Mechanisms of exercise intolerance in Cystic Fibrosis: Role of PDE5 Inhibition

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A. HYPOTHESES AND SPECIFIC AIMS

Cystic Fibrosis (CF) is the most common fatal genetic disease in North America. The predicted median life expectancy age for patients with CF is 47.7 years compared to 78.8 years in the general U.S. population. **Exercise intolerance, evaluated as a reduction in exercise capacity (VO_2 peak), has been shown to predict mortality in patients with CF independent of lung function.** A **critical barrier** to improving exercise tolerance in CF is our lack of knowledge regarding the different physiological mechanisms which contribute to decreased exercise capacity. The present investigation will not only evaluate the impact that sildenafil has on clinically relevant and patient oriented outcomes, it will also provide mechanistic insight.

Phosphodiesterase type 5 (PDE5) inhibitors reduce inflammation, improve vascular health, increase microvascular O_2 delivery and improve skeletal muscle function. Accordingly, our **central hypothesis** is that treatment with the PDE5 inhibitor, sildenafil, can improve exercise capacity, vascular and cardiac function, and overall quality of life, all of which may contribute to improvement in exercise tolerance in people with CF (**Figure 1**). Drs. Harris and Taylor-Cousar have independently conducted small, single center investigations that have demonstrated the safety of PDE5 inhibition in patients with CF of varying lung disease severity, as well as the efficacy of this novel therapy to improve exercise capacity in CF. In addition, our proof-of-concept studies have provided compelling preliminary data to support that sildenafil 1) improves clinically important measures of exercise tolerance, cardiac function, and quality of life, 2) improves vascular endothelial function, 3) reduces both sputum and systemic inflammation, and 4) improves oxygen utilization and skeletal muscle function. Thus, the **overall goal** of this proposal is to evaluate the impact that sildenafil has on clinically relevant and patient oriented outcomes and to increase our understanding of the potential mechanisms which contribute to improvements in exercise capacity following PDE5 inhibition in people with moderate to severe CF lung disease. Using a double blind, placebo controlled experimental design; clinically relevant and experimental outcomes will be evaluated at baseline, and following 6, and 12 weeks of sildenafil therapy (40 mg, thrice daily) in patients with moderate to severe CF lung disease regardless of mutation or modulator therapy. We will test our central hypothesis with the following specific aims:

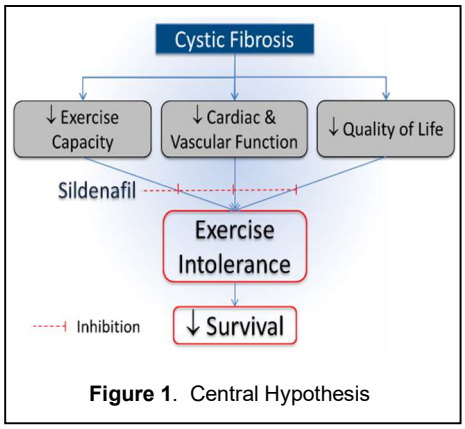


Figure 1. Central Hypothesis

Aim 1. Test the hypothesis that PDE5 inhibition improves clinically relevant and patient oriented outcomes in patients with moderate to severe CF lung disease. Exercise capacity [6MWD and complete cardiopulmonary exercise testing (CPET)]; **Aim 1A**), Pulmonary artery blood flow and right heart structure and function (**Aim 1B**), and the cystic fibrosis related quality of life (QOL)-revised (CFQ-R); **Aim 1C**) will be assessed at baseline and following treatment with sildenafil or placebo. Based on our preliminary data, we predict that sildenafil therapy will improve exercise capacity, cardiac strain (improve cardiac performance), and QOL.

Aim 2. Test the hypothesis that PDE5 inhibition will increase vascular endothelial function and contribute to an increase in exercise capacity in patients with moderate to severe CF lung disease. At each experimental time point, brachial artery flow-mediated dilation (FMD) will be determined as a bioassay of vascular endothelial function. To provide mechanistic insight into the action of sildenafil on exercise intolerance, plasma from each patient will be incubated with human aortic endothelial cells to determine the change in ex-vivo endothelial cell NOS3 expression following sildenafil or placebo administration. Based on our preliminary data, we predict that 12 weeks of sildenafil therapy will increase FMD through an increase in NOS3 phosphorylation.

Exploratory Aim. Test the hypothesis that PDE5 inhibition will improve skeletal muscle function and decrease pulmonary and systemic inflammation and contribute to an increase in exercise capacity in patients with CF. At each experimental time point, indices of gas exchange (O_2 uptake kinetics, expired CO_2, V_E/VO_2, V_E/VCO_2) will be determined and spontaneous or induced sputum will be collected. NOS2 expression will also be determined in nasal epithelial cells. Based on our preliminary findings, we predict that sildenafil will 1) improve skeletal muscle function and 2) decrease both lung and systemic inflammation, both of which may contribute to improvements in exercise capacity in CF. Furthermore, we predict that sildenafil will decrease NOS2 expression in nasal epithelial cells.

B. BACKGROUND AND SIGNIFICANCE

B1. OVERVIEW. Cystic fibrosis (CF) is the most common, life-shortening, inherited disease in Caucasians, affecting approximately 30,000 people in the United States[1]. Perhaps most disturbing, the predicted median age of survival for patients with CF is 47.7 years compared to 78.8 years in the general U.S. population. Due to the ubiquitous expression of the mutated cystic fibrosis transmembrane conductance regulator (CFTR) gene in many different tissues, patients with CF not only present with pulmonary dysfunction but with systemic manifestations of disease, including those of the gastrointestinal, immune, endocrine, and musculoskeletal systems. In addition, a CF community survey performed through research voice demonstrated that **47% of people with CF and their families were interested in research related to the manifestation of exercise intolerance**.

B2. EXERCISE INTOLERANCE CONTRIBUTES TO EARLY MORTALITY IN CF.

Exercise capacity is an objective measure of exercise tolerance. Over 25 years ago, exercise capacity was shown to predict mortality in patients with CF, independent of lung function[2], and higher exercise capacity is a marker for longer survival in this patient population[3]. Even in the setting of early pulmonary disease, exercise intolerance is a prevalent phenotype in patients with CF[4]. Thus, it is important to emphasize that **decreases in lung function (FEV_1) do not always explain reductions in VO_2 peak[5].**

Due to the prognostic value in CF, routine exercise tests to evaluate exercise capacity are now recommended by the European Respiratory Society (ERS) and the Cystic Fibrosis Foundation (CFF)[6]. **Lower exercise capacity is associated with greater mortality, steeper decline in pulmonary function, reduced quality of life, and an increased number of pulmonary exacerbations**[7]. There are a number of factors that have been hypothesized to play a role in exercise intolerance including ventilatory limitation, poor nutritional status, systemic inflammation, direct effects of CFTR mutations, decreased muscle mass, and peripheral muscle dysfunction[8-10]. However, a **critical barrier** to improving exercise capacity in patients with CF is our lack of knowledge regarding the different physiological mechanisms that contribute to exercise intolerance.

B3. EVIDENCE FOR CARDIAC AND VASCULAR DYSFUNCTION IN CF.

B3.1. Cardiac Dysfunction Is Present In CF And May Lead To Exercise Intolerance. Despite substantial improvements in care, there is a clear link between right heart failure and high mortality in CF[11]. However, even in the setting of mild to moderate pulmonary disease, exercise intolerance is observed in patients with CF[4]. Increasing evidence implicates the contribution of right ventricular (RV) structural changes on diminished functional capacity in patients with airway disease[12]. At autopsy, the pulmonary circulation in patients with CF is often characterized by the presence of RV hypertrophy with abnormal muscularization of medium and small pulmonary arteries[13]. Recently, increased right ventricular torsion and strain have been identified in cystic fibrosis transmembrane conductance regulator (CFTR) knockout mice[14]. Characterization of dysfunctional RV mechanics and their contribution to exercise intolerance in patients with CF is unexplored, but could be evaluated through the proposed combination of exercise testing (CPET) and cardiac magnetic resonance (CMR) imaging.

B3.2. Vascular Dysfunction Is Present In CF And May Lead To Exercise Intolerance. Several studies have shown that endogenous production or bioactivity of NO is impaired in the airways of patients with CF. Animal and clinical data have shown 1) marked down regulation of inducible NO synthase (NOS) in airway epithelium, 2) a strong association between severe lung disease and endothelial NOS polymorphisms, 3) increased degradation of NO metabolites, and 4) decreased amounts of NO in exhaled breath condensate of patients with CF[15-19]. Thus, CF lung disease can be characterized by a relative NO deficiency with down-regulation of the NO-cGMP signaling cascade. More recently, our group was the first to describe both conduit[20] and microvascular endothelial dysfunction[21] in people with CF who exhibit preserved spirometric function. Naturally, impaired nutritive blood flow to the working musculature during exercise is suggested to contribute to exercise intolerance in several clinical populations[22, 23]. In support, we have recently published that patients with CF exhibit impaired blood flow regulation compared to controls and this impairment was proportional to exercise capacity and inversely related to oxidative stress[24]. **Whether or not changes in endothelial function contribute to changes in exercise capacity in CF has yet to be explored.**

B4. CLINICAL RELEVANCE OF PATIENT ORIENTED OUTCOMES IN CF.

The CFQ-R, the associated scores of which correlate with survival in CF[25], is designed to measure CF-specific patient-reported health-related quality of life. The CFQ-R has been validated in CF, and the minimally clinically important difference for the respiratory score has been established as 4[26, 27]. Importantly, the Federal Drug Administration expects that benefits of a new therapy including improvement in how a patient survives, functions or feels. A patient reported outcome measure such as the CFQ-R score fulfills this expectation.

B5. INFLAMMATION AND SKELETAL MUSCLE DYSFUNCTION ARE COMMON PHENOTYPES IN PEOPLE WITH CF.

B5.1. Systemic Inflammation Is Present In CF And May Lead To Exercise Intolerance. Although debate exists between the cause or consequence[28], it is clear that chronic inflammation contributes significantly to the progression of CF disease. Even in patients with mild asymptomatic disease, neutrophil count, active elastase, and immunoglobulin levels are substantially higher compared to normal controls[29, 30]. Elevated systemic inflammation increases oxidative stress which can impair blood flow regulation and reduce skeletal muscle function[31, 32]. Consequently, it is reasonable to believe that greater inflammation in CF reduces exercise capacity. In fact, a negative relationship between C-reactive protein (CRP) and exercise capacity has been reported in CF[33] and compelling preliminary data from our group indicates that patients with elevated systemic inflammation have lower exercise capacity (**Figure 9**).

B5.2. Skeletal Muscle Dysfunction Is Present In CF And May Lead To Exercise Intolerance. Numerous studies, including published data from our team[34], have described that patients with CF exhibit intrinsic skeletal muscle defects, including mitochondrial dysfunction[35-38], independent of muscle mass, lung function, or physical activity status[39-44]. The manifestation of these skeletal muscle impairments in patients with CF has been closely related to the abnormal function of CFTR in muscle tissue[43, 45] that is not observed in other respiratory pathologies[43, 45, 46]. In addition, we have previously demonstrated that young patients with CF have impaired skeletal muscle oxygen uptake kinetics[47], which has recently been proposed as a mechanistic link to impaired muscle oxygen extraction and utilization[48]. Unequivocally, mitochondrial dysfunction coupled with impaired oxygen extraction and utilization would reduce exercise capacity; a likely scenario in people with CF that will be tested in the current proposal.

B6. PDE5 INHIBITION OFFERS MULTIPLE MECHANISMS TO IMPROVE EXERCISE CAPACITY IN CF.

*S*ildenafil, a commonly used PDE5 inhibitor, increases exercise capacity in Fontan patients[49], systolic heart failure[50] and congestive heart failure[51]. However, work using PDE5 inhibitors in patients with CF is scant and a recent review on the topic highlights the **potential** use for PDE5 inhibitors in CF treatment[52].

B6.1. PDE5 Inhibition Improves Vascular Function. The majority of studies evaluating sildenafil are focused on the vascular system. In fact, chronic PDE5 inhibition is routinely shown to improve endothelial function (in as little as 4 weeks) in patients with vascular dysfunction[53-55]. A recent publication from our team demonstrates that 4 weeks of sildenafil can significantly improve vascular endothelial function in patients with CF that exhibit mild to moderate disease severity (**Figure 6**)[56]. In support of our hypothesis, the improvement in endothelial function is associated with an increase in exercise capacity (**Figure 7**).

B6.2. PDE5 Inhibition Decreases Inflammation. Although the exact role of NO signaling in the CF airway is unclear, several studies have suggested that the NO-cGMP cascade plays an important role in airway inflammation and bacterial infection[57, 58]. Pretreatment with sildenafil inhibits LPS-induced airway hyper reactivity, white cell influx, and NO dysfunction in two guinea pig models of airway disease[59]. In addition, the excessive pro-inflammatory response to *P. aeruginosa* exposure in CF respiratory epithelial cells could be reversed by treatment with sildenafil[57]. Preliminary data from our team demonstrates that sildenafil reduces sputum elastase (**Figure 10**), which is a sensitive measure of CF airway inflammation and has been shown to predict lung function decline in patients with CF[60]. In addition, preliminary data from our team demonstrates that sildenafil can also reduce systemic pro-inflammatory cytokines (**Figure 11**).

