Losartan as anti-inflammatory therapy to augment F508del CFTR recovery

PROTOCOL

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Background: The life span of patients with Cystic fibrosis (CF) continues to be shortened by progressive lung disease. CF can be caused by ~2000 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. These mutations can be, even though imperfectly, classified into 5 groups, permitting the development of small molecules that rescue group-specific CFTR mutants. These small molecules made remarkable impacts on patients. The CFTR potentiator ivacaftor (Kalydeco™), approved by the FDA mainly for class III mutations, has shown improvements in ion transport (large decrease in sweat chloride), clinical outcome (increase in FEV1 and weight, decrease in exacerbation rates), and quality of life (1). However, ivacaftor did not immediately eliminate airway inflammation, despite improvement in CFTR conductance, at least not within 12 month (2). Later time points may indicate anti-inflammatory effects. *However, airway inflammation is associated with morbidity in CF (reviewed in 3) and additional anti-inflammatory treatment options are needed.*

F508del, a class II mutation, is the most common mutation in the US CF population. The FDA recently approved Orkambi™ and Symdeco™, both combinations of a corrector and potentiator (lumacaftor/tezacaftor and ivacaftor), to reduce exacerbations in F508del homozygous patients. However the effect of both medications on lung function and sweat chloride was modest at best (4-6). One explanation for this suboptimal efficacy *in vivo* could be the ongoing airway inflammation as outlined above. In this context, TGF-β1 has been identified as a disease modifier in CF (7). Increased production of TGF-β1 is common in CF airways and has been associated with worse pulmonary outcome (8,9). In particular, TGF-β1 has been shown to decrease the ability of lumacaftor to rescue CFTR function and airway surface liquid (ASL) volume *in vitro* (10,11). Since tezacaftor has a similar mode of action compared to lumacaftor, it is not surprising that TGF-β1 also decreases the ability of tezacaftor to rescue CFTR currents in vitro (our own published observations). TGF-β1-associated inflammation also decreases the function of other ion channels important for ASL volume regulation: Calcium activated chloride channels (CaCC) and the apical potassium channel BK (11,12). We therefore propose that ***airway inflammation, especially via TGF-β1, leads to a lower CaCC and BK activity, worsening mucociliary dysfunction in CF. Importantly, TGF-β1 also causes a decreased ability of lumacaftor to rescue F508del CFTR to the membrane, explaining at least part of the suboptimal efficacy of correctors in inflamed airways.***

IN VIVO PRELIMINARY EVIDENCE FOR THE ABILITY OF LOSARTAN TO REVERSE MUCOCILIARY DYSFUNCTION IN SHEEP

We developed a large animal model, at least in part simulating CF airway disease by exposing sheep to an inhalation challenge with $CFTR_{inh}172$ and human neutrophil elastase (HNE) or TGF-β1 (to produce inflammation). Either combination led to a significant reduction in tracheal mucus velocity (TMV), a surrogate marker of mucociliary clearance. Importantly, inhaled losartan reversed the $CFTR_{inh}172$+HNE- or $CFTR_{inh}172$+ TGF-β1-induced mucociliary dysfunction *in vivo* (Fig. 1).

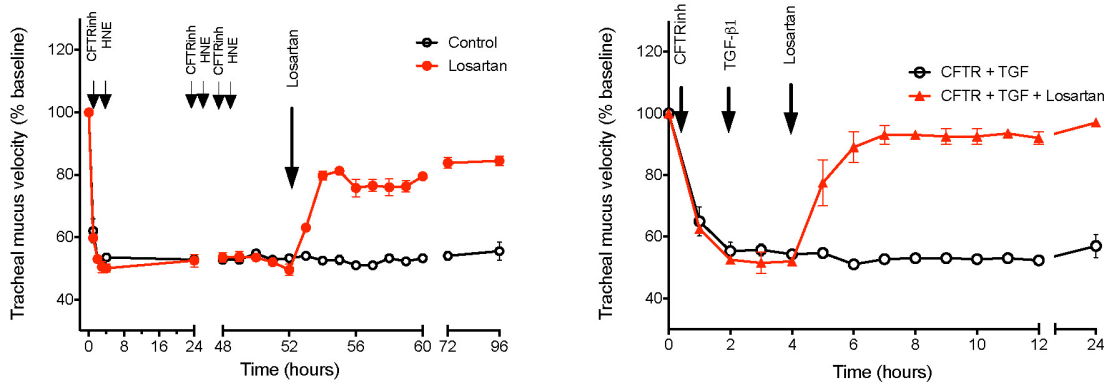

Figure 1. *Effects of losartan on a 3-day challenge model of mucociliary dysfunction.* Left: 3-day challenge with $CFTR_{inh}172$ (10 mg) and HNE (2380 mU) leads to prolonged (at least lasting 48h) mucociliary dysfunction (reduction in TMV) in sheep. Inhaled losartan (50 mg) reversed TMV decreases (solvent of losartan had no effect). Right: Challenge with $CFTR_{inh}172$ and TGF-β1 (25 µg) reduces TMV that is again rescued by inhaled losartan (50 mg). All n = 3. Mean ± SE are shown.

ADDITIONAL PRELIMINARY DATA

Additional preliminary data in patients show that Orkambi™ alone does not improve CFTR function *in vivo* as measured by nasal potential difference (NPD), possibly because of ongoing inflammation in CF patients. Given the small changes in sweat chloride, the same is true for Symdeco™. *In vitro* data show that:

- Losartan reverses the detrimental effects of TGF-β1 on ASL volume
- Losartan reverses the detrimental effects of TGF-β1 on CaCC and BK currents
- Losartan reverses the detrimental effects of TGF-β1 on lumacaftor-mediated F508del CFTR current recovery
- TGF-β1 has detrimental effects on tezacaftor-mediated F508del CFTR current recovery
- Decreases in ATP-induced PD are a measure of both CaCC and BK dysfunction.

