Inhaled Sodium Nitrite for Cystic Fibrosis

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PROTOCOL SYNOPSIS

Protocol Title:	Inhaled Sodium Nitrite for Cystic Fibrosis	
Protocol Number:	PRO15040062	
NCT Number:	NCT02694393	
Version # and Date:	Version 5.0 /January 5, 2018	
Clinical Phase:	Phase I/II clinical investigation	
Investigational Drug:	Sodium Nitrite (nebulized)	
Trial Site:	Single-Center Trial	
Sponsor:	Mark Schmidhofer, MD Associate Professor of Medicine University of Pittsburgh Heart and Vascular Institute	
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Clinical Laboratories:	UPMC CLSI Lab 3477 Euler Way Pittsburgh, PA. 15213	Automated Testing Laboratory Children's Hospital of Pittsburgh UPMC One Children's Hospital Drive Pittsburgh, PA 15224

Manufacturer:	Hope Pharmaceuticals.
Study Rationale:	Cystic fibrosis (CF) is the most common severely life-shortening genetic disease of Caucasians. Tenacious mucus plugs the airways and allows for bacterial biofilms to grow. Ongoing infections lead to bronchiectasis and ultimately death from chronic respiratory failure (reviewed in (1–3)). The most common pathogen in adults with CF is *Pseudomonas aeruginosa,* which affects 80% of the CF population (4). Nebulized sodium nitrite may represent a new therapeutic approach for the treatment of *P. aeruginosa* infection. Sodium nitrite has *in vitro* antimicrobial activity against *P. aeruginosa* and other airway pathogens (5–7). Nebulized sodium nitrite has an excellent safety profile from ongoing studies in pulmonary hypertension. In CF, there is the potential for bronchospasm in subjects with CF-related airway hyperactivity, thus a safety study such as the one proposed is warranted.
Study Objectives:	The objective of this research study is to determine whether nebulized sodium nitrite is safe in adults with cystic fibrosis.
Study Hypothesis:	The hypothesis of this study is: Nebulized sodium nitrite will be well tolerated by adults with CF.
Study Aims:	Specific Aim: ***1.*** Determine the safety of nebulized sodium nitrite administered in two doses to patients with CF. ***2.*** Explore the effects of inhaled sodium nitrite on measures of lung function, exhaled airway nitric oxide, and bacterial burden as measured by quantitative sputum cultures.
Study type	This is an experimental study of a new inhalation solution for cystic fibrosis. The initial study is a Phase I/II open-label design enrolling a total of 35 subjects that meet all the eligibility criteria.
Planned Sample Size:	35 Subjects
Duration of Treatment:	56 days
Inclusion Criteria:	1. Subjects 18 years or older with CF as documented by clinical features of CF, and documentation of a positive sweat test or two diseasing causing mutations in the CF gene, will be eligible.

Exclusion Criteria:	1. Inability to provide consent 2. Use of supplemental oxygen or non-invasive ventilatory support 3. $FEV_1 < 40\%$ 4. Awaiting lung transplantation 5. Pregnancy or unwilling to comply with birth control during the intervention period 6. History of glucose-6-phosphate dehydrogenase (G6PD) deficiency or any contraindication to receiving methylene blue 7. Hospitalization within the past 4 weeks prior to enrollment 8. Subject requires additional antibiotics or steroids within the past 4 weeks prior to enrollment 9. Change in maintenance CF therapies in the past 4 weeks prior to enrollment 10. Baseline systemic hypotension (systolic BP < 90 mm Hg) 11. History of orthostatic hypotension or syncope 12. Chronic kidney disease (creatinine > 2.5 or requiring dialysis 13. Severe anemia (Hgb <9 gm/dL in the last six months) 14. 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) 15. History of abusing alcohol or illicit drugs within 28 days prior to study 16. Severe pulmonary hypertension (mean PA pressure > 25) 17. Use of sildenafil or other pulmonary vasodilator 18. History of any organ transplantation 19. Participation in another interventional trial in the previous 28 days
Study Endpoints:	Primary endpoint: The primary safety and efficacy endpoint is FEV_1. A sustained decrease in FEV_1 of >15% will be considered intolerable. Secondary endpoints include exhaled NO (FE_{NO}), sputum nitrite concentration, quantitative bacterial cultures, percutaneous methemoglobin levels, blood pressure, and subject symptoms as measured by the CF Questionnaire-R.

1. OBJECTIVE, SPECIFIC AIMS, BACKGROUND, AND SIGNIFICANCE

1.1 OBJECTIVE

The objective of this research study is to determine the safety of nebulized sodium nitrite administered in a dose escalation manner in adults with cystic.

1.2 SPECIFIC AIMS

Hypotheses:
(1) Nebulized sodium nitrite will be well tolerated by adults with CF.

To determine the therapeutic potential of sodium nitrite for CF, we propose two specific aims:

Specific Aims:

Aim 1: Determine the safety of nebulized sodium nitrite administered in two doses to patients with CF.

Aim 2: Explore the effects of nebulized sodium nitrite on measures of lung function, exhaled airway nitric oxide, and bacterial burden.

1.3 BACKGROUND

1.3.1 Cystic Fibrosis and Chronic *Airway Infections*

Cystic fibrosis (CF) is the most common severely life-shortening genetic disease of Caucasians. Tenacious mucus plugs the airways and becomes chronically infected (reviewed in 1-3). Ongoing infections lead to bronchiectasis and ultimately death from chronic respiratory failure. The most common pathogen in adults with CF is *Pseudomonas aeruginosa*, which affects 80% of the CF population (4). Current treatment focuses on monthly cycled of inhaled antibiotics such as tobramycin and aztreonam(8). However, over decades bacteria become resistant to all existing antibiotics. There is an additional 10% of the CF population infected with organisms such as *B. cepacia complex* for which there are no existing inhaled antimicrobial therapies(4). Despite the magnitude of disease caused by *P. aeruginosa* and other airway pathogens, we do not have adequate antibiotic therapies to either keep colonization in check or eradicate the organism. This will continue to be an unmet therapeutic need as the CF population ages and is exposed to additional decades of antibiotics.

