Identifiers: NCT02626182

Brief Title: Evaluation and Treatment of Pulmonary Vascular Disease in Moderate to Severe CF

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NOTICE OF RENEWAL APPROVAL

CPA# 20601

To: Jennifer Taylor-Cousar, MD

Re: HS-2910 "EVALUATION AND TREATMENT OF PULMONARY VASCULAR DISEASE IN MODERATE TO SEVERE CYSTIC FIBROSIS LUNG DISEASE"

Date: Wednesday, October 31, 2018

This is to inform you the National Jewish Health IRB has renewed its approval of the above research study.

The IRB has approved the study for 12 months.
The study has been approved until 10/24/2019.

All conditions for continued approval during the prior approval period remain in effect. These include, but are not necessarily limited to the following requirements:

- Prior written approval must be obtained from the IRB for any modifications to the study, including changes in procedures, co-investigators, funding agencies, etc.
- Unexpected or otherwise significant adverse events, unanticipated problems or incidents that may occur in the course of this study must be promptly reported to the IRB.
- Significant new findings that develop during the course of this study that may affect the risks and benefits to participation must be promptly reported to the IRB.
- Records of this research must be maintained according to IRB guidelines.
- Status reports must be submitted to the IRB upon request.
- The study cannot continue after **10/24/2019** until re-approved by the IRB. Complete and submit progress reports to the IRB as follows:
    - Renewal of the study - submit the Continuing Review Report approximately 6 weeks prior to the expiration of the approval period. The IRB provides a continuing review notice as a courtesy, however the Principal Investigator is responsible for ensuring timely submission of renewal documents.
    - Completion of the study - submit the Completion Report for study closure.

Please call the IRB office if you have any questions about the terms of this approval.

Thank you ,
National Jewish Health IRB

Enclosures:
Continuing Review Report dated 7/3/18

HS-2910 Evaluation and Treatment of Pulmonary Vascular Disease in Moderate to Severe Cystic Fibrosis Lung Disease

1. Specific Aims

Over time, patients with Cystic Fibrosis (CF) develop disabling lung disease that progresses to chronic respiratory failure, exercise intolerance with marked limitation of physical activity, and premature death. Despite substantial improvements in care, patients with CF often develop pulmonary vascular disease (PVD) that leads to pulmonary hypertension. Previous studies have clearly linked severe pulmonary hypertension and right heart failure with high mortality in CF. Early clinical manifestations of PVD prior to the development of *cor pulmonale* include shortness of breath and dyspnea with exertion, but the extent to which PVD contributes to the decline in exercise tolerance and quality of life in patients with CF is not known. Early evidence of PVD could be recognized in CF patients through standardized exercise testing and echocardiographic evaluation. Identifying those CF patients with PVD prior to the onset of right ventricular dysfunction may allow pharmacologic intervention to attenuate the progression of cardiovascular disease and improve quality of life. Clinical trials have demonstrated that treatment with the phosphodiesterase type 5 inhibitor, sildenafil, can decrease pulmonary vascular resistance and improve exercise tolerance in non-CF patients with pulmonary hypertension. Because experimental and clinical studies have implicated impaired NO-cGMP signaling in the pathophysiology of lung disease in CF, sildenafil may provide a novel pharmacological approach for treating PVD in patients with CF lung disease.

I hypothesize that PVD contributes significantly to exercise intolerance in patients with moderate to severe CF lung disease, and that pulmonary vasodilator therapy will increase exercise capacity, improve cardiac performance during exercise, and enhance quality of life in patients with CF. To test these hypotheses, I propose the following specific aims:

Specific Aim #1: To evaluate the contribution of PVD to exercise intolerance in CF patients with moderate to severe lung disease utilizing cardiac magnetic resonance imaging (MRI), six-minute walk testing (6MWT), cardiopulmonary exercise testing and pulmonary function testing.

Specific Aim #2: To assess whether chronic treatment with the selective pulmonary vasodilator sildenafil citrate will be safe, improve cardiac output, increase ventilatory function, decrease symptoms of exercise intolerance and improve quality of life in CF patients with moderate to severe lung disease.

2. Background and Significance

Cystic fibrosis (CF) is the most common life-shortening inherited disease in Caucasians, currently affecting approximately 30,000 people in the United States.[1] Although previously considered a disease of childhood, approximately 50% of U.S. patients are over the age of 18.[2] CF lung disease is characterized by persistent airway inflammation that leads to a progressive decline in pulmonary function and early death.

