

**RESEARCH PLAN****Title: Effects of sildenafil on CFTR-dependent ion transport activity in subjects with mild-moderate CF lung disease**

Sildenafil is an attractive agent for further study in cystic fibrosis (CF). Preclinical studies have demonstrated that sildenafil can correct the pH and sodium abnormality in CF cells, improve F508del CFTR trafficking and potentiate CFTR dependent Cl<sup>-</sup> transport, and may also exhibit anti-inflammatory properties. We have shown it is well-tolerated in a pilot trial of CF patients<sup>1</sup>, and it also has a well-established safety profile in severe lung disease.<sup>2,3</sup> We propose to test the *in vitro* ability of sildenafil to improve ion transport in the setting of abnormal CFTR in human CF subjects.

**Hypothesis:** Systemic administration of sildenafil to patients with mild-to-moderate CF lung disease will correct CFTR-mediated sodium (Na) hyperabsorption, and CFTR-dependent anion secretion, resulting in improvements in sodium measurements in nasal potential difference and sweat electrolyte analysis collected by pilocarpine iontophoresis.

**Aim 1. Test if systemically administered phosphodiesterase inhibitors improve ion transport in CF**

- a. Measure Na<sup>+</sup> and Cl<sup>-</sup> conductance by NPD before and after therapy
- b. Measure Na<sup>+</sup> and Cl<sup>-</sup> concentration in sweat utilizing pilocarpine iontophoresis before and after therapy

**Aim 2. Evaluate the effect of sildenafil on clinically relevant outcomes in subjects with CF**

- a. Evaluate the effect of sildenafil on lung function in subjects with CF
- b. Evaluate the effect of sildenafil on weight in subjects with CF
- c. Evaluate the effect of sildenafil on pulmonary exacerbations in subjects with CF
- d. Evaluate the effect of sildenafil on quality of life in subjects with CF

**Aim 3. Further evaluate the safety and tolerability of sildenafil in subjects with CF**

- a. Evaluate the effect of sildenafil on treatment emergent adverse events
- b. Evaluate the effect of sildenafil on change in clinical laboratory measures, vital signs and physical exam findings

**Background*****In vitro* effects of phosphodiesterase inhibitors on ion transport, pH and inflammation in CF**

CF is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which encodes a protein of the same name. CFTR belongs to a family of proteins involved in ion transport. It is known to act as a chloride transport channel, and also to regulate other ion channels, including the epithelial sodium channel, ENaC.<sup>4</sup> Defective transport of sodium and chloride across the respiratory epithelial cell membrane leads to dehydration of the periciliary liquid and mucous layers overlying the surface epithelium, resulting in defective mucociliary and cough clearance in the lung.<sup>5</sup>

Alterations in ion transport due to absent or deficient CFTR function have also been shown to effect organelle pH. Poschet *et al* demonstrated that increased Na<sup>+</sup> efflux (resulting from loss of inhibition by dysfunctional CFTR) results in intracellular positive charge loss. To compensate for this charge loss and neutralize the membrane potential, there is excessive H<sup>+</sup>-ATPase action, resulting in trans-Golgi network (TGN) acidification (Figure 1).<sup>6</sup> Inhibition of ENaC by amiloride or the Na<sup>+</sup>/H<sup>+</sup> pump (by acetylthiocholine with H<sup>+</sup> ion substitution) lead to improvements in pH, thus providing evidence that Na<sup>+</sup> transport is crucial in organellar hyperacidification.<sup>6</sup>

One recently described consequence of intracellular hyperacidification in CF is increased activity of the proprotein convertase, furin.<sup>7</sup> Furin, found both in the TGN and at the plasma membrane, activates ENaC by direct proteolytic processing of ENaC subunits.<sup>8</sup> Proteolytic cleavage of ENaC is requisite for ENaC mediated sodium transport thought to play a causative role in CF lung disease.<sup>9</sup> In addition to effects on furin and ENaC, trans-Golgi acidification has also been postulated to contribute to CF pathogenesis by altering the function of glycosylation enzymes key to normal processing of the CFTR protein. The glycosylation enzymes such as sialyltransferases and fucosyltransferases are pH sensitive and are crucial to the processing of wtCFTR. As