Nebulized sodium nitrite may represent a new therapeutic approach for the treatment of *P. aeruginosa* infection. Previous work by Dr. Hassett demonstrated that nitrite has bactericidal activity against *P. aeruginosa* growing in liquid culture, with mucoid strains and anaerobically growing bacteria being 2-5 fold more sensitive(5, 6). We have extended this work and shown that nitrite prevents biofilm growth by *P. aeruginosa* growing on the surface of primary human

airway epithelial cells(7). The effect ceiling is 50 mM, which is near the estimated airway concentration of nitrite after nebulization. In addition to anti-Pseudomonal activity, nitrite has antimicrobial activity against other CF pathogens including *Staphylococcus aureus, Burkholderia spp* and *Achromobacter spp*(5, 7, 9). The broad activity of nitrite is a unique strength of the agent as an antimicrobial agent.

Within the lung, the chemistries of nitrite and nitric oxide (NO) are closely related. Nitrite is reduced to NO both enzymatically and nonenzymatically. NO is formed when nitrite reacts with metal containing proteins such as hemoglobin and cytochrome c oxidase (mitochondrial complex 4)(10). These reactions are influenced by both pH and oxygen tension, and occur more rapidly under acidic conditions with a low oxygen tension, such as those found in the CF lung (6). NO has a biologic half-life of milliseconds and is the principle regulator of pulmonary vascular tone. With a half life of 51 minutes when delivered IV (and potentially longer when inhaled), nitrite can be viewed as a long acting source of NO(11, 12). In addition to its role as a vasodilator, nitrite plays a role in innate immunity. In the epithelium NO is produced by inducible nitric oxide synthetase (iNOS) from L-Arginine. In the healthy lung, both the airway epithelium and immune cells produce NO. However, in CF the airway epithelium expresses much less iNOS leading to decreased exhaled nitric oxide (13). Low exhaled nitric oxide levels are correlated with worse lung function as well as *P. aeruginosa* colonization in CF (14). Because NO has strong antimicrobial effects, low NO levels in the airway may contribute to the susceptibility to bacterial colonization in CF. Nitrite has the theoretical added benefit of restoring normal airway NO levels in patients, such as those with CF, who have an endogenous NO production defect. The antimicrobial activity of nitrite derives from its ability to inhibit bacterial respiration(7, 15). The precise molecular targets are an area of active investigation, but nitrite can inactivate heme-containing proteins (such as cytochrome c oxidases), disrupt iron-sulfur containing proteins (such as NADH dehydrogenase and succinate dehydrogenase), and S-nitrosate redox active cysteine residues impairing protein function (16). Nitrite was initially proposed as an inhaled antimicrobial agent in 2006 by Dr. Hassett's group, however compound to perform clinical trials was not available until recently and preclinical safety data was not previously available.

1.3.2 Investigational Drug

The study material (nebulized sodium nitrite) is sodium nitrite in a sterile aqueous solution. It is FDA-approved for intravenous use. We will be nebulizing the solution in the current study. The solution is packaged at 30 mg/mL. Thus 1.5 ml will be nebulized for a 45 mg dose and 3ml will be nebulized for a 90mg dose.

1.3.3 Rationale for Dose of Nitrite

The doses of nitrite proposed (45 mg and 90mg) were chosen based on existing human safety data and *in-vitro* antimicrobial data. In healthy volunteers, the maximum tolerated dose of nitrite was 90 mg(12). In the initial study CS01, 2/6 subjects experienced symptomatic hypotension and tachycardia at a dose of 176 mg. This effect occurred immediately after nebulization, and thus for CS01 the MTD was 125mg. In study CS04, 2/3 subjects had transient, but symptomatic, orthostasis with 120 mg, thus the MTD was lowered to 90mg. No other dose limiting toxicity

was seen at 90 mg. This study included dosing subjects with mild hypoxia (such as might be seen in patients with end stage lung disease) and no interaction was seen between hypoxia and nebulized nitrite. Methemoglobin did not exceed 3% in these studies. Subequently, in a study of 36 participants with pulmonary hypertenions, no increase in methemoglobin was seen with 90 mg doses (17). Another study on heart failure with preserved ejection fraction found no increase in methemoglobin as compared to control (18). Methemoglobin levels above 30% of total hemoglobin can cause shortness of breath due to the reduced oxygen carrying capacity, and levels above 50% can lead to seizures and death(19). In these studies, nebulized sodium nitrite was dosed up to every 8 hours for 6 days. Thus at doses up to 90mg, nebulized sodium nitrite appears safe in healthy subjects for dosing. Additional, unpublished safety data is available from Phase 2b clinical trials that were terminated with the sale of of the sponsor company. More than 30 subjects with pulmonary arterial hypertension have been treated for as long as 12 months with no treatment related serious adverse events. In the recently completed studies of inhaled nitrite for pulmonary hypertension, doses of 46 or 80 mg four times daily were reportedly well tolerated for up to 16 weeks. Since initial submission of this protocol, additional safety data in 36 patients with pulmonary hypertension and heart failure with preserved ejection fraction have been published. In these studies, one subject was withdrawn due to asymptomatic hypotension, and the only side effect reported was occasional self-limited cough (17, 18). Regarding the doses where nitrite may have antimicrobial activity, we estimate that with a dose of 46 mg in a high efficiency nebulizer no less than 50% of the dose will reach the lower airways. Assuming a volume of distribution in the airway surface liquid of at most 7 mL, we estimated that the initial sodium nitrite concentration in the airway after nebulization of a 46 mg dose will be 47 mM, and with 80 mg, 82 mM. In the CF airway, assuming surface liquid depletion, these are likely conservative estimates and the immediate post-deposition concentration may be higher. Importantly, these estimated concentrations are above the concentrations that we found reduce biofilm growth of *P. aeruginosa* (1 log reduction at 15 mM)(7). Based on these considerations, in this safety and proof of concept study, we will target dosing above the concentrations necessary for an anti-microbial effect and two fold below the maximal tolerated dose in normal volunteers. Dosing will be 45 mg (controlled by the nebulizer) for the first week to provide multiple day safety data prior to observed escalation to the target 90 mg dose for the remaining three weeks of the study

1.4 SIGNIFICANCE

Chronic airway infections with multi-drug resistant Gram-negative organisms are common in patients with structural lung diseases, particularly CF but also including those with non-CF bronchiectasis, COPD, following lung transplant, and those with chronic airway hardware such as tracheostomies. The current FDA approved inhaled antibiotics are limited to tobramycin and aztreonam, to which many organisms are resistant. We hypothesize that inhaled nitrite provides an innovative therapeutic approach for patients with chronic airway infections because:

1. Nitrite has broad antimicrobial activity against all organisms tested, including respiratory isolates of *Burkholderia cepcacia* complex, *Staphylococcus aureus,*

Achromobacter complex, and *Klebsiella pneumoniae*(5, 9). This study will provide proof-of-concept efficacy data that nitrite inhibits bacterial growth.