Even in the setting of early pulmonary disease, exercise intolerance is a prevalent symptom in patients with CF.[3]Additionally, the rate of loss of exercise capacity in patients with CF occurs at a higher rate than in patients without CF.[4] It is known that peak VO_2 predicts mortality in patients with CF; higher peak VO_2 is a marker for longer survival.[5]

There are a number of factors that are hypothesized to play a role in exercise intolerance including ventilatory limitation, decreased muscle mass and peripheral muscle dysfunction, and poor nutritional status.[6] It is also possible that Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations directly contribute to exercise intolerance. Jiang et al developed mouse models of global CFTR knock out and selective cardiac myocyte knock-out.[7] In both knockout models, mice had increased myocardial contractility, and as well as increased ventricular torsion and strain. They hypothesized that such baseline changes might lead to cardiac remodeling that was independent of lung function. This question has not been explored in patients with CF.

Pulmonary vascular disease in CF

In addition to abnormalities of the airways, patients with CF who develop significant respiratory impairment are at marked risk for the development of PVD, characterized by pulmonary hypertension (PH) and subsequent *cor pulmonale*. [8-12] At autopsy, the pulmonary circulation is often characterized by the presence of right ventricular hypertrophy with abnormal muscularization of medium and small pulmonary arteries, with some intimal proliferation and increased extracellular matrix production within the adventitia. With advanced disease, pulmonary artery density is reduced, and subintimal fibrosis and muscularization of pulmonary veins have been noted at autopsy.[12] In addition to these structural changes, past studies have also shown that CF patients have a marked elevation of pulmonary artery pressure with brief exposure to acute hypoxia even in the absence of severe pulmonary hypertension at rest, suggesting that the pulmonary circulation in CF is characterized by abnormal pulmonary vasoreactivity.[11]

Florea et al examined right ventricular (RV) function in 103 adults with severe CF (mean FEV_1 20.3 ± 5.3%), and compared their RV function to that of healthy controls. Both RV diastolic and systolic dysfunction were seen in the CF patients. Importantly, while 22 patients had documented pulmonary hypertension (by Doppler echocardiography), only 7 of those patients demonstrated signs and symptoms of overt heart failure.[13] To further assess the prevalence and impact of subclinical pulmonary hypertension (PH) in patients with CF, Fraser et al evaluated cardiac function in 33 adults with moderately severe (FEV_1 40-65%, n=13) to severe (FEV_1 <40% predicted, n=15) CF lung disease who did not have clinical signs of right heart failure.[9] The authors found no qualitative evidence of RV dysfunction in any of the patients, however 41% of the patients with severe disease, and 20% of those with moderate disease had PH. In a multivariate analysis that included FEV_1, awake resting oxygen saturation was found to be the best predictor of pulmonary artery systolic pressure. Importantly, patients with PH had a significantly higher mortality after 5 years than those without PH. In a more recent study, Hayes and colleagues reviewed the United Network of Organ Sharing database to evaluate the effects of mild pulmonary hypertension (mean pulmonary artery pressure greater than or equal to 25 mm Hg as measured on right heart catheterization) on mortality in patients with CF. Both univariate and multivariate Cox models showed increased risk of death even in patients with mild PH (hazard ratio, 1.747; 95% CI 1.387-2.201; P < 0.001; and hazard ratio, 1.757; 95% CI, 1.367-2.258; P < 0.001, respectively).[14]

Mechanisms that contribute to PH and progressive PVD in CF are poorly understood, but are likely due to the long-term effects of inflammation, loss of lung parenchyma and intermittent or prolonged alveolar hypoxia.[8-12] Although it is clear that patients with more significant lung function impairment are at higher risk for PH, declining FEV_1 is not an entirely reliable predictor for the development of PVD in CF.[9] Early clinical manifestations of PVD prior to the development of *cor pulmonale* include shortness of breath and dyspnea with exertion, but the extent to which PVD contributes to these symptoms, and the decline in exercise tolerance and lower quality of life in patients with CF is unknown. Diagnosing PVD in patients with CF prior to overt clinical signs of *cor pulmonale* has historically been difficult as non-invasive evaluation has been limited to echocardiographic estimates of right ventricular (RV) pressure at rest. This diagnostic tool has been shown to underestimate the degree of PVD until significant RV dysfunction has occurred.[10] Identifying CF patients with PVD prior to the onset of significant RV hypertrophy or dysfunction may allow for the earlier application of pharmacologic interventions to improve symptoms and attenuate the progression of disease.

Early evidence of PVD may be recognized in CF patients through standardized exercise testing and echocardiographic evaluation. Abnormal structure and function of the pulmonary vascular bed in CF patients may worsen ventilation/perfusion mismatch, especially during physical activity. Increased pulmonary vascular resistance impedes the normal increase in cardiac output from the RV during exercise, due to the failure to adequately recruit or dilate the distal vasculature, which can lead to more marked rises in pulmonary artery pressure or less ability to increase cardiac output with exercise.[15, 16] With more advanced disease, pulmonary vascular abnormalities become manifest even at rest, leading to increasing strain on the RV, and ultimately, overt RV failure.[11, 12] Early detection would ideally allow for the use of therapeutic interventions to halt the progression of PVD to PH and RV dysfunction, thereby improving the overall quality of life and perhaps survival. Such a treatment strategy would represent a significant improvement over our current approach of awaiting more severe signs of PH with RV failure. Currently, our major approach to the treatment of established pulmonary hypertension is the avoidance of hypoxemia by aggressive monitoring of oxygenation while awake and during sleep, and providing oxygen as needed.[17] Pharmacologic therapies, such as calcium channel blockers, diuretics, and tolazoline have previously been investigated for treatment of PH in CF.[18-20]

However, these interventions have significant systemic side effects and generally lack long-term benefit, thereby limiting their use. In addition, treatment has generally been initiated late in the clinical course.