- Losartan improved mucociliary dysfunction in a sheep model with CFTR inhibition and airway inflammation

These data make a compelling case for testing losartan as an anti-inflammatory medication in patients homozygous for F508del to improve the effects of Orkambi™ or Symdeco™ therapy on ion transport. Losartan will be given at least 12 weeks to assure that possible anti-inflammatory effects will have a chance to change the airway environment, since 4 weeks of treatment was insufficient in previous trials with other anti-inflammatory medications.

Study Design and Methods

We propose to conduct a proof of concept clinical trial to test if administration of losartan augments CFTR function *in vivo* in patients who are on Orkambi™ or Symdeco™. The goal of our pilot clinical trial is to examine the ability of losartan on CFTR (primary hypothesis) and possibly BK rescue/augmentation (secondary hypothesis) in patients homozygous for F508del on Orkambi™ or Symdeco™ therapy. The additional benefit for BK channels could be important but is harder to measure *in vivo*. Towards this end, however, we will assess CaCC/BK activity together as the response to ATP during NPD measurements, even though this is not an accurate reflection of either channel on its own.

Note that ATP-induced changes in NPD in CF patients are higher than in normal subjects because in vitro data show that this response is increased in the *absence* of BK activity. Thus, the expectation here is that the rescue of BK activity with losartan will *DECREASE* NPD responses to ATP.

Primary hypothesis: Losartan treatment for 12 weeks will improve CFTR conductance as measured by NPD in patients on Orkambi or Symdeco therapy. We expect that compared to placebo, ΔNPD in response to 0 Cl^- /isoproterenol will change by more than -4 mV using the most polarized nostril each time.

Secondary hypotheses: Losartan treatment for 12 weeks will improve BK and CaCC activity as assessed by a decrease of changes in NPD to ATP exposure. Furthermore, losartan will reduce nasal cytokine levels and systemic inflammatory markers.

Study plan: Details are seen in Figure 2 and the study table. Briefly, 36 patients with CF, > 12 years of age, who are homozygous for F508del and have been on treatment with Orkambi™ or Symdeco™ for at least 3 months will be recruited for this trial. This trial will be randomized 1:1 with placebo (n=18 for each group). After signing informed consent at the screening visit, we will perform spirometry, determine sweat chloride, take blood (see below), and test for pregnancy where applicable. Since losartan has teratogenic effects, we will enforce strict birth control in female participants (see Potential Risk section.)

Eligible patients will complete visits as outlined below. Quality of life will be assessed by CFQ-R (CF quality of life questionnaire - revised, 13). NPD will use standard protocols and so will nasal cell harvest (see below for assessments). Cytokines will be measured from nasal fluid recovered by Leukosorb filter paper (14). There will be two visits for these different nasal assessments to assure that nasal cell fluid and cell collection will not influence NPD and vice versa. After assessing baselines, a daily dose of 50 mg losartan or placebo will be started. If patient's weight is less than 55 kg dose will be adjusted to a daily dose of 25 mg losartan or placebo A safety follow up phone call will occur after 7 days of treatment (± 2 days) and the losartan dose (or placebo) increased to 25 mg or 50 mg twice a day as higher dose is likely needed to get anti-inflammatory effect in airways if present. Since this trial assesses anti-inflammatory effects of losartan (and lack of placebo), the total duration will be 14 weeks to achieve at least 12 weeks of treatment with the higher dose of losartan (or placebo).

Endpoints

- Primary endpoint
  - ΔNPD with 0 Cl^-/isoproterenol as a measure of CFTR activity in losartan treatment compared to placebo (15), done according to standard procedures (16); for power calculation, see below. All traces will be read blinded at the Cincinnati Children's Hospital Medical Center and University of Alabama at Birmingham for the active or placebo arm.
- Secondary endpoints
  - CaCC and BK activity as measured by change in NPD upon ATP application (see above).
  - Change in sweat chloride concentration
  - FEV_1 (absolute and relative changes)
  - Changes in serum inflammatory markers (hsCRP, WBC including absolute neutrophil count, % PMNs, SAA, calprotectin, GM-CSF, TGF-β active and total)
  - Nasal cytokine changes (IL-1β, TGF-β active and total, IL-6, IL-8 IL-13)
  - Quality of life questionnaire (CFQ-R)
  - Blood levels of losartan, EXP3179, and EXP3174 (17,18)
  - LRRC26 mRNA (correlates with BK activity *in vitro*) and TGF-β mRNA expression in nasal cells by qPCR (while some contamination with other cells is possible, "purification" with for instance cell sorting

is not feasible given the small number of cells; we will assess purity after cytospin with epithelial and other cell markers using immunofluorescence).

Intervention

- After establishing baseline parameters, patients will be started on oral losartan, 50 mg or 25 mg (based on patient's weight) daily in am for 1 week before dose will be increased to twice daily in am and pm. If participant present any relevant AE related to the study medication, at the PI discretion, the dose will not be increased to BID and participant will be withdrawn from the study. .
- Trial is randomized 1:1 to active drug and placebo (pharmacy will provide drug and placebo; randomization occurs through the biostatistics core at University of Miami).

