Effect of losartan on Mucociliary dysfunction in patients with Chronic Obstructive Pulmonary Disease (COPD) and Chronic Bronchitis

PROTOCOL

VERSION 5.0

DATE: February 1, 2019

NCT Number: NCT02416102

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Funding: Flight Attendant Medical Research Institute

Objectives

On this study we will test the hypothesis that inhibiting TGF-ß signaling with losartan in smokers and ex-smokers with chronic obstructive pulmonary disease (COPD) leads to increased Cl^- conductance through CaCC in vivo by assessing nasal potential difference (NPD), a surrogate for potential differences in epithelia of the lower airways and therefore an appropriate outcome variable for a proof of concept clinical trial, avoiding the long trial durations typical for COPD studies. NPD measurements will be done in the untreated cohort and repeated while giving 50 mg and subsequently 100 mg losartan for 4 weeks each. The study design will allow intra-individual comparisons of the effects of losartan on BK channel function as measured by Cl^- conductance through CaCC.

Background

Mucociliary clearance (MCC), a major airway host defense system, is dysfunctional in patients with smoking-associated lung diseases such as chronic bronchitis and COPD. Mucociliary dysfunction accounts for the clinical hallmarks of these diseases, namely chronic productive cough and recurrent respiratory infections. For appropriate MCC, motile cilia beat in a highly regulated airway surface liquid (ASL) that optimizes ciliary beat frequency (CBF) and efficiency and provides hydration to mucus. Airway epithelia use ion transport (absorption of Na^+ and secretion of K^+ and Cl^-) to regulate ASL volume and composition to optimize MCC.

We recently found that ATP-dependent, apical Cl^- secretion and ASL fluid regulation depend on the activation of apically expressed large conductance, Ca^{2+} activated and voltage-dependent potassium (BK) channels. By secreting K^+, apical BK channels provide a driving force for apical Cl^- secretion via Cl^- channels, forming a so-called apical loop current. This BK function is critically important since inhibition of BK channels reduces ASL volume significantly (in the presence or absence of functional CFTR), thus shifting the focus of ASL volume regulation upstream from Cl^- channels to BK channels.

Increased airway TGF-ß1 expression and signaling is common in active cigarette smokers and ex-smokers with COPD. Our preliminary data show that TGF-ß1 and cigarette smoke exposure decrease BK activity and cause ASL volume depletion. We therefore propose that smoke exposure, via TGF-ß1 production, leads to apical BK channel dysfunction to cause ASL volume depletion and mucociliary dysfunction in smokers and ex-smokers with COPD. BK rescue is associated with increased ASL volume and CBF. Therefore, restoring ASL volume requires BK channel rescue or inhibition of TGF-ß signaling. Clinically used Angiotensin II Receptor Blockers (ARBs) are known to inhibit TGF-ß signaling and present a “low-hanging-fruit” approach (medication already in clinical use) for intervention in COPD. In a murine model, losartan has previously been shown to attenuate cigarette smoke-induced lung injury and to rescue lung architecture. Our preliminary data suggest that losartan partially rescues BK channel function leading to increased ASL volume and CBF in airway epithelial cells exposed to smoke. The **long-term goal** of this study is therefore to evaluate the effect of currently available and clinically useful TGF-ß1 signaling inhibitors on mucociliary function in individuals with smoke-associated airway diseases, such as chronic bronchitis and COPD.

Study Design

This is a proof of concept, open label protocol to evaluate the effect of ARB on cigarette smoke-induced lung injury in smokers, ex-smokers with and without COPD, giving 50 mg and subsequently 100 mg losartan for 4 weeks

Study Population

Group I, COPD:

10 ex-smokers not treated with ARBs **with** evidence of fixed airflow obstruction by pulmonary function testing (PFT), decreased DLco **and** with the clinical diagnosis of chronic bronchitis.

Group II, non-COPD:
20 active smokers not treated with ARBs **without** evidence of airflow obstruction by PFT and **normal** DLco but **with** the clinical diagnosis of chronic bronchitis.