*Pseudomonas aeruginosa* is thought to exhibit increased affinity for respiratory mucins due to disordered glycosylation pathways (undersialylated glycoconjugates), altered glycosylation due to absent CFTR activity may also contribute to the predisposition to *P. aeruginosa* highly characteristic of CF lung disease.<sup>10, 11</sup>

Given the multiple detrimental effects of TGN hyperacidification, including excess furin activity, disordered glycosylation (leading to *P. aeruginosa* predisposition), and possibly dysregulated inflammation (see also below), the TGN pathway represents a potential therapeutic target in CF to reconcile a number of the downstream effects of CFTR dysfunction. As correction of organelle pH would be expected to reduce excessive Na<sup>+</sup> transport on the airway surface by blocking ENaC mediated sodium transport through inhibition of furin, this strategy represents a means to block excessive sodium absorption thought to contribute to disease pathogenesis.

PDE inhibitors (PDEi) have been reported to reduce excessive sodium transport in the CF cell by increasing cellular cGMP and therefore, restoring ENaC inhibition; use of this drug class therefore represents an attractive approach to address both TGN hyperacidification and the sodium transport abnormality found in the CF airway. Recently, our laboratory demonstrated that the treatment of CF cell lines (including those from  $\Delta F508$  homozygous and heterozygous patients) with the archetype PDE 5 inhibitor sildenafil led to increased intracellular cyclic guanosine monophosphate (cGMP), and corrected endosomal hyperacidification in CF respiratory epithelial cells (Figure 1).<sup>12</sup> As PDEis have demonstrated other effects likely beneficial in CF (see below), and are available by systemic administration, this class of agents may have inherent advantages over other strategies (such as inhaled medications) intended to inhibit ENaC function.<sup>13</sup>

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.<sup>14</sup> 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.<sup>15</sup> In related work, Robert *et al* demonstrated that a structural analog of sildenafil optimized for cell culture experiments, KM11060, partially restored  $\Delta F508$  trafficking in baby hamster kidney cells.<sup>16</sup> In our lab, using nasal mucosal tissue from  $\Delta F508$ del mice, we showed that transepithelial Cl<sup>-</sup> currents improved following treatment with sildenafil.<sup>17</sup> 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.<sup>18</sup>

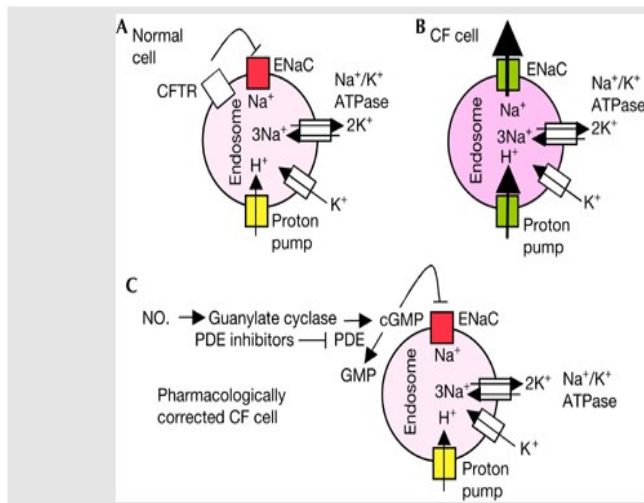
Sildenafil has also been shown to have anti-inflammatory effects that may be beneficial in the context of the pro-inflammatory environment of the CF lung. For example, in CF respiratory epithelial cells, we demonstrated that the excessive proinflammatory response to *P. aeruginosa* exposure could be reversed by treatment with sildenafil.<sup>12</sup> Toward *et al* demonstrated that pretreatment with sildenafil inhibited LPS-induced airway hyperreactivity, white cell influx and NO dysfunction in two guinea pig models of airway disease.<sup>19</sup> Finally, DBA/2 mice sensitive to *P. aeruginosa* were fed a diet with or without sildenafil. Following aerosol-delivered *P. aeruginosa* respiratory infection, myeloperoxidase in lung homogenates (a reflection of neutrophil infiltration) was reduced by  $42 \pm 11\%$  ( $p=0.047$ ) in animals who received sildenafil-containing diets.<sup>17</sup>

In concert, these studies suggest that PDEis might be a way to reconcile both the sodium and chloride ion transport defects present in the CF lung, and may also address other downstream effects resulting from TGN hyperacidification found in CF, including disordered CFTR glycosylation, a predisposition to *P. aeruginosa*, and excessive inflammation. Based on the *in vitro* data suggesting a possible role for PDEi as anti-inflammatories in CF, we 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.<sup>1</sup> 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.<sup>20</sup> 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).