2. The antimicrobial activity of nitrite increases under anaerobic and acidotic conditions such as those found in the CF airway. Both low pH and oxygen tension decrease the activity of many antibiotics.
3. This study will provide evidence that nebulize nitrite can reconstitute the NO deficiency found in CF.
4. The results obtained in this study will lay the groundwork for future large scale clinical trials needed to determine the efficacy of inhaled nitrite as an antibiotic in patients with cystic fibrosis and other patient populations with chronic airway infections.

2. STUDY DESIGN AND METHODS

2.1 CLASSIFICATION AND METHODOLOGICAL DESIGN

This is a single-center, open label phase I/II study to determine the safety of inhaled nitrite in adults with cystic fibrosis.

2.2 STUDY DESIGN AND METHODS

2.2.1 Study Design

A total of 35 subjects with a confirmed diagnosis of cystic fibrosis meeting all the eligibility criteria will receive the investigative drug, nebulized sodium nitrite. The starting dose of nebulized sodium nitrite is 45 mg for the first week to provide multiple dose safety data and subject tolerability prior to the observed escalation target dose of 90 mg for the remaining three weeks of the study.

Screening (day -28 to -2): Potential subjects will be recruited from the CF Center at Children's Hospital of Pittsburgh and/or the UPMC Comprehensive Lung Center (CLC). In these locations, individuals with CF are followed on a routine basis and are well known to the study investigators. Initial screening evaluations including urine pregnancy test (only on females of childbearing potential), physical examination, medical history, and clinical laboratory assessments will be conducted to determine study eligibilities during a routine clinic visit. If a patient has had the required screening laboratory assessments within the past 6 months, results of those tests will be used for screening purposes and only repeated if clinically significant abnormalities were identified. In addition, vital signs (pulse, non-invasive blood pressure measurement, temperature, respiratory rate and oxygen saturation) will be performed. Subjects who meet the inclusion criteria and none of the exclusion criteria will be entered into the study and baseline spirometry will be obtained with the study spirometer. If the participant is on inhaled antibiotics, Visit 1 will be scheduled 2-7 days after the participant initiates the next scheduled inhaled antibiotic. Subjects are to remain on the same inhaled antibiotic through the end of Visit 5. Remaining on the same inhaled antibiotic for more than one month is quite common in CF clnical care.

Experimental Procedures (visit 1/day 0 (+/- 1day)): This visit will take approximately 5 hours. Subjects will arrive and complete the CFQ-R CF Questionnaire. Vital signs will be recorded and a physical examination performed, subjects will provide a sputum sample if able, and complete an oral wash specimen. The fractional excretion of nitric oxide (FE_{NO}) will be measured using a NIOX device, and baseline spirometry will be obtained. If subjects are unable to spontaneously expectorate sputum, then sputum induction will be done, followed by at least a 30 minute observation period because sputum induction is associated with temporary bronchoconstriction which might confound further measurements. The observation period will be omitted in subjects that spontaneously expectorated sputum. Next, an electronic nebulizer will be used to deliver 45 mg of nebulized sodium nitrite. Subjects will be monitored closely for the next two hours, including percutaneous measurements of oxygen saturation and methemoglobin levels, recorded at minutes 15, 30, 60 and 120, and noninvasive systemic blood pressure measurements every 15 minutes. Spirometry will be repeated at 30 and 120 minutes following nebulized sodium nitrite administration. If percutaneous methemoglobin measurements exceed 5%, they will be confirmed by venous blood measurement. If nebulized sodium nitrite is tolerated, then the subject will be dispensed an 8 day supply of 45 mg nebulized sodium nitrite dosed two times daily and discharged from the CTRC, CHP or the Emphysema Research Center in Montefiore. If the 45mg dose of nebulized sodium nitrite is not tolerated, the subject will be observed until stable and then discharged from the CTRC, CHP or the Emphysema Research Center in Montefiore.

Experimental Procedure (visit 2/day 7 (+/- 1day)): This visit will take approximately 5-7 hours. Subjects will arrive and complete the CFQ-R CF Questionnaire. FENO will be measured as above. If the subject had no serious adverse events for the first seven days of the 45 mg dose of nebulized sodium nitrite, and has stable systemic blood pressure and FEV_1 on presentation to the Day 7 visit, an electronic nebulizer will be used to deliver 90 mg of nebulized sodium nitrite. Subjects will be monitored closely for the next two hours, including percutaneous measurements of oxygen saturation and methemoglobin levels, recorded at minutes 15, 30, 60 and 120, and noninvasive systemic blood pressure measurements every 15 minutes. If percutaneous methemoglobin measurements exceed 5%, they will be confirmed by venous blood measurement. Spirometry will be repeated at 30 and 120 minutes following nebulized sodium nitrite administration. If nebulized sodium nitrite is tolerated, then the subject will be dispensed an 8 day supply of 90 mg nebulized sodium nitrite dosed two times daily and discharged from the CTRC, CHP or the Emphysema Research Center in Montefiore. If the 90 mg dose of nebulized sodium nitrite was not tolerated, the subject will be observed. If FEV_1 returns to baseline in 4 hours and subject's SaO2 is not greater than 5% drop from baseline and systolic BP is not greater than 40 mm Hg drop from pre-dose baseline, the subject will be re-challenged at this visit with a 45 mg dose of nebulized sodium nitrite and the observation protocol of blood pressure measurements, spirometry and percutaneous oxygen saturation measurement will be repeated.