Group III, lung healthy, life-time non-smoker:
20 never smokers not treated with ARBs **without** evidence of airflow obstruction by PFT, a **normal** DLco **and no** evidence of the clinical diagnosis of chronic bronchitis

Groups will be appropriately balanced considering COPD stage, smoking status, lung function and age.

Inclusion and Exclusion Criteria

Inclusion criteria

- Age between 35 and 75 years old

- ex-smokers (>10 pack-years of cigarette smoking) not treated with ARBs and **with** evidence of fixed airflow obstruction by pulmonary function testing (PFT), decreased DLco **and** with the clinical diagnosis of chronic bronchitis.
  Or
  active smokers (>10 pack-years of cigarette smoking) not treated with ARBs **without** evidence of airflow obstruction by PFT and **normal** DLco but **with** the clinical diagnosis of chronic bronchitis.
  Or
  never smokers not treated with ARBs **without** evidence of airflow obstruction by PFT, a **normal** DLco **and no** evidence of the clinical diagnosis of chronic bronchitis

- Ability to understand and willingness to sign consent documents

Exclusion criteria:

- Current therapy with ACE inhibitor, intolerance to ARB
- Women of child bearing potential
- Regular use of NSAIDs or potassium supplementation, treatment with aliskiren, anticoagulation
- COPD exacerbation requiring treatment within 6 weeks
- Oral corticosteroid use within 6 weeks
- Significant hypoxemia (oxygen saturation <90% on room air), chronic respiratory failure by history (pCO_2 > 45 mmHg) and forced expiratory volume in 1 second (FEV_1) below 40%, clinical evidence of cor pulmonale

- Untreated arterial hypertension (systolic blood pressure >140 mm Hg, diastolic blood pressure > 90 mm Hg)
- Blood pressure less than 100 mm Hg systolic or 70 mm Hg diastolic at Screening visit and Visit 2.
- Cardiac, renal, hepatic (LFTs > 3x normal upper limit), neurological, psychiatric, endocrine or neoplastic diseases that are judged to interfere with participation in study
- History of renal artery stenosis
- Concomitant airway disorders other than COPD and chronic bronchitis, such as bronchiectasis and asthma (history and reversible airflow obstruction by ATS criteria)
- History of pulmonary malignancies, and any other malignancies in the last 5 years
- History of thoracic surgery.
- Acute pulmonary exacerbation within 6 weeks from the Screening Visit.
- Subjects with no airflow obstruction by spirometry but with a decrease in DLco < 70% possibly indicating emphysema.
- Significant exposure to environmental tobacco smoke or atmospheric or occupational pollutants for the non-smokers group.
- Urine pregnancy positive test at the Screening Visit.

Treatment arms

Patients will be given four weeks of 50 mg of losartan daily and consequently an additional four weeks of 100 mg of losartan (divided in two doses of 50 mg twice daily). This higher losartan dose was used safely in other clinical trials (www.clinicaltrials.gov; search: losartan and COPD). At the end of each four-week period, participants will be re-evaluated. We will compare treatments using baseline measurements of each individual as his/her own control.

Study Drug:

Cozaar®, the commercial form of losartan will be obtained from the UMHC Pharmacy and given to the subjects by the PI or study coordinators free of charges. FDA has granted IND exemption for this study. Study drug will be stored as per package instructions in a locked cabined on the research room at UMH 6th floor west room 607. Only the study team has access to this room. The storage area at the study center will be monitored by the staff for temperature consistency with the acceptable storage conditions.

The Investigator is responsible for maintaining accurate study drug accountability records throughout the study.

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 clinical site as per the Institution regulations.

Study Procedures

Once informed consent is signed, the subjects will have an initial visit during which they will have a physical exam (including vital signs), complete study questionnaires and PFTs. All study subjects will undergo the following procedures during a single visit:

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

Laboratory Samples: Urine will be collected for a pregnancy test if applicable and blood test for CMP and CRP will be drawn, if results are not available within 6 months of the Screening visit.

