JOHN V. FAHY, MD, MSC
Clinical Research Protocol
INPATIENT CLINICAL TRIAL OF NAC IN ASTHMA
NCT03581084

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Version Date:	11/2/2018
Investigational Product:	n-acetylcysteine
Development Phase:	4
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Approval:

, Professor

1/24/2018

PI or Sponsor Signature (Name and Title)

Date

This confidential information about an investigational product is provided for the exclusive use of investigators of this product and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Airway Clinical Research Center Quality Assurance Committee and the UCSF Committee on Human Research with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: 17-24231

Protocol Title: Inpatient Clinical Trial of NAC in Asthma

Protocol Date: 01/24/2018

1/24/2018

Investigator Signature *Date*

John V. Fahy, Professor of Medicine

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LIST OF ABBREVIATIONS

AE	adverse event
CBC	complete blood count
CFR	Code of Federal Regulations
CRF	case report form
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	forced expiratory flow
FeNO	fractional exhaled nitric oxide
FEV_1	forced expiratory volume over one second
FVC	forced vital capacity
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
mEq	milliequivalent
NAC	n-acetylcysteine
PEF	peak expiratory flow
PI	Principal Investigator
RV	residual volume
SAE	serious adverse experience
TLC	total lung capacity

PROTOCOL SYNOPSIS

TITLE	Inpatient Clinical Trial of NAC in Asthma
SPONSOR	John V. Fahy, MD, MSc
FUNDING ORGANIZATION	Departmental funds, Division of Pulmonary and Critical Care Medicine, Department of Medicine, UCSF
NUMBER OF SITES	1
RATIONALE	We show that airway mucus plugs are strongly associated with measures of airflow obstruction in chronic severe asthma, and this association provides the rationale for testing whether a mucolytic drug (n-acetylcysteine, "Mucomyst") can decrease mucus plug scores and improve FEV1 values in patients with asthma. It is perhaps surprising that inhaled NAC has not been tested in a clinical trial in asthma, but a significant reason has been the uncertainty outlined above for the role of mucus in chronic disease. Another factor has been that clinical trials of NAC in COPD and cystic fibrosis have not been consistently encouraging. However, these trials have not had a biomarker to select patients who might benefit and they have used orally administered NAC, which does not achieve detectable drug concentration in airway lining fluid[6]. Our proposal to test the efficacy of NAC in a specific patient subgroup identified by a biomarker (CT imaging) is timely and addresses a novel approach to asthma treatment.
STUDY DESIGN	This is a single arm, non-randomized interventional phase 4 study.
PRIMARY OBJECTIVE	The primary objective is to determine if inhaled NAC decreases mucus plugs and improves lung function in patients with asthma.
SECONDARY OBJECTIVES	The secondary objective is to determine the duration of benefit of inhaled NAC in asthma.
NUMBER OF SUBJECTS	30

SUBJECT SELECTION CRITERIA	**Inclusion Criteria** 1. Male or female between the ages of 18 and 80 years of age at Visit 1 2. Written informed consent obtained from subject and ability for subject to comply with the requirements of the study. 3. Able to perform reproducible spirometry according to ATS criteria 4. Physiological evidence of airflow obstruction (FEV1 bronchodilator reversibility of ≥ 12% or hyperreactivity to methacholine reflected by a methacholine PC20 ≤ 16 mg/mL) 5. Clinical history of asthma per patient report or medical record 6. Pre-bronchodilator FEV1 > 35% predicted 7. Post-bronchodilator FEV1 > 40% but < 90% predicted 8. Asthma requiring treatment with inhaled corticosteroids (ICS) for 3 months or greater 9. CT mucus score ≥ 5 **Exclusion Criteria** 1. Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study. 2. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data. 3. Smoking of tobacco or other recreational inhalants in last year and/or >10 pack-year smoking history 4. Current participation in an investigational drug trial 5. Other chronic pulmonary disorders, including (but not limited to) cystic fibrosis, chronic obstructive pulmonary disease, chronic bronchitis, vocal cord dysfunction (that is the sole cause of respiratory symptoms and at the PI's discretion), severe scoliosis or chest wall deformities that affect lung function, or congenital disorders of the lungs or airways 6. Unwillingness to follow study procedures 7. History of allergy or intolerance to study drug 8. Any other criteria that places the subject at unnecessary risk according to the judgment of the Principal Investigator
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	20% NAC (3 mL) and albuterol (.5 mL) Product will be administered every 4-6 hours for 5 days during an inpatient stay at the hospital. Medication will be delivered by inhalation via nebulizer.
CONTROL PRODUCT, DOSE	No control product