Pharmacologic treatment of PVD

Over the past ten years, new advances in vascular biology have led to remarkable improvements in the clinical care of patients with severe pulmonary hypertension.[21, 22] The use of several interventions, such as prostacyclin analogues, endothelin receptor antagonists, and inhaled nitric oxide (NO), have led to increased survival in patients with idiopathic and secondary causes of PH.[12] Extensive studies in diverse animal models have implicated disruption of NO-cGMP signaling as a major factor in the abnormal vascular tone, reactivity and structure in development of PH. Increased activity of the cGMP specific phosphodiesterase type 5 (PDE5) isoform in vascular smooth muscle inactivates cGMP, thereby worsening vasoconstriction, smooth muscle cell proliferation and adventitial growth.

Inhibition of phosphodiesterase type 5 (PDE-5,) which is responsible for degradation of cGMP, or stimulation with nitric oxide (NO) can cause protein kinase G-dependent smooth muscle relaxation. Sildenafil-induced increased cGMP concentration leads to pulmonary vasculature smooth muscle relaxation, and it is this effect that is key in treatment of pulmonary hypertension. Sildenafil citrate is FDA-approved for treatment of pulmonary hypertension, and has been used to treat both adult and pediatric patients with pulmonary hypertension.[23-27] In a large trial of sildenafil use for pulmonary hypertension, escalating doses of sildenafil were used for 12 weeks. Although the initial group of subjects was treated for 12 weeks, improvements in the primary outcome were seen at 4 weeks.[23] Whether sildenafil therapy can improve pulmonary hemodynamics at rest and during exercise, and enhance the quality of life in patients with CF have not been studied.

In addition to its potential effects on the pulmonary circulation, sildenafil may potentially improve lung function in CF patients through other mechanisms effected by the NO-cGMP signaling cascade. Several studies have shown that endogenous production or bioactivity of NO is markedly impaired in the airways of patients with CF.[28-32] Animal and clinical data have shown marked down regulation of inducible NO synthase (NOS) in airway epithelium, strong associations of severe lung disease with endothelial NOS polymorphisms, and increased degradation of NO metabolites, and decreased amounts of NO in exhaled breath condensate of patients with CF.[28-32] Thus, CF lung disease can be characterized by a relative NO deficiency with down-regulation of the NO-cGMP signaling cascade.

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.[33, 34] Sildenafil has also been shown to have anti-inflammatory effects. For example, Toward et al demonstrated that pretreatment with sildenafil inhibited LPS-induced airway hyper reactivity, white cell influx and NO dysfunction in two guinea pig models of airway disease.[35] In CF respiratory epithelial cells, Poschet et al demonstrated that the excessive pro-inflammatory response to *P. aeruginosa* exposure could be reversed by treatment with sildenafil.[33] Based on the *in vitro* data suggesting a possible role for PDEi as anti-inflammatories in CF, I designed a single site open-label dose escalation study (Clinicaltrials.gov No.: NCT00659529) to evaluate the safety, efficacy and pharmacokinetics of sildenafil in subjects with mild to moderate CF lung disease. On study day 1, subjects underwent exhaled breath condensate (EBC) measurement, lung function and routine laboratory testing, completed the CF Health Related Quality of life questionnaire (CFQ-R), and had sputum collected for bacterial counts and sputum biomarkers. Subjects received oral sildenafil 20 or 40 mg p.o. t.i.d. for 6 weeks, and all evaluations were repeated at the end of 6 weeks. Twenty subjects completed the study. There were no drug-related serious adverse events, and side effects were generally mild and consistent with those previously reported. There was improvement in the primary endpoint, mean sputum elastase ($p<0.03$), which is a sensitive measure of CF airway inflammation and has been shown to predict lung function decline in patients with CF.[36] There was also a non-significant trend towards improvement in sputum IL-8 ($p=0.13$). There was no difference in EBC pH, the respiratory score of the CFQ-R, or sputum microbiology ($p=0.44$, $=0.88$ and 1.0, respectively). With recent data showing a correlation between systemic markers of inflammation and pulmonary artery pressure in adults with CF[37], this aspect of sildenafil therapy may also be important in patients with advanced disease.