Experimental Procedures (visit 3/day 14(+/- 1day)): This is a safety and tolerability visit and will take approximately 1 hour. Subjects will complete the CFQ-R and spirometry. An oral wash will be collected, and then a sputum sample will be collected. If the subject cannot spontaneously expectorate, then sputum induction will be done. The subject will then be discharged with a 15 day supply of nebulized sodium nitrite.

Experimental Procedures (visit 4/day 28(+/- 1day)): This visit will take approximately 1.5 hours. Subjects will complete the CFQ-R and spirometry. Expectorated or induced sputum will be collected, an oral wash will be collected, and FE_{NO} measured as above. Sputum will be used to measure nitrite concentration and quantitative bacterial load.

Experimental Procedure (visit 5/day 42(+/- 5day)): This visit will take approximately 1 hour. Subjects will complete the CFQ-R and have spirometry, an oral wash will be collected, expectorated or induced sputum will be collected, physical exam, vital signs (pulse, non-invasive blood pressure measurement, temperature, respiratory rate and oxygen saturation) and interval history assessment will be performed.

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 TREATMENT

The study treatment will be delivery of nebulized sodium nitrite by nebulization at the dose of 45 or 90 mg.

Table 1 Dose Limiting Toxicity Criteria

Sign or Symptom	Moderate	Severe[1]
Bronchospasm/ wheezing	Symptomatic but not requiring therapy	Symptomatic and requiring therapy
Decrease in FEV_1	>15%	>25%
SBP[2]	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[3]	SaO2 > 5% drop from baseline AND symptomatic	SaO_2 ≥ 10% drop from baseline or SaO_2< 88% AND symptomatic

Percutaneous methemoglobin[4,5]	>5% and <7%	≥7%
Other drug-related signs or symptoms[6]	Causes more discomfort (than a mild, easily tolerated AE) and interrupts the subject's usual daily activities OR CTCAE v.4[6] Grade 2 toxicity	Incapacitating and causes considerable interference with the subject's usual daily activities OR CTCAE v.4[6] Grade 3 or 4 toxicity

AE = adverse event; CTCAE = Common Toxicity Criteria for Adverse Events; DLT = dose limiting toxicity; SBP = systolic blood pressure; SaO_2 = oxygen saturation (hemoglobin)

1. Severe DLT seen in any subject which is deemed to be study drug-related will preclude further dosing with nebulized sodium nitrite for that subject.
2. 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 or dose escalation will be based upon follow up blood pressure measurements, subject symptoms and physician-investigator discretion (see Stopping Rules).
3. Moderate or Severe SaO2 without symptoms will be continuously monitored and recorded every 5 minutes until return of saturation > 92%.
4. 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.
5. 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.
6. 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.

2.4 DURATION OF FOLLOW-UP: All subjects will be followed for 14 days +/- a 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. The presence of severe DLT deemed to be study-drug related will lead to a discontinuation of the study treatment.

Following study drug treatment, subjects will be evaluated as an outpatient at the clinic on Day 42 (+/- 5 day window). Interval study visits (days 7, 14 and 28) have been included in the study design to ensure subject safety. 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 beginning of visit 1 through final study visit. Study subjects will be routinely questioned about adverse events at study visits. The study investigators will evaluate all subject reported and observed AEs. 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. AEs occurring pre-treatment, during active treatment and post-treatment will be summarized separately. 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

Two distinct scenarios in which subjects must be removed from the study are anticipated.

First, the subject experiences a CF respiratory exacerbation marked by a persistent drop in FEV1 >15% that is not temporally associated with nebulized sodium nitrite administration. The drop in FEV1 would be seen on the first set of spirometry at a visit, prior to dosing of nebulized sodium nitrite for that day. In this case, subjects will be removed from the study and referred to their pulmonologist for treatment of the CF respiratory exacerbation.

Drop in FEV1 >15% on the first set of spirometry, prior to drug administration, on any study visit when compared with baseline spirometry collected at screening.

In the second scenario, nebulized sodium nitrite is associated with a dose-dependent intolerance that will be seen at the dose escalation visit 2, or after receiving the first dose of drug on visit 1. Specific intolerances of concern are acute bronchospasm, hypotension or methemoglobinemia. In this case, if any one of the below occurs at the 45 mg dose, subject will be removed from the study; if any occur at the 90 mg dose, the subject will return to the previously tolerated dose of 45 mg.

Specific criteria for dose-dependent intolerance are:

Decrease in FEV1 by >15% between measurements on an individual study visit.

A decrease in systolic BP > 40 mmHg drop from pre-dose baseline or to <80mmHg and symptomatic.

SaO2 drop below 88% and symptomatic or SaO2 desaturation > 5% from baseline and symptomatic

A single occurrence of venous methemoglobin level >5%

Any serious adverse event thought to be possibly related to the study treatment

2.7 STUDY INVESTIGATIONAL THERAPY SUPPLIES

2.8.1 Formulation and Packaging

Formulation:

The active ingredient of the Sodium Nitrite Inhalation Solution (nebulized sodium nitrite Inhalation Solution) is sodium nitrite in a sterile aqueous solution approved for intravenous use. It is packaged at a 30 mg/mL in 10ml vials.

Packaging:

Vials will be purchased from Hope Pharmaceuticals and patients will withdraw the appropriate volume using a 3 mL sterile syringe with needle and place in nebulizer. Study subjects have extensive experience administering maintenance CF medications using this technique.

2.7.2 Availability

Nebulized sodium nitrite will be purchased from Hope Pharmaceuticals. We will ensure that enough compound is on site to complete the study prior to enrolling any individual subject

2.7.3 Preparing and Dispensing

The study drug will dispensed by the research pharmacist and the research coordinator will provide instructions for withdrawing the appropriate volume via a syringe with needle and delivering to the nebulizer device.

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 nebulized sodium nitrite placed into the nebulizer cup. We will be using Aerogen Solo nebulizers, as was done in the study by Simon, *et al* (17).