B6.3. PDE5 Inhibition Improves Skeletal Muscle Function. In otherwise healthy men, daily administration of sildenafil increases skeletal muscle protein synthesis and reduces fatigue[61]. In addition, sildenafil not only enhances proliferation of skeletal myoblasts and increases Ca^{2+} availability in skeletal myotubes[62], it improves exercise O_2 uptake kinetics in patients with heart failure[63]. For the first time in patients with CF, preliminary data from our group demonstrates that 4 weeks of sildenafil improves oxygen uptake kinetics and skeletal muscle O_2 extraction (**Figures 12 & 14**), improvements that are related to increased exercise capacity.

B7. INNOVATION AND CLINICAL RELEVANCE

An understanding of the etiology of exercise intolerance and its treatment **is a significant unmet need** in people with CF lung disease. Innovation in this proposal is evident in the topic of the research, the concepts and hypotheses to be tested, and the approaches to be used. This proposal:

- represents **a paradigm shift and focuses on non-pulmonary factors** related to CF lung disease.
- **provides insight into potential mechanisms** that contribute to exercise intolerance in people with CF.
- is based on the combined concepts and rationale established in cell, animal, and our own pilot studies, and therefore demonstrates the **true model of translational research**.

- would be **the first study** to rigorously investigate the therapeutic potential and offer mechanistic insight of PDE5 inhibition to improve exercise capacity in people with CF.
- incorporates near infrared spectroscopy on the vastus lateralis coupled with the patented technology of the Physioflow Enduro provides a **state-of-the-art,** non-invasive methodology to evaluate skeletal muscle function **during exercise** in people with CF.
- **utilizes oral administration of sildenafil which is mutation agnostic and would not significantly increase the therapy burden for patients with CF.** Sildenafil offers the advantage of a drug that is already FDA-approved. It can be administered, independent of mutation, to people with CF on modulators and even more importantly, **to the last 7-10% of patients who are unlikely to have access to a highly effective modulator in the near future.** In addition, given the significant burden of care that impacts the lives of CF patients[64, 65], and continues to substantially increase over time[66], the use of an oral medication, rather than an inhaled one, is more desirable to modify disease and/or combat symptoms.

C. PRELIMINARY STUDIES

In this section, we provide the essential proof of concept support for the current proposal followed by strong preliminary data that supports the rationale and feasibility of the hypothesis to be tested in each specific aim.

SILDENAFIL IS SAFE AND EFFECTIVE IN MILD TO SEVERE CF LUNG DISEASE: Drs. Harris and Taylor-Cousar each have FDA INDs (INDs #102639 and #119923) for use of sildenafil in people with CF. Our team has published the **tolerability and safety of both 20 mg and 40 mg of sildenafil thrice daily[67]**. In Dr. Taylor-Cousar's published pilot study, 20 patients with mild-moderate CF were given either 20 or 40 mg of sildenafil TID for 6 weeks. Side effects were generally mild and consistent with the product insert. There were no drug-related SAEs and **this study was the first to demonstrate that sildenafil was safe and well tolerable in patients with CF**. Importantly, the safety review of Dr. Taylor-Cousar's completed study in moderate to severe CF lung disease presented at NACFC showed no safety concerns. In Dr. Harris' study, 20 mg of sildenafil TID for 4 weeks was given in mild to moderate CF lung disease. Treatment was also tolerated well. These 2 pilot studies provide the foundation for the proposed investigation to provide additional insight into the potential mechanisms which contribute to the improvement in exercise capacity following treatment with sildenafil in CF.

Aim 1. Test the hypothesis that PDE5 inhibition improves clinically relevant and patient oriented outcomes in patients with moderate to severe CF lung disease.

C1.1. SILDENAFIL TREATMENT IMPROVES EXERCISE CAPACITY IN PEOPLE WITH CF.

New compelling preliminary data from our team support the efficacy of sildenafil to improve indices of exercise capacity in patients with CF. In Dr. Taylor-Cousar's pilot study of patients with moderate to severe CF pulmonary disease (mean ppFEV$_1$ was 59.2 [range 35-68]), **Figure 2** illustrates the 25 m improvement in 6MWD (25.16 [SD 18.78]) following 12 weeks of sildenafil therapy. **Figure 3** illustrates the significant improvement in exercise capacity in patients with CF following Dr. Harris' 4 weeks of sildenafil.

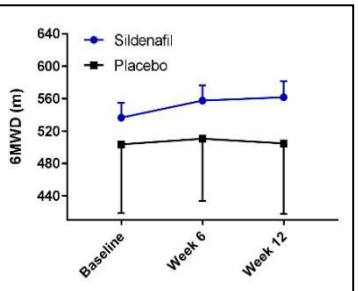


Figure 2. Progressive increase in exercise capacity (6MWD) throughout 12 weeks of sildenafil in patients with CF. Extreme outlier removed from placebo.

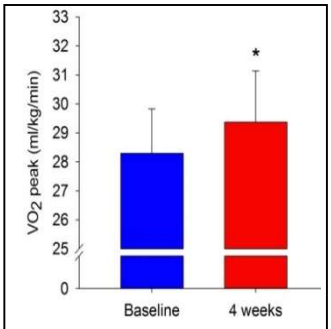


Figure 3. Significant increase in VO_2 peak following 4 weeks of sildenafil in patients with CF.

C1.2. SILDENAFIL TREATMENT IMPROVES GLOBAL LONGITUDINAL STRAIN (CARDIAC FUNCTION).

In Dr. Taylor-Cousar's pilot study of patients with moderate to severe CF pulmonary disease cardiac function was evaluated using cardiac MRI. **Figure 4** illustrates the cardiac images and the calculated strain from those images. (More negative strain corresponds with improved cardiac performance.) Following 12 weeks of sildenafil, the global cardiac strain decreased (-4.77±5.5) in the sildenafil group and slightly increased in the placebo group (1.44±4.59).

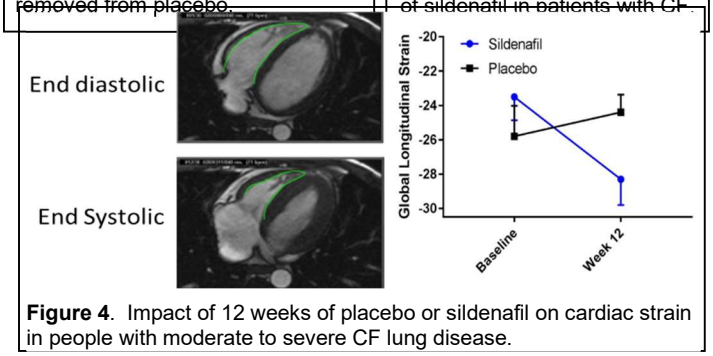


Figure 4. Impact of 12 weeks of placebo or sildenafil on cardiac strain in people with moderate to severe CF lung disease.

C1.3. SILDENAFIL TREATMENT IMPROVES PATIENT REPORTED RESPIRATORY QUALITY OF LIFE DOMAIN.

In Dr. Taylor-Cousar's pilot study of patients with moderate to severe CF pulmonary disease the CFQ-R was administered during week 1, and 6 weeks and 12 weeks following either sildenafil or placebo. **Figure 5** illustrates the 8.6 point improvement in the CFQ-R respiratory domain following sildenafil and the -9 point reduction following placebo. Importantly, this improvement was greater than what was observed during the Orkambi® clinical trial[68].

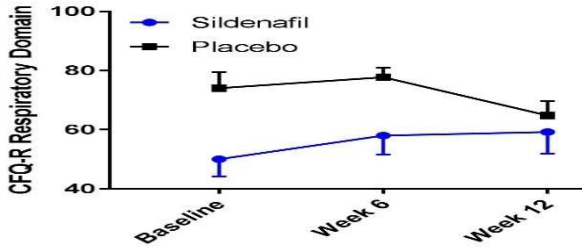


Figure 5. Impact of 12 weeks of placebo or sildenafil on CFQ-R respiratory domain in patients with CF.

Aim 2. Test the hypothesis that PDE5 inhibition will increase vascular endothelial function via greater endothelial nitric oxide synthase (NOS3) expression and contribute to an increase in exercise capacity in patients with CF.

C2.1. SILDENAFIL TREATMENT IMPROVES VASCULAR ENDOTHELIAL FUNCTION. In Dr. Harris' 4-week open label study, ultrasonic assessment of endothelial function using the FMD test was determined at baseline and following treatment. **Figure 6** illustrates a significant increase in FMD following 4 weeks of sildenafil, data that were recently published[56]. In addition, a positive association between the change in endothelial function (ΔFMD) and the change in exercise capacity were observed controlling for baseline vascular function (**Figure 7**). These data support the role of sildenafil to improve vascular health and contribute to an increase in exercise capacity in CF.

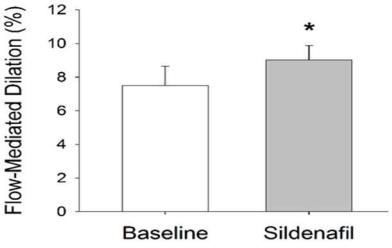


Figure 6. Significant increase in flow-mediated dilation following 4 weeks of sildenafil therapy.

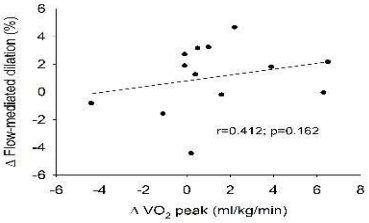


Figure 7. Positive relationship between the change in flow-mediated dilation and the change in exercise capacity.

C2.2. SILDENAFIL TREATMENT INCREASES ENDOTHELIAL CELL EXPRESSION OF NOS3. As described in detail below (section D17), human aortic endothelial cells were incubated with plasma taken from the 15 patients with CF at baseline and following 4 weeks of open-label sildenafil treatment. **Figure 8** illustrates a significant increase in phospho-NOS3 (panel A) with plasma treated with sildenafil and no change in NOS3 protein (panel B); resulting in a significant increase in the ratio of pNOS3:NOS3 (panel C). These recently published[56] molecular data provide mechanistic insight into how sildenafil can increase NO bioavailability (i.e. increase in NOS activation) and impact vascular endothelial function and exercise capacity in patients with CF.

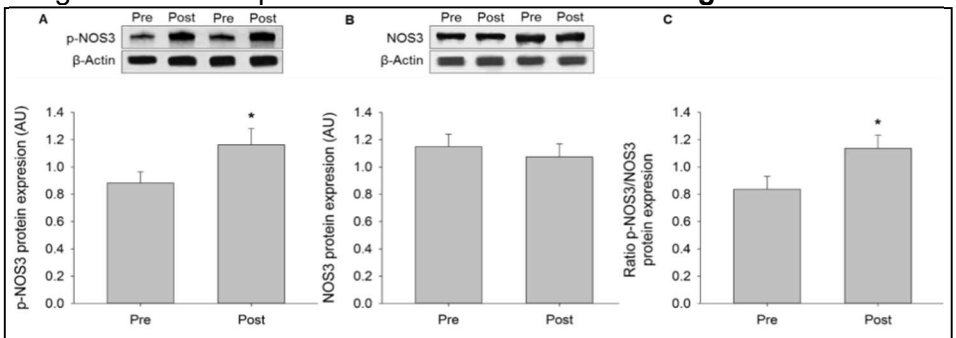


Figure 8. Pre and post 4 weeks of sildenafil for **A**. representative Western blot and mean phosphorylated NOS3 (serine 632), **B**. representative western blot and NOS3, and **C**. ratio of phospho-NOS3 and total NOS3. Protein loading was verified by β-actin. *Significant from pre.

Secondary/Exploratory Aim. Test the hypothesis that PDE5 inhibition will improve skeletal muscle function and decrease pulmonary and systemic inflammation and contribute to an increase in exercise capacity in patients with CF.

C3. PATIENTS WITH ELEVATED INFLAMMATION HAVE LOWER EXERCISE CAPACITY. Using between group, cross-sectional analysis, the impact of exercise capacity level on circulating hsCRP was evaluated in 33 patients with mild to moderate CF lung disease. **Figure 9** illustrates that patients with an exercise capacity (VO_2 peak) greater than 65% predicted have a lower CRP compared to those patients with a VO_2 peak <65% predicted. Although these data cannot confirm a causation, they do provide support that inflammation may contribute to exercise intolerance in people with CF.

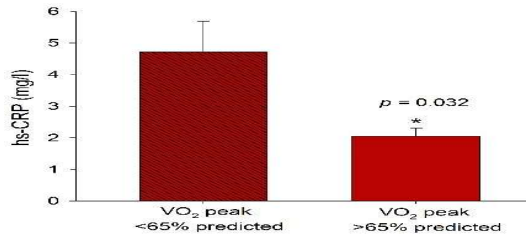


Figure 9. Patients with a higher VO_2 peak exhibit lower CRP compared to those patients with a lower exercise capacity.