AND ROUTE OF ADMINISTRATION	
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be in the study for up to 91 days. **Screening:** up to 14 days **Treatment:** 5 days (subjects to be admitted to the hospital) **Follow-up:** 3 months The total duration of the study is expected to be 24 months. Subject recruitment will happen on a rolling basis.
CONCOMMITANT MEDICATIONS	Prohibited: • thiols and thiol derivatives • activated charcoal • inhaled insulin
EFFICACY EVALUATIONS	
PRIMARY ENDPOINT	The primary endpoint is the FEV1. Post-treatment FEV1 will be compared to pre-treatment baseline values.
SECONDARY ENDPOINTS	Secondary efficacy endpoints include: • CT mucus score • FVC • PEF (AM and PM) • Air trapping (RV/TLC ratio) • FeNO • Blood eosinophils
OTHER EVALUATIONS	
SAFETY EVALUATIONS	Incidence of adverse events
PLANNED INTERIM ANALYSES	No interim analysis is planned.
STATISTICS **Primary Analysis Plan**	The primary analysis is a paired t-test analysis of the % change in FEV1 from the start to the end of the one week treatment period with four times daily NAC (Mucomyst-20). The secondary analysis will be to determine how the change in FEV1 relates to the change in CT mucus score.
Rationale for Number of Subjects	We propose a sample size of 30, which will provide us with the power to examine the effect of NAC in a subgroup of individuals with asthma who have CT evidence of intraluminal mucus and to identify the CT mucus score that performs best as a biomarker of treatment response to NAC. Participants will be enrolled if their CT mucus scores are ≥5.0. To calculate sample size for the one-week treatment period study, we used FEV1 measures from 219 adults with asthma published in Corren et al. NEJM, 2011[1]. The authors found that the standard deviation for the change in FEV1 in liters from day 1 to day

	7 was 19%. Using a standard deviation of 19 and a two-sided alpha of 0.05, we calculate a sample size of 30 to have 80% power to detect a change in FEV1 of 10% (a reasonable effect size because early studies of Pulmozyme in CF reported a 10% increase in FEV1 at day 3 of treatment).[7]

1 BACKGROUND

Mucus plugging of the airway is consistently found in fatal asthma[2]. Decades ago, Dunnill provided graphic descriptions in 20 cases of fatal asthma[3] noting that "the cut surface of the lung showed a striking picture with numerous grey, glistening, mucous plugs scattered throughout the airway passages." He summarized that "pathologically, the outstanding feature of the asthmatic lung lies in the failure of clearance of the bronchial secretions." Others have confirmed these findings, and it is only a small minority of asthma deaths that are not associated with airway mucus impaction.

In non-fatal or near-fatal asthma exacerbations, segmental collapse of lung lobes due to luminal occlusion is common. Lavage of these cases yields abnormal mucus plugs in the form of airway casts[4]. The combination of airway narrowing from concentric smooth muscle contraction with luminal obstruction by mucus marks asthma as uniquely dangerous among airway diseases in its propensity for sudden and sometimes fatal exacerbations. The role of mucus as a cause of airflow obstruction in acute severe asthma suggested to us that mucus plugs plays a role in the pathophysiology of airflow obstruction in chronic severe asthma as well. This role has been hard to prove, however, in large part because of difficulty in showing that mucus occludes the lumen in chronic severe disease. Lungs are available at autopsy to show mucus occlusion in fatal asthma, and it is notable that there is evidence of occlusion in asthmatics who die with asthma (rather than because of it)[5]. But the field has yet to be persuaded of a role for mucus in airway dysfunction in chronic forms of severe asthma.

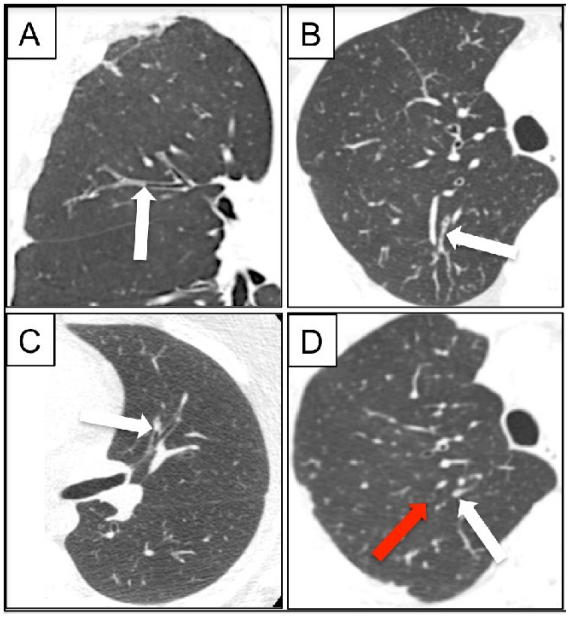

Figure 1: Intraluminal mucus in CT lung images of asthmatics. Mucus plugs in CT scan images (white arrows) were defined as complete occlusion of an airway lumen by mucus and were identified as tubular structures in longitudinal section with branching (A & B) or without branching (C) or as rounded opacities in cross-section (D). The latter were traced cephalad or caudad on adjacent slices to confirm their continuity with unoccluded bronchi (red arrow).