2.8 STUDY INVESTIGATIONAL THERAPY STORAGE

The investigational drug will be kept in its original packaging and stored in a locked, secure area at controlled room temperature (20 – 25º 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 STUDY INVESTIGATIONAL THERAPY 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

Screening: Potential subjects will be recruited from the CF Center at Children's Hospital of Pittsburgh and/or the UPMC Comprehensive Lung Center (CLC). In these locations, individuals with CF are followed on a routine basis, and well known to one or more of the study investigators. After informed consent is obtained, screening evaluations, including urine

pregnancy test (only on females of childbearing potential), physical examination, medical history, and clinical laboratory assessments, will be conducted to determine study eligibilities during a routine clinic visit. In addition, vital signs (pulse, non-invasive blood pressure measurement, temperature, respiratory rate and oxygen saturation) will be performed and baseline spirometry will be obtained. Screening is further discussed in Section 2.2.1.

Spirometry: FEV_1 will be measured with a handheld spirometer as the primary endpoint. Spirometry is performed for patients with CF at every outpatient visit, thus the subjects will have had experience performing spirometry since they were children. A baseline spirometry will be done as well as spirometry at 30 and 120 minutes following nebulization of nebulized sodium nitrite on days 0 and 7. Spirometry will also be performed at subsequent visits on days 14, 28 and 42.

Sputum Induction: Sputum induction will be performed per the CF Foundation Therapeutic Development Network SOP. See detailed protocol in appendix. In brief, the Sputum Induction (SI) procedure consists of four 3-minute cycles during which the participant breathes aerosolized 3% hypertonic saline and then expectorates sputum into a sample container. The participant's peak flow is monitored at the end of each cycle. As long as the peak flow remains ≥80% of pre-induction baseline, the cycle is repeated four (4) times. An optional fifth 3-minute cycle may be employed for those participants who have not yet expectorated an adequate sample and are tolerating the procedure. The complete procedure includes several preparatory steps, discharge monitoring, and clean up. For the participant, the entire sputum induction procedure from the time of initial assessment (vital signs and peak flow) to the end of the induction with associated monitoring is expected to last approximately 25-35 minutes. Total saline inhalation time should not exceed 15 minutes.

If subjects spontaneously expectorate, then sputum induction will not be done. After sputum induction, subjects will wait a minimum of 30 minutes prior to nebulization of nebulized sodium nitrite because sputum induction can cause temporary bronchoconstriction and this might confound the safety assessment of nebulized sodium nitrite. The amount of wait time will be determined by the physician investigator at the time of visit. Sputum induction will be done on Study days 0, 14, 28, and 42. If no further administration of nebulized sodium nitrite is planned during the visit (Days 14, 28, and 42), subjects may leave after sputum induction if they are not symptomatic.

Nebulization of Drug**:** nebulized sodium nitrite will be nebulized via electronic nebulizer with direct observation by a study physician or nurse coordinator on day 0, and at dose escalation by a study physician or nurse coordinator on day 7 in the CTRC, CHP or an Emphysema Research Center room in Montefiore. Subsequent doses will be self-administered by the subjects two times daily at home.

CFQ-R Questionnaire: Subjects will complete this standard and validated questionnaire on paper. Results of the CFQ-R will be confidentially stored electronically. Original paper will be maintained and stored in the locked Pulmonary Medicine Allergy and Immunology office.

NIOX device: Subjects exhale slowly through the device as guided by a small video screen with a smiling cloud icon. Disposable filters are used for each subject to minimize infection control concerns with the device. NIOX data will be recorded on days 0, 7 and 28.

Phlebotomy: Blood will be drawn by trained personnel at the screening visit (and day 0 if labs require repeating). Peripheral blood for confirmatory methemoglobin level will be obtained if percutaneous methemoglobin levels exceed 5%.

Vital sign measurements: Pulse, systemic blood pressure, temperature, respiratory rate and pulse oximetry will be measured noninvasively by trained study personnel using standard clinical equipment. All these values will be measured on screening visit and days 0, 7 and 42 (exit exam). On days 0 and 7 when nebulized sodium nitrite is being administered with observation, percutaneous measurements of oxygen saturation and methemoglobin levels will be measured for two hours after delivery. Noninvasive systemic blood pressure measurements will be taken every 15 minutes for the first two hours.

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

2.11 SCHEDULE OF ACTIVITIES

Study Phase	**Screening**	**Treatment**				**Follow-Up Monitoring**
Visit Type	Outpatient	Outpatient				
Study Day	**day -28 to -2**	**(visit 1) day 0 - +/- 1 day**	**(visit 2) day 7 - +/- 1 day**	**(visit 3) day 14 - +/- 1 day**	**(visit 4) day 28 - +/- 1 day**	**(visit 5) day 42 - +/- 5 day**
Informed Consent	x					
Medical History and Demographics	x					
I/E Criteria	x					
Tolerability Determination for Dose Escalation			x			
Physical Exam	x	x				x
Vital Signs (Pulse, systemic blood pressure, temperature, respiratory rate and pulse oximetry)	x	x	x			x
Non-invasive blood pressure measurement		x	x			
Oxygen Saturation		x	x			
Laboratory Tests***	x					
Urine Pregnancy Test Δ	x					
Spirometry	X	x	x	x	x	x
Sputum Collection		x		x	x	x
FeNO		x	x		x	
oral wash		x		x	x	X
Percutaneous metHgb Level§		x	x		x	
CFQ-R symptom assessment		x	x	x	x	x
AE Assessment		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 § Methemoglobin

(metHgb) levels: will be measured and recorded at the following time points: pre-dose, approx. 5, 15, 30, 60, and 120 minutes post each dose of nebulized sodium nitrite. If percutaneous measurements exceed 5%, then a venous blood sample will be drawn for confirmation. Peripheral blood for confirmatory methemoglobin level will be obtained 60 minutes after nebulization on study days 0 and 7 and for a final methemoglobin level on day 28.

2.12 DESCRIPTION OF STUDY PROCEDURES

2.12.1 Sputum Induction

Please see description in 2.10 above and in the SOP in the appendix.

2.12.2 Spirometry

Spirometry will be used to measure FEV_1. All CF subjects undergo spirometry at every outpatient visit, and have extensive experience with the procedure. A handheld spirometer will be used.

2.12.3 Oral Washes

Subjects will be asked to brush teeth with a clean, single use toothbrush. Next subjects will be given 10 ml of 0.9% salt water solution and instructed to swish and gargle for 1 full minute before spitting the salt water solution into a sterile specimen cup.