C3.1. SILDENAFIL TREATMENT DECREASES SPUTUM ELASTASE IN PATIENTS WITH CF. In an open label study, 20 patients with CF were treated with sildenafil (40 mg TID) for 6 weeks. Spontaneous or induced sputum (as per the CF TDN research protocol) expectoration was collected and placed on ice and sent overnight to the CF TDN Inflammatory Mediator Core Laboratory (Aurora, CO) for batch analysis of active elastase as previously described[69]. **Figure 10** illustrates a significant reduction in sputum elastase following 6 weeks of sildenafil.

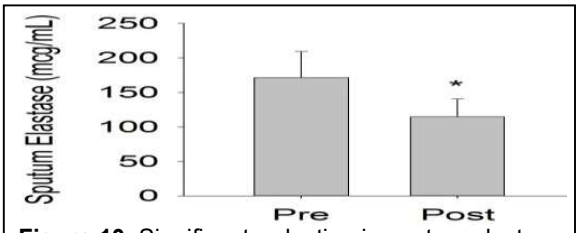


Figure 10. Significant reduction in sputum elastase following 6 weeks of sildenafil in 20 patients with CF.

C3.2. SILDENAFIL TREATMENT DECREASES BIOMARKERS OF SYSTEMIC INFLAMMATION IN PATIENTS WITH CF. Using an open-label proof-of-concept design, 15 patients with CF were treated with sildenafil (20 mg TID) for 4 weeks. Circulating concentrations of inflammatory biomarkers were assessed at baseline and following treatment. **Figure 11** below illustrates the tendency for sildenafil to reduce circulating concentrations of TNF-α (panel A), IL-1β (panel B), and IL-6 (panel C).

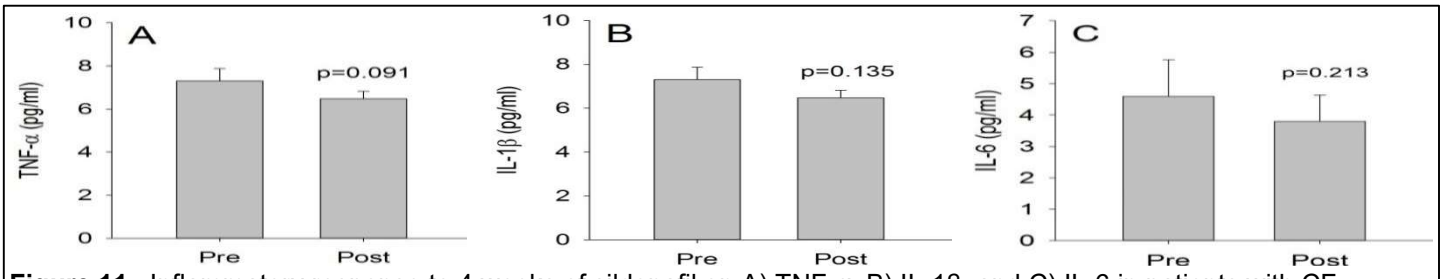


Figure 11. Inflammatory response to 4 weeks of sildenafil on A) TNF-α, B) IL-1β, and C) IL-6 in patients with CF.

C3.3. SILDENAFIL IMPROVES MUSCLE OXYGEN EXTRACTION DURING MAXIMAL EXERCISE IN CF.

Following 4 weeks of sildenafil therapy in patients with CF, muscle oxygen extraction (mO_2 extraction) during exercise was determined using a derivation of the Fick equation and calculated as mO_2EX = 100- [SpO_2 – VO_2 / (13.9 x CO x Hb)]. **Figure 12** illustrates the significant increase in mO_2 extraction following sildenafil therapy. Additionally, the change in mO_2 extraction following treatment was significantly associated with the change in exercise capacity (**Figure 13**). These data not only support the efficacy of sildenafil to improve skeletal muscle function, they demonstrate that the increase in O_2 extraction may contribute to improvements in exercise capacity.

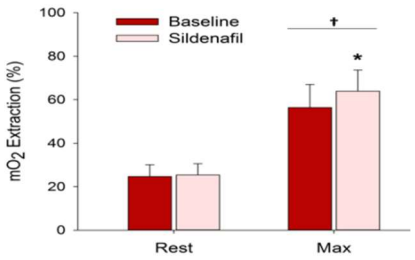


Figure 12. Muscle oxygen extraction at rest and during maximal exercise at baseline and following sildenafil treatment. *Significant from baseline; † Significant from rest.

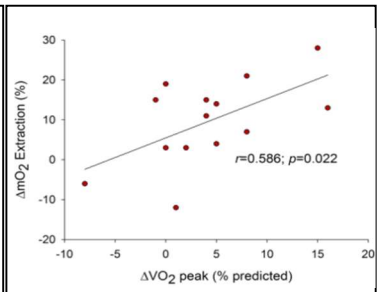


Figure13. Significant relationship between the change (Δ) in mO_2 and the change in exercise capacity (VO_2 peak) following sildenafil treatment.

C3.4. SILDENAFIL IMPROVES MUSCLE FUNCTION DURING EXERCISE. Near infrared spectroscopy (NIRS) was placed over the vastus lateralis to measure changes in skeletal muscle O_2 concentrations and consumption during exercise as previously described[70-72]. **Figure 14** illustrates an individual representation of the concentration changes of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb) in the vastus lateralis during a maximal cycling exercise test in A) a healthy control, B) a patient with CF, and C) the same patient treated with sildenafil for 4 weeks.

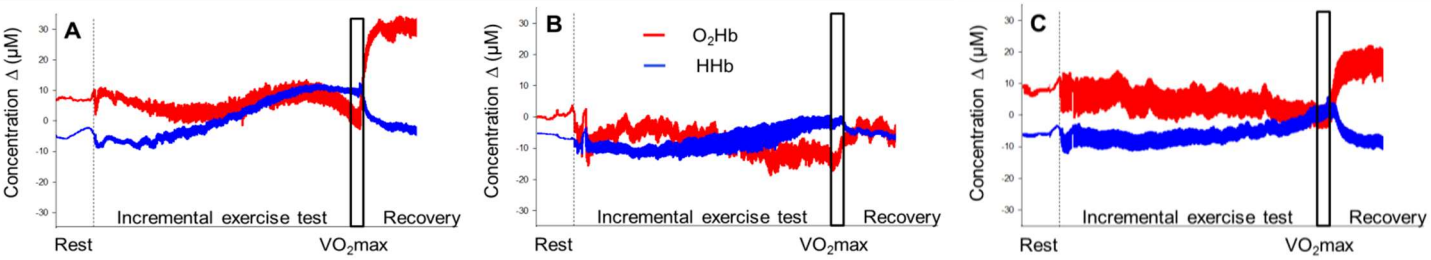


Figure 14. An individual representation of the concentration change response of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (*HHb*) throughout an incremental cycling exercise test in A) a healthy control, B) a patient with CF, and C) a patient with CF following 4 weeks of sildenafil.

Importantly, 1) despite similar SpO_2, resting concentrations of O_2Hb are higher in the control (Panel A) compared to the patient (Panel B), and 2) the hyperemic reperfusion response during recovery is impaired in the patient compared to the control. Four weeks of sildenafil 1) restores resting concentrations of O_2Hb, 2) delays the increased concentration change of HHb, and 3) restores the rebound reperfusion response during recovery.

D. Experimental Design and Methods

D1. Overview This section is organized to provide a complete overview of our experimental design (**Figure 15**) and the particular details for each specific aim. Based on our proof-of-concept pilot studies using sildenafil in CF, we are proposing a randomized, double blind, placebo controlled investigation to evaluate the potential of sildenafil to improve exercise tolerance and other clinically relevant outcomes in CF and provide mechanistic insight into the improvement in exercise capacity. Patients will be randomized in a 1:1 (sildenafil:placebo) fashion, and exercise capacity, cardiac and vascular health, quality of life, inflammation, and skeletal muscle function will be investigated at the same time of day during baseline, 6 weeks (interim assessment), and 12 weeks following treatment. The length of participation for each patient will be ~16 weeks and will consist of a screening visit, dose initiation and escalation visits, 3 experimental testing days, and 3 phone call evaluations.

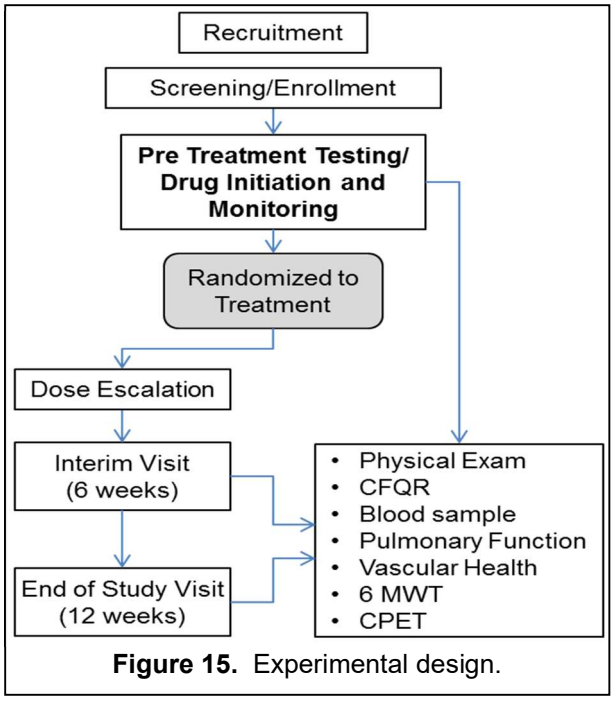


Figure 15. Experimental design.

D3. Study Participants. A total of 40 clinically stable patients with moderate to severe CF lung disease ≥ 9 years of age will be recruited and enrolled from the pediatric and adult outpatient CF clinics at Augusta University and National Jewish Health. Both children and adults of any CFTR genotype will be included. All patients will be instructed to maintain their daily routine for airway clearance, inhaled therapies and exercise. Patient safety is a priority; therefore inclusion/exclusion criteria will be based on disease severity, past medical history, microbiology, and current medications.

Inclusion Criteria

- Confirmed diagnosis of CF based on the following criteria: Positive sweat chloride concentration ≥60mEq/liter (by pilocarpine iontophoresis) and/or genotype with two identifiable disease-causing mutations consistent with CF, and accompanied by one or more clinical features consistent with the CF phenotype
- Male or female patients ≥ 9 years of age
- FEV_1 ≥ 30% predicted and ≤ 80% for patients ≥ 18 years of age and ≤ 85% for patients < 18 years of age

- Clinically stable without evidence of acute upper or lower respiratory tract infection or current pulmonary exacerbation within the 14 days prior to the screening visit
- Resting oxygen saturation (room air) ≥85%
- Patients with or without CF related diabetes
- Ability to perform spirometry reproducibly (according to ATS criteria)
- Willingness to maintain chronic CF medication schedule (e.g. alternating month inhaled antibiotics)

Exclusion Criteria:

- Children 8 yrs. old and younger
- Subjects who weigh < 20 Kgs
- History of hypersensitivity to sildenafil
- Use of an investigational agent within the 4-week period prior to Visit 1 (Day 0)
- Breastfeeding, pregnant, or verbal expression of unwillingness to practice an acceptable birth control method (abstinence, hormonal or barrier methods, partner sterilization or intrauterine device) during participation in the study for women of child-bearing potential.
- History of significant hepatic disease (AST or ALT > 3 times the upper limit of normal at screening, documented biliary cirrhosis, or portal hypertension),
- History of significant cardiovascular disease (history of aortic stenosis, coronary artery disease, or life-threatening arrhythmia),
- History of severe neurological disease (e.g. history of stroke),
- History of severe hematologic disease (e.g. history of bleeding diathesis; current INR > 2.0
- History of severe ophthalmologic disease (e.g. history of retinal impairment or non-arteritic ischemic optic neuritis)
- History of severe renal impairment (creatinine >1.8 mg/dL.)
- Inability to swallow pills
- Previous organ transplantation
- Use of concomitant nitrates, α-blocker, or Ca channel blocker (currently or within one month of Visit 1)
- Use of concomitant medications known to be potent inhibitors of CYP3A4 [e.g. ketoconazole, itraconazole, ritonavir, clarithromycin, erythromycin, rifampin (currently or within one month of initiation of study drug)] NOTE: use of azithromycin is NOT a cause for exclusion
- History of sputum or throat swab culture yielding *Burkholderia cepacia* or ***Mycobacteria massiliense*** within 2 years of screening
- History of migraine headaches.
- Presence of a condition or abnormality that in the opinion of the investigator would compromise the safety of the subject or the quality of the data
- Initiation of CFTR modulator therapy less than 1 month prior to first dose of sildenafil or placebo
- Use of anticoagulants
- Frank pulmonary hypertension (RVSP >45 mm Hg by echocardiography)

D3.1. Additional Enrollment Considerations. People on CFTR modulators may participate if they have been on a stable dose for at least 4 weeks prior to study drug initiation. It is possible that circulating sildenafil levels would be decreased because of the effects of lumacaftor/ivacaftor on sildenafil metabolism; however, we are using the 40 mg TID dosing for this study and will check sildenafil levels at the interim and end of study visits. There was no impact on outcomes in people with CF who were taking modulators versus those who were not taking modulators in Dr. Taylor-Cousar's study in people with moderate to severe lung disease. Patients who do not meet the enrollment criteria at the time of the screening visit may be re-screened at a later date at the discretion of the site investigator.