Urine cotinine levels will be measured at the time of study visits to assess smoking status (active or not).

Questionnaires: Will collect basic demographic data, history of other medical conditions, detailed occupational history (focusing on environmental exposures), prior exposures (including biomass, smoke and air pollution), medications, chronic respiratory symptoms and exacerbation history. A second part will include standardized questionnaires (Appendix I) for subjects on the COPD group. Participants will have an objective assessment of cough and sputum production with the Breathlessness, Cough and Sputum Scale (BCSS). For patients with COPD we will record dyspnea with the Medical Research Council (MRC) dyspnea scale, co-morbidities with the Charlson co-morbidity index and health-related quality of life with the St. George's Respiratory Questionnaire and the COPD assessment test (CAT) for accurate COPD staging

6-Minute walk test (6MWT) will be done for participants on the COPD group as per American Thoracic Society guidelines.(Apendix II)

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. Spirometry will be repeated 15 min after the administration of 180 μg (two puffs) of albuterol from an MDI through a spacer. Lung volumes and DLco will be performed following ATS recommendations. Height, weight and oxygen saturation will be measured prior to these tests. Since smoking has both biologic and technical effect on DLco and there is not yet a appropriate method of adjusting pulmonary function indices for the effect of smoking, the DLco Normal Range (Low 95th percentiles of percent predicted) criteria for this study is ≥70% of Crapo predicted values .

BODE score will be calculated from BMI, FEV_1, 6-MWT and the MRC dyspnea scale.

NPD measurement will be performed according to standard procedures. In this aim, we will use one side of the nose for the evaluation of the function of CFTR and CaCC by measuring the potential differences by initial blocking ENaC with amiloride, then using chloride free solution and isoproterenol to evaluate CFTR function, followed by ATP to evaluate CaCC function. In the other nostril, the same experiment will be repeated as mentioned above, except that after blocking ENaC with amiloride and perfusion with chloride-free solution the CFTR will not be stimulated with isoproterenol, but ATP will be used to maximize the CaCC signal. We will adjust the way of measuring the NPD for aim 2.2 depending of the results in aim 2.1.

TGF-ß and citokines will be measured using Leukosorb, a noninvasive sampling method of the nasal mucosa as previously reported. In addition, we will obtain a small amount of nasal epithelial cells to measure TGF-ß by qPCR.

Study Visits:

V1 (Screening Visit): This will be a single visit for the purpose of determining eligibility, obtaining informed consent, and recording the necessary data will include:

- Assessment of general health status and physical examination (incl. vital signs)
- Urine pregnancy test if indicated (female, child bearing age)
- Spirometry pre- and post-bronchodilators, DL_{CO}, lung volumes
- Pulse oximetry
- Review of laboratory values, or if not available: venous blood sampling to assess for renal and liver abnormalities (then the measurements will be done within a week in an additional visit), CRP
- If inclusion criteria fulfilled and no exclusion criteria found:
    - Enrollment

- Completion of respiratory questionnaires (COPD only)
- Six minute walk test (COPD only)
- BODE index calculation

V2 (week 4): This visit (and the two following ones) will only done for the groups not treated with any ARBs. This visit will occur 4 weeks (± 4 days) after V1.

- We will again review laboratory values (CMP, CRP), repeat urine pregnancy test if indicated (female, child bearing age).
- The following procedures will be executed:
  - Completion of respiratory questionnaires (COPD only)
  - Six minute walk test (COPD only)
  - BODE index calculation
  - NPD measurement followed by nasal fluid recovered by Leukosorb filter paper and nasal cell collection for TGF-ß
  - Subjects will be provided with a 4-week supply of 50 mg losartan for daily use (first dose given in clinic; observation with blood pressure monitoring for 2 hours).

V3 (week 5 ± 4 days):
Telephone visit to follow up on symptoms and tolerability of medication.