Given the relative ease of testing this approach due to the clinical availability of sildenafil and other PDEis, these results suggest a strong need to also study the ion transport effects of sildenafil in CF. This study represents an excellent opportunity to capitalize on the safety and tolerability assessments already completed at our institution.

**Figure 1. Model of endosomal hyperacidification in cystic fibrosis respiratory epithelial cells and its correction by NO and phosphodiesterase 5 inhibitors.** Shades of pink, endosome luminal acidification levels (darker color—lower pH); green boxes, active pumps or open channels; red boxes, inactive channels; yellow boxes, normal pump or channel activity.



**(A)** In normal human respiratory epithelial cells, the CFTR inhibits sodium channels (ENaC), resulting in positive charge build-up as vacuolar  $H^+$  ATPase pumps protons into the endosomal lumen. The proton pump is sensitive to transmembrane potential build-up, and shuts down, resulting in physiologically normal, mild luminal acidification of the endosome. **(B)** In CF respiratory epithelial cells, with defective CFTR, ENaC is no longer inhibited by CFTR, which allows efflux of sodium as the protons are being pumped into the endosomal lumen by the proton pump. The sodium efflux dissipates membrane potential, allowing the vacuolar  $H^+$  ATPase to extend its proton-pumping action, thus causing mild hyperacidification. **(C)** As shown in this work, nitric oxide (NO) can correct endosomal hyperacidification in CF respiratory epithelial cells, by cGMP-dependent block of sodium transport through amiloride-sensitive channel ENaC. Hyperacidification can be corrected using sildenafil or other inhibitors specific for cGMP PDE5. CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; cGMP, cyclic GMP; PDE5, phosphodiesterase<sup>5</sup>.

### Sildenafil as candidate drug

Protein kinase G-dependent smooth muscle relaxation occurs in response to elevated levels of cGMP. Inhibition of phosphodiesterase type 5 (PDE-5,) which is responsible for degradation of cGMP, or stimulation with nitric oxide (NO) can cause this effect. Sildenafil (Revatio, Pfizer) is a PDE-5 inhibitor. Sildenafil-induced increased cGMP concentration leads to pulmonary vasculature smooth muscle relaxation. It is this effect that is key in treatment of pulmonary hypertension by sildenafil.

A case study reported on the use of sildenafil in a patient with severe CF lung disease (genotype  $\Delta F508/G551D$ , FEV<sub>1</sub> 1.051, 23% predicted) complicated by exercise-induced pulmonary hypertension. Treatment with 1 week of oral sildenafil 25 mg twice a day, followed by 4 months of 25 mg four times a day, lead to physiological improvement, and decreased subjective complaints of dyspnea and chest pain with exertion within 2 weeks of starting therapy. Improvements in exercise tolerance were increased further after 4 months of therapy. Sildenafil was well-tolerated with no adverse events.<sup>21</sup> Although these effects were attributed to the reduction in pulmonary artery pressure, the more prolonged and sustained improvement suggests the possibility for a more global effect on the ion transport abnormality, and also demonstrates the relative safety of this agent even in individuals with severe lung disease.

### Pharmacokinetics of sildenafil

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, CYP3A4 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 (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.<sup>22</sup>

In summary, laboratory evidence that sildenafil can correct the pH and sodium abnormality in CF cells, improve F508del CFTR trafficking and potentiate CFTR dependent Cl<sup>-</sup> transport, and may also exhibit anti inflammatory properties, combined with the well-established safety profile of sildenafil in severe lung disease make it an attractive agent to study in patients with CF. To date, studies have examined the *in vitro* ability of sildenafil to improve ion transport in the setting of abnormal CFTR; we propose to evaluate effects in human CF subjects.