D4. Rationale For 40 mg TID Drug Dose/Frequency. The approved/standard dosing of sildenafil for pulmonary vascular disease (marketed as Revatio® for this indication) is 20 mg p.o. TID. In the pivotal study of PH patients without CF, doses up to 80 mg p.o. TID were given without improvement in the primary outcome measure (6MWD). Our pilot study identified a modest, yet significant increase in exercise capacity following 20 mg TID.

However, in our team's published pilot study evaluating sildenafil as an anti-inflammatory in patients with mild-moderate CF, **10/12 of the subjects who had a decrease in sputum elastase were in the 40 mg group**. Although there was more flushing and rhinorrhea in the 40 mg group, there were no drug related serious adverse events nor an increased rate of discontinuation in the 40 mg group. There were no study drug related discontinuations in Dr.Taylor-Cousar's study in moderate-severe CF disease. Furthermore, we did estimate an increased rate of sildenafil elimination in people with CF compared to healthy controls. Accordingly, based on the combined safety, efficacy and pharmacokinetic data from our preliminary investigations, and the likelihood of a higher dose having a greater impact, we have chosen to use the 40 mg p.o. TID dosing for this study. Dosing in children is described in the Protection of Human Subjects.

D5. Study Medication, Randomization, and Distribution Plan. Subjects will be randomized to treatment or placebo groups. Study statistician, Dr. Doug Everett will generate a random sequence number for each participant which he will provide directly to the central pharmacy. Investigators, subjects, and laboratory personnel will be blinded to the treatment. Blinded study drug kits will be packaged, labeled and shipped from a contracted central pharmacy based on sequence number. The study site pharmacist must receive the study drug, record its receipt, and assure that the study drug is handled properly and stored safely. An accurate accounting of dispensing and return of the study drug will be maintained by the research pharmacist at each site. Subjects will be instructed to return all unused study drug at the End of Study or Early Withdrawal visits. The PIs each hold an IND for the use of sildenafil in CF (IND #102639, IND #119923) and FDA reports will continue to be submitted annually.

D6. Exercise Testing and Physical Activity Evaluation. All participants will perform the six minute walk test[73] and undergo maximal exercise testing on an electronically braked cycle ergometer using the Godfrey protocol at baseline, 6 weeks and 12 weeks following treatment with sildenafil or placebo. In addition, all patients will be receive a Fitbit wrist accelerometer and instructed on its use from pre-dose through the end of the study. Fitbit data will provide objective assessments of daily physical activity and sleep quantity and efficiency. Data will be used to ensure baseline physical activity is similar between treatment groups as well as the effect that treatment may have on daily physical activity, which can impact exercise capacity. Interestingly, preliminary data from Dr. Taylor-Cousar's randomized pilot study not only observed a statistically significant change ($p=0.02$) in Fitbit steps following 12 weeks of sildenafil when compared to placebo, the participants gave very positive feedback about the use of Fitbit during the study. All testing will be conducted at the same time of day to avoid the impact of diurnal variations.

Aim 1. Test the hypothesis that PDE5 inhibition improves clinically relevant and patient oriented outcomes in patients with moderate to severe CF lung disease.

D7. Aim 1 Rationale. Patients with CF exhibit exercise intolerance, independent of lung function. Exercise capacity, an objective measurement of exercise tolerance, predicts mortality in patients with CF. The mechanisms for exercise intolerance in CF have yet to be fully elucidated and further understanding could improve clinical outcomes and survival in CF. Preliminary data from two independent proof-of-concept clinical trials support the use of sildenafil to improve exercise capacity, cardiac function, and quality of life in CF.

D7.1. Aim 1 Experimental Design. Exercise capacity, cardiac function, and quality of life will be evaluated in all patients prior to treatment and 6 weeks and 12 weeks following treatment with either sildenafil or placebo. Exercise capacity will be assessed using 6MWD, whereas cardiac MRI and the CFQ-R will be used to determine global cardiac strain and patient quality of life, respectively

Aim 2. Test the hypothesis that PDE5 inhibition will increase vascular endothelial function and contribute to an increase in exercise capacity in patients with moderate to severe CF lung disease.

D8. Aim 2 Rationale The mechanisms for exercise intolerance in CF have yet to be fully elucidated. The ability to maintain adequate oxygen delivery to the working musculature is of critical importance, particularly under conditions of increased demand, such as during exercise. Our group was the first to describe both conduit and microvascular endothelial dysfunction in patients with CF[20, 21]. In addition, recent published data from our lab indicate that patients with CF exhibit an impairment in blood flow regulation during exercise[74] Indeed, impaired nutritive flow to the working musculature during exercise is suggested to contribute to exercise intolerance in several clinical populations[22, 23], and has yet to be investigated as a potential mechanism for exercise intolerance in CF. PDE5 inhibition therapy is routinely shown to improve vascular health (in as little as 4 weeks) in patients

with vascular dysfunction[53-55] and we have recently published the efficacy of 4 weeks of sildenafil to improve vascular function in patients with CF[56]. Therefore, we hypothesize that vascular health plays a role in exercise intolerance in CF and sildenafil can improve vascular health.

D8.1. Aim 2 Experimental Design. Endothelial function assessed via brachial artery FMD will be evaluated in all patients prior to treatment and 6 weeks and 12 weeks following either sildenafil or placebo. In addition, plasma from each time point will be incubated with human aortic endothelial cells as previously described[56]. *in vitro* total NOS3 protein and phosphorylated NOS3 protein expression will be determined from endothelial cells incubated with participants' plasma and used as a surrogate for the underlying mechanisms of the FMD response.

Aim 3. Secondary/Exploratory Aim. Test the hypothesis that PDE5 inhibition will improve skeletal muscle function and decrease pulmonary and systemic inflammation and contribute to an increase in exercise capacity in patients with CF.

D9. Aim 3 Rationale. The mechanisms for exercise intolerance in CF have yet to be fully elucidated. Our group and others have reported impairments in both skeletal muscle oxidative capacity[34, 40] and oxygen uptake kinetics[47] in patients with CF, both of which can decrease exercise capacity. Pulmonary and systemic inflammation are also central to the pathophysiology of CF[75-77]. Notably, systemic inflammation also negatively impacts exercise capacity, mediated in part by deleterious effects on skeletal muscle function[31, 32]. In fact, our group has identified a link between inflammation and exercise capacity in patients with CF. Sildenafil has anti-inflammatory effects that may be beneficial in the context of the skeletal muscle function and the CF pro-inflammatory environment. Improvements in muscle function following PDE5 inhibition have also been documented following treatment[61, 78]. In addition, the excessive proinflammatory response to *P. aeruginosa* exposure in CF respiratory epithelial cells could be reversed by treatment with sildenafil[57]. These published findings coupled with our preliminary data provide compelling support that sildenafil can reduce inflammation and improve skeletal muscle function.

D9.1. Aim 3 Experimental Design. Under fasting conditions, blood and sputum samples will be obtained from all participants prior to treatment and 6 weeks and 12 weeks following treatment. Sputum elastase, IL-8, calprotectin, high-mobility group box 1 (HMGB-1), and myeloperoxidase will be determined as well as serum assessments of hsCRP, calprotectin, serum amyloid A (SAA), TNF-α, IL-6, IL-8, and IL-1β. Oxygen uptake kinetics, skeletal muscle oxygen extraction and consumption during exercise will be determined as indices of skeletal muscle function prior to treatment and 6 weeks and 12 weeks following treatment.

D10. Overall Power Analysis. Based on our new double-blind, placebo-controlled pilot study, we have calculated power to detect differences in clinically relevant and patient-oriented outcomes. For each Aim, we used our preliminary data to estimate power based on the time-averaged difference between two means (PASS 15). Given the linear mixed model with which we plan to analyze our data, we expect our power estimates to be conservative. Each sample size estimate assumes an independent two-sample *t* test, a two-sided significance level of 0.05, unequal variances in the two groups, and power of 90% (PASS 15). **Power for Aim 1** is based on the anticipated effect size (see **Table 1** below) for the change in 6MWD, cardiac strain, and CFQ-R respiratory domain. For each of the proposed outcomes for Aim 1, with placebo control and treatment group sizes of 20 (40 subjects total), we expect a conservative 90–100% power to detect a difference between treatments. **Power for Aim 2** is based on the anticipated effect size for the change in FMD that we observed in our pilot study. FMD increased by ~2% after 4 weeks of sildenafil treatment; FMD decreased by ~1% unit (SD 3) in the placebo group. With placebo control and treatment group sizes of 20 (40 subjects total), we expect a conservative 87% power to detect a similar difference. We have not estimated sample size or power for our secondary, exploratory aims.

Table 1. Power Analysis for primary outcomes in Aim 1.

	Placebo		*Sildenafil*		*Sample size*		*Power*
Change in Outcome	*Ave*	*SD*	*Ave*	*SD*	*Initial**	*Target*	*with n=17†*
6-min walk distance	1	5	25	19	11	9	100
CFQ-R	–9	3	9	18	16	13	97
Cardiac strain	1.4	5	–4.8	5	20	17	90

* The initial sample size is the target sample size / 0.85 in order to account for expected dropout over the course of the study. † Target sample size of $n=17$.

D11. Overall Statistical Analysis Plan. Our statistical analysis plan is identical for Aims 1–3. Descriptive statistics and graphical methods, including box plots, will be used to examine the distribution of all variables. For all analyses, an intent-to-treat analysis will be utilized regardless of any changes to treatment during the course of the study. Assumptions for the statistical analysis performed, including normality of residuals and equality of variance will be examined. If violations to the assumptions are found, different transformations will be examined.

Imputation of missing values will not be performed. Physical activity accelerometry will be used to control for basal cardiovascular conditioning. The impact of site, age, gender, CFRD, pancreatic function status, colonization of bacteria, and HbA1c on the change in each outcome (for example, sputum elastase, FMD, and oxygen uptake kinetics) will be evaluated. We will analyze each outcome using a longitudinal statistical model—a linear mixed model—that can incorporate covariates (i.e. change in FEV_1,) that may vary at different time points of the study (baseline, 6 weeks, and 12 weeks). The linear mixed model will include a random subject effect that accounts for the repeated measurements made in each subject. Moreover, this approach can account for the expected result that treatment with sildenafil will impact inflammation, vascular function, and skeletal muscle function in addition to exercise capacity: we plan to include metrics of inflammation, vascular function, and skeletal muscle function in the statistical model for exercise capacity. We can also look at center as a random effect and we can perform a sensitivity analysis as suggested by the reviewer. Nonetheless, this model will allow us, for the control and treatment groups, to estimate each outcome at each time point and to compare those estimates. We plan to assess the model with proc mixed (SAS v9.3).

D12. Expected Results and Interpretation. Based on our preliminary data, we predict that 12 weeks of sildenafil treatment will elicit favorable outcomes on exercise capacity, cardiac and vascular function, and quality of life; all of which may potentially contribute to improvements in exercise capacity. All patients will be screened for frank PAH and severe pulmonary hypertension that may be observed with echo. Improvements in all the proposed mechanisms can be viewed as inter-related and since we predict sildenafil to have a global impact, interpretation may be difficult. We will not only statistically control for other mechanistic confounders, we expect to identify the proposed mechanisms which contribute to a patient being a responder or non-responder to therapy. For example, we will be able to make sub-group comparisons and determine phenotypical characteristics (i.e. pulmonary function, CFRD, pancreatic function, etc.) of patients who exhibit the greatest change in exercise capacity. In addition, the recording of daily physical activity (via Fitbit accelerometry) throughout the investigation will be essential and aid in discerning the contribution of daily physical activity on the proposed mechanisms for the improvement in exercise capacity. Based on the reviewer's suggestion, we will only include patients with moderate to severe CF lung disease. Findings from this investigation are critical to identify which sub-group of patients will benefit from therapy and to guide the design of large-multicenter clinical trials of various therapies to improve exercise tolerance. It is also noteworthy that recently published data from the European CF exercise group, of which Dr. Harris is a part, demonstrates **no effect of CFTR genotype on exercise capacity in CF, even when comparing class III and V patients with preserved lung function**[79]. These data suggest that consequences of CF, and not genetic variants of CFTR, contribute to exercise intolerance in CF; data which increase the significance of the present investigation.