V4 (week 8 ± 4 days):

- The following procedures will be executed:
  - Monitor for adverse events.
  - Completion of respiratory questionnaires (COPD only)
  - Six minute walk test (COPD only)
  - Venous blood sampling to assess for renal and liver abnormalities and CMP, CRP
  - Urine pregnancy test (if applicable)
  - BODE index calculation
  - Nasal fluid recovered by Leukosorb filter paper and nasal cell collection for TGF-ß
  - Subjects will be provided with a 4-week supply of 50 mg losartan for twice daily use (100 mg)

V5 (week 9 ± 4 days):
Telephone visit to follow up on symptoms and tolerability of medication.

V6 (week 12 ± 4 days) or Early Termination Visit:

- We will draw laboratory (CMP, CRP). Repeat pregnancy test if indicated (female, child bearing age).
- The following procedures will be executed:
  - Monitor for adverse events.
  - Completion of respiratory questionnaires(COPD only)
  - Venous blood sampling to assess for renal and liver abnormalities and CMP, CRP
  - Urine pregnancy test (if applicable)
  - Six minute walk test (COPD only)

- BODE index calculation
- NPD measurement followed by nasal fluid recovered by Leukosorb filter paper and nasal cell collection for TGF-ß

Unscheduled visit (up to 2 visits) will apply if there is a need to repeat study procedures.
Subjects are allowed to be re-screened one time as per PI discretion.

Specimen Banking
Not applicable.

Data Management
Data collected will include demographics, clinical symptoms, health-related quality of life, and pulmonary function test data. Samples to be collected include NPD measurement followed by nasal fluid and cell collection for TGF-ß. We will obtain blood for safety reasons (liver and renal function) and for evaluation of an inflammatory marker. No personal identifiers will be collected and each patient's data will be coded.

Safety Reporting
DSMB: the PI and the co-investigators will continuously monitor the study for side effects. If any AE or SAE occur, the physicians will assess the event and report them to the appropriate authorities if needed as per Good Clinical Practice. Subjects might be withdrawn from the study if they experience intolerance to the study medication and/or the NPD testing. Furthermore, a COPD exacerbation will be a reason for subject withdrawal. We expect few dropouts for exacerbation given the three-month duration of the study. IND: because this study will use a medication released on the market but not for this indication, we will file an IND with the FDA for use of losartan in this population. Given our experience with INDs and the long experience with use of losartan, we do not believe that this will be a hurdle for the trial.

Assessment of efficacy
N/A

Statistical analysis
Differences between groups and doses will be analyzed with regression analysis. Multivariate analysis will be done to evaluate differences in the tests results between different groups and correlations between different measures will be examined using the Pearson's correlation coefficient. Biostatistics Collaboration and Consulting Core services will be used on a fee for service

Risks to Subjects
Potential Risks: All procedures are performed during this study are routine clinical procedures and are associated with minimal risks.

Pulmonary Function Testing 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. The risk is minimized by having the PFT done in a sitting position.

NPD measurement: is a non-invasive method with minor risks, which are 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 very 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 and can, when given in a higher dose, increase urine production, which is not anticipated here as the used dose is low and systemic absorption is not anticipated. Isoproterenol is a beta 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.

Nasal fluid and cell sampling: this procedure is routine and is sometimes associated with an unpleasant feeling due to the fluid, minimal risk of aspiration of part the 5 ml of sterile saline solution, and coughing. To prevent this from happening, the head of the patient will be bend forward during the collection, so the fluid will be absorbed by Leukosorb and not get into the pharynx where it can be swallowed or aspirated. Cell sampling can cause a mild discomfort and a mild nosebleed.

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 to not look directly at the site of blood draw.

Treatment with losartan: Cozaar ® 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 every 30 minutes. If systolic blood pressure falls below 100 mmHg, the patient will be discharged from the study and dosing not repeated.

If the patient has symptomatic hypotension, the patient will be put into a supine position until recovered. If needed additional interventions will be undertaken (including button limited to fluid administration) according to the supervising physician’s instructions.