Measurements

1. Visits will occur for screening, baseline and then treatment according to the attached schedule.

2. Sample collection will include NPD, sweat chloride, nasal cell and nasal fluid collection, spirometry, CFQ-R, serum C-reactive protein (hsCRP), WBC (including absolute neutrophil count, % PMNs), serum alpha-SAA, calprotectin, and GM-CSF. In addition, losartan and EXP3179/3174 levels will be assessed (17,18).. Nasal airway epithelial cells will be taken by brush for mRNA expression levels of TGF-β and LRRC26. Exacerbation assessment, defined according to the TRAFFIC/TRANSPORT studies (19) will occur during study visits and unscheduled visits if needed

Inclusion criteria:

- CF patients homozygous for F508del and on current treatment with Orkambi™ or Symdeco™ for at least 3 months
- Age >12 years
- $FEV_1 \geq 40\%$ of predicted

Exclusion criteria

- When enrolling female patients
  - Not willing to adhere to strict birth control (combination of two methods)
  - Pregnancy
- History of intolerance to angiotensin receptor blockers (ARBs)
- Treatment with ACE inhibitor
- Regular use of NSAIDs or potassium supplementation, treatment with aliskiren, or on anticoagulation
- Oral corticosteroid use within 6 weeks
- Exacerbation requiring treatment within 4 weeks
- Current treatment for mycobacterial infections
- Significant hypoxemia (oxygen saturation <90% on room air and rest or use of continuous oxygen treatment), chronic respiratory failure by history (pCO_2 > 45 mmHg), clinical evidence of cor pulmonale
- Untreated arterial hypertension (systolic blood pressure >140 mm Hg, diastolic blood pressure > 90 mmHg)
- Blood pressure less than 90 mm Hg systolic while standing
- Cardiac, renal (creatinine 1.5 times normal limit), hepatic (LFTs > 3x normal upper limit), neurological, psychiatric, endocrine or neoplastic diseases that are judged to interfere with participation in study
- Known renal artery stenosis
- Concomitant airway disorders other than CF, such as ABPA.
- Subjects with prior thoracic surgery

The visits are detailed in the attached table. The timeline and randomization is given in Figure 2 below.

Measures of Compliance: This will be accomplished in two ways: analysis of blood levels of losartan and the metabolites EXP3179/EXP3174; pill count (patients will bring their medication supply to the visits).

Timeline: We will start up the trial in April 2017 with database creation for data capturing. We expect to start enrollment within 3 months. We expect to enroll each year 18 patients and be able to publish the results by the end of the 3.5-year funding request.

Other issues: Efficacy and safety are addressed in the DSMB section. Data collection will occur by clinical research coordinators. Data monitoring and quality control will be provided by the Clinical Research Operations & Regulatory Support (CRORS) monitoring group at the University of Miami.

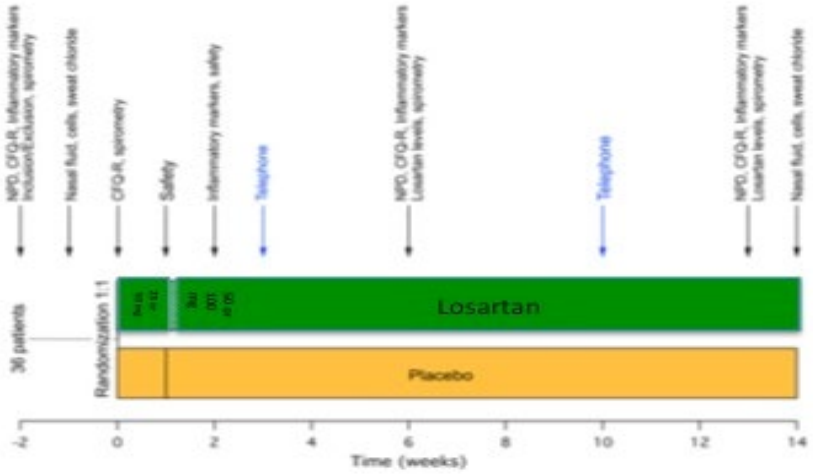

Figure 2. *Timeline and interventions.* The enrollment and interventions are depicted graphically for an easier understanding. More detailed visit procedures are provided in the attached table.

Study Procedures

Once informed consent is signed, the subjects will undergo the following procedures:

General and questionnaire: Data collected will include demographics, clinical symptoms, CFQ-R, and blood work. We will also collect history of other medical conditions, medications, and exacerbation history.

History and physical examination will be performed including vital signs and body mass index (BMI) will be recorded.

Pulmonary function testing will be performed according to American Thoracic Society recommendations. Participants will be asked to refrain from using short-acting bronchodilator drugs for at least 4 hours before testing and not take any long acting bronchodilator in the morning. Height, weight and oxygen saturation will be measured prior to these tests.

Blood and urine samples: Urine pregnancy test will be performed at all site visits. If positive, confirmation with serum pregnancy test will be obtained. Blood test for liver and renal function, inflammatory markers and blood levels of losartan and the metabolites EXP3179/EXP3174 will be drawn. A total of 30 cc (2 tablespoons) of blood will be collect at Visit 1 and visits 4, 5, and 7.

NPD measurement will be performed according to standard procedures (latest SOP of the Therapeutic Development Network of the CF Foundation). Double lumen nasal catheters with an external diameter of 2.5 mm will be used for measurements. One lumen will be filled with freshly prepared agar; the other lumen will be used for perfusion with different solutions. The agar catheter and subcutaneous bridges (metal butterfly needle system) will be made prior to use by warming 3% agar gel and injecting the solution into the catheter. They will be attached to 3M KCl/calomel reference electrodes (Baxter; Deerfield, IL) and connected to a bioamplifier system (AD Instruments; Colorado Springs, CO) for measuring and recording. The initial potential difference (PD) will be measured at the anterior tip of the inferior meatus. Then, the basal PD will be measured at various distances within the inferior meatus in each nostril. The nasal catheter will be fixed at the most negative potential in one nostril, and the test will be initiated using perfusion

with Ringer solutions until a stable value (± 0.5 mV over 30 s) is obtained. Then, the nose will be rinsed with different solutions containing amiloride (to block ENaC), no chloride (stimulate flow through CFTR), and isoproterenol (activate CFTR). Finally, we will use ATP to increase the intracellular calcium concentration and activate CaCC. These tests will be repeated in the other nostril. De-identified NPD traces will be read at a central laboratory (Dr Clancy's lab at Cincinnati Children's Hospital Medical Center (CCHMC) and Dr Harris at University of Alabama at Birmingham (UAB)) blinded for the active or placebo arm.