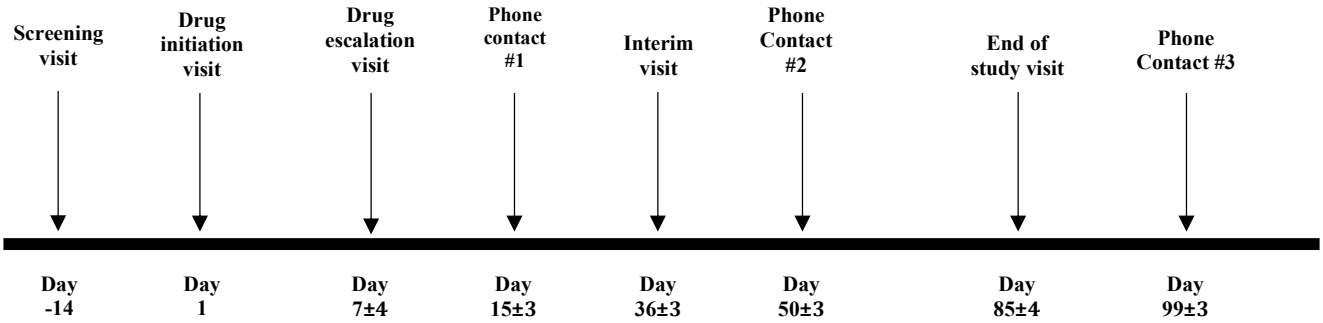
D13. Anticipated Problems and Solutions. Although safety is always a concern, our team has conducted proof-of-concept studies and demonstrated both safety and efficacy of sildenafil treatment in both children and adults with mild to moderate to severe CF lung disease. We will utilize standardized guidelines for conducting exercise tests (6MWD and CPETs) in patients with CF and assessing cardiac function with MRI, vascular endothelial function using the FMD test, and quality of life using the CFQ-R. Dr. Harris has spearheaded the current recommendations of conducting brachial artery FMD in humans[80] and will work closely with Dr. Morreau, Director of Cardiovascular Imaging at Colorado University (CU), who has expertise and a strong publication record using FMD). Drs. Harris and Taylor-Cousar have developed a strong MPI plan and all specific data for individual outcome analyses will be analyzed by a single site. For example, all sputum samples will be analyzed at the Colorado CF TDN Center, whereas plasma for evaluation of NOS2, NOS3, and circulating inflammatory biomarkers will be sent to Augusta University for analysis. This process will increase rigor and eliminate the between-site variability in data analysis. In addition, the REDCap secure (part 11 compliant) web based database, will be customized to allow for data entry and sharing between investigators. We have detailed the coordination of data collection and analysis in section D15 below. We have identified 3 clinically relevant outcomes that may make interpretation difficult. Although the present study cannot determine cause and effect, we will be able to tease out the effects of sildenafil on the systems described and provide insight into the role of each potential mechanism. We will explore the relative contribution of each potential mechanism using a variety of techniques: multiple logistic regression and classification techniques (classification and regression trees) to identify responders to therapy and to identify mechanisms (i.e. changes in cardiac function and NOS phosphorylation) that may impact clinically relevant outcomes such as exercise capacity. This is a phase IIb investigation and therefore we expect that this study will identify potential mechanisms and magnitudes of changes to inform larger future studies aimed at improving exercise capacity in patients with CF. Omitting muscle biopsies to evaluate skeletal muscle function may be viewed as a potential problem/weakness. However, our

preliminary data using state-of-the-art, non-invasive methods support the sensitivity to detect changes in skeletal muscle function following sildenafil treatment without performing muscle biopsies. In addition, the NIRS methodology is similar to phosphorous magnetic resonance spectroscopy (^{31}P-MRS)[81, 82], which is the gold standard for measuring the rate of recovery of phosphocreatine (PCr) during exercise[83, 84]. Based on reviewer's concerns, muscle function will only be evaluated in the Augusta cohort.

D15. Coordination of Data Collection and Analysis. Briefly, we will meet as a complete research team via Webex once a month to 1) maintain an open line of communication among the research team, 2) to keep everyone motivated and informed of our progress, and 3) provide a forum for discussion of any minor changes in direction. All personnel will be invited to give an update on their progress and share their research strengths, opportunities, and challenges with the full team. We will collaborate with the Research Informatics Integrated Core at NJH to develop the research database in the Research Electronic Data Capture (REDCap) program. REDCap will be customized to accept all study related data (i.e. case report forms, inflammation, vascular function, and exercise capacity data). Because REDCap is a secured database accessible from any computer with internet access, use of REDCap will facilitate communication during the study between the investigators, research coordinators, and technicians and with statisticians for data analysis at the conclusion of the study.

D14. Proposed Timeline. The proposed testing/assessment timeline is presented in the **study timeline and table** below.

Study Timeline



Screening visit (Day -14-0): Prior to conducting any study-related activities, written informed consent/assent will be obtained, signed, and dated by the subject. A medical history including diagnosis of CF, current medications and other relevant past medical history will be obtained, and a complete physical exam will be performed by a study investigator. Height, weight, temperature, blood pressure, respirations and baseline pulse oximetry will be recorded. Participants will be lead through six-minute walk test (6MWT) to prepare for the subsequent visit. Any abnormal findings will be documented. An ECG and an ECHO will be done. Routine laboratory studies will be performed. Because sildenafil citrate is a class B drug, a urine pregnancy test will be performed for females of child-bearing potential. Female enrollees will be counseled to utilize appropriate contraceptive methods while participating in this study. Subjects will undergo spirometry according to ATS criteria.[85]

Study visit 2 (Day 1): Subjects meeting entry criteria will be evaluated in the outpatient research unit. CFQ-R (see description below) will be administered prior to all other assessments. Vital signs will be obtained. A physical exam will be performed by a study investigator. Routine laboratory studies will be performed. An exhaled nitric oxide test will be done prior to any lung function testing. Complete lung function will be performed. Cardiac MRI will be performed to classify subjects' estimated right heart pressures and right heart function. After completing spirometry and baseline MRI examination, a 6MWT and cycle ergometry test will be performed. The cycle ergometry will be the last procedure performed (see description of each procedure below). Additionally, a DEXA scan will be done to measure fat free body mass. Fitbits will be distributed, created with the subjects personal email account and linked to the participants cellphone. Following setup, the Fitbit account will be connected to the Fitabase account, and the subject will be educationed on use. At the completion of the visit, each subject will be randomized and instructed to begin daily treatment with sildenafil citrate or placebo (Belmar

Pharmacy) an oral dose of 20 mg, three times daily. Blood pressure will be checked at 30 minutes, 1 hour, and 2 hours after the first dose.

Study visit 3 (Day 7±4): After seven days of therapy, the subject will return to the study site for vital signs, review of concomitant medications, and discussion of concerns related to sildenafil therapy. If tolerated (see **Subject and Study Stopping Criteria)**, the oral sildenafil or placebo dose will be increased to 40mg, three times daily. If there is evidence of sildenafil intolerance (blood pressure will be checked at 30 minutes, 1 hour, and 2 hours after the first increased dose), the dose will be decreased to the previous dose or discontinued. If sildenafil is discontinued, the patient will be removed from the study. Oral sildenafil therapy will continue at 40mg, three times daily for eleven weeks. Study drug will be dispensed for five-weeks. (Total sildenafil or placebo therapy will last twelve weeks.)

Phone contact 1 (Day 15±3): Phone contact will be made with each subject to review dosing, drug compliance and side effect profiles. The study coordinator will call to review adverse events and concomitant medications. The visit will be recorded on case report forms.

Study visit 4 (Day 36±3): The subject will return to the study site for CFQ—R, vital signs, height, weight, safety labs, sputum collection, review of concomitant medications, adverse events, and discussion of concerns related to sildenafil therapy. A study investigator will perform a brief physical exam. An exhaled nitric oxide test will be done prior to any lung function testing. Complete lung function will be performed and the subject will complete a 6MWT followed by a cycle ergometry test. Subjects will return any study drug from visit 3 and six-weeks of study drug will be dispensed. Dosing at site is not required.

Phone contact 2 (Day 50±3): Phone contact will be made with each subject to review dosing, drug compliance and side effect profiles. The study coordinator will call to review adverse events and concomitant medications. The visit will be recorded on case report forms.

Study visit 5 (Day 85±4): Subjects will return to the outpatient research unit, where the CFQ-R (see description below) will be administered prior to all other assessments. CFQ-R (see description below) will be administered prior to all other assessments. Vital signs will be obtained. A physical exam will be performed by a study investigator. Routine laboratory studies will be performed. An exhaled nitric oxide test will be done prior to any lung function testing. Complete lung function will be performed. Cardiac MRI will be performed to classify subjects' estimated right heart pressures and right heart function. After completing spirometry and the MRI examination, a 6MWT and cycle ergometry test will be performed. The cycle ergometry will be the last procedure performed (see description of each procedure below). Additionally, a DEXA scan will be done to measure fat free body mass. The Fitbit will no longer be tracked through Fitabase but the participant will be allowed to keep the wrist accelerometer.. At the conclusion of this visit, subjects will be instructed to return any remaining study drug and discontinue sildenafil or placebo therapy.

Phone contact 3 (Day 99±3): The study coordinator will call to review adverse events and concomitant medications. If there are new abnormalities or changes in medication felt to be study-related, these abnormalities will be followed up with weekly visits to the study site until resolution.

Exacerbations: During the course of the study, any enrolled subject requiring hospitalization and/or intravenous antibiotics for a cystic fibrosis-related pulmonary exacerbation will be withdrawn from the study.

Adverse Events: AEs reported at or between visits will be recorded in source documents. The PI will be notified and the site pharmacist may be asked to review. Participant may be encouraged to reach out to their PCP and follow up with study team.

Early Withdrawal: In case of early withdrawal, an Early Withdrawal Visit will be scheduled within 7 days following the last dose of study medication. Unless medically contraindicated, CFQR, safety labs, urine pregnancy for all women, spirometry, a 6MWT and cycle ergometry test will be performed. Physical exam will be performed by a study investigator including weight, temperature, blood pressure, respirations and pulse oximetry. Any abnormal findings will be documented. All remaining study drug will be collected.

Concomitant medications: All subjects should continue the same medications throughout the study period, as medically feasible, with no introduction of new chronic therapies during or <4 weeks prior to enrollment. If a change in concomitant medications is required, the reason(s) for the change(s) will be recorded on the subject's CRF.

	Screening	Pre-treatment/ Drug initiation	Dose escalation	Phone contact #1	Interim visit #1	Phone Contact #2	End of study visit	Phone contact #3	Early Term Visit
Visit	1	2	3		4		5		
Day(s) Weeks(s)	0	1 Week 1	7±4 Week 2	15±3 Week 3	36±3 Week 6	50±3 Week 8	85±4 Week 13	99±3 Week 15	
Informed consent	x								
Continued consent		x	x	x	x	x	x	x	x
Eligibility assessment	x								
Randomization	x								
CFQ-R		x			x		x		x
Demographics	x								
Medical history	x								
Con meds and ACT	x	x	x	x	x	x	x	x	x
Bacterial infection (historical)	x								
Vitals signs	x	x	x		x		x		x
Pulse Oximetry	x	x			x		x		x
Spirometry	x								x
Complete lung function test		x			x		x		
Height	x	x	x		x		x		x
Weight	x	x	x		x		x		x
Complete physical exam	x						x		x
EKG	x								
ECHO	x								
Limited physical examination (HEENT, Chest, Lungs)		x			x				
Vascular function (Augusta only)		x			x		x		x
DEXA		x					x		
Inflammatory biomarkers and sputum samples		x			x		x		x
Circulating Sildenafil		x			x		x		x
Exhaled NO		x			x		x		x
6MWT		x			x		x		x
CPET		x			x		x		x
Cardiac MRI		x					x		x
Laboratory serology (CBC, liver function, chemistry, INR)	x	x			x		x		x
Pregnancy test	x	x			x		x		x
Study drug dispensed		x	x		x				
Study drug bottle return			x		x		x		x
Adverse events		x	x	x	x	x	x	x	x

D17. Specific Techniques (Primary Methodology).

Cardiac MRI: Imaging to evaluate pulmonary artery blood flow and right heart structure and function with MRI will be performed using standardized methods. Following image acquisitions, volumetric and functional analyses will be performed using commercially available software [4D V-Function (TomTech, Munich, Germany)].

Clinical laboratory Serology: Routine clinical serology including CBC with differential, liver function, chemistry, and INR will be performed to evaluate safety.

Clinical laboratory Urology: urine pregnancy test (for females of child-bearing potential) will be performed at the screening, pre-drug initiation, interim visit, and end of study visits for safety.

Comprehensive Assessment of Lung Function: Spirometry (FVC, FEV_1, FEF_{25-75}), MEP, MIP, and MVV will be performed according to ATS standards and the National Health and Nutrition Examination Survey (NHANES) III spirometric reference standards will be used to determine the % predicted data set. All values will be presented as % predicted and normalized for TLC.

DEXA Scan: Dual Energy X-ray Absorptiometry, a scan used to measure bone density, the scan takes about six and a half minutes. The process requires you to lay flat on an x-ray table completely still so your body can be scanned. Females of child-bearing potential will complete urine pregnancy test before DEXA.