One final aspect of PDEi therapy that may be important in CF is its effect on CFTR function. Recent studies have also shown that PDEi can potentiate CFTR-mediated chloride transport activity and also correct surface

localization of F508del CFTR. Cobb *et al* showed that PDEis, including sildenafil, stimulate CFTR-dependent chloride transport in polarized airway cell monolayers expressing wild-type CFTR. Importantly, this effect was dose-dependent, and for two of the agents tested, achievable at concentrations below peak serum levels that occur with dosing used in systemic treatment of pulmonary hypertension.[38] Researchers have also studied the effects of sildenafil on CFTR trafficking in airway epithelial cells from CF and non-CF patients. Exposure to sildenafil led to rescue of F508del CFTR to the apical membrane as measured by immunofluorescence localization.[39] In related work, Robert *et al* demonstrated that a structural analog of sildenafil optimized for cell culture experiments, KM11060, partially restored ΔF508 trafficking in baby hamster kidney cells.[40] In our lab, using nasal mucosal tissue from ΔF508del mice, we showed that transepithelial Cl- currents improved following treatment with sildenafil.[34] More recently, Lubamba *et al* restored CFTR-dependent chloride transport using the nasal potential difference (NPD) assay in F508del mice, substantiating the approach that PDEi can restore both the cellular localization and activity of F508del CFTR in the airway.[41]

3. Previous Clinical Experience Using Sildenafil in Patients with Moderate to Severe CF Lung Disease: The CF center at TCH treated a single patient with severe lung disease with a combination of sildenafil citrate and inhaled nitric oxide for treatment of severe pulmonary hypertension. During four months of therapy the patient had a significant improvement in exercise tolerance with a 38% increase in distance walked during six-minute walk testing.[42]

Echocardiographic measures of pulmonary arterial pressures were also improved in this patient. Therapy was stopped at the time of lung transplantation.

Dr. Taylor-Cousar also treated a patient with moderate severe CF lung disease and exercise intolerance with sildenafil. Following 5 months of treatment, the patient reported marked reduction in his symptoms and demonstrated a 44% improvement in his six-minute walk distance as well as improvements in hemodynamic measurements. (Figure 1) Therapy was well-tolerated with no adverse events.

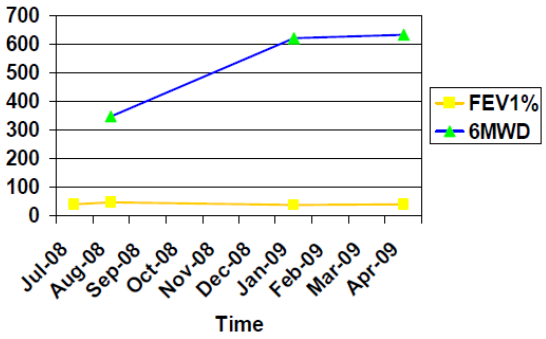


Figure 1. Serial changes in lung function (FEV1%; yellow line) and six minute walk distance (6MWD, blue line) over time in a patient with advanced CF lung disease. As shown, despite no improvement in lung function, the 6MWD increased during the treatment period.

In summary, based on exciting laboratory and clinical data, I hypothesize that pulmonary vascular disease contributes to exercise intolerance in patients with advanced CF lung disease, and that pulmonary vasodilator therapy can increase exercise capacity and improve cardiac performance without worsening gas exchange, and improve the quality of life in patients with CF. To test these hypotheses, I propose a pilot study to evaluate exercise tolerance and pulmonary function in patients with moderate to severe CF lung disease before and after oral sildenafil therapy. I will first determine the degree of pulmonary vascular disease that exists in this population and its effect on sub-maximal and maximal exercise in patients with moderate to severe CF as measured by cardiac magnetic resonance imaging, six-minute walk tests, and formal cardiopulmonary exercise tests. I will then study the effects of sildenafil therapy on these assessments of exercise tolerance, as well as on quality of life utilizing a validated CF-specific quality of life questionnaire. If positive, these pilot data will be used to plan a multicenter randomized controlled trial to determine the cardiopulmonary effects of sildenafil in CF patients with moderate to severe CF lung disease.[43]

4. Research Design and Methods

A. Study Design

This study is blinded pilot study that examines the use of sildenafil citrate in clinically stable patients with moderate to severe CF lung disease. Subjects will be randomized in a 3:1 (sildenafil:placebo) fashion. Subjects will perform exercise, pulmonary function and quality of life evaluations before and after 12 weeks of

therapy with sildenafil or placebo. The length of participation for each subject will be approximately 16 weeks, and will consist of a screening visit, three clinical evaluations and four phone evaluations over the study period.