Nasal sample collection: Nasal sample will be collected using Leukosorb as previously reported (14). In addition, we will obtain a small amount of nasal epithelial cells to measure TGF-ß by qPCR. De-identified samples will be sent to the Human Research Laboratory at University of Kansas Medical Center (KUMC) for analysis.

Sweat test. The sweat chloride test is a standard diagnostic tool for CF, serving as a biomarker of CFTR activity. Sweat samples will be sent to a central laboratory (Dr. Clancy's lab at CCHMC) for testing and interpretation of results. Individual sweat chloride test results will not be disclosed to the participants, with the exception of screening results. Specific instructions for collection, handling, processing, and shipping of sweat chloride samples to the local laboratory will be according to CFF guidelines.

List of assessments (see Appendix 1)

EXPECTED RESULTS, LIMITATION AND DIFFICULTIES

Given our preliminary data *in vitro* and *in vivo*, we expect that losartan increases functional CFTR recovery by Orkambi™ or Symdeco™ and possibly CaCC and BK function. We expect to see meaningful improvements not only in NPD but also in sweat chloride as well as decreases in hsCRP levels and other inflammatory markers locally and systemically (if elevated). We also expect improvements in CFQ-R scores.

The investigators have experience with executing clinical trials and with the measurements of the proposed endpoints. It is clear that there are several limitations: Even though power calculations (see below) indicate that the sample number should be sufficient to detect meaningful changes at least in the primary endpoint, the NPD and secondary endpoint signals need to be high enough to detect changes. Given the *in vitro* data, we are moderately confident that we will be able to detect these if present. The duration of the trial could be an issue (attrition), but our experience with adherence in clinical trials of this duration with CF patients indicate that this is not a limiting factor. We believe that allowing losartan to work as an anti-inflammatory for 3 months is important to counteract chronic inflammation in the airways. We will collect data that will indicate whether or not such an anti-inflammatory action actually occurs (even locally) and whether it is related to CFTR conductance changes. Secondary endpoints are purely exploratory however. If only trends are established, the data will lend itself to the design of a larger trial to fully explore the anti-inflammatory actions of losartan in CF patients.

Potential Benefits of the Proposed Research to the Subjects and Others

There is no direct benefit to the subjects for participating on this study. Benefits for others may arise from data obtained in these studies to prevent and/or treat mucociliary dysfunction.

At the conceptual level, this project innovates by addressing airway inflammation with known properties of the commonly used, well tolerated, FDA approved medication losartan. At the therapeutic level, this project has the potential to add medical approaches for CF patients: the project could identify ARBs, and particularly losartan, as a "low hanging fruit" for treating CF patients by inhibiting TGF-β1 signaling to try to restore mucociliary transport and improve ΔF508 CFTR restoring therapies. This approach is innovative since it avoids lengthy toxicity studies of new molecules and can be introduced into the clinic rapidly if successfully tested in clinical trials. Furthermore, the anti-inflammatory metabolite of losartan could stimulate the development of other, more potent anti-inflammatory molecules, and used for other airway diseases.

Recruitment and Informed Consent: Each site will recruit approximately 9 patients at the CF clinics. The study and informed consent form will be approved by the University of Miami's IRB. University of Miami IRB will serve as Central IRB and other Relaying IRBs will be the CCHMC, KUMC and UAB. Subjects will have sufficient time to meet with the PI and co-PIs to address all their questions about the research.

Protection of Confidentiality: No personal identifiers will be collected and each patient's data will be coded There will be no names or other identifiers recorded on study data guaranteeing the success of these confidentiality measures (we will know age, gender and CF mutations).

STUDY DRUG: LOSARTAN

Cozaar®, the commercial form of losartan is an FDA-approved ARB widely prescribed for treatment of hypertension, heart failure, and for renal protection in patients with diabetes. Losartan and matching placebo (25 mg or 50 mg pills/ placebo) will be compounded at each site by the research pharmacist and distributed to the subject based on the randomization table provided by the Biostatistician at University of Miami. Losartan/placebo will be given to the subjects by the PI or study coordinators free of charge. FDA has granted IND exemption for this study. The research pharmacist is responsible for maintaining accurate study drug accountability records throughout the study. Dose will be adjusted based on participant's weight at visit 1 as following: if participant's weight is < 55 kg, participant will be randomized to either 25 mg or placebo. If participant's weight is ≥ 55 kg, participant will be randomized to either 50 mg or placebo.

Each dispensing of study drug will be documented in the subject's study records and in the drug accountability log. Subjects will return all unused study drug for drug accountability. Study drug will be destroyed at the research site as per the Institution regulations at the end of study.

Research pharmacists, UM biostatistician core and the assign unblinded monitor will be unblinded. Emergency plan for unblinding will be discussed with PI with consulting with the medical monitor and DSMB if necessary.

STATISTICS AND POWER ANALYSIS

Randomization: Upon eligibility, a statistician randomizes all subjects in a 1:1 ratio to the placebo or losartan treatment group and informs the research pharmacy. Participants will be stratified by site during randomization.

Design: The basic study design consists of random assignment of patients to one of two treatment arms: losartan or placebo. Data will be collected at the visits as outlines above.

Analysis: Primary endpoint: A t-test will be used as preliminary data analysis. For the formal analysis, mixed effects models model will be used for analysis to accommodate the paired measures nature of the design, as well as missing values under the MAR assumption. We will determine the final analytic approach based on the final available data. Examination of baseline differences on key variables between subjects remaining and those lost-to-follow-up will also be conducted. The first set of analyses will not adjust for dropouts. Only cases with complete data will be included.