Subjects with hyperkalemia, renal dysfunction, or who are taking potassium supplements will not be enrolled into the study. Participants will be given at the screening visit written descriptions of potential side effects and participants wiil be instructed to contact the study doctor if any new symptoms occur. 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.

Six Minute Walk Test: the participant walks for six minutes and the distance traveled is measured. As with any exercise test, there is a theoretical risk but in multiple clinical trials conducted at our site, we had no adverse events. Risks are minimized by medical supervision and trained medical personnel.

Questionnaires, Health History and Physical Examination 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. Filling out the questionnaires could cause

psychological distress. Participant will be informed to escape questions if he/she feels uncomfortable or upset doing that.

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

Definitions

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.

Unexpected AE: is any adverse drug event, which is not consistent with the current Losartan US Package Insert.

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

• 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 COPD 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.

Potential Benefits to Subjects

Although there will be no direct benefit for subjects taking part in this study, the researcher may learn more about COPD. The study has the potential for an overall benefit in advancing knowledge of the disease studied. Because the study drug is well tolerated, the risks to subjects are reasonable in relation to the anticipated benefits of knowledge gained.

Vulnerable Populations

COPD is more common among men than women, although the prevalence is increasing in women. COPD is rare among African-Americans. Less information is available about Hispanics, which are a heterogeneous group. The planned accrual of minority groups reflects the prevalence of COPD in our region in South Florida.

Setting

The Human Research Laboratory, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, is located on the 6th floor of the Doctor's Office West Building at the University of Miami on the Medical School Campus. It has 2,200 square feet of space for evaluation of patients for pulmonary research. This space is equipped with spirometers, a nSpire® HDpft plethysmograph including DLco measurement, , blood pressure machines, pulseoximeters, 12-lead ECG machine, ACLS equipment and setup for 6-minute walk tests, including O_2 tanks, an HP 78352A monitor for heart rate and ECG monitoring (3 lead ECG cables). Other equipment includes a DeVilbiss nebulizer, Ultrasonic nebulizer and - 20°C freezer.

We have the ability to draw blood, prepare specimens for laboratory analysis. Our staff is BLS, GCP, OSHA and IATA certified and follows UN 1845 and 3373 regulation for the guidelines of shipping biological materials. Physicians are BLS and ACLS certified. We have a fully functional nasal potential difference (NPD) measurement unit (use certified by Cystic Fibrosis Foundation Therapeutic Development Network: CFF TDN). Dr. Schmid has an office within this setting.

Recruitment Methods

Forty subjects will be enrolled from two outpatient clinics run by members of the Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Medicine, at the University of Miami Miller School of Medicine considering 20% of screening failure to enroll 30 patients on the treatment phases. Collectively, ~400 patients with COPD are seen at the two sites annually. Smokers without COPD and lung-healthy lifetime non-smokers will be recruited from the pre-operative clinics at the University of Miami Hospitals and 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 PI or co-investigators to address all their questions about the research and take the informed consent home to discuss with family and possibly PCP.

Subjects will be paid $100.00 for each completed study visit and $ 25.00 for each of the phone calls. The total amount you could receive for participating in all of the study visits would be $450.00. Unscheduled visit if applicable will be paid $50.00

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. If a patient becomes pregnant during administration of the drug, treatment should generally be discontinued.
- Severe COPD exacerbation during the study.

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 be replaced.

Subjects who are discontinued from the study will be requested to return for Early Discontinuation and perform procedures as for visit 6

Provisions to Protect the Privacy Interests of Subjects

All patient data will be stored in locked file cabinets or a room that is locked when unattended. All electronic data will be secured using password protection. Access to these data will be restricted to the research staff only. Because this proposal is a proof-of-principle study, a formal data and safety monitoring board will not be created.

Consent Process

The informed consent document (in English and Spanish) and the protocol will be submitted to the University of Miami's Institutional Review Board for review and approval. Subjects interested in volunteering for the study will have a lengthy discussion with the investigators about the risks and benefits of their participation. Patients need to agree to practice effective pregnancy prevention for the duration of the study.