1.1 Overview of Non-Clinical Studies

Against this background, the preliminary data we show in Figures 1 and 2 are very important, because we show how CT lung images can reveal mucus plugs in chronic severe asthma and how these plugs are associated with lower lung function. Specifically, we use CT imaging to uncover that a majority (58%) of asthmatics in the NHLBI Severe Asthma Research Program (SARP) have at least one lung segment with a mucus plug and 27% have more than four lung segments with mucus plugs. Notably, asthmatics with a high mucus score achieve a post albuterol FEV1 > 80% much less frequently than asthmatics who have a zero mucus score (Fig 3).

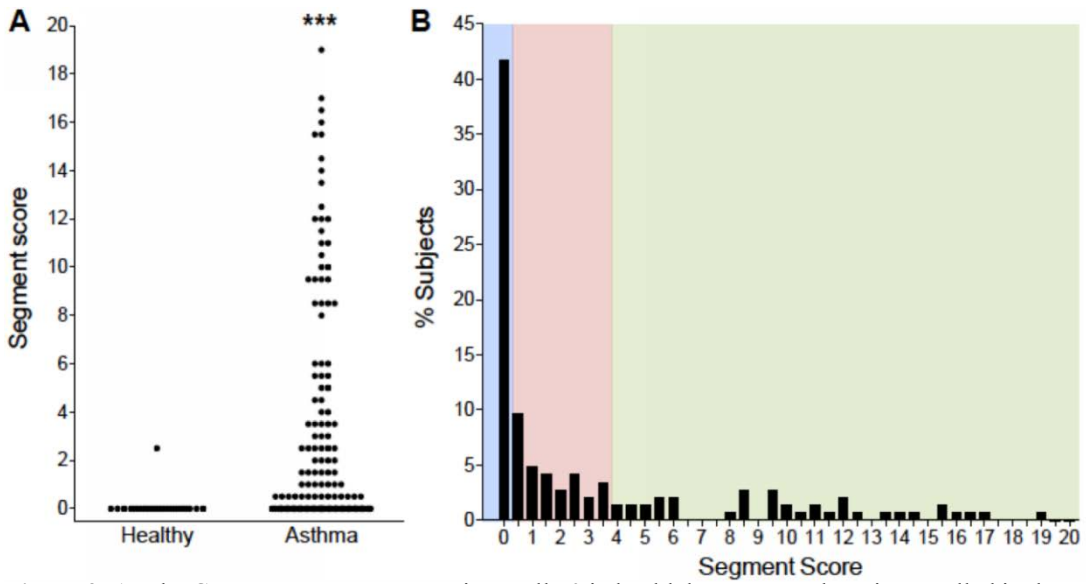

Figure 2. A. The CT mucus segment score is usually 0 in health but many asthmatics enrolled in the NHLBI Severe Asthma Research Program have scores above 0 with some as high as 15-19. B. The frequency distribution of mucus score in asthma shows that 42% of patients have a score of 0 (zero mucus score); among the remaining 58% of asthmatics, the median mucus score was 3.5, and we classified score between 0.5 and 3.5 as "low" and above 4.0 as "high".

Examined another way, we find that all asthmatics with an FEV1 < 60% after albuterol treatment have abnormal mucus scores, whereas the majority of asthmatics with an FEV1 >80% with albuterol treatment have zero or low mucus scores (Fig 3). These data clearly implicate mucus plugs in the pathogenesis of airflow obstruction in asthma. One reason why this role for mucus plugging has been underappreciated in chronic severe asthma is that mucus-related symptoms are both insensitive and non-specific indicators of mucus.

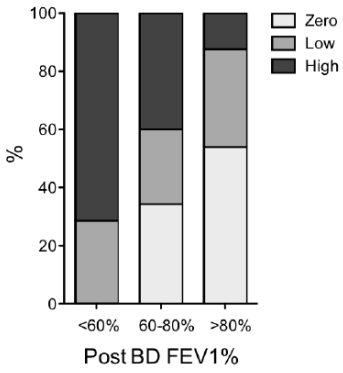

Figure 3. Mucus scores in asthmatics with different FEV1 values (post 6-8 puffs of albuterol). Note that all asthmatics with an FEV1 <60% have abnormal mucus scores.