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Primary Endpoints

Na<sup>+</sup> conductance by nasal potential difference (within subject change in maximum basal PD)

Secondary Endpoints

Other measures of Na transport (mean basal PD, end-ringers profusion PD, change in PD with amiloride)  
 Cl<sup>-</sup> conductance by NPD (total Cl conductance, change in PD with chloride free solution, change in PD with isoproterenol, total change in NPD (delta NPD)  
 Sweat Na<sup>+</sup> by pilocarpine iontophoresis  
 Sweat Cl<sup>-</sup> concentration by pilocarpine iontophoresis  
 Pulmonary function by spirometry  
 Lung clearance index  
 Exhaled nitric oxide (eNO)  
 Sputum nitric oxide metabolites  
 Safety and tolerability (including laboratory studies, CFQ-R)

**Experimental Design and Methods**

Study population: Total of 18 adults with CF will be recruited from the Colorado CF program at National Jewish Health. Subjects who withdraw early will be replaced until the target n of 18 is achieved.

**Inclusion criteria:**

- 1) Confirmed diagnosis of CF based on the following criteria: Positive sweat chloride ≥60mEq/liter (by pilocarpine iontophoresis) and genotype with two F508del CFTR mutations, and accompanied by one or more clinical features consistent with the CF phenotype
- 2) Male or female subjects ≥ 18 years of age
- 3) FEV<sub>1</sub> ≥ 50% 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) Willing and able to perform nasal potential difference testing
- 8) No changes in use of nasal medications within 2 weeks of screening visit
- 9) If on Orkambi, has been on stable Orkambi dose for at least 4 weeks at day 1.

#### **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
- 4) History of significant hepatic (SGOT or SGPT > 3 times the upper limit of normal at screening, documented biliary cirrhosis, or portal hypertension), cardiovascular (history of aortic stenosis, coronary artery disease, pulmonary hypertension with right ventricular systolic pressure >55 mmHg or life-threatening arrhythmia), neurological (history of stroke), hematologic (history of bleeding diathesis), ophthalmologic (history of retinal impairment or non-arteritic ischemic optic neuritis) or renal impairment (creatinine >1.8 mg/dL.)
- 5) Inability to swallow pills
- 6) Previous lung transplantation
- 7) Use of concomitant nitrates,  $\alpha$ -blocker, or Ca channel blocker
- 8) Use of concomitant medications known to be potent inhibitors of CYP3A4 (e.g. ketoconazole, itraconazole, ritonavir, clarithromycin, erythromycin, rifampin, verapamil)
- 9) 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
- 10) Weight less than 40 kg
- 11) History of sputum or throat swab culture yielding *Burkholderia cepacia* within 2 years of screening
- 12) History of nasal disease or nasal surgery that would, in the opinion of the investigator, impede accurate measurements of NPD
- 13) Use of anticoagulant medication (e.g. heparin, coumadin)
- 14) Resting room air oxygen saturation <93%
- 15) Use of nighttime oxygen
- 15) History of migraine headaches
- 16) Baseline BP of < 90/50 mm Hg

#### Study Design

This study is a randomized, placebo-controlled, double blind study of sildenafil (Revatio) in clinically stable patients with mild-moderate CF lung disease. The length of participation for each subject will be approximately 6 weeks, and will consist of a screening visit, 4 out-patient visits, and a follow-up phone assessment 2 weeks after subject's study completion.

#### Randomization

Subjects will be randomized in a 2:1 fashion to sildenafil or placebo. The randomization protocol will be created by Dr. Douglas Everett (Professor and Head, Division of Biostatistics and Bioinformatics, National Jewish Health). The protocol will be provided directly to the study pharmacist, who will communicate randomization to the coordinator at the research site.

#### Dosing

During the course of the study, patients will receive 4 weeks of therapy: 28 days of placebo orally t.i.d. or 28 days of days of sildenafil orally t.i.d. Dosing of sildenafil will be escalated weekly (20 mg orally t.i.d for the first week, 40 mg orally t.i.d. for the second through fourth weeks.) Patients receiving placebo will have sham dose escalation to maintain blinding. Patients not tolerating dose escalation will be discontinued from the study.

#### Screening visit (visit 1; Day -14-0):

Prior to conducting any study-related activities, written informed consent/assent will be obtained, signed and dated by the subject and/or parent/guardian.