Secondary endpoints: Prior to analysis, we will investigate the distributional properties of the defined outcome measures - such as CaCC/BK activity, change in sweat chloride concentration, FEV1, changes in serum inflammatory markers, etc. Necessary transformations such as logarithmic or square root will be applied to satisfy normality assumptions if necessary. Baseline values of all variables will be examined, and estimates of baseline means and changes over time will be provided. The statistical analysis will consist of descriptive statistics associated with outcome measure (mean change or mean difference pre and post treatment) for two treatments. Means at baseline, 6 and 13/14 weeks, for each outcome, as well as the change over time will be provided, as well as the corresponding estimates of variability, and 95% confidence intervals will be constructed. t-tests for comparisons of the changes over the period between treatments will be conducted. However, the primary focus for this analysis is to obtain reliable estimates of the mean changes and variability, which will be useful for designing further investigation focused and specifically powered for comparing these outcomes. P-value will be significant if < 0.05. All tests will be two-tailed. The SAS statistical software package version 9.3 (Cary, NC) will be used for all statistical analyses.

Consultant Arrangements

Biostatistics support will be provided by Dr. Hua Li from the biostatistics core. The presented statistics and power analysis was developed with Dr. Hua Li. A specialized lab will provide blood level measurements of losartan and its metabolites in the blood (Dr. Peloquin at the University of Florida).

Data management

Data collected will include demographics, clinical symptoms, CFQ-R, pulmonary function tests and NPD. Samples to be collected include nasal fluid (Leukosorb) and nasal cells at the beginning, during and at the end of the study. We will obtain blood for safety reasons (liver and renal function), for evaluation of an inflammatory marker (CBC w/ differential; CMP; CPK, CRP and other inflammatory markers). Blood for levels of losartan and metabolites (before next morning dose) will be also collected at the end of each treatment phase.

No personal identifiers will be collected and each patient's data will be coded. Data will be captured at secure password protected website (Red-Cap). Sites will be granted access to this database.

Monitoring will be provided by Clinical Research Operations & Regulatory Support (CRORS) monitoring group at the University of Miami.

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Potential Risks

All procedures are performed during this study are routine procedures and are associated with minimal risks.

Pulmonary Function Testing (Spirometry) is a routine clinical procedure with few risks. Patients are coached to make repeated forceful breathing efforts. The subjects might have chest soreness. Unusually, subjects may become lightheaded during these efforts. We will minimize this risk by having the PFT done in a sitting position.

NPD measurement: is a non-invasive method with minor risks and will be performed according to standard procedures (21-25).
Minor risks include swallowing and aspiration of the solution used for rinsing the nose. Having the patient bending the head forward will minimize this risk, so that the solution is dripping out of the nose and not in the pharynx. The perfusion rate will be set at 5 ml/min, indicating that the nose will be rinsed with low volumes. Even in case of accidental swallowing or aspiration, there are limited risks of a severe reaction, even in a respiratory-wise impaired individual. Amiloride is a diuretic medication and can increase urine production when given in a higher dose, which is not expected here as the used dose is low and systemic absorption is not anticipated. Isoproterenol is a β-mimetic and can lead to tachycardia, hypertension and migraine. The dose used is low and the systemic absorption negligible. Other potential side effects include nosebleed, runny nose and coughing. For the study, a subcutaneous insertion of a butterfly needle is required, which brings a minimal risk of bruising, bleeding and infection. The butterfly is only used as a control electrode. No medications are injected through it into the skin or vascular system.

Nasal sample collection and cell sampling: this procedure is routine and is sometimes associated with an unpleasant feeling due to the use of the Leukosorb filter, itching and coughing. Cell sampling can cause a mild discomfort and a mild nosebleed due to brushing.

Phlebotomy: The risks from blood draw from a vein are minimal but include discomfort at the site of puncture, possible bruising around the puncture site, rarely an infection, and uncommonly, fainting from the procedure. Blood draws will be performed with as much care as possible to minimize local complications. Subjects will be sitting during the process for their comfort and will be asked not to look directly at the site of blood draw.

Treatment with losartan (IND exemption for CF approved): Losartan is an FDA-approved ARB widely prescribed for treatment of hypertension, heart failure, and for renal protection in patients with diabetes. The doses proposed in this study are well tolerated by non-hypertensive patients without significant effect on blood pressure. The most common side effects are fatigue and dizziness. Serious adverse events that are rare include angioedema, hypotension, renal dysfunction or failure, blood dyscrasias, hepatitis, or rhabdomyolysis (clinical assessment of muscle pain). The following should lower the risks to the subjects: The first dose of medication will be administered in the clinic and the patient observed for two hours with blood pressure monitoring. Subjects with hyperkalemia, renal dysfunction, or who are taking potassium supplements will not be enrolled into the study. Participants will be given written descriptions of potential side effects and what to do. For example, mild postural hypotension can be minimized by getting up slowly and maintaining adequate hydration. Skin rashes, palpitations or other moderate or severe adverse events (interference with usual daily activities) without other clear explanation should warrant immediate cessation of treatment and notification of study personnel. There are no drug interactions between losartan and Orkambi™. Nor are there relevant drug interactions between losartan and or Symdeco™ (by Vertex' drug interaction guidance in their 661 and later clinical trials).

Sweat chloride collection: this is a routine procedure in a CF center and uses a macroduct to collect sweat secretions stimulated by pilocarpine iontophoresis, done according to CFF and CLSI guidelines. The test is well tolerated. A small risk of complications includes mild reddening of the skin. Damage to the skin, such as blistering or burns, occurs very infrequently and this risk can be minimized by careful attention to technique.

Questionnaires, Health History and Physical Examination acquire information that is routinely collected. No significant physical risks arise from these procedures. There is always the risk of psychological distress and breach of confidentiality. In order to minimize this risk, electronic medical records are held in HIPAA compliant password-protected databases, and written information is stored in locked files or file-rooms when not attended by study personnel.