2 STUDY RATIONALE

We show that airway mucus plugs are strongly associated with measures of airflow obstruction in chronic severe asthma, and this association provides the rationale for testing whether a mucolytic drug (n-acetylcysteine, "Mucomyst") can decrease mucus plug scores and improve FEV1 values in patients with asthma. It is perhaps surprising that inhaled NAC has not been tested in a clinical trial in asthma, but a significant reason has been the uncertainty outlined above for the role of mucus in chronic disease. Another factor has been that clinical trials of NAC in COPD and cystic fibrosis have not been consistently encouraging. However, these trials have not had a biomarker to select patients who might benefit and they have used orally administered NAC, which does not achieve detectable drug concentration in airway lining fluid[6]. Our proposal to test the efficacy of NAC in a specific patient subgroup identified

by a biomarker (CT imaging) is timely and addresses a novel approach to asthma treatment.

2.1 Risk / Benefit Assessment

Risk: Inhaled n-acetylcysteine (NAC) is approved for the treatment of mucus associated airway disease, including asthma. The main side effect from inhaled n-acetylcysteine (NAC) is bronchospasm. We find that co-administering NAC and albuterol effectively prevents excessive bronchoconstriction in asthmatic patients. Specifically, in ten asthmatics that were given doses of 20% NAC as high as 3 mL together with 3 mL of albuterol, the largest FEV1 decline was 6%.

Benefit: Mucus plugs cause airway obstruction and symptoms of dyspnea. The role of mucolytics in decreasing plugs and increasing FEV1 has not been well studied. Our preliminary data, utilizing CT imaging and a novel scoring system, can identify and target a subgroup of patients who may benefit from inhaled mucolytic treatment more so than the asthmatic population as a whole.

To minimize risk, study participants will be admitted to a medical-surgical ward in the hospital and given all treatments under the supervision of a registered nurse and the covering medical team. Our study team of experienced clinicians will be available as needed.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to determine if inhaled NAC decreases mucus plugs and improves lung function in patients with asthma.

3.2 Secondary Objectives

The secondary objective is to determine the duration of benefit of inhaled NAC in asthma and identify characteristics of those subjects who benefit from mucolytic treatment.

4 STUDY DESIGN

4.1 Study Overview

This is a single center, single arm, non-randomized trial in 30 patients with asthma who demonstrate mucus plugging on a screening CT lung scan. Following outpatient screening studies, each subject will be admitted for 6 days/5 nights and receive NAC mixed with albuterol by nebulizer four times daily. After the active treatment phase, the subjects will be followed for 3 additional months as outpatient to track changes in CT lung scans in the off treatment period. Total duration of participation will be four months. Total duration of the study is expected to be 2 years.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

FEV1 will be the primary endpoint because it is the most robust measure of airflow obstruction in asthma. We will compare post-treatment FEV1 to pre-treatment baseline values.

5.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints will include:

- CT mucus score
- FVC
- PEF (AM and PM)
- Air trapping (RV/TLC ratio)
- FeNO
- Blood eosinophils

5.3 Safety Evaluations

The most important safety evaluations will be those that monitor for evidence of NAC-induced bronchoconstriction. In this regard, our plan is to measure peak flow (PEF) before and after each treatment.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of asthma who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Male or female between the ages of 18 and 80 years of age at Visit 1
2. Written informed consent obtained from subject and ability for subject to comply with the requirements of the study.
3. Able to perform reproducible spirometry according to ATS criteria
4. Physiological evidence of airflow obstruction (FEV1 bronchodilator reversibility of ≥ 12% or hyperreactivity to methacholine reflected by a methacholine PC20 ≤ 16 mg/mL)
5. Clinical history of asthma per patient report or medical record
6. Pre-bronchodilator FEV1 > 35% predicted
7. Post-bronchodilator FEV1 > 40% but < 90% predicted
8. Asthma requiring treatment with inhaled corticosteroids (ICS) for 3 months or greater

9. CT mucus score ≥ 5

6.3 Exclusion Criteria

1. Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study.
2. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.
3. Smoking of tobacco or other recreational inhalants in last year and/or >10 pack-year smoking history
4. Current participation in an investigational drug trial
5. Other chronic pulmonary disorders, including (but not limited to) cystic fibrosis, chronic obstructive pulmonary disease, chronic bronchitis, vocal cord dysfunction (that is the sole cause of respiratory symptoms and at the PI's discretion), severe scoliosis or chest wall deformities that affect lung function, or congenital disorders of the lungs or airways
6. Unwillingness to follow study procedures
7. History of allergy or intolerance to study drug
8. Any other criteria that places the subject at unnecessary risk according to the judgment of the Principal Investigator

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

Standard therapy for asthma is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

7.2 Prohibited Medications and Treatments

The following medications are prohibited during the study and administration will be considered a protocol violation.

- Thiols and thiol derivatives
- Activated charcoal
- Inhaled insulin

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

There is one treatment group all subjects will be assigned to.

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will be known to investigators, research staff, and patients. Study treatments will not be blinded.

8.3 Formulation of Test and Control Products

NAC (trade name: Mucomyst) is manufactured by American Regent. The active drug studied here is 20% NAC. It will be delivered via nebulizer.