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_2 and methemoglobin levels under conditions of hypoxia and methemoglobinemia. SaO_2 and methemoglobin monitoring will occur prior to study drug administration until 2 hours post-dose. Measurements will be recorded pre-dose, approx. 5, 15, 30,60, and 120 minutes post each dose of nebulized sodium nitrite.

2.12.5 CFQ-R Questionnaire

Subject symptoms will be measured using the Cystic Fibrosis Questionnaire – Revised (CFQ-R), which is the best validated and most widely used disease specific instrument for measuring health-related quality of life in CF(20). The CFQ-R was used during evaluation of inhaled amikacin, inhaled aztreonam, and ivacaftor. The Minimal Clinically Important Difference for CFQ-R is 4.0 for stable patients and 8.5 during an exacerbation, thus a threshold of 4.0 point change will be used to define a meaningful change(21).

2.12.6 Exhaled NO measurements

FE_{NO} measurement will be done with a NIOX MINO device. This is a noninvasive test that involves the subject slowly exhaling through the measurement device for a 10-second period of time. The device is in widespread clinical use for the assessment of asthma severity.

2.12.7 Laboratory Testing Peripheral blood samples will be evaluated for evaluation of study eligibility and includes complete blood count with differential, platelets, electrolytes, glucose, BUN, serum creatinine, and liver function tests (total bilirubin, ALT, AST, alkaline phosphatase). Urine pregnancy test may be performed only on female of child-bearing potential at screening. 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. Except for urine pregnancy tests, 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.

2.12.8 Specimen collection and management

Specimen Collection / Documentation:

Oral washes: Washes will be collected as described, de-identified and frozen for future microbiome studies.

Sputum Collection and Handling: Sputum will be collected by spontaneous expectoration if possible, or induction with hypertonic saline if needed as described above. After de-identification, sputum samples will be divided for nitrite measurements, microbiome studies, and quantitative CFU measurements. Sputum for nitrite measurements and microbiome studies will be immediately frozen. Sputum for CFU measurements will be packaged for shipping to the CF Foundation Microbiology Core Laboratory at University of Washington.

Blood Collection and Handling: Venous blood for screening labs and methemoglobin levels will be drawn by trained personnel. Samples will be sent to the hospital laboratory for analysis. Remaining sample will be bar coded and stored.

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, sputum and oral wash 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 endpoint: The primary endpoint is FEV_1 to assess for bronchoconstriction induced by the study compound. We will compare FEV_1 before and at 30, and 120 minutes after dosing on day 0 with 45 mg dose, and day 7 with 90 mg dose. A sustained decrease in FEV1 > 15% with the 45 mg dose will be considered intolerability and the study subject will be removed from the protocol. If a subject tolerates one week of the 45 mg dose but has a sustained decrease in FEV1 after getting the first 90 mg dose, the subject will remain on the 45 mg dose for the duration of the study. If a subject experiences a CF respiratory exacerbation as defined by a drop in FEV_1 >15% on the first set of spirometry at any visit compared with baseline spirometry, then the subject will be removed from the study.

2.13.2 Secondary endpoints:

- FE_{NO} (exhaled NO)
- sputum nitrite concentrations
- CF Questionnaire-R subsection
- quantitative bacterial cultures for *P. aeruginosa* and/or *S. aureus*
- methemoglobin levels measured both non invasively and in whole blood
- systolic blood pressure

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
- Subject requires the use of new inhaled antibiotics between day -2 and study visit 4.
- 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.

2.14.2 WITHDRAWL CRITERIA

Study treatment will be discontinued in individual subjects during the day 0 inhalation if any of the following occurs regardless of symptoms:

- ***Decrease in FEV_1 by >15%*** between measurements on an individual study visit.
- A decrease in systolic BP > 40 mm Hg drop from pre-dose baseline or to <80mmHg and symptomatic;
- SaO2 drop below 88% and symptomatic or SaO2 desaturation > 5% from baseline and symptomatic
- A single occurrence of venous methemoglobin level >5%;
- Any serious adverse event thought to be possibly related to the study treatment

Any serious adverse event thought to be possibly related to the study treatment

If a study subject tolerates the 45 mg dose but develops hypotension, sustained > 15% decrease in FEV_1 (as measured at time points (30min and 120 min), or oxygen desaturation with the 90 mg dose on day 7, the subject will be observed. If after 4 hours of observation the subject's FEV_1 returns to baseline, the subject's SaO2 is not greater than 5% drop from baseline, and the subject's systolic BP is not greater than 40 mm Hg drop from pre-dose baseline, the subject will be re-challenged with the 45mg dose; if the lower dose is tolerated, the subject will remain in the study at the lower dose.

Subjects will be withdrawn if at any time the investigator feels that it is not in the subject's best interest to continue.

2.15 STATISTICAL ANALYSIS

2.15.1 Power Analysis and Sample Size:

The main adverse events reported in CF antibiotic trials are increased cough and change in respiratory symptoms. The overall subject driven discontinuation rate for these symptoms in both the placebo and treatment arms of recent antibiotics studies has ranged from 10-18%, although these symptoms do not necessarily correlate with bronchoconstriction (22, 23). Hypertonic saline is the most osmotically similar agent in use, and it causes 4.5% of children with severe CF lung disease to have an acute drop in FEV_1 of >15% (our stopping threshold) when used for sputum induction(24). Over a 4 week period, recent antibiotic studies have showed a decrease in FEV_1 of 3 to 6.5% in the placebo arms (19, 20, 22), so we are unlikely to have subjects reach the FEV_1 discontinuation threshold if nebulized sodium nitrite is safe but ineffective. Considering these data, we expect a 5-10% rate of discontinuation due to FEV_1 drop >15%, and we would consider up to 20% discontinuation acceptable. At a significance level of 0.05, a sample size of 33 is required to achieve 82% power to detect a difference of 0.14 using a one-sided binomial test, assuming that the population proportion under the null hypothesis is 0.06.

2.15.2 Safety Analysis

Adverse events (AEs) will be grouped by body system. 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. All adverse events experienced by subjects will be collected from beginning of visit 1 through final study visit. Study subjects will be routinely questioned about adverse events at study visits. The study investigators will evaluate all subject reported and observed AEs. 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. AEs occurring pre-treatment, during active treatment and post-treatment will be summarized separately.