Inflammatory Biomarkers: Spontaneously expectorated or induced sputum will be collected at the screening, interim, and end of treatment visit (at least 1 mL). Sputum will be labeled, processed and the cell count and differential analyzed according to the CF TDN Inflammatory Mediator Core Laboratory. The remaining portion of the sample will be frozen and subsequently shipped in batch on dry ice shipped to the CF TDN Inflammatory Mediator Core Laboratory (Aurora, CO) for analysis of inflammatory markers [elastase, IL-8, calprotectin, high-mobility group box 1 (HMGB-1), and myeloperoxidase] If subjects are unable to produce sputum spontaneously, sputum induction will be performed by having the subject inhale 3% hypertonic saline (as per the CF TDN research SOP for Sputum Induction using the Nouvag Nebulizer or institution specific Sputum Induction SOP.) An inflammatory mediator simpleplex will be conducted in plasma samples for evaluation of inflammatory biomarkers [hsCRP, calprotectin, serum amyloid A (SAA), TNF-α, IL-6, IL-8, and IL-1β] using the ELLA assay system (Protein Simple and RnD systems). Blood will be collected at the screening, interim, and end of treatment visits and plasma will be separated and placed in a -80°C freezer until it is shipped on dry ice in batches via overnight mail to Augusta University and Dr Harris' lab for analysis.

Circulating Concentrations of Sildenafil: Plasma samples will be shipped frozen to the advanced diagnostics laboratory at NJH and sildenafil levels will be determined by high-performance liquid chromatography as previously described[86, 87].

Health Related Quality of Life: The CFQ-R, which has been shown to correlate with survival in CF[25], is designed to measure CF-specific patient-reported health-related quality of life. The 48 questions encompass five domains including physical symptoms, role functioning (e.g. school/work), psychological and emotional functioning, energy/fatigue, and social functioning. The four domains specific to CF that are measured are: eating disturbances, body image, embarrassment caused by symptoms, and treatment burden. The CFQ-R, which has been validated in patients with CF[26] and shown to correlate with survival in CF[25], will be administered at the first, interim, and end of treatment visits.

Vascular Endothelial Function: The FMD test will be performed as previously described by our team[20, 56, 80]. **(Augusta only)**

NOS3 and NOS2 expression: Human aortic endothelial cells (HAEC) will be purchased (PromoCell, Germany), cultured, and incubated with 20% plasma to evaluate NOS3 as previously described[56]. Nasal epithelial cells will be collected as previously described. (TDN SOP) NOS2 and NOS3 protein expression and phosphorylation will be determined using standard Western blot technique.

Echo: An echocardiogram will be performed at the screening visit to rule out frank pulmonary hypertension.

Six Minute Walk Test: 6MWT will be performed according to ATS standards[73].

Exercise Capacity Testing: A maximal exercise test on a cycle ergometer using the Godfrey protocol. CPET will always be the last procedure of the appointment.

Physioflow Enduro: The Physioflow Enduro device uses the patented technology of signal Morphology-based thoracic impedance cardiography and produces reproducible assessments of cardiovascular hemodynamics (i.e. HR, SV, CO, SVR, and EF) that have been validated against the invasive thermodilution Swan-Ganz catheter technique. Using CPET and Physioflow data together, muscle oxygen extraction during exercise will be determined using a derivation of the Fick equation: $mO_2EX = 100- [SpO_2 – VO_2 / (13.9 \times CO \times Hb)]$. **(Augusta only)**

Near Infrared Spectroscopy: A NIRS device (Artinis, The Netherlands) will be placed on the vastus lateralis to assess skeletal muscle function during exercise as previously described[70-72]. **(Augusta only)**

Accelerometry Monitoring: The Fitbit wrist-based accelerometer will be used to collect information about physical activity on a day-to-day basis, even during evenings and weekends. All data will be downloaded and incorporated into Fitabase, a web-based solution that enables participants to upload their individual data on a daily or weekly basis so the investigators can keep track. Physical activity status will be used as a covariate in the analyses.

ENO: Exhaled nitric oxide will be completed before any assessments of lung function.

Subject Reimbursement

In accordance with TDN recommendations, subjects will receive payment according to the schedule below:

Visit	Visit 1	Visit 2	Visit 3	Call 1	Visit 4	Call 2	Visit 5	Call 3	Total
Payment	$120	$250	$90	$15	$105	$15	$250	$15	$860

Subjects who complete the screening visit (Visit 1), will receive the $120 payment regardless of whether they qualify for the study to compensate for their time and effort.

Subjects who live greater than 50 miles from the study site, will be reimbursed at the current standard mileage rate for medical purposes.

Mechanism for Monitoring Patient Safety

Protections of Human Subjects

1. **Risks to Human Subjects**

A. Human Subjects Involvement, Characteristics, and Design

Patients with Cystic Fibrosis (CF) will be recruited for the proposed investigations. Both children (9 years or older) and adults will be recruited. The **overall goals** of this proposal is to evaluate the impact that sildenafil has on clinically relevant and patient oriented outcomes and to increase our understanding of the potential mechanisms which contribute to improvements in exercise capacity following PDE5 inhibition in patients with CF.

Following written consent/assent and screening, all eligible subjects (and parents of minors) will come to one of the two research sites to complete study visits and procedures. 40 patients with CF will be enrolled (both children and adults). Each patient will be enrolled for 12 weeks. Study participation will be comprised of 5 study visits and 3 phone calls. Accordingly, a total of 40 patients will result in 200 study visits and 120 phone calls that will be split amongst the 2 centers.

Inclusion Criteria:

- Confirmed diagnosis of CF based on the following criteria: Positive sweat chloride concentration ≥40mEq/liter (by pilocarpine iontophoresis) and/or genotype with two identifiable disease-causing mutations consistent with CF, and accompanied by one or more clinical features consistent with the CF phenotype
- Male or female patients ≥ 9 years of age
- FEV_1 ≥ 30% predicted and ≤ 80% for patients ≥ 18 years of age and ≤ 85% for patients < 18 years of age
- Clinically stable without evidence of acute upper or lower respiratory tract infection or current pulmonary exacerbation within the 14 days prior to the screening visit
- Resting oxygen saturation (room air) ≥85%
- Patients with or without CF related diabetes
- Ability to perform spirometry reproducibly (according to ATS criteria)
- Willingness to maintain chronic CF medication schedule (e.g. alternating month inhaled antibiotics)

Exclusion Criteria:

- Children 8 yrs. old and younger
- Subjects who weigh < 20 Kgs
- History of hypersensitivity to sildenafil
- Use of an investigational agent within the 4-week period prior to Visit 1 (Day 0)

- Breastfeeding, pregnant, or verbal expression of unwillingness to practice an acceptable birth control method (abstinence, hormonal or barrier methods, partner sterilization or intrauterine device) during participation in the study for women of child-bearing potential.
- History of significant hepatic disease (AST or ALT > 3 times the upper limit of normal at screening, documented biliary cirrhosis, or portal hypertension),
- History of significant cardiovascular disease (history of aortic stenosis, coronary artery disease, or life-threatening arrhythmia),
- History of severe neurological disease (e.g. history of stroke),
- History of severe hematologic disease (e.g. history of bleeding diathesis; current INR > 2.0
- History of severe ophthalmologic disease (e.g. history of retinal impairment or non-arteritic ischemic optic neuritis)
- History of severe renal impairment (creatinine >1.8 mg/dL.)
- Inability to swallow pills
- Previous organ transplantation
- Use of concomitant nitrates, α-blocker, or Ca channel blocker (currently or within one month of Visit 1)
- Use of concomitant medications known to be potent inhibitors of CYP3A4 [e.g. ketoconazole, itraconazole, ritonavir, clarithromycin, erythromycin, rifampin (currently or within one month of initiation of study drug)] NOTE: use of azithromycin is NOT a cause for exclusion
- History of sputum or throat swab culture yielding *Burkholderia cepacia* or ***Mycobacteria massiliense*** within 2 years of screening
- History of migraine headaches.
- Presence of a condition or abnormality that in the opinion of the investigator would compromise the safety of the subject or the quality of the data
- Initiation of CFTR modulator therapy less than 1 month prior to first dose of sildenafil or placebo
- Use of anticoagulants
- Frank pulmonary hypertension (RVSP >45 mm Hg by echocardiography)

Additional Enrollment Considerations:

Patients on cystic fibrosis transmembrane conductance regulator (CFTR) modulators may participate if they have been on a stable dose for at least 4 weeks prior to study drug initiation. Patients who do not meet the enrollment criteria at the time of the screening visit may be re-screened at a later date at the discretion of the site investigator.

Because many patients who would participate in the proposed trial could be on ivacaftor (Kalydeco®) or ivacaftor/lumacaftor (Orkambi®), it is possible that sildenafil levels would be decreased because of the effects of ivacaftor and lumacaftor on sildenafil metabolism. We are using the 40 mg TID dosing for this study, and will check sildenafil levels at interim and end of study visits.

Regardless of the findings, **the current proposal will shift current understanding and introduce new knowledge about the mechanisms associated with exercise intolerance in patients with CF**.

B. Sources of Materials

All study procedures will be carried out at Augusta University and National Jewish Health.

Prior to any procedures written informed consent/assent will be obtained from all participants and parents of minors. Health related quality of life data will be obtained by administration of a questionnaire. The CFQ-R is designed to measure CF-specific patient-reported health-related quality of life. The 48 questions encompass five domains including physical symptoms, role functioning (e.g. school/work), psychological and emotional functioning, energy/fatigue, and social functioning. The four domains specific to CF that are measured are: eating disturbances, body image, embarrassment caused by symptoms, and treatment burden. This questionnaire has been validated in CF patients. The questionnaire will be administered at the first, interim, and end of treatment visits.

Clinical laboratory Serology: Routine clinical serology will be performed including complete blood count with differential, comprehensive chemistry profile [including sodium, potassium, bicarbonate, blood urea nitrogen, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and total bilirubin) PT/INR

Clinical laboratory Urology: urine pregnancy test (for females of child-bearing potential) will be performed at the screening, pre-drug initiation, interim visit, and end of study visits for safety.

Inflammatory Biomarkers: Spontaneously expectorated or induced sputum will be collected at the screening, interim, and end of treatment visit (at least 1 mL). Sputum will be labeled, processed and the cell count and differential analyzed according to the CF TDN Inflammatory Mediator Core Laboratory. The remaining portion of the sample will be frozen and subsequently shipped in batch on dry ice shipped to the CF TDN Inflammatory Mediator Core Laboratory (Aurora, CO) for analysis of inflammatory markers [elastase, IL-8, calprotectin, high-mobility group box 1 (HMGB-1), and myeloperoxidase] If subjects are unable to produce sputum spontaneously, sputum induction will be performed by having the subject inhale 3% hypertonic saline (as per the CF TDN research SOP for Sputum Induction using the Nouvag Nebulizer or institution specific Sputum Induction SOP.) An inflammatory mediator simpleplex will be conducted in plasma samples for evaluation of inflammatory biomarkers [hsCRP, calprotectin, serum amyloid A (SAA), TNF-α, IL-6, IL-8, and IL-1β] using the ELLA assay system (Protein Simple and RnD systems). Blood will be collected at the screening, interim, and end of treatment visits and plasma will be separated and placed in a -80°C freezer until it is shipped on dry ice in batches via overnight mail to Augusta University and Dr Harris' lab for analysis.

Comprehensive Assessment of Lung Function: Spirometry (FVC, FEV_1, FEF_{25-75}) will be performed at the screening, interim, and end of treatment visits according to ATS standards. The National Health and Nutrition Examination Survey (NHANES) III spirometric reference standards will be used to determine the % predicted data set. All values will be presented as % predicted and normalized for TLC and hemoglobin. Maximum inspiratory and expiratory pressures and maximal voluntary ventilation will also be measured.

Vascular Endothelial Function: The brachial artery FMD test will be performed according to the recent tutorial on the ultrasonic assessment of FMD[80] and shear rate will be calculated as the stimulus of the vasodilatory response. Cryopreserved human aortic endothelial cells (HAEC) will be purchased from PromoCell (Heidelberg, Germany). Cells will be cultured in 24-well plates (100,000 cells per well) coated with 2% gelatin (Corning Inc., Corning, NY) at 37 °C in 5% CO_2 and 95% humidity and will not be used beyond passage six and allowed to reach confluence before experiments. HAEC will be incubated in ECGM-MV2 medium with 20% plasma at 37 °C in 5% CO_2 environment for 8 h. HAEC cultured without plasma will be used as a negative control. After incubation, cells will be prepared for measuring NOS3 expression using Western blot analysis. Specifically, HAEC will be homogenized in 50 µl of RIPA buffer containing a protease (Complete Mini EDTA free, Roche, Switzerland) and a phosphatase (PhosStop, Roche) inhibitor cocktail with an ultrasonic processor. Samples containing 40 µg of protein will be separated by SDS precast gels (Bio-Rad, Hercules, CA) and transferred to commercial nitrocellulose-transfer packs using the Trans-Blot Turbo Transfer System (Bio-Rad). Blocking will be completed using fluorescent blocking buffer (Odyssey, LI-COR Biotechnology, NE, USA) during 60 min at room temperature. Membranes will be incubated overnight at 4°C with specific primary antibodies. Bound primary antibody will be detected using IRDye secondary antibody (LI-COR Biotechnology). Membranes will be scanned with an infrared imaging system (Odyssey CLx, LI-COR Biotechnology). The blots will subsequently be quantified using imaging densitometer (ImageJ, Bethesda, MD, USA). Equal protein loading will be verified by total β-actin (1:20,000; Sigma-Aldrich.)