8.3.1 Formulation of Test Product

20% NAC is an existing formulation of Mucomyst (Mucomyst-20), manufactured by American Regent, for aerosol administration in the management of patients with chronic bronchopulmonary disease. 20% NAC is a colorless solution that requires no reconstitution.

Albuterol sulfate inhalation solution is an existing formulation of Proventil Nebules, manufactured by Nephron Pharmaceuticals Corporation for oral inhalation in the management of patients with bronchospasm. Albuterol sulfate inhalation solution is a clear, colorless solution.

Table 1: Formulation and Measured pH of 20% NAC and albuterol

	20% NAC	**Albuterol**
Active Ingredient, mg/mL	Acetylcysteine 200mg/mL	Albuterol sulfate 0.83mg/mL
Other ingredient, mg/mL	Edetate disodium, sodium hydroxide, purified water	Water, sodium chloride, sulfuric acid
pH	7	3-5

8.3.2 Packaging and Labeling

The test product will be in its original packaging and is provided by the UCSF Pharmacy. It will be ordered as a medication through the patient's electronic medical record (EMR).

8.4 Supply of Study Drug at the Site

The UCSF Pharmacy will order the study drug and albuterol per the patient's medication administration record (MAR). It will be located in the Pyxis MedStation on the medical-surgical ward the patient is admitted on.

8.4.1 Dosage/Dosage Regimen

During each treatment, participants will receive a mixture of 3 mL of study drug and .5 mL of albuterol. It will be delivered via nebulizer. Subjects will take four treatments a day, spaced at approximately 4 - 6 hour intervals. The treatment period is five days. No adjustments will be made based on weight or age.

8.4.2 Dispensing

Study medication will be dispensed from the Pyxis MedStation by the floor nurse.

8.4.3 Administration Instructions

3 mL of study drug and .5 mL of albuterol sulfate should be carefully added to the nebulizer.

8.4.4 Storage

Study drug supply will be maintained by the UCSF Pharmacy and will be supplied following hospital pharmacy regulations according to the stocking and storage of the Pyxis Medstation.

Study drug should be stored by the study site pharmacy at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of study drug storage in the pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee and captured as a deviation. Subjects and study staff will be instructed to store the medication in original packaging at room temperature according to the instructions outlined on the Drug Administration Instructions until ready for administration.

8.5 Study Drug Accountability

An accurate and current accounting of the dispensing of study drug for each participant will be maintained on an ongoing basis by the study nurse or study site staff. The number of study drug dispensed will be recorded. The study monitor will verify these documents throughout the course of the study.

8.6 Measures of Treatment Compliance

Participants will be monitored by a nurse or study staff. The nurse administering the study treatment will record the date and time the study drug was administered as well as any adverse events on a study case report form. Study staff will verify these documents throughout the course of the study.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Visits 1-4 and at early termination when applicable. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Medication Withholding

Maximum bronchodilator reversibility will be used to establish eligibility at Visit 2 and post-bronchodilator reversibility will be assessed at Visits 3, 4, 9, 10, 11. Since some asthma medications can blunt the response to bronchodilators, we will ask that participants withhold these medications according to the guidance provided in Table 2.

Table 2. Medication Withholding Parameters

Medication	**Withholding Period**
Leukotriene modifiers	24 hrs
Ultra-long-acting bronchodilators (indacaterol, tiotropium)	24 hrs
Long-acting beta-agonists	12 hrs
Theophylline	12 hrs
Short-acting anticholinergic	6 hrs
Short-acting beta-agonists	4 hrs

Participants will be evaluated at Visit 1, without any medication holds, to establish the safety of withholding asthma medications. If a study clinicians feels the participant is not clinically stable enough to withhold medications, they may shorten the guidance (with appropriate documentation on the Visit 1 CRF Physician Attestation section) or they may excuse the participant from some or all of the medication withholds.

Participants are reminded of these medication holds by phone 24 hours prior to their medication withholding visits and instructed to resume all medications should symptoms develop in the period prior to the visit.

Associated risks of medication withholding include a worsening of asthma control or asthma exacerbation. To mitigate these risks, participants are given clear instructions to resume asthma medications if symptoms arise.

9.1.3 Food and Beverage Withholding

Participants are asked to withhold eating for one hour prior to the sputum induction at visit 3 since recently digested food can contaminate sputum samples. Participants are also asked to withhold caffeine and alcohol-containing products for 6 hours prior to all study visits as these substances can influence lung function values.

9.1.4 Demographics

Demographic information (date of birth, gender, race) will be recorded at Visit 1.

9.1.5 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Visit 1.

Associated risks include discomfort evoked with some of the questions asked. To minimize risk, participants will be informed that they can defer answering any questions that make them feel uncomfortable.

9.1.6 Physical Examination

A complete physical examination will be performed by either the investigator or a study clinician at Visit 1. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.7 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at all study visits.