Direct Access To Source Data/Documents
Qualified representatives of regulatory agencies, internal auditors or monitors will have the right, both during and after this study, to conduct inspections and to audit and review medical records and/or study documents (CRFs, source data/documents, etc.) pertinent to the clinical study as permitted by the regulations (21 CFR 312.58; 312.68, ICH-GCP E6; 6.10).

Quality Control & Quality Assurance

Monitoring of the study will be provided through the University of Miami's Office of Clinical Research Operations and Regulatory Support (CRORS).
Medical Monitoring: the PI and the co-investigators will continuously monitor the study for side effects. If any AE or SAE occur, the physicians will assess the event and report them to the appropriate authorities if needed as per federal regulations, IRB policies and procedures and ICH-Good Clinical Practice. Subjects might be withdrawn from the study if they experience intolerance to the study medication and/or the NPD testing. Furthermore, a COPD exacerbation will be a reason for subject withdrawal. We expect few dropouts for exacerbation given the three-month duration of the study.

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 the FDA, 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, we will file an IND with the FDA for use of losartan in this population. Given our experience with INDs and the long experience with use of losartan, we do not believe that this will be a hurdle for the trial.

Record Keeping
Accurate and complete study records will be maintained and kept at Iron Mountain for a minimum of 10 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.

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Appendix I

	For all subjects	For subjects who are not on ARBs treatment				
	Visit 1 Screening	Visit 2[g] 4 weeks (± 4 days) after V1	Visit 3 Phone Call 1 weeks (± 4 days) after V2	Visit 4 8 weeks (± 4 days) after V2	Visit 5- Phone Call 9 weeks (± 4 days) after V2	Visit 6 12 weeks (± 4 days) after V2
Informed Consent	X					
Assessment of health status	X	X		X		X
Medical History	X					
Occupational history	X					
Smoking History	X					
Concomitant medication	X		X		X	
Urine cotinine test	X	X		X		X
AE and SAE		X	X	X	X	X
Physical exam	X	X		X		X
Vital signs	X	X		X		X
Urine pregnancy test[a]	X	X		X		X
β–HGC[b]	X	X		X		X
Complete Pulmonary function test pre and post	X					
Renal and liver blood test[c]	X	X		X		X
CRP[d]	X	X		X		X
Respiratory Questionnaires[e]	X	X		X		X
6 Minute Walk Test[f]	X	X		X		X
NPD measurement		X		X		X
Nasal fluid for TGF -β measurement	X	X		X		X
Drug Dispensation (losartan 50 mg or 100 mg)		X		X		
Drug Compliance				X		X

a- Only for female
b- If urine pregnancy test is positive
c- Only if results are not available on the last 6 month
d- Only if results are not available on the last 6 month
e- Respiratory Questionnaire (BCSS, MRC, Charlson co-morbidity index, SGRQ, CAT) will be performed at each visit before any study procedure (COPD group only)
f- 6MWT will be performed for participants on the COPD group only
g- Visit 1 and 2 could be combined if the subject is not on ARBs treatment and has renal and liver blood test results available on the last 6 month.

Appendix II
Questionnaires used on this protocol could be found at the following links**:**

St George's RespiratoryQuestionnaire:
http://www.thoracic.org/assemblies/srn/questionaires/sgrq.php

COPD assessment test (CAT)
http://www.thoracic.org/assemblies/srn/questionaires/copd.php

Medical Research Council (MRC) dyspnea scale
http://www.thoracic.org/statements/resources/archive/rrdquacer.pdf

Breathlessness, Cough and Sputum Scale (BCSS).
http://journal.publications.chestnet.org/data/Journals/CHEST/22001/2182.pdf

Appendix III
Changes from version 1.2 of the protocol

1.Version and date to the protocol were updated
2.The address for the PI and the study site were updated.
3. The address for Drug storage was updated to at UMH 6th floor west room 607.
4. to clarify footnote “a” on the Appendix I : “child bearing age” was deleted from the footnote of Urine pregnancy test. Woman in child bearing age were excluded from the study