research*. 2007;75:327-338
26. Sugimoto R, Nakao A, Kohmoto J, Wang Y, Gladwin MT, Billiar TR, McCurry KR. 32: Nitrite protects rat lung grafts from ischemia/reperfusion injury via xanthine oxidoreductase (xor). *The Journal of Heart and Lung Transplantation*. 2008;27:S71
27. Dejam A, Hunter CJ, Tremonti C, Pluta RM, Hon YY, Grimes G, Partovi K, Pelletier MM, Oldfield EH, Cannon RO, 3rd, Schechter AN, Gladwin MT. Nitrite infusion in humans and nonhuman primates: Endocrine effects, pharmacokinetics, and tolerance formation. *Circulation*. 2007;116:1821-1831
28. Blood AB, Power GG. In vitro and in vivo kinetic handling of nitrite in blood: Effects of varying hemoglobin oxygen saturation. *American journal of physiology. Heart and circulatory physiology*. 2007;293:H1508-1517
29. Zuckerbraun BS, Shiva S, Ifedigbo E, Mathier MA, Mollen KP, Rao J, Bauer PM, Choi JJ, Curtis E, Choi AM, Gladwin MT. Nitrite potently inhibits hypoxic and inflammatory pulmonary arterial hypertension and smooth muscle proliferation via xanthine oxidoreductase-dependent nitric oxide generation. *Circulation*. 2010;121:98-109
30. Fujita H, Fukumoto Y, Saji K, Sugimura K, Demachi J, Nawata J, Shimokawa H. Acute vasodilator effects of inhaled fasudil, a specific rho-kinase inhibitor, in patients with pulmonary arterial hypertension. *Heart and vessels*. 2010;25:144-149
31. Fukumoto Y, Matoba T, Ito A, Tanaka H, Kishi T, Hayashidani S, Abe K, Takeshita A, Shimokawa H. Acute vasodilator effects of a rho-kinase inhibitor, fasudil, in patients with severe pulmonary hypertension. *Heart*. 2005;91:391-392
32. Voswinckel R, Enke B, Reichenberger F, Kohstall M, Kreckel A, Krick S, Gall H, Gessler T, Schmehl T, Ghofrani HA, Schermuly RT, Grimminger F, Rubin LJ, Seeger W, Olschewski H. Favorable effects of inhaled treprostinil in severe pulmonary hypertension: Results from randomized controlled pilot studies. *Journal of the American College of Cardiology*. 2006;48:1672-1681