B. Patient Population

Twelve clinically stable adult CF patients ≥ to 18 years of age will be recruited and enrolled from the adult oupatient CF clinic at NJH. Additional subjects (up to 20) may be enrolled if any patient leaves the study. We expect to enroll an approximately equal number of males and females. Most CF patients in the Colorado CF clinics are of white, non-Hispanic origin and I anticipate this ethnic mix to persist in this study. Because sildenafil has not been formally studied in CF patients with moderate to severe CF lung disease, and it is now uncommon for children to develop severe disease I will limit enrollment to adult patients for this pilot study.

C. Inclusion and Exclusion Criteria

Inclusion Criteria

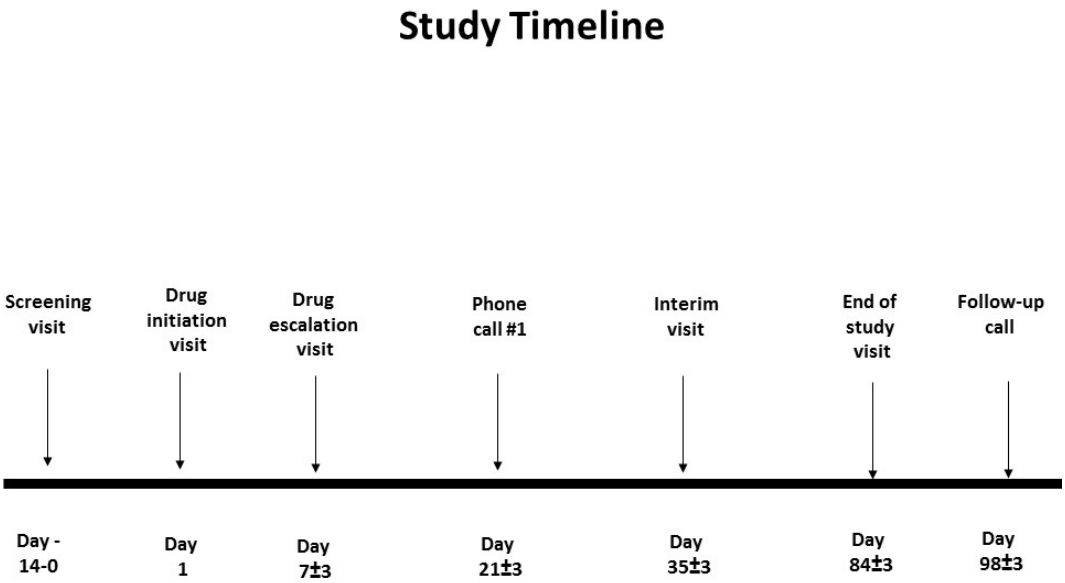
1) Confirmed diagnosis of CF based on the following criteria: Positive sweat chloride ≥60mEq/liter (by pilocarpine iontophoresis) and/or Genotype with two identifiable mutations consistent with CF, and accompanied by one or more clinical features consistent with the CF phenotype
2) Male or female patients ≥ 18 years of age
3) FEV_1 ≥ 20% predicted and ≤ 70% predicted (Hankinson)
4) 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
5) Ability to reproducibly perform spirometry (according to ATS criteria)
6) Ability to understand and sign a written informed consent or assent and comply with the requirements of the study
7) Willingness to maintain chronic CF medication schedule (e.g. alternating month inhaled antibiotics)

Exclusion Criteria

1) History of hypersensitivity to sildenafil
2) Use of an investigational agent within the 4-week period prior to Visit 1 (Day 0)
3) 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.
4) History of significant hepatic disease (AST or ALT > 3 times the upper limit of normal at screening, documented biliary cirrhosis, or portal hypertension),
5) History of significant cardiovascular disease (history of aortic stenosis, coronary artery disease, or life-threatening arrhythmia),
6) History of severe neurological disease (e.g. history of stroke),
7) History of severe hematologic disease (e.g. history of bleeding diathesis; current INR > 2.0
8) History of severe ophthalmologic disease (e.g. history of retinal impairment or non-arteritic ischemic optic neuritis)
9) History of severe renal impairment (creatinine >1.8 mg/dL.)
10) Inability to swallow pills
11) Previous organ transplantation
12) Use of concomitant nitrates, α-blocker, or Ca channel blocker (currently or within one month of Visit 1)
13) 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
14) History of sputum or throat swab culture yielding *Burkholderia cepacia* or *Mycobacteria massiliense* within 2 years of screening
15) Weight less than 40 kg at Screening
16) History of migraine headaches.
17) Resting room air oxygen saturation <80% without supplemental oxygen
18) 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
19) Start of CFTR modulator therapy less than 1 month prior to first dose of sildenafil or placebo
20) Use of anticoagulants

Frank pulmonary hypertension (RVSP >40 mmHg by ECHO)

D. Study visits



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. 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.[44]

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. Sweat test will be performed. 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 six-minute walk test and cycle ergometry test will be performed (see description of each procedure below). Tests will be performed in that order. Subjects will have a 30 minute rest period between the six-minute walk and the CPET. Subjects will be educated on the use of the fitbit. At the completion of the visit, each subject will begin daily treatment with sildenafil citrate or placebo (Belmar Pharmacy) at 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±3): After 7 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. (Total sildenafil or placebo therapy will last twelve weeks.)