2.15.3 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 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. No exclusion criteria shall be based on race, ethnicity or gender. Because cystic fibrosis is largely a disease of Caucasians, our pool of potential subjects is >95% Caucasian reflecting the incidence of the disease across various ethnic and racial groups.

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:

Subjects 18 years or older with cystic fibrosis as documented by clinical features of CF, and documentation of a positive sweat test or two diseasing causing mutations in the CF gene, will be eligible..

3.3 EXCLUSION CRITERIA:

FEV_1<40%, use of supplemental oxygen or non-invasive ventilatory support, awaiting lung transplant, pregnancy or unwilling to comply with birth control during the intervention period, history of glucose-6-phosphate dehydrogenase (G6PD) deficiency or any contraindication to receiving methylene blue, hospitalization within the past 4 weeks prior to enrollment, need for additional antibiotics or steroids within the past 4 weeks prior to enrollment, change in maintenance CF therapies in the prior 4 weeks, inability to provide consent, baseline systemic hypotension (systolic BP<90mm Hg), chronic kidney disease (Cr >2.5 or requiring dialysis), severe anemia (Hgb <9 gm/dL in the last six months), 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); history of abusing alcohol or illicit drugs within 28 days prior to study, severe pulmonary hypertension (mean PA pressure > 25), use of sildenafil or other pulmonary vasodilator, organ transplant of any kind, history of orthostatic hypotension or syncope, participation in another interventional trial in the previous 28 days.

4. RECRUITMENT AND INFORMED CONSENT PROCEDURES

4.1 RECRUITEMENT METHODS:

Patients will be recruited from the CF Center at Children's Hospital of Pittsburgh and the University of Pittsburgh Medical Center. A list of eligible patients will be generated using a query of the Pittsburgh CF Center patient registry and patients who meet the lung function and bacterial pathogen criteria will be approached in clinic by one of the physicians or nurse coordinators to discuss the study and review the informed consent. A consent form will be given to the individual for review. The investigator and/or research coordinator will answer any questions and review study procedures and the potential risks and benefits of the study. The subject will be given a copy of the consent form to take home and read and may be called in approximately one week by the department research coordinator to answer questions and assess interest in participation. If the subject would like to enroll themselves, a visit will be arranged and written informed consent will be obtained by the investigator (pulmonologist) after any questions are answered and before any study procedures are performed. Patients meeting all inclusion/exclusion criteria and providing informed consent will be enrolled.

4.2 INFORMED CONSENT PROCEDURES:

Subjects will be recruited from the cystic fibrosis clinics at Children's Hospital of Pittsburgh and the Lung Center at Falk Clinic. Potential subjects will be identified by querying the CF Registry (IRB# 0405219). Patients must have given their consent for participation in the CF Registry to be included. The study will be introduced to eligible subjects by their primary pulmonologist or the research coordinator. The subject, if interested, will speak to the research coordinator. The subject will be given a copy of the consent form to take home and read and may be called in approximately one week by the department research coordinator to answer questions and assess interest in participation. If the subject is interested in participation, a visit will be arranged and written informed consent will be obtained by the investigator (pulmonologist) after any questions are answered and before any study procedures are performed.

The subject will be told research is voluntary and they are not obligated to participate if they are not interested. The subject will be presented with information on all studies they may qualify for prior to assessing for interest in a particular study.

If circumstances arise which necessitate that new information be provided to a subject, relevant subjects will be verbally notified by clinical staff as soon as possible. PI will be available to answer questions. Verbal notification will be documented in the subject's research chart. Upon approval of a revised consent, if applicable, all relevant subjects will be re-consented using the same process as outlined above as soon as possible.

5. 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.

5.1.1 Risks of Sodium Nitrite Inhalation Solution:

The first-in-man clinical study of nebulized sodium nitrite 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 nebulized sodium nitrite 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(12).

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 nebulized sodium nitrite 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.

The Hope Pharmaceuticals package insert includes the following warnings and precautions: hypotension, methemoglobin formation, risk for interaction with carbon monoxide (in the setting of smoke inhalation), use in the setting of known anemia (causing further decreased oxygen carrying capacity in the setting of methemoglobinemia), G6PD Deficiency, and use with PDE5 inhibitors and diuretics. Adverse reactions listed include syncope, hypotension, tachycardia, palpitations, dysrhythmia, methemoglobinemia, headache, dizziness, blurred vision, seizures, confusion, coma, nausea, vomiting, abdominal pain, tachypnea, dyspnea, anxiety, diaphoresis,

lightheadedness, injection site tingling, cyanosis, acidosis, fatigue, weakness, urticaria, generalized body numbness and tingling. Note that these reactions were reported with intravenous formulation of sodium nitrite being used to treat life-threating cyanide poisoning, the total dose of nitrite given is 3-6 fold higher than in our study, and that the intravenous route may have more abrupt onset of effects. These adverse reactions are manifestations of systemic hypoperfusion or severe methemoglobinemia. The severe hypotension, methemoglobinemia, cardiac dysrhythmias and coma were reported in patients without life-threatening cyanide poising that were treated with intravenous doses less than twice those recommended for treatment of cyanide poisoning.

5.1.2 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 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.

5.1.3 Risks of sputum induction

Sputum induction has been a standard study intervention in patients with CF for almost ten years and is generally well tolerated. Sputum induction may be associated with any of the following symptoms: You may experience: a salt water aftertaste, coughing, a need to swallow, sore throat, shortness of breath, wheeze, chest tightness, lightheadedness, nausea, and headache. Patients will be asked to report any symptoms during the procedure, and measures to assess symptoms will be taken as described in the Standard Operating Procedure for sputum induction (see Appendix).

5.1.4 *Risks of withholding medications:*

Participants will not be asked to withhold medications during the study.

5.1.5 *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 or become pregnant, 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.

5.1.6 *Risks of Exhaled Nitric Oxide Measurement:*

Exhaled Nitric Oxide Measurement is a non-invasive test commonly performed on asthma patients. There are no significant risks. Subjects may experience coughing during the test.

5.1.7 *Risks of Spirometry:*

Subjects may experience brief mild shortness of breath, wheezing, an increase in cough, or lightheadedness while doing spirometry.