Pregnancy

Female subjects will have to adhere to strict birth control. They will be counseled to immediately inform the Investigator if there was non-compliance or if pregnancy occurs during study treatment and within 90 days after the last dose of the study drug.

If a female subject becomes pregnant while participating in the study, study drug must be immediately and permanently discontinued. Positive pregnancy test results, for minors under the age of 18, will be shared with parents. The subject will be counseled and if pregnancy is continued until delivery, the subject if followed and the infant for 1 year after the birth, provided informed consent/assent is obtained. A separate informed consent form will be provided

to explain these follow-up activities. The investigator must notify the DSMB and IRB within 24 hours of the site's knowledge of the subject's pregnancy and other
agencies as by law. Pregnancy itself does not constitute an AE.

Adequacy of Protection Against Risks

Recruitment and Informed Consent: Subjects will be recruited from the respective CF clinics. First, the subjects will be briefed about the study and if interested will be invited to come on a separate day with ample time just to discuss the study objectives, procedure risks and benefits with the study investigators. The study and informed consent form will be approved by the University of Miami's IRB. Subjects will have sufficient time to meet with the co-PIs to address all their questions about the research and take the informed consent home to discuss with family.

Protection against Risk: General approaches for each procedure to minimize risk were described above. We will carefully select subjects that can tolerate the procedures of the study. All participant sites are fully equipped to handle possible fainting and other, more serious types of medical emergencies (CPR cart physicians and study staff are trained in BLS and ACLS) to handle any potential emergency.

All research data will be secured using password in Universities approved databases. Access to these data will be restricted to the research staff only.

Vulnerable Populations: The study intends to enroll subjects from the following "vulnerable" categories: minor. In this case, subjects will be asked to sign the IRB approved assent form.

Potential Benefits of the Proposed Research to Human Subjects and Others

Subjects will likely not receive any long-term benefit from participating in this project, but might experience a potential benefit from participating (improved clearance). They always have the alternative not to participate. By participating, they will allow the performance of the proposed studies. The studies have the potential for an overall benefit in advancing knowledge of the disease studied. Because the study drug is usually well tolerated, the risks to subjects are reasonable in relation to the anticipated benefits, including knowledge to be gained.

Importance of the Knowledge to Be Gained

The proposed clinical research has the potential of providing important information that could be used as base for future studies and eventually change the way CF is treated and improving not only the quality of life of patients with CF but also their lung function, at least in combination with other treatments. If the study is negative, we will have gained improved understanding of why and might be able to deliver these kind of medications via a different route *in vitro* and *in vivo*.

Communication between sites

There will be several lines of communications established between the sites. The investigators, Drs. Salathe, Kramer, Harris and Tupayachi-Ortiz will have monthly teleconference meetings to set up the study at all sites including use of case report forms (CRFs) and databases; establishing route of communications and shipping by pharmacy etc. When the trial is ongoing the meetings will be at least bi-monthly to discuss the progress of the study and bring up issues that need resolution. More frequent informal communications will occur via email and phone calls if necessary. The conference calls will be attended by additional members of the team as needed to resolve any issues. Cystic Fibrosis Foundation Therapeutics (CFFT) will serve as DMC and DSMB on this study.

Safety Reporting and Monitoring Plan

Data monitoring plans will be implemented as follows:

The study will be monitored by Clinical Research Operations & Regulatory Support (CRORS) monitoring group at the University of Miami.

3. Monitoring will occur every 3 months or more frequently if needed.
4. IND exemption for use of losartan in CF patients was granted by the FDA
5. Safety monitoring will be done by the DSMB of the CFFT.
6. Adverse Event Monitoring

- Subjects will be carefully monitored for adverse events (AEs) and serious adverse events (SAEs). Adverse events will be assessed in terms of their seriousness, severity, and relationship to the administration of Losartan.
- All adverse effects will be reported to the co-PIs and discussed with the monitoring group
  - If deemed severe the data will be shared with the DSMB. They will make the decision whether or not to stop the trial.
  - All SAEs will be reported to the DSMB and the appropriate agencies as required.
  - All adverse effects will be reported according to the rules to the FDA, the IRB and to the DMSB

Definitions

7. Adverse Event (AE): An AE is any untoward medical occurrence in a clinical study participant administered with a pharmaceutical product. The AE does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the medicinal product.
8. Unexpected AE: is any adverse drug event, which is not consistent with the current Losartan US Package Insert.
9. Serious Adverse Event (SAE): A SAE is any untoward medical occurrence that at any dose:

• results in death
• is life-threatening
• requires unanticipated in-patient hospitalization or prolongation of existing hospitalization
• results in persistent or significant disability or incapacity
• results in a congenital anomaly or birth defect
• any significant medical event as per the investigator's discretion

Relationship of Adverse Event to Losartan

The assessment of the relationship of an AE to the administration of study drug is a clinical decision based on all available information. An assessment of 'No' would include choices of "not related" or "unlikely related" in the following cases:

1. The existence of a clear alternative explanation, or
2. Non-plausibility (clearly an unrelated circumstance).

An assessment of 'Yes' includes choices of "possibly related or "related" and indicates that there is a reasonable suspicion that the AE is associated with the use of the investigational drug.

Factors to be considered in assessing the relationship of the AE to study drug include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event. We will consider a significant time interval up to 2 hours.
- Underlying, concomitant, intercurrent diseases: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have (i.e. a pulmonary exacerbation)
- Concomitant medication or treatment: The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be suspected to cause the event in question
- Concurrent study procedures: Study procedures should also be considered as possible causes of an AE.

In this study, the investigator will make a separate and independent assessment of every AE's relationship not just to the study drug, but also to concurrent study procedures and to the subject's pre-existing medical condition(s).