9.1.8 Oximetry

Oximetry will be measured on room air with the subject at rest at all study visits at the Airway Clinical Research Center.

9.1.9 Spirometry

Spirometry will be performed at all study visits and in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests.

Associated risks include the following:

- Likely (>10%): Shortness of breath or cough during the six-second-exhalation
- Less Likely (<5%): Wheezing and chest tightness
- Rare but serious (<1%): Syncope

In an effort to minimize risk, participants will be seated during the procedure to reduce any injury should a participant become dizzy and fall. Study staff will have albuterol available to administer to participants who develop wheeze or shortness of breath following spirometry.

9.1.10 Post-Bronchodilator / Maximum Reversibility

Spirometry will be repeated 15 minutes following the administration of four to eight puffs of albuterol to assess bronchodilator reversibility. Maximal bronchodilation will be performed at Visit 2. Post-bronchodilator reversibility will be performed at Visit 3, 4, 9, 10, 11 and 12.

Associated risks include the following:

- Likely (>10%): Transient tachycardia, tremor, feeling nervous, rhinitis, pharyngitis, and nausea
- Less Likely (<5%): Headache, cough, upper respiratory infection

- Rare but serious (<1%): Chest pain, atrial fibrillation, hypertension, hypotension, diabetic ketoacidosis, hyperglycemia, hypersensitivity reactions, paradoxical bronchospasm

Given that the study population is made up of individuals with moderate-to-severe asthma, and that albuterol is a standard of care treatment for asthma, it is unlikely that participants will experience any of the side effects described above. However, if a potential participant describes previous sensitivity or side effects to treatment with albuterol, they will be excluded from participation for safety. Furthermore, albuterol will only be administered after a physician-administered history and physical, to ensure that it is okay for the participant to receive albuterol.

9.1.11 Methacholine Challenge Testing

Methacholine challenge testing is performed to assess airway responsiveness. Only participants that are unable to achieve ≥ 12% improvement in FEV1% following administration of four to eight puffs of albuterol and who have a baseline FEV1 > 50% predicted will undergo methacholine challenge testing at Visit 2b. In the methacholine challenge test, increasing concentrations of methacholine are delivered until the FEV1 falls by 20% or more compared to the reference (post-diluent baseline) level. When the provocative concentration that causes a 20% fall (PC20) is identified, the challenge is stopped.

Associated risks include the following:

- Likely (>10%): Shortness of breath
- Less Likely (<5%): Wheezing, chest tightness, or cough
- Rare but serious (<1%): Rarely, patients may have severe bronchoconstriction during or following methacholine challenge

Additionally, methacholine has been shown to increase tone of the uterus, which could lead to preterm labor, in pregnancy laboratory animals; there are no human studies to verify this effect during pregnancy. However, because of this, methacholine has been placed in FDA Category C, meaning that exposure during pregnancy should be avoided. As such, pregnant women or women of reproductive age who are unwilling to practice pregnancy prevention strategies will be excluded from participation.

In an effort to minimize risk to participants, only those participants with a pre-diluent FEV1 of > 50% predicted and at least one liter will undergo the methacholine challenge testing, and only in the instance that they are not able to show 12% or greater improvement in FEV1 following bronchodilator administration. A study physician will be available during the challenge. Participants will not be discharged until their FEV1 is within 10% of their pre-diluent FEV1. Medications and personnel will be available to manage and treat bronchoconstriction.

9.1.12 Sputum Induction

A 12-minute sputum induction using nebulized 3% hypertonic saline will be performed at Visits 3 and 11. All participants will perform baseline spirometry after 4 or more puffs of albuterol administration prior to sputum induction. Peak flow measurements are done

every 2 minutes during the induced sputum collection to assess for excessive bronchoconstriction.

Associated risks include the following:

- Likely (>10%): Salty after taste in the mouth, coughing, or a feeling of needing to swallow
- Less Likely (<5%): Sore throat, shortness of breath, wheezing, chest tightness, light-headedness, nausea, or headache, worsening of lung function
- Rare but serious (<1%): Some patients have had a severe asthma attack or a reaction to the salty water that they breathe in

In an effort to minimize risk to participants, bronchodilator treatment will be available if sputum induction induces a worsening of asthma symptoms. The following safety procedures will be followed for the sputum induction procedure: only participants with a post-bronchodilator FEV1 of > 50% predicted will undergo sputum induction; a physician will be available during the induction; study staff will calculate and record the peak flow and FEV1 value that equals both a 10% and 20% fall in lung function based upon the recorded post-bronchodilator peak flow and FEV1 values; and participants will not be discharged until their FEV1 is within 10% of their post-bronchodilator FEV1.