5.1.8 *Risks of Breach of Confidentiality*:

Participation in this study also exposes subjects to risks associated with medical record review, specimen banking, and the completion of the CF respiratory symptom questionnaire. Some subjects may feel the questionnaire is too personal. To minimize this risk, privacy will be assured during completion of the questionnaire. Review of medical records and specimen banking are associated with the risk of loss of privacy or confidentiality There is the possibility that if results of the medical record were generally known, the information may impact future insurability, employability, reproductive planning, or have a negative impact on family relationships. Note that the risk of breach of confidentiality exists during regular medical treatment. To minimize the risk of breach of confidential patient information we will do the following: All study visits are conducted in a private room. Samples will be bar coded as described above. Only IRB-approved staff will have access to any samples or information that is not de-identified. All research records will be kept in the locked office in the Pulmonary Medicine Allergy and Immunology office. Initials and identification number only will be used identify patients. All databases are password protected.

5.2 PROTECTIONS AGAINST RISK

5.2.1 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 study medications.
- Specific holding criteria related to study adverse events.
- Frequent monitoring of vital signs and spirometry over the duration of the study.

5.2.2 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 cystic fibrosis 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. All adverse events experienced by subjects will be collected from beginning of visit 1 through final study visit. Study subjects will be routinely questioned about adverse events at study visits. The study investigators will evaluate all subject reported and observed AEs. 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. AEs occurring pre-treatment, during active treatment and post-treatment will be summarized separately. 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**:
Two distinct scenarios in which subjects must be removed from the study are anticipated.

First, the subject experiences a CF respiratory exacerbation marked by a persistent drop in FEV1 >15% that is not temporally associated with nebulized sodium nitrite administration. The drop in FEV1 would be seen on the first set of spirometry at a visit, prior to dosing of nebulized sodium nitrite for that day. In this case, subjects will be removed from the study and referred to their pulmonologist for treatment of the CF respiratory exacerbation.

- Drop in FEV1 >15% on the first set of spirometry, prior to drug administration, on any study visit when compared with baseline spirometry collected at screening.

In the second scenario, nebulized sodium nitrite is associated with a dose-dependent intolerance that will be seen at the dose escalation visit 2, or after receiving the first dose of drug on visit 1. Specific intolerances of concern are acute bronchospasm, hypotension or methemoglobinemia. In this case, if any one of the below occurs at the 45 mg dose, subject will be removed from the study; if any occur at the 90 mg dose, the subject will return to the previously tolerated dose of 45 mg.

- Decrease in FEV_1 by >15% between measurements on an individual study visit.
- A decrease in systolic BP > 40 mmHg drop from pre-dose baseline or to <80mmHg and symptomatic
- SaO2 drop below 88% and symptomatic or SaO2 desaturation > 5% from baseline and symptomatic
- A single occurrence of venous methemoglobin level >5%
- Any serious adverse event thought to be possibly related to the study treatment

The 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.
- We will comply with the reporting of adverse events to the IRB per the Human Research Protection Office Policies and Procedures Manual.

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 as define below:

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.

5.5.6 Recording/Reporting requirements

5.5.6.1 Eliciting adverse event information

All adverse events experienced by subjects will be collected from beginning of visit 1 through final study visit. Clinical study subjects will be routinely questioned about adverse events at study visits.

5.5.6.2 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 casual relationship between the adverse event and the investigational drug or study treatment(s).

Adverse events or abnormal test findings felt to be associated with the investigational drug or 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.

5.5.6.3 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
- 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

Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.

5.5.6.4 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.

5.6 Reporting of adverse events

5.6.1 Reporting of adverse events to the FDA

5.6.1.1 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.

5.6.1.2 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.

5.6.2 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

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 DATA HANDLING AND RECORD-KEEPING

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 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

6.3.1 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 I/II 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.

6.3.2 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 Cystic Fibrosis Foundation and NIH. The Cystic Fibrosis Foundation supports the costs of subject payment, analysis of samples, procedures such as spirometry, and costs associated with use of the clinical research center. No study related costs will be billed to patients or their insurance providers.

7.1 PAYMENTS

Subjects will be paid as follows:
Screening $30
Visit 1 / Day 0 $150
Visit 2 / Day 7 $150
Visit 3 / Day 14 $50
Visit 4 / Day 28 $75
Visit 5 / Day 42 $50
In addition, subjects will also be reimbursed for parking and travel for study visits at the standard government rate.

8. QUALIFICATIONS AND SOURCE OF SUPPORT

8.1 QUALIFICATION OF 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.

Principal Investigator

Joseph Pilewski, MD is an Associate Professor of Medicine, Pediatrics, and Cell Biology, and Co-director of the Adult CF program in the CF Center at CHP, and has been principal investigator on numerous large clinical trials involving children and adults with CF as well as serving on the CF Foundation Therapeutics Development Network. Dr. Pilewski treats adult cystic fibrosis patients as part of his practice, and will aid with the recruitment and consenting process. He will also provide medical supervision for the studies being performed and will be extensively involved in the interpretation of the results of the study.

Sub-investigators:

Michael Myerburg, MD is an Assistant Professor in the Pulmonary, Allergy and Critical Care Medicine Division who has a clinical practice at the Cystic Fibrosis Center and conducts basic and clinical research related to CF. Dr. Myerburg sees adult CF patients as part of his practice, and he will aid with recruitment, consenting, and study execution.

Keven Robinson, MD is an Assistant Professor of Medicine in Pulmonary, Allergy and Critical Care Medicine Division who has joined Dr. Pilewski in his clinical practice at the Cystic Fibrosis Center. Dr. Robinson sees adult CF patients as part of her practice, and she will aid with recruitment, consenting and study execution.

Joel Weinberg, MD is Co-director of the CF Center and an adult CF clinician, and has experience as principal or co-investigator on numerous clinical trials for cystic fibrosis. He will aid with recruitment and consenting.

Anna Zemke, MD. PhD. is Assistant Professor of Medicine in Pulmonary, Allergy and Critical Care Medicine Division. She currently practices ICU medicine at UPMC East and UPMC Presbyterian-Shadyside.

8.2 SOURCE OF SUPPORT

Grant support from the Cystic Fibrosis Foundation and NIDDK P30 Core.

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