A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an AE. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

Each AE should be followed up until resolution or stabilization as determined by the investigator.

Severity of the Adverse Event

The severity of AEs, except for AEs for exacerbations, should be graded as follows:

- Mild – usually transient in nature and generally not interfering with normal activities
- Moderate – sufficiently discomforting to interfere with normal activities
- Severe – prevents normal activities.

Pulmonary Exacerbation: A pulmonary exacerbation is defined as a new or change in antibiotic therapy (IV, inhaled, or oral) for any 4 or more of the following signs/symptoms:

- Change in sputum
- New or increased hemoptysis
- Increased cough
- Increased dyspnea
- Malaise, fatigue, or lethargy
- Temperature above 38°C (equivalent to approximately 100.4°F)
- Anorexia or weight loss
- Sinus pain or tenderness
- Change in sinus discharge
- Change in physical examination of the chest
- Decrease in pulmonary function by 10%
- Radiographic changes indicative of pulmonary infection

Removal of Subjects from Study

A subject withdrawal is defined as a discontinuation from the study for any reason. Subjects may withdraw or be withdrawn from this study for the following reasons:

- At their own request or at the request of their authorized representative at any time for any reason
- If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being

Also subjects must be withdrawn from the study for the following reasons:

- Subjects with an occurrence of a concomitant disease, or any medical condition which, either because of its severity or duration or necessary change in treatment, contravenes the condition of the study or puts the patient at unnecessary risk or harm.
- Subjects with an occurrence of an AE/SAE which in the opinion of the investigator and/or subject requires termination of treatment. For example, skin rashes, palpitations or other moderate or severe adverse events (interference with usual daily activities) without other clear explanation should warrant immediate cessation of treatment and notification of study personnel.
- Subjects who are noncompliant with the protocol per the investigator’s discretion
- Pregnancy
- Severe CF exacerbation during the study as by investigators’’ discretion.

Subjects who terminate the clinical study prematurely, either at their own request or on the recommendation of the clinical investigator, will be considered early terminations. Subjects who withdraw from the study will not be replaced. Subjects who are discontinued from the study will be requested to return for Early Discontinuation and perform procedures as for visit 8.

Assessment of efficacy

N/A

Quality Control & Quality Assurance

Monitoring of the study will be provided through the Clinical Research Operations & Regulatory Support (CRORS) monitoring group at the University of Miami.

Ethics

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the GCP and applicable regulatory requirements.

The clinical protocol will be submitted to and approved by the UM IRB and will not be initiated until written notification of approval of this study has been received.

IND: because this study will use a medication released on the market but not for this indication, IND exemption was granted for use of losartan in this population.

Record Keeping

Accurate and complete study records from all sites will be maintained and kept at storage facility (Iron Mountain) for a minimum of 6 years after completion of the study.

ADMINISTRATIVE SECTION

Protocol Amendments

Any amendments to the protocol will be approved by the PI and co-investigators. All amendments will be submitted to the IRBs for review and approval prior to implementation unless the amendment details changes related to safety.

Protocol Deviations

The investigator will only make changes to the protocol procedures when necessary to protect the safety, rights, and welfare of the subjects. In such an event where protocol procedures are changed, the investigators are responsible for notifying the IRBs and the internal DSMB.
In the event that an isolated, unforeseen instance occurs resulting in a protocol deviation, the investigators are to document this deviation and notify IRBs as by the rules of the University of Miami, the Cincinnati Children's Hospital Medical Center, the University of Alabama, Birmingham and University of Kansas Medical Center.

Case Report Form (CRF)

All clinical data will be recorded on the CRFs for this study, including their electronics versions (Red Cap).

Study Documentation and Records Retention

The Good Clinical Practice guidelines issued by the Committee for Proprietary Medicinal Products state that "The investigator must arrange for retention in strict confidence of the subjects' identification codes, names and addresses for at least 15 years after the completion or discontinuation of the trial." Subjects' files and other pertinent documentation (i.e., study protocol, signed informed consent forms, drug dispensing logs, correspondence, and other documents pertaining to the conduct of the study) must be kept for the maximum period permitted by the hospital or private office in accordance with the local requirements, but not less than 15 years.

Publication policy

The results of this research will be disseminated to the scientific community. These results will include results of primary study outcomes and secondary analyses. Priorities in selecting journals and forums for publications submission will be given to peer-reviewed journals as well as presentations and publications of abstracts at national and international scientific meetings. All individuals who have made substantial intellectual, scientific and practical

contributions to this research protocol and the manuscript should, where possible, be credited as authors; all individuals credited as authors should deserve that designation.

ClinicalTrials.gov Registration
This trial will be registered at ClinicalTrials.gov

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Appendix 1

List of assessments

VISIT	Visit 1	Visit 2	Visit 3 Phone call	Visit 3.a Phone call	Visit 4	Visit 5	Visit 6 Phnne call	Visit 7	Visit 8
		25 mg or 50 mg losartan/placebo	50 mg or 100 mg losartan/placebo						1.4 mg/kg or 100 mg losartan/placebo
	−1 weeks	0 week ± 2 days	1 week ± 2 days	2 days ± 1 day from Visit 3	3 weeks ± 2 days	6 weeks ± 2 days	10 weeks ± 2 days	13 weeks ± 2 days	14 weeks ± 2 days
Informed consent	X								
Full medical history	X								
Intermittent history		X			X	X		X	X
Physical exam	X	X			X	X		X	X
Vitals	X	X			X	X		X	X
Pulse oximetry	X	X			X	X		X	X
Concomitant medications	X								
Changes in medications		X	X	X	X	X	X	X	X
CFQ-R	X					X		X	
Review adverse events, exacerbations		X	X	X	X	X	X	X	X
Spirometry	X					X		X	
CBC w/ differential; CMP; CPK, CRP and other inflammatory markers	X				X	X		X	
Blood for levels of losartan and metabolites (before next morning dose)						X		X	
NPD	X					X		X	
Leukosorb cytokine levels		X							X
Nasal cells		X							X
Sweat chloride		X							X
Serum or urine pregnancy test	X	X			X	X		X	X

IP dispensation: Visit 2 (3-week supply), visit 4 (3-week supply) and visit 5 (8-week supply)

Appendix 2

CFQR Questionnaire:
More information:
http://qol.thoracic.org/sections/instruments/ae/pages/cfq-cfq-r.html

SUMMARY OF CHANGES: Protocol 2.0 dated July 2017:

This amendment is to make the following modifications on the protocol version 1.0 dated march 2017.