9.1.13 Point of Care Urine Pregnancy Test

A point of care urine pregnancy test will be obtained from female participants who are of childbearing potential prior to their participation in the study and routinely throughout their participation (see Appendix 1).

9.1.14 CT Imaging of the Thorax

A single inspiratory low dose CT scan of the thorax using a model based iterative reconstruction (MBIR) approach will be taken at the baseline eligibility visit (Visit 2) and following the treatment period over 3 months (Visit 9, 11 and 12).

The risks associated with CT scanning are that of the additional amount of radiation exposure. The additional amount of radiation that each participant will receive as a result of participating in this study will be approximately 5 mSv, which is slightly greater than the yearly natural background of radiation in the US, which is 3 mSv. This amount of radiation may involve a low risk of cancer. A participant should not participate in this study if she is pregnant or breastfeeding.

Additional risks are associated with the uncovering of abnormal findings. There is a risk of possible detection of an abnormality in the lung, which after testing or treatment, is found not to be disease causing. This includes possible misdiagnosis of lung cancer. Such findings may result in unnecessary anxiety for the participant, and increase his or her chance that an outpatient physician may believe that the abnormality is lung cancer and order further testing. This testing could include additional CT scans with additional radiation exposure, other types of scans to determine if the abnormalities are rapidly growing, a needle biopsy (taking a sample of the abnormality with a needle) or a lung biopsy, which requires surgery. Whether these additional studies would be performed would be a decision that the participant would make with his or her regular physician.

In an effort to minimize risk to participants, scans will be read by radiologists that have expertise in interpreting findings of chest CTs. If there are any abnormalities, other than those that are usually found in asthmatic patients, observed by the clinical center radiologists, these would be reported to the principal investigator, who will in turn communicate these findings to the participant. The most likely abnormal result will be the identification of a spot on the lung that might be cancer.

9.1.15 Body Plethysmography

Body plethysmography will be done to determine functional residual capacity (FRC), residual volume (RV), total lung capacity (TLC), and slow vital capacity (SVC) at Visit 3 and 9. Plethysmography will be done with bronchodilation. If bronchodilation procedures are done, participants will follow the medication hold times as described in Section 9.1.2.

In an effort to minimize risk to participants, patients with severe claustrophobia, inability to sit upright in a chamber, inability to perform the panting maneuver, inability to perform maximal inspiratory and expiratory efforts, and known perforated tympanic membrane without a snug-fitting earplug will be excluded from participation in the study.

9.1.16 Measurement of Fractionated Exhaled Nitric Oxide (FeNO)

Fractional exhaled nitric oxide measurement test will be performed at Visits 3-12. Subjects will inhale a full breath of air and then exhale it slowly through a mouthpiece while a device measures the concentration of nitric oxide in the air exhaled. Nitric oxide is a gas normally present in the air exhaled from the lungs.

9.1.17 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood will be obtained and sent to the clinical hematology lab for a complete blood count with 5-part differential (CBC-D) (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count) and serum IgE for assessment of systemic evidence for infection and inflammation. A CBC-D will be obtained at Visits 3, 9 and 11. A serum IgE will be obtained at Visit 3 only.

Risks associated with hematology include the following risks associated with venipuncture:

- Lightheadedness or nausea while having blood drawn
- Bruising at the site where the needle enters the skin and a remote risk of infection

In an effort to minimize risk to participants, aseptic technique will be used and pressure applied to site to prevent infection/bruising. Participants who have previously identified

that they experience lightheadedness during blood draws will have blood drawn while lying supine on an exam room table.

9.3 Research Laboratory Measurements

9.3.1 Sputum Measurements

Sputum for determination of airway inflammation will be collected and processed. Airway inflammation measurements may include cell count and differential, cytokine gene expression measurements, and rheology.

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Day -14)

1. Review the study with the subject and obtain written informed consent and HIPAA authorization.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record medical history, including a history of asthma, diagnosis date, and asthma-related healthcare utilization and exacerbation frequency.
5. Record concomitant medications.
6. Perform a complete physical examination.
7. Perform and record vital signs.
8. Perform and record oximetry.
9. Perform and record urine pregnancy test.
10. Perform and record spirometry.
11. Schedule participant for Visit 2.

10.2 Visit 2 (Day -10)

1. Record changes to concomitant medications.
2. Perform and record vital signs.
3. Perform and record oximetry.
4. Perform and record urine pregnancy test.
5. Perform and record spirometry.
6. Perform and record maximum bronchodilator spirometry.
7. Obtain a CT scan of the thorax.
8. Schedule participant for Visit 3 if CT mucus plug sore is equal to or greater than 5.

10.3 Visit 2b (Day -7) ***Optional**

1. Record changes to concomitant medications.
2. Perform and record vital signs.
3. Perform and record oximetry.
4. Perform and record urine pregnancy test.
5. Perform and record spirometry.
6. Perform methacholine challenge testing.
7. Obtain a CT scan of the thorax.
8. Schedule participant for Visit 3 if CT mucus plug score is equal to or greater than 5.