Echo: Echocardiogram will be performed at the screening visit to insure that the patient does not meet the exclusion criterion of frank pulmonary hypertension. If the subject has undergone echo within 3 months of study enrollment, it will not be repeated.

Six Minute Walk Test: 6MWT will be performed according to ATS standards.[73]

Exercise Capacity Testing: Previous studies of cardiopulmonary exercise testing (CPET) have consistently demonstrated decreased maximal work effort, peak oxygen consumption, and ventilatory reserve in patients with CF lung disease when compared to healthy, age-matched controls.[88, 89] Trained personnel will perform the test. Patients will perform a maximal exercise test on an electronically braked cycle ergometer using the Godfrey protocol, a standardized incremental protocol validated in CF. Breath-by-breath respiratory gas exchange will be collected. Expired gasses will be collected using a Parvo Medics True One metabolic cart for determination of exercise capacity (VO_2 peak), CO_2 retention, O_2 uptake kinetics, ventilatory equivalents, and AT. Pulse-oximetry (SpO_2), ECG, heart rate, and blood pressure will also be measured throughout the test. The American College of Sports Medicine and the American Thoracic Society/American College of Chest Physicians guidelines for contraindications to cardiopulmonary exercise testing and exercise test termination criteria will be used. [90,91] Patients with an FEV_1 <40% predicted will be directly supervised by a physician during the exercise test. A physician will be immediately available for all other tests.

Physioflow Enduro: The Physioflow Enduro will be utilized to monitor cardiovascular hemodynamics during the maximal exercise tests in all patients. The Physioflow Enduro device uses the patented technology of signal Morphology-based impedance cardiography and produces continuous, sensitive, and reproducible assessments of cardiovascular hemodynamics (i.e. HR, SV, CO, SVR, and EF) that have been validated against the invasive thermodilution Swan-Ganz catheter technique. Measurements will only occur at Dr. Harris' site.

Near Infrared Spectroscopy (NIRS): The NIRS will remain on the vastus lateralis during the maximal exercise capacity test and will be used to assess skeletal muscle oxidative capacity during exercise as previously described[70-72]. Real time O_2Hb and HHb will be recorded at a frequency of 10 Hz during baseline, warm-up and throughout maximal exercise. Hb difference (Hb difference =O_2Hb - HHb), and total blood volume (tHb) (tHb = O_2Hb + HHb) according to the Beer-Lambert law will be obtained during baseline, warm-up and throughout maximal exercise. Since subcutaneous adipose tissue thickness (ATT) influences the NIRS signal[92], ATT will be measured at the site of the NIRS optode using B-mode ultrasound (LOGIQ 7, GE HealthCare).

Accelerometry monitoring: Participants will be given and asked to wear a Fitbit wrist based accelerometer throughout the study period. The Fitbit will be used to collect information about exercise tolerance on a day-to-day basis, even during evenings and weekends. All data will be downloaded and incorporated into a fitabase database, a web-based solution that enables participants to upload their individual data on a daily or weekly basis so the investigators can keep track. Physical activity status will be used as a covariate in the analyses.

Case report forms will be created in conjunction with the CCTSI bioinformatics staff using the Research Electronic Data Capture (REDCap) program. The case report forms, labeled with subject's study number, will contain history (including CF genotype) and physical exam data, laboratory results, inflammatory biomarker data, exercise testing information, FMD and spirometry data. Investigators and coordinators involved in the study will have secured passwords to enter information/access the electronic case report forms.

The patient's name, address and social security numbers will be maintained in a paper-based log at the patient's home site for the length of the study in order to create and make compensation for study participation, and also to contact patients in the event of the need for study termination. Hard copies and links to the online data will be kept in a locked file in the PI/study coordinator's office at each site. Only investigators and coordinators directly involved in the study will have access (through the PIs/site study coordinator) to the linking information.

Research specimens and data are being collected solely for the purpose of this study and will also be de-identified.

C. Potential Risks

The two main risks associated with these studies include CF exacerbations (spontaneously or associated with the study,) and side effects from the medication.

In trials of sildenafil, adverse effects were generally mild to moderate and transient in nature. The overall frequency of discontinuation in Revatio® -treated patients at the recommended dose of 20 mg TID was low (3%) and the same as placebo (3%). Side effects that occurred in more than 2%, and more frequently by patients on study drug than on placebo included headache (67% vs. 56%) epistaxis (13% vs. 2%) flushing (15% vs. 6%), dyspepsia (19% vs. 10%), nasal congestion (6% vs. 0%), insomnia (11% vs. 2%), erythema (9% vs. 2%), exacerbation of dyspnea (11% vs. 5%), diarrhea (13% vs. 9%), myalgia (11% vs. 6%), pyrexia (9% vs. 5%), gastritis (5% vs. 0%), sinusitis (5% vs. 0%) and paresthesia (5% vs. 0%).

At doses higher than 20 mg TID, incidence of adverse events of some symptoms was increased including flushing, diarrhea, myalgia, and visual disturbances (mild and transient color-tinge, sensitivity to light or blurred vision.) Incidence of retinal hemorrhage in subjects taking the recommended dose was 1.9% vs. placebo and occurred in patients on anti-coagulation. **It is noteworthy that patients in this study will not be on anticoagulation**. There was no report of priaprism in the placebo controlled trial of Revatio, nor in Dr. Harris and Taylor-Cousar's studies.

Post-marketing experience of Viagra® (dosed once daily as 50-100 mg for erectile dysfunction), has demonstrated serious cardiovascular, cerebrovascular and vascular events, as well as priaprism and ischemic retinopathy. These adverse events were not seen in the randomized, placebo-controlled trial of Revatio® for pulmonary hypertension. The majority of these events occurred in patients with preexisting cardiovascular risk factors. **Patients with cardiovascular risk factors will not be included in the study**. Revatio did cause transient decreases in supine blood pressure in healthy volunteers (mean maximum decrease in systolic/diastolic blood pressure 8.4/5.5mmHg). Subjects in this study will have monitoring of their blood pressure following administration of the initial dose of study drug. Furthermore, they will be instructed not to take more than the prescribed dose, and will be excluded from the study if it is determined that they are taking more than the prescribed dose.

Cases of sudden decrease or loss of hearing have been reported post-marketing in temporal association with the use of phosphodiesterase inhibitors. In some cases, medical and other factors were reported that may also have played a role in the otologic adverse events. It was not possible to determine whether these reported events are directly related to the use of phosphodiesterase inhibitors, to the patient's underlying risk factors for hearing loss, or a combination of these factors. Hearing was monitored in Dr. Taylor-Cousar's pilot study of sildenafil as an anti-inflammatory; there were no occurrences of study drug related hearing loss.

There is a risk of allergic reaction to the drug. Patients with known history of reaction to this class of drugs will not be included in the study. There is a low likelihood of such events.

The study drug is pregnancy category B, thus fetal harm is a risk of the study, although is unlikely to occur. Lactation safety is unknown, and therefore caution is advised. Breastfeeding, pregnancy, or verbal expression of unwillingness to practice an acceptable birth control method (abstinence, hormonal or barrier methods, partner sterilization or intrauterine device) during participation in the study are criteria for exclusion.

Risks from study spirometry and sputum collection (which are routinely performed at quarterly CF clinic evaluations) include coughing, breathlessness, chest pain and lightheadedness. There is a moderate chance that patients will experience these transient symptoms after performing spirometry or sputum collection.

The risks of drawing blood include temporary pain and discomfort from the needle sticks, occasional bruising, sweating, feeling faint or lightheaded and in rare cases infection. It is likely that all patients will experience mild discomfort from blood draws. To minimize discomfort from blood draws, for patients with indwelling catheters, the catheter will be used for blood draws. For patients who do not have indwelling catheters, a temporary intravenous catheter will be placed for multiple timed draws.

Risks from EKG and FMD testing and the required electrode placement are skin irritation and the minor, transient discomfort from removal of the leads.

There are risks of stress and emotional distress from being asked questions about how one copes with living with CF on the questionnaire. It is possible that some subjects may experience mild distress from being answering questions on the questionnaire; however, a visit from the CF social worker, who addresses the impact of disease on social function, is standard of care for CF patients during quarterly visits. Furthermore, subjects will be told that they may skip questions that cause significant distress.

Although maximal exercise tests are routinely conducted, the risk of death during a maximal CPET in adults is 1 in 10,000 tests. This risk reflects the incidence of coronary artery disease in adults undergoing cardiac stress testing. This risk is further minimized in our patient population by excluding patients with possible coronary artery disease. The risk of adverse events during maximal CPET in the pediatric population is minimal.

The American College of Sports Medicine and the American Thoracic Society/American College of Chest Physicians guidelines for contraindications to cardiopulmonary exercise testing and exercise test termination criteria will be used. Patients with an FEV_1 <40% predicted will be directly supervised by a physician during the exercise test. A physician will be immediately available for all other tests

Loss of privacy is a risk of the study. We will minimize this risk by keeping all study documents, including any link between subject name and study number in a locked office in the office of the PI/study coordinator. Sputum samples and other biomarker specimens will be de-identified. Case report forms will contain study numbers only.

The only alternative to study participation is not to participate in the study while continuing on standard of care therapies for CF lung disease associated (including inhaled antibiotics, inhaled mucolytics, azithromycin, and airway clearance therapies).

2. Adequacy of Protection Against Risks

A. Recruitment and Informed Consent

After satisfactorily preparing for active recruitment, we will begin the screening and recruitment process of patients with CF. At Augusta University, Dr. Harris has developed a strong collaboration with Drs. McKie and Forseen, the Augusta University CF center directors and both Dr. McKie and Dr. Forseen are very enthusiastic about the current proposal (See letters of collaboration from Drs. McKie and Foreseen). The CF center has approximately 95 children and 85 adult patients with CF, majority of whom would fit our inclusion criteria. At National Jewish Health, Dr. Taylor-Cousar is the Co-Director and Director of Clinical Research of the Adult CF Program. The Adult CF Program at NJH has 491 patients registered in PortCF of whom 377 meet the basic inclusion/exclusion criteria. For Drs. Harris and Taylor-Cousar's pilot studies, we were able to recruit even those patients who traveled 2-3 hours away by coordinating some of their research visits with their routine CF clinic visits, and by offering gas mileage reimbursement. In some instances patients spent the night on the inpatient unit at NJH or in local hotels so that they can arrive to the research site early the next morning to participate in the studies.

Only the principal investigators or sub investigators named on the informed consent documents for this study will seek consent/assent. The potential participant will have the study explained to them in a language that they can understand, be asked to read each page of the documents and initial at the bottom of each page after reading it. The burden of participation, potential risks, and any costs that may be incurred as a result of their participation will be discussed with the prospective participant. After reading the detailed consent documents and explaining all aspects of the study they will be given an opportunity to ask questions and have them answered. The participant will acknowledge that participation in the study is voluntary and that he/she may withdraw at any time and for any reason.

We will minimize the possibility of coercion or undue influence by not highlighting reimbursement, and by explaining that participation in the study or lack of participation in the study will have no effect on the routine care of the patient.

After having all their questions answered, and if the potential participant chooses to participate, then they will sign and date the consent form. In the event the subject is under 18, a parental consent will be obtained in addition to the child's written and verbal assent. The consent document(s) will then be signed by a witness and signed by the investigator seeking consent. Each participant will be asked to provide their signature, to indicate they agree to release private information and are authorizing us to use their test data and perform testing on biological specimens. However, in the event a participant elects to not allow certain procedures, all information will be kept confidential utilizing all safeguards to protect their information. The location for this authorization will be in the second to last page of the ICD just prior to consenting to be involved in the research study. If the environment is perceived by the participant to not provide adequate privacy, accommodations will be made to ensure participant feels and knows sensitive materials are safeguarded. After signing consent forms, the subjects will undergo the aforementioned preliminary visit testing. Subsequently, subjects will be scheduled for their experimental testing sessions to complete the proposed aims (described above).

The consent process will be documented in the medical record with a dated/timed note that describes the discussion with the subject/family and questions that were answered. A copy of the consent will be placed in the patient's medical record, and the patient will be informed of such.

We will use the portCF data base to identify potential subjects. Medical charts will be reviewed to determine if eligibility criteria are met. If a patient consents to be in the study, his or her medical records will also be reviewed to determine CF sputum microbiology as required for study participation.