Phone contact 1 (Day 28±3): Phone contact will be made with each subject to review dosing, compliance and side effect profiles. Phone contacts will be recorded on case report forms.

Study visit 4 (Day 42±3): The subject will return to the study site for CFQ—R vital signs, review of concomitant medications, and discussion of concerns related to sildenafil therapy. A study investigator will perform a brief physical exam. The subject will perform spirometry and will complete a 6MWT.

Study visit 5 (Day 84±3): Subjects will return to the outpatient research unit, where the CFQ-R (see description below) will be administered prior to all other assessments. A physical exam will be performed by a study investigator. Vital signs, weight, safety labs, and urine pregnancy in all women, will be done. Spirometry will be performed. Sweat test will be performed. After completing spirometry, cardiac MRI will be performed, a six-minute walk test and cycle ergometry test will be performed (see description of each procedure below). Tests will be performed in that order. Subjects will have a 30 minute rest period between the six-minute walk and the cycle ergometry test. The fit-bit will be removed. At the conclusion of this visit, subjects will be instructed to discontinue sildenafil or placebo therapy.

Phone contact 2 (Day 98±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.

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, serum pregnancy for all women, spirometry, a six-minute walk test 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.

Allocation and blinding

Patients will be randomized to sildenafil or placebo in a 3:1 fashion on study entry. The randomization sequence will be generated by Dr. Everett and provided directly to Belmar pharmacy. Investigators, subjects, laboratory, and study personnel will be blinded to treatment group.

Study Drug

Sildenafil (Revatio®) is FDA-approved for treatment of pulmonary hypertension at an oral dose of 20 mg three times per day. In a large trial (n=278) of use of sildenafil for pulmonary hypertension published in the New England Journal of Medicine, escalating doses of sildenafil were used for 12 weeks (20 mg three times a day, 40 mg three times a day, and 80 mg three times per day.) There was no dose-related effect on the primary outcome. Although the initial group of subjects was treated for 12 weeks, improvements in the primary outcome were seen at 4 weeks.[23]

Sildenafil is rapidly absorbed following oral administration in the fasting state with peak plasma concentrations occurring within 30-120 minutes (median 60 minutes) of administration. It is metabolized by the hepatic microsomal isoenzymes, CYP34A and cytochrome p450 2C9 (CYP2C9) to its major metabolite N-desmethyl

sildenafil. The metabolite accounts for approximately 20% of the pharmacologic effects of sildenafil. Both sildenafil and N-desmethyl sildenafil have a half-life of approximately 4 hours.

Sildenafil was the first PDEi approved by the FDA for treatment of pulmonary/cardiovascular disease. Tadalafil has the advantage of once daily dosing, and has been recently approved by the FDA for the treatment of PH. However, the short half-life of sildenafil makes it more appealing for study in a new patient population as exposure would be limited if short-term adverse reactions are observed.

Blinded study drug bottles will be packaged, labeled and shipped from Belmar pharmacy based on sequence number. Sildenafil will be stored and dispensed through the research pharmacy at National Jewish Health. An accurate accounting of dispensing and return of the study drug will be maintained by research personnel. Subjects will be instructed to return all unused study drug at the End of Study or Early Withdrawal visits. The PI holds the IND for the use of sildenafil in CF (IND #102639) and FDA reports will continue to be submitted annually.

E. Outcome measures

Clinical laboratory tests 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, and serum pregnancy test (for females of child-bearing potential) will be performed at the screening and end of study visits for safety. Add HS-CRP

Assessment of lung function: Spirometry will be performed at the screening and end of treatment visits by a CF research coordinator according to ATS standards.

Assessment of health related quality of life: The CFQ-R, which has been shown to correlate with survival in CF[45], 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.[46] The questionnaire will be administered at the first, interim and end of treatment visits.

ECHO: Echocardiogram will be performed at the screening visit to insure that the subject does not meet the exclusion criterion of frank pulmonary hypertension.

Six Minute Walk Test: 6MWT will be performed according to ATS standards.[47]

Cardiac MRI: Cardiac MRI is a non-invasive imaging method to evaluate pulmonary artery blood flow and right heart structure and function. Pulmonary artery blood and right heart structure and function will be measured before and after treatment with sildenafil or placebo.

Fitbit monitoring: In order to determine whether sildenafil improves exercise tolerance on a day-to-day basis, subjects will wear a Fitbit for the 12 weeks of the study for monitoring.