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 the principal investigator including nasal exam rating (NER), height, weight, temperature, blood pressure, respirations and baseline pulse oximetry will be recorded. Any abnormal findings will be documented. Clinical laboratory tests will be performed including CBC with differential, chemistry profile, INR (total of 10cc of blood), and a urine pregnancy test (for females of child-bearing potential). A baseline EKG will be obtained. Subjects will undergo spirometry according to ATS criteria.<sup>23</sup>

Initiation visit period 1 (visit 2; Day 1): Subjects meeting entry criteria will be evaluated in the outpatient research unit. CFR-Q will be administered. A limited physical exam will be performed to include vital signs and NER. A urine pregnancy test will be done for females of child-bearing potential. eNO and spirometry will be performed. Spontaneously expectorated sputum will be collected. Safety labs and a sildenafil trough level will be drawn. Each subject will undergo pilocarpine iontophoresis with sweat collection, nasal potential difference testing, and lung clearance index testing. Patient will then receive study medication (placebo or sildenafil.) Subsequent to medication dosing, vital signs will be assessed at 1 hour and 2 hours post-dose. A drop in systolic blood pressure of  $\geq 20$ mmHg will be considered intolerance, and the subject will be discontinued from the study.

Subjects tolerating the medication will be discharged with a 7-day supply of placebo or sildenafil 20 mg p.o. t.i.d. Subjects will be instructed to call the study staff (during business hours) or the pulmonary attending on call (after business hours) for any significant adverse events that occur (e.g. severe headache, dizziness, or persistent visual changes.)

Study visit (visit 3; Day 8 $\pm$ 3): After 7 days of therapy, the subject will return to the study site. Vitals signs and adverse events will be assessed. Safety labs and a sildenafil trough level will be drawn. Each subject will undergo pilocarpine iontophoresis with sweat collection, nasal potential difference testing, and lung clearance index testing. Medication bottles will be collected. Subject will then receive study medication (placebo or sildenafil 40 mg). Subsequent to medication dosing, vital signs will be assessed 1 hour and 2 hours post-dose. A drop in systolic blood pressure of  $\geq 20$ mmHg will be considered intolerance. Subjects with intolerance of dose escalation will be discontinued from the study. Subjects tolerating medication will be given 21 days of placebo or sildenafil 40 mg p.o. t.i.d.

Study visit (visit 4; Day 29 $\pm$ 3): Subjects will return to the study site for visit 4. CFR-Q will be administered. A complete physical exam will be performed to include vital signs and NER, and adverse events will be assessed. eNO and spirometry will be performed. Spontaneously expectorated sputum will be collected. Safety labs and a sildenafil trough level will be drawn. Each subject will undergo pilocarpine iontophoresis with sweat collection, nasal potential difference testing, and lung clearance index testing. Clinical laboratory tests will be performed including CBC with differential, chemistry profile, INR (total of 10cc of blood), and a urine pregnancy test (for females of child-bearing potential). Medication bottles will be collected.

Study visit (visit 5; Day 43 $\pm$ 3): Two weeks following study completion, the research coordinator will call subjects to determine if any adverse events have occurred or if there have been any changes in concomitant medications.

### Early Withdrawal

In case of early withdrawal, an Early Withdrawal Visit will be scheduled within 7 days following the last dose of study medication. Physical exam will be performed by the primary investigator including weight, temperature, blood pressure, respirations and pulse oximetry. Spirometry will be performed. Subject will undergo pilocarpine iontophoresis with sweat collection, nasal potential difference testing, and lung clearance index testing. Any abnormal findings will be documented. Clinical laboratory tests will be performed including CBC with differential, chemistry profile, and urine pregnancy test (for females of child-bearing potential.) Medication bottles 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. Subjects who

require hospitalization for the treatment of a pulmonary exacerbation during the course of the trial will be withdrawn from the study.

### Outcome measurements and Procedures

Nasal exam rating score will be performed at each visit using the validated system established by the CF TDN Center for CFTR Detection.

Spirometry will be performed at the visits according to ATS standards.

Spontaneously expectorated sputum will be collected for measurement of NO metabolites at the initiation and final visits.

Exhaled nitric oxide will be collected prior to spirometry according to ATS standards. Subjects will be instructed not to eat or drink for 1 hour prior to eNO measurements.

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

Nasal potential difference will be performed according to CF TDN standard operating procedure (SOP 528.01) Interpretation and analysis of results will be performed by a blinded investigator at the University of Alabama.