At each visit, the plan for the testing procedures will be explained to the participant (continued consent) and he/she will have an opportunity to ask questions about the procedure and decline participation. The sub-investigator obtaining continuing consent will document this discussion on a continuing consent form and sign the form at each testing visit. During phone screenings, consenting process, and throughout the testing process all participants will be spoken to in private or by letter of any details related to the study where continued communication was necessary. In this way privacy would be safeguarded.

B. Protections Against Risk

Co-PI, Dr. Ryan A Harris, PhD, CES, FACSM is a clinical exercise physiologist with extensive vascular training. Co-PI, Dr. Jennifer L. Taylor-Cousar, MD, MSCS is an adult and pediatric trained pulmonologist with training in exercise physiology. Drs. Harris and Taylor-Cousar will monitor the study on a daily basis at their respective sites. During testing days, Drs. Harris and Dr. Taylor-Cousar, or his/her designee, will then decide the course of action, if any, that needs to be taken. In the event that Drs. Harris or Taylor-Cousar are not available, their respective on-site study Co-Investigators, will be notified immediately, if a serious or potentially serious adverse event or unanticipated problem occurs or an injury requiring medical treatment.

Subjects will be receiving regular medical care as outlined in standard texts and the published guidelines of the U.S. Cystic Fibrosis Foundation (CF Foundation Center Committee and Guidelines Subcommittee 1990.) Subjects will continue all other standard of care therapies during the trial.

All subjects should continue the same medications throughout the study period, as medically feasible, with no introduction of new chronic therapies during or <4 weeks prior to enrollment. If a change is concomitant medications is required, the reason(s) for the change(s) will be recorded on the subject's CRF. Subjects who require treatment for a pulmonary exacerbation during the course of the trial will be withdrawn from the study.

To monitor closely for immediate side effects of the medication (particularly, hypotension,) subjects will be monitored following administration of the first dose of sildenafil. Blood pressure monitoring will occur prior to the administration of sildenafil administration, and up to 2 hours after administration.

To further assess for medication side effects, and reduce the risk of delaying diagnosis or treatment of a respiratory exacerbation, there will be close monitoring and follow-up. Subjects will be monitored for safety with the collection and recording of serious adverse events during the course of the study.

All blood draws will be performed by trained personnel using sterile technique. To minimize risk from blood draw, only a nurse or trained and certified research ream member will be allowed to draw blood, and aseptic techniques will always be employed.

All women 12 years and older will have a urine pregnancy test prior to study enrollment to rule out pregnancy. In the event of a positive test, we will re-test to ensure a false positive was not identified. If the second test is still positive, we will exclude the subject from the study. Efforts will be made to minimize psychological stress from anthropometric and other measurements by explaining procedures and protecting participant's privacy during weighing, measuring, testing, and completing questionnaires and forms.

Sildenafil is safe and FDA approved. We will educate subjects and parents on the more common side effects and associated frequency unwanted stimulated blood flow (i.e. clitoral and penile erections).

The risks from PFTs, sputum collection and blood drawing are low risks that CF patients encounter in standard care for their disease. The side effects associated with sildenafil are generally mild and transient. Thus, patients will agree to participate in a study in which the tests are low risk, and the drug is FDA approved, and associated with possible benefits of personal symptomatic improvement as well as benefit from the knowledge that they have contributed to the understanding and treatment of CF lung disease, a benefit discussed on the CFF website. (http://www.cff.org/research/ClinicalResearch/) To minimize the risk to participants' confidentiality, none of the participants will be listed by name, SSN, medical record number, or any other identifiable markers. Instead, each participant will be assigned a random, but consecutively distributed, identification number. Those unique ID numbers will be used in the phenotypic data file. A list of participants' names and their ID numbers is kept in a separate file and only the Principal Investigator and the project coordinator will have access to this file.

All data collection records will be kept in a locked file cabinet when not in use. Upon completion of the study all files will be stored in a locked secured filing room. All data will be numerically coded and will not contain any personal identifiers (i.e. names, addresses, etc.). The information being collected will not be shared or disclosed to any third party, the participant, or their children. The participant will be notified only if tests indicate serious health problems that may threaten the health of the participant. All tubes with blood specimens will be coded with unique numbers to further protect confidentiality. After all data are collected, the unique ID numbers used as the means of initially bringing the phenotypic file together with the genetic information will be erased after the files are merged.

Datasets will be stored on the secured password protected network drive assigned by GRU IT. Only approved sub-investigators who need access to confidential information for the purpose of data entry and participant payment will have access to this information and access is further limited by password protected computer access.

Each risk described has been assessed for threats to confidentiality. We believe our protective procedures currently implemented are adequate to ensure protection of the data both as it is managed, stored, and accessed. These methods have been utilized by previous studies without issue. The system in place is a product of the work implemented by institutional IT departments and the protocols put in place at each institution for protecting material documents, data, and information.

Additional Protections for Children Involved as Subjects in Research

Khan et al performed bronchoalveolar lavage (BAL) in infants with CF. Even in those patients who had negative cultures, BAL neutrophil count and IL-8 were elevated; thus, demonstrating that inflammation occurs even prior to colonization with pathogenic bacteria in CF lung disease.[29] Additionally, Dr. Harris' group showed that even children with CF have endothelial dysfunction[20]. Children are being included in this study because they are most likely to benefit from early intervention.

Sildenafil dosing in children is suggested to be 1 mg/kg TID. Accordingly, children between 9-17 years old who weigh < 20 Kg will be excluded from the study. **In addition, children who weigh between 20-**

40 Kgs will not have their dose increased from 20-40 mg TID, rather they will remain on 20 mg TID for the duration of the study. Use of sildenafil has been studied in both healthy adults, and in infants, children and adults with cardiopulmonary disease, and is generally well tolerated.[93-97] Additionally, a case report of the use of sildenafil in a patient with severe lung disease described symptomatic improvement in the patient with no serious adverse events.[10] However, because sildenafil has not been systematically studied in patients with CF, it is possible that there would be unanticipated events. Therefore, with respect for risk for children, the study falls into category B: it involves greater than minimal risks but presents the prospects of direct benefit to the individual subjects.

Because cystic fibrosis patients are generally diagnosed in infancy, parents and patients are usually seen simultaneously even through the teenage years because of comfort level of both parent and child. If there are sensitive issues (i.e. sexuality) to be discussed, the parent and child are asked if the patient should be interviewed by him/herself. Parent and child will be asked if they would like explanation of the study to occur simultaneously or separately. Consent of both parents and assent of the child will be required for study participation.

For subjects who are under 18, one parent will be required to attend all study visits with the participating child

3. Potential Benefits of the Propose Research to Human Subjects and Others

Exercise intolerance is an independent risk factor for death in patients with CF, independent of lung function. A critical barrier to improving exercise tolerance in patients with CF is our lack of knowledge regarding the different mechanisms which contribute to the lower exercise capacity. Accordingly, the **overall goal** of this proposal is to increase our understanding of the potential mechanisms which contribute to improvements in exercise capacity following PDE5 inhibition in patients with CF, and to determine if sildenafil therapy increases exercise capacity. This project represents **a paradigm shift** by focusing on systemic complications, rather than pulmonary, as the cause for exercise intolerance in CF. In addition, based on our pilot studies, PDE5 inhibition can increase exercise capacity and overall quality of life in patients with CF. Findings from the proposed investigation will provide the essential evidence necessary to guide the design of a large, multi-center clinical trial to support sildenafil and other systemic targeted therapies to improve exercise capacity, health, and survival in CF.

4. Importance of the Knowledge to be Gained

The evaluation of exercise capacity has tremendous prognostic value in patients with CF, recognition that has recently prompted the European Respiratory Society and the Cystic Fibrosis Foundation to publish a joint statement recommending that routine exercise testing be conducted in all patients with CF. There is a desperate need to understand the mechanisms which contribute to exercise intolerance in CF, and our preliminary studies offer strong, compelling support for the use of PDE5 inhibitors, specifically sildenafil, as a therapeutic option to increase exercise capacity in this patient population. The proposed investigation will identify 3 potential mechanisms which contribute to exercise intolerance in CF. The **impact** of our research will be to provide mechanistic insight into exercise intolerance in CF and the effectiveness of sildenafil in improving exercise capacity. In addition, findings will provide the essential evidence necessary to guide the design of a large, multi-center clinical trial to support sildenafil and other targeted therapies to improve exercise capacity, health, and survival in CF.

5. Data and Safety Monitoring Plan (DSMP)

The individuals responsible for the data to day safety monitoring will be the PIs, Dr. Ryan Harris and Dr. Jennifer Taylor-Cousar. In addition, Dr. Greg Harshfield, PhD will serve as the outside study monitor. Dr Doug Everett, un-blinded statistician will compile all of the serology and adverse events (if any) and provide an unbiased report to Dr. Harshfield every 6 months. Dr Harshfield will then provide a report to Drs. Harris and Taylor-Cousar that will be included in the annual progress report sent to the FDA. If funded, we would also request review by the CFFT DMC.

The PIs (Ryan A Harris, PhD and Jennifer Taylor-Cousar, MD, MSCS) will monitor the study on a daily basis. If adverse events should arise, Drs. Harris and/or Taylor-Cousar, or his or her designee, will be notified immediately.

In order to determine if there are changes needed to the protocol, Drs. Harris and Taylor-Cousar will notify the human assurance committee as soon as possible per their policies and procedures.

The site PIs will monitor adverse event(s) continuously. Each PI will be responsible for ensuring that any adverse events are reported to the Institutional Review Board at each institution in compliance with their requirements. Adverse events will be reported to the IRB within 72 hours of any member of the research team becoming aware of the event. Serious adverse events will be reported within 24 hours to the IRB and to the FDA.

Ongoing Quality Control will include regular data verification and protocol compliance checks to be performed by Drs. Harris and Taylor-Cousar. Required administrative reports that describe study progress to include accrual, demographics, and participants' status will be prepared and reviewed. These reports will also identify adherence to inclusion/exclusion criteria, study protocol, and any adverse events. Summary reports that are reviewed will always be void of personal identifiers to protect confidentiality.

An adverse event will be defined as any unfavorable and unintended sign, symptom, or disease temporally associated with the use of Revatio® or other protocol-imposed intervention, regardless of attribution.

This definition will include the following:

•AEs not previously observed in the subject that emerge during the study period, including signs or symptoms associated with CF that were not present prior to the study period

• Complications that occur as a result of protocol-mandate interventions (e.g. sputum induction)

• Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period

Subjects will be questioned and/or examined by the Investigator or her designee for evidence of adverse events.

Subjects having adverse events will be monitored with relevant clinical assessments and laboratory tests as determined by the investigator. All adverse events will be followed to satisfactory resolution or stabilization of the event(s.) Any actions taken and follow-up results will be recorded on the appropriate page of the CRF, as well as in the subject's source documentation. Follow-up laboratory results will be filed with the subject's source documentation.

For all adverse events that require the subject to be discontinued from the study, relevant clinical assessments and laboratory tests will be repeated until satisfactory resolution or stabilization of the event(s.)

A serious event will be determined as follows:

- It results in death
- It is life threatening
- It requires or prolongs inpatient hospitalization
- It results in persistent or significant disability/incapacity
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product
- It is considered a significant medical event by the investigator based on medical judgment

Grading of AEs will be as follows:

- Mild: Transient or mild discomfort (<48 hours); no interference with the subject's daily activities; no medical intervention/therapy required
- Moderate: Mild to moderate interference with the subject's daily activities; no or minimal medical intervention/therapy required
- Severe: Considerable interference with the subject's daily activities; medical intervention/therapy required; hospitalization possible

To determine the causality of AE and SAE the follow guidelines will be assessed:

- Yes

There is a plausible temporal relationship between the onset of the AE and administration of Revatio®, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE abates or resolves upon discontinuation Revatio®.

• No

Evidence exists that the AE has an etiology other than administration of Revatio® (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to administration of Revatio® (e.g., cancer diagnosed 2 days after first dose of study drug.)

In this pilot study, it is unlikely that there would be early (i.e. before study completion) demonstration of efficacy or futility. However, unanticipated serious adverse events would result in early termination of the study.

Criteria for Participant Discontinuation:

- Decrease in FEV_1 of ≥20% from baseline
- Severe dyspnea at rest
- Two-fold increase in creatinine
- Two-fold increase in liver function tests
- Pregnancy
- Subject refusal to continue in study
- Development of an adverse event that the investigator feels is a preclusion to continued subject participation
- Development of a pulmonary exacerbation requiring hospitalization and/or intravenous antibiotics.

As per standard consent, emergency care will be provided, but at the cost of the patient/patient's provider.

Safety and Efficacy Review Personnel

- The IRB will review enrollment data and individual AE reports.
- If the study is funded, we will request the use of the CF TDN DSMB. A DMC charter will be created and the DMC and the principal investigator, will be responsible for reviewing enrollment data and individual AE reports, as well as for periodic safety analysis (every 6 months.)

6. IND

Both Drs. Taylor-Cousar and Harris hold INDs for the study of sildenafil in patients with CF: IND # 102639 and IND # 119923. Each will continue to submit annual FDA reports.

7. Clinical Trials.gov Requirements

The trial will be registered on Clinical Trials.gov prior to study enrollment.

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