Sweat test: Pilocarpine iontophoresis will be performed and sweat collected according to CFF standards. Sweat volume will be recorded. Sweat will be collected using the Macroduct® collection system. Samples will be shipped to the TDN Core laboratory in Aurora, CO for chloride concentration measurement.

Cycle Ergometry Exercise 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.[48, 49] Trained personnel will perform the test. Subjects will perform a standard ramped protocol on dynamically braked cycle ergometer. A SensorMedics metabolic cart will be used to measure breath-by-breath respiratory gas exchange with nose clip in place. Pulse-oximetry, ECG and heart rate 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. [50,51] 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.

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	Visit 5	Call 2	Total
Payment	$125	$250	$100	$25	$100	$250	$25	$875

Subjects who complete the screening visit, will receive the $100 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.

F. Data Analysis

Primary outcome measures:

Change in distance walked during six-minute walk test after sildenafil therapy
Change in maximum work

Secondary outcome measures:

Change in gas exchange and heart rate kinetics (VO_2, VO2 VCO_2, VE)
Change in average daily activity (steps per day as measured by Fitbit)
Change in average heart rate after study drug therapy (as measured by Fitbit)
Change in cardiac hemodynamics
Change in measures of pulmonary function (FEV_1)
Change in quality of life as measured by the CFQ-R
Change in sweat test
Changes in antibiotic regimen during study period

Data Collection Tools

The site investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with study drug.

Designated study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific Research Electronic Data Capture (REDCap) electronic case report form (eCRF) as soon as the information corresponding to the visit is available. Each subject will be identified by site number, subject number and subject initials; subjects will not be identified by name in the study database or any study documents to be sent or transmitted out of National Jewish. All data entered during the course of the study must be reviewed and verified for completeness and accuracy by the site investigator.

The CFQR and CFRSD-CRISS will be completed by the subject at the visits outlined above. Data from the CFQ-R will be entered into the corresponding form for the subject's visit.

Statistical analysis:

As this is a pilot study, many parameters for establishing the rates of events and the degree of response to sildenafil therapy of our primary and secondary endpoints for patients with moderate to severe CF are unknown. In fact, defining these effects and obtaining data for the purpose of designing a large, multicenter randomzied trial is one of the major goals of this study. A 3:1 allocation is being utilized in this proof of concept study so that adverse events can be observed in the drug group versus the placebo group, and so that some sense of the natural variability in the previosly unexplore outcome measures (e.g. cardiac MRI findings) can be observed in the placebo group. The degree of change in the means of primary outcome measures (distance

walked during 6MWT, VO_2 peak, and VE/VCO_2) will be described with emphasis toward a future study, but the study data will be analyzed with stringent statistical methods.

The data analysis for this study will be performed by the prinicpal investigator with assistance from biostatistician, Douglas Everett, PhD. This protocol is a pilot study designed to evaluate physiologic changes after a therapeutic intervention following a therapeutic intervention. Primary and secondary outcome measures will be reported as means +/- standard deviations. Patient data will be assumed to comprise a normal distribution. Paired t tests will be used to compare primary and secondary outcome measures between pre- and post-treatment. Values of $P < 0.05$ will be considered significant. Repeated measures analysis using a mixed effect model to compare results between the different time points. Non-parametric data analysis may also be performed if subject data is found to not be normally distributed. The counted subjects will be compared with treated subjects using a mixed model to allow for the different time points and the correlation within subjects.

G. Mechanism of monitoring patient safety

Medical Monitoring and Adverse Events: The medical monitor will perform interim analyses of the safety data. The first interim analysis will occur after five patients have been enrolled in the study protocol. Data will be reviewed with the principal investigator on a monthly basis. Adverse events that may occur within this study population are most likely to be related to the underlying disease process as patients with cystic fibrosis are frequently hospitalized for intravenous antibiotic administration during periods of pulmonary exacerbation. Therefore, hospitalization for worsening pulmonary function will not be considered an adverse event or serious adverse event in this study protocol. However, those patients will be withdrawn from the study. Study related adverse events that will occur will most likely be related to the vasodilator effects of oral sildenafil citrate therapy. We expect the incidence of severe headache or uncomfortable symptoms of vasodilation (flushing, nasal congestion, etc.) to be self-limited.

Serious adverse events and reporting: Serious adverse events relating to this sildenafil therapy would include myocardial infarction, stroke, death, or anaphylactic reaction related to sildenafil citrate therapy. No patients with known cardiac disease or sildenafil allergy will be included in the study. Serious adverse events relating to exercise testing would include life-threatening arrhythmia or death during exercise testing. If any serious adverse event occurs, the principal investigator or one of the other investigators will report the incident (and any decision to suspend or halt the protocol) to the medical monitor and NJH IRB immediately (within 24 hours of becoming aware). Protocol stopping criteria will include any sildenafil citrate-related death.

Confidentiality: Confidentiality will be maintained through the duration of the study via strict adherence to all HIPAA compliance regulations.