Lung clearance index (LCI) is a measure of ventilation inhomogeneity that is obtained by multiple breath washout. It has been shown to be more sensitive than FEV<sub>1</sub> in detecting mild lung function abnormalities<sup>24-26</sup>, and has been endorsed by the European CF Society Clinical Trial Network Standardization Committee.<sup>27</sup> LCI measurement will be performed on an Eco Medics device using 100% O<sub>2</sub> as described by the ECSF-TDN.<sup>24</sup>

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.<sup>28</sup> The questionnaire will be administered at the initial and end of treatment visits for each treatment period prior to any procedures.

There is no drug interaction between ivacaftor and sildenafil. However, because the lumacaftor portion of Orkambi is a strong inducer of CYP3A, use of Orkambi with sildenafil may decrease sildenafil levels. Based on our previous work, average trough values at steady state for subjects with CF receiving 40 mg of sildenafil t.i.d. were approximately 39 ng/mL. Sildenafil trough levels will be performed at visits 2, 3, and 4 to confirm non-interference of metabolism with co-administration with Orkambi.

### Study Drug

Blinded study drug bottles will be packaged, labeled and shipped from a contracted pharmacy (Belmar Pharmacy, Lakewood, CO) based on sequence number. Sildenafil and placebo will be stored and dispensed through the research pharmacy at NJH. An accurate accounting of dispensing and return of the study drug will be maintained by the research pharmacist at each institution.

### Patient reimbursement

In accordance with TDN guidelines, patients will be reimbursed according to the following schedule:

Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Total
Payment	\$75	\$200	\$200	\$200	\$25	\$700

Subjects who complete the screening visit, will receive the \$75 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.

## Statistical analysis

### Sample size

Using PASS 13, data from two consecutive within subject NPD measurements following treatment with placebo in a trial of the Na transport inhibitor QAU145 were used to perform power calculations for this study. Based on a 2.59 mV standard deviation of the change in conductance of the maximal basal PD with placebo, enrollment of 12 (sildenafil) and 6 (placebo) subjects will provide 100% power to detect a 10.4 mV change in maximal basal PD at the 5% significance level.<sup>29</sup> The reported results from the VX-770-101 trial reported at NACFC in 2008 also indicate that this study is well-powered to detect changes in sweat chloride.<sup>30</sup> Based on a standard deviation of 2.6 mEq, enrollment of 12 (sildenafil) and 6 (placebo) subjects will provide 95% power to detect at least a 5 mEq change from placebo at the 5% significance level.

### Data Analysis

Primary outcome, baseline change in maximal basal PD measurement (mean of 2 nostrils), will be assessed as well as change with amiloride and total change in PD. Secondary outcomes include the mean basal PD, end Ringer's perfusion PD, change with amiloride, change with low chloride, change with isoproterenol, change with low chloride plus isoproterenol, and total change in NPD (delta NPD). Analysis of NPD results will be performed at the Center for CFTR Detection at UAB. All NPD tracings will be interpreted by an independent, blinded investigator, and no scoring will be conducted by the site investigator to prevent biased estimates. All tracings will be scored with electronic assisted data analysis (developed by the UAB Center for CFTR detection). Data for both the primary and the secondary endpoints (other Na and Cl conductance measures by NPD and the concentration of Na and Cl in sweat) will be analyzed separately as a 2X2 crossover design using a repeated measures analysis.

If emerging data from ongoing CFTR modulator trials and NPD measurements obtained using the latest NPD methodology suggests alternative analytic methods (including log transformation) should be used, statistical analysis methods will be updated prior to data base lock and analysis initiation. If these analysis suggests non-normality, rank based analyses (e.g. Mann-Whitney-Wilcoxon rank-sum test or other appropriate test) will be used. Any change in the statistical analysis plan will be finalized before database lock, and reported to the DSMB and CF-TDN PRC. Data will be also be presented using descriptive analyses including individual patient line plots of outcome over time. All patients will be plotted across part one and part two to discern carryover effects.

Data from pharmacokinetic analysis will be analyzed using ANOVA in SAS v.9.2. P-values  $\leq 0.05$  will be regarded as significant. Data will be screened for nonnormality, and appropriate transformations as needed (such as log) will be applied; careful residual analysis will be performed to detect outliers and other anomalies. Non-parametric procedures (Proc NPAR1WAY in SAS v9.2) will be applied in cases where transformations do not normalize the data. Carryover effects in the crossover design will be fit initially but discarded if they are not (as expected) significant. Correlations between NPD, sweat chloride and clinical parameters will also be conducted. All statistical analysis will be performed in conjunction with Dr. Steven Rowe and CCTSI statisticians.