1. Losartan and matching placebo (25 mg or 50 mg pills/ placebo) will be obtained from Hunt Valley Research Pharmacy (Cockeysville, MD) and distributed to the 3 sites, and not from University of Miami Research Pharmacy

2. Dose will be adjusted based on participant's weight at visit 1 as following: If participant's weight is < 55 kg, participant will be randomized to either 25 mg or placebo. If participant's weight is ≥ 55 kg, participant will be randomized to either 50 mg or placebo.

3. Blood for levels of losartan and metabolites will be collected before next morning dose and 2 and 6 hours post dose)

4. Monitoring of the study will be provided through the Clinical Research Operations & Regulatory Support (CRORS) monitoring group at the University of Miami.

SUMMARY OF CHANGES Protocol 2.1 dated September 2017

This modification is to make the following changes on the protocol version 2.0 dated July 2017.

1. Inclusion criterion FEV1: It was changed to **FEV1 ≥40% of predicted**. Upper limit was deleted.

2. Study medication provider: To change study medication provider from Hunt Valley to Research Pharmacists at University of Miami, University of Alabama at Birmingham and Cincinnati Children's Hospital Medical Center. New text on page 7: **Losartan and matching placebo (25 mg or 50 mg pills/ placebo) will be compounded at each site by the research pharmacist and distributed to the subject based on the randomization table provided by the Biostatistician at University of Miami**.

3. Destruction of the Study drug: New text on page 7: Study drug will be destroyed at the research site as per the Institution regulations **at the end of study**.

4. Clarification about unblinded study team: New text page 7: **Research pharmacists,** UM biostatistician core **and the assigned unblinded monitor** will be unblinded**.**

5. Clarification about plan of unblinding: New text page 7: Emergency plan for unblinding will be discussed with PI **with consulting with the medical monitor** and DSMB if necessary.

6. Record Keeping: 6 years after study completion instead of 10 years. (on page 13).

7. Visit 3 time-window: ± 2 days was deleted on table of assessment page 17.

8. Version and date of the protocol were updated to version 2.1 dated September 2017 on page 1.

SUMMARY OF CHANGES Protocol 3.0 dated April 2018

This modification is to make the following changes on the protocol version 2.1 dated September 2017

1. Added PI at University of Miami: Maria Gabriela Tupaychi-Ortiz
2. Version and date of the protocol were updated
3. Urine pregnancy test was added to all site visits.
4. Asthma was delete from exclusion criteria list
5. Positive pregnancy tests will be shared with parents for participants under 18 years of age.
6. Table of assessments was updated to add urine pregnancy test at all site visits and dispensation of study drug.

SUMMARY OF CHANGES Protocol 3.1 dated June 2018

This modification is to include patients treated with Symdeko™ (tezacaftor) for more than 3 month on the study. Orkambi™ and Symdeco™ are both FDA approved combinations of a corrector and potentiator (lumacaftor/tezacaftor and ivacaftor), to reduce exacerbations in F508del homozygous patients. Tezacaftor has a similar mode of action compared to Lumacaftor. Symdeko™ information was added on the Background, Study Design, Inclusion criteria and on related sections of the revised protocol.
There are no relevant drug interactions between losartan and Symdeco™ (by Vertex' drug interaction guidance in their 661 and later clinical trials), therefore this modification doesn't increase participant risks.

SUMMARY OF CHANGES Protocol 4.0 dated August 2018

This modification is to include University of Kansas Medical Center (KUMC) as a study site. Dr Matthias Salathe will serve as PI at KUMC.

Co-PIs information were updated on page 1.

Nasal Samples: De-identified samples will be sent to the Pulmonary Research Laboratory at KUMC for analysis.

Number of participants per site: number was changed to 9 participants per site.

SUMMARY OF CHANGES Protocol 5.0 dated February 2019

This modification is to make the following changes on the protocol version 4.0 dated August 2018:

1. Exclusion of NPD response to 0Cl/Iso > -6.6 mV **was removed.**
2. Exacerbation requiring treatment within the past 6 weeks- changed to **4 weeks**
3. NTM exclusion criteria changed to: **Current** treatment for mycobacterial infections
4. Added New schedule of the visits. **Appendix 1** Study includes 6 site visits (visits 1,2,4,5,7 and 8) and 2 phone calls (visits 3,3a and 6). Study duration and treatment phases still the same.
5. Extend the timeline for publish the results by the end of the **3.5**-year funding request instead 2.5 years.
6. Blood and urine samples were modified to match new study procedures schedule as: A total of 30 cc (2 tablespoons) of blood will be collect at **Visit 1 and visits 4, 5 and 7**.
7. Secondary endpoints were changed to match new study procedures schedule as following: Means at baseline, **6** and 13/14 weeks, for each outcome, as well as the change over time will be provided, as well as the corresponding estimates of variability, and 95% confidence intervals will be constructed.
8. Early Discontinuation procedures were changed to match new study procedures schedule as following: Subjects who are discontinued from the study will be requested to return for Early Discontinuation and perform procedures **as for visit 8**.