Mechanism for Monitoring Patient Safety

Study Oversight

The CFFT Data Safety Monitoring Board (DSMB) at the University of Arizona will provide trial oversight. A Data Monitoring Committee (DMC) a subcommittee of the DSMB will be formed for this trial in order to review the protocol, patient informed consent, and safety monitoring plan. During the study, the DMC will be responsible for real-time serious adverse event monitoring and reviewing interim safety reports to ensure the continued safety, scientific validity and merit of the trial and to make recommendations concerning continuation, termination or modification of the trial. The DMC will include at least two CF clinicians, and a biostatistician.

.Adverse Events (AE)

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 (with the exception of pulmonary exacerbation unrelated to study drug, see section G)

• 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:

• Definitely: There is a plausible temporal relationship between the onset of the AE and administration of sildenafil, 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 of sildenafil.

•Probably: There is a plausible temporal relationship between the onset of the AE and administration of sildenafil, and the AE follows a typical response to sildenafil, but a potential alternative cause may be present.

- Unlikely: There is a reasonable possibility that the onset of the AE is related to the administration of sildenafil, but an alternative cause seems more likely.

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

Subject and Study Stopping Criteria

Criteria for Study Termination

There will be no interim evaluation for demonstration of efficacy or futility. Formal stopping rules for safety are will be outlined in the DMC Charter. The study may be terminated by the DMC if they have concerns about the safety data.

Criteria for Study drug Discontinuation

- •Symptomatic drop in systolic blood pressure of >20mmHg at drug initiation
- • Severe dyspnea at rest
- • Two-fold increase in creatinine from baseline
- • Five-fold increase in liver function tests (AST, ALT, Alkaline phosphatase, total bilirubin)
- • Pregnancy
- • Subject refusal to continue in study
- • Development of an adverse event that the investigator feels is preclusion to continued subject participation

Subjects who discontinue study drug will be asked to complete all study visits.

Criteria for drug reduction

- •Any subject with a symptomatic drop in systolic blood pressure of >20mmHg following the first dose of 40mg of sildenafil, or recurrent nausea, vomiting or headaches will remain on 20 mg of sildenafil for the remainder of the study.

Known Potential Toxicity of Study Drug

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 t.i.d. 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 the recommended dose* of 20mg t.i.d., 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. Patients in this study will not be on anticoagulation. There was no report of priaprism in the placebo controlled trial of Revatio®.

Post-marketing experience of Viagra® (dosed as 50-100 mg once daily 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 doses of study drug (30 minutes, 60 minutes, and 120 minutes after the initial dose of 20 mg at visit 2, and subsequent to the first 40 mg dose at visit 3).

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. In our previously conducted study of sildenafil in subjects with CF, there was no evidence of hearing loss following treatment with sildenafil.

Pregnancy
If a subject becomes pregnant during the study, the subject will be discontinued from the study.

Medical Monitor
The medical monitor (Dr. Scott Sagel) should be contacted directly for any medical or safety concerns. The medical monitor will review all SAE. If an SAE qualifies for expedited reporting to regulatory agencies (classified as unexpected and related to study drug), the medical monitor will immediately notify Dr. Taylor-Cousar by phone or pager. Within one business day of medical monitor review, the SAE report will be distributed to the Data Monitoring Committee (DMC) chair.

DMC
The Cystic Fibrosis Foundation Therapeutics Data Safety Monitoring Board at the University of Arizona will provide a DMC for oversight of this trial.

Interim safety analysis will occur when half the study patients have been enrolled. Additional interim analyses may be requested at any time by the DMC. Serious adverse events will be reviewed by the DMC as they occur.

	Screening	Drug initiate visit	Dose escalate visit	Follow-up contact #1	Interim visit #1	End of study visit	Follow-up contact #2	Early Term Visit
Visit	1	2	3		4	5		
Day(s)	0	1 Week 1	7±3 Week 2	21±3 Week 4	35±3 Week 6	84±3 Week 13	98±3 Week 15	
Informed consent	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
Bacterial infection (historical)	x							
Vitals signs	x	x	x		x	x		x
Pulse Oximetry	x					x		x
Spirometry	x				x	x		x
Complete lung function test		x				x		
Height	x							
Weight	x	x				x		x
Complete physical exam	x					x		x
EKG	x							
ECHO	x							
Limited physical examination (HEENT, Chest, Lungs)		x			x			
Sweat test		x				x		
Exhaled NO		x				x		
6MWT		x			x	x		x
CPET		x				x		x
Cardiac MRI		x				x		
Laboratory tests (CBC, liver function, chemistry, INR, CRP)	x	x				x		x
Pregnancy test	x					x		x
Study drug dispensed		x	x		x	x		
Study drug bottle return			x		x	x		x
Adverse events		x	x	x	x	x	x	x

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