## Mechanism for Monitoring Patient Safety

### Known Potential Toxicity of Study Drug

In trials of sildenafil in patients without contraindications to drug administration, 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 priapism 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 priapism and ischemic retinopathy. Very rarely, cases of decreased hearing have been reported in patients taking Viagra. It is not clear if this was related to taking Viagra or the patients' medical conditions. 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. 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. No hearing loss related to sildenafil administration was observed in CF subjects who participated in the pilot study performed at our institution.

#### 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
- 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:

- Probably related

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®.

- Possibly related

There is a plausible temporal relationship between the onset of the AE and administration of Revatio®, and an alternative etiology (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication) for the AE is equally or more likely than the potential relationship to administration of Revatio®.

- Not related

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

- Severe dyspnea at rest
- Pregnancy
- Subject refusal to continue in study
- Development of an adverse event that the investigator feels is a preclusion to continued subject participation

#### Data Safety Monitoring Board (DSMB.)

Oversight for this trial will be conducted by the Cystic Fibrosis Foundation Therapeutics Development Network DSMB

#### Pregnancy

If a subject or subject's partner becomes pregnant during the study, the subject will be discontinued from the study. We will not request to follow the pregnancy or the offspring.

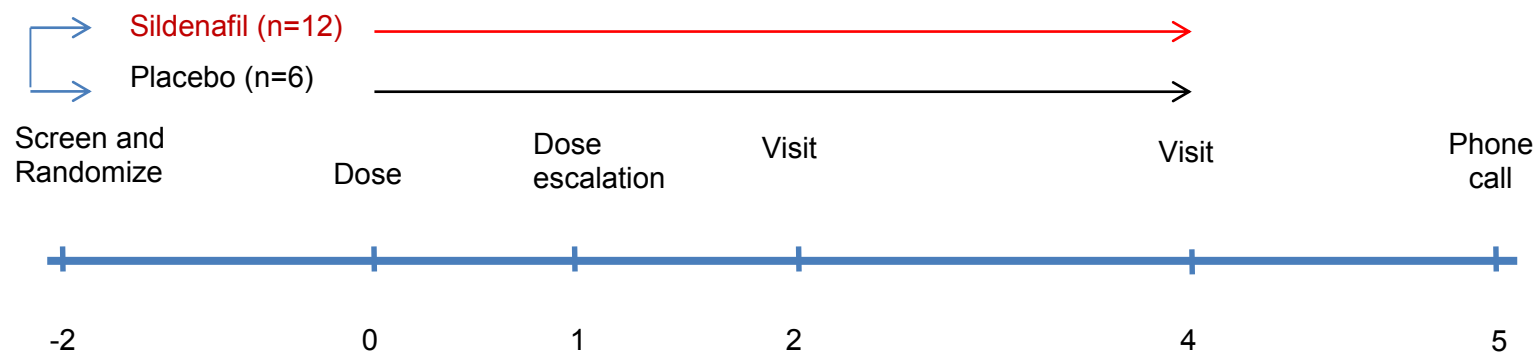
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## STUDY TIMELINE



## STUDY FLOWCHART

	Screening/ Pretherapy	Baseline	Treatment	Treatment	Follow-up call
Visit	1	2	3	4	5
Day(s)	0	1	8±3	29+3	43±3
Informed consent	x				
Demographics	x				
Medical history	x				
Concomitant medications and airway clearance	x	x	x	x	x
Bacterial colonization	x				
Vitals signs	x	x	x	x	
Spirometry	x	x		x	
Height	x				
Weight	x	x		x	
CFQ-R		x		x	
Complete physical examination	x	x		x	
Safety labs	x	x		x	
Sildenafil Trough		x	x	x	
Pregnancy Test	x			x	
Nasal Potential Difference		x	x	x	
Sweat testing		x	x	x	
Lung clearance index		x	x	x	
EKG	x				
Study drug dispensed		x	x		
Study drug bottle return			x	x	
Adverse events		x	x	x	x