10.4 Visit 3 (Day -5)

1. Record changes to concomitant medications.
2. Perform and record vital signs.
3. Perform and record oximetry.
4. Perform and record urine pregnancy test.
5. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
6. Perform and record spirometry.
7. Collect blood sample for clinical laboratory tests.
8. Perform and record plethysmography with bronchodilation.
9. Collect induced sputum.
10. Schedule participant for Visit 4.

10.5 Visit 4 (Day 1)

1. Record changes to concomitant medications.
2. Perform and record vital signs.
3. Perform and record oximetry.
4. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
5. Perform and record spirometry.
6. Perform and record 3 peak flow AM measurements before first treatment of the day.
7. Perform and record post-bronchodilator reversibility.
8. Admit patient to medical-surgical floor.
9. Administer study drug treatment three times over the course of the day, spaced every 4-6 hours. Peak flow measurements should be taken before and after each treatment.
10. Perform and record 3 peak flow PM measurements after all treatments have been completed.

10.6 Visit 5 (Day 2)

1. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
2. Perform and record spirometry.
3. Perform and record 3 peak flow AM measurements before first treatment of the day.
4. Administer study drug treatment four times over the course of the day, spaced every 4-6 hours. Perform peak flow measurements before and after each treatment.
5. Perform and record 3 peak flow PM measurements after all treatments have been completed.

10.7 Visit 6 (Day 3)

1. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
2. Perform and record spirometry.
3. Perform and record 3 peak flow AM measurements before first treatment of the day.
4. Administer study drug treatment four times over the course of the day, spaced every 4-6 hours. Perform peak flow measurements before and after each treatment.
5. Perform and record 3 peak flow PM measurements after all treatments have been completed.

10.8 Visit 7 (Day 4)

1. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
2. Perform and record spirometry.
3. Perform and record 3 peak flow AM measurements before first treatment of the day.
4. Administer study drug treatment four times over the course of the day, spaced every 4-6 hours. Perform peak flow measurements before and after each treatment.
5. Perform and record 3 peak flow PM measurements after all treatments have been completed.

10.9 Visit 8 (Day 5)

1. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
2. Perform and record spirometry.
3. Perform and record 3 peak flow AM measurements before first treatment of the day.
4. Administer study drug treatment four times over the course of the day, spaced every 4-6 hours. Perform peak flow measurements before and after each treatment.
5. Perform and record 3 peak flow PM measurements after all treatments have been completed.

10.10 Visit 9 (Day 6)

1. Discharge patient from medical-surgical floor.
2. Perform and record vital signs.
3. Perform and record oximetry.
4. Perform abbreviated physical exam.
5. Perform and record urine pregnancy test.
6. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
7. Perform and record spirometry.
8. Perform and record post-bronchodilator reversibility.
9. Perform and record plethysmography with bronchodilation.
10. Obtain a CT scan of the thorax.
11. Collect blood sample for clinical laboratory tests

10.11 Visit 10 (Day 13)

1. Perform and record vital signs.
2. Perform and record oximetry.
3. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
4. Perform and record spirometry.
5. Perform and record post-bronchodilator reversibility.

10.12 Visit 11 (Day 48)

1. Perform and record vital signs.
2. Perform and record oximetry.
3. Perform and record urine pregnancy test.
4. Perform and record fractional exhaled nitric oxide (FeNO) measurement.

5. Perform and record spirometry.
6. Perform and record post-bronchodilator reversibility.
7. Obtain a CT scan of the thorax.
8. Collect blood sample for clinical laboratory tests.
9. Collect induced sputum.

10.13 Visit 12 (Day 91)

1. Perform and record vital signs.
2. Perform and record oximetry.
3. Perform and record urine pregnancy test.
4. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
5. Perform and record spirometry.
6. Perform and record post-bronchodilator reversibility.
7. Obtain a CT scan of the thorax.

10.14 Early Withdrawal Visit (Before end of Visit 3)

1. Record any adverse experiences.
2. Record changes to concomitant medications.
3. Perform and record vital signs.
4. Perform and record oximetry.
5. Perform abbreviated physical examination.
6. Perform and record spirometry.

10.15 Late Withdrawal Visit (After Visit 3 Completion)

1. Record any adverse experiences.
2. Record changes to concomitant medications.
3. Perform and record vital signs.
4. Perform and record oximetry.
5. Perform abbreviated physical examination.
6. Perform and record fractional exhaled nitric oxide (FeNO) measurement.
7. Perform and record spirometry.
8. Perform and record post-bronchodilator reversibility.
9. Collect blood sample for clinical laboratory tests.
10. Obtain a CT scan of the thorax.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature,

severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 3 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 3. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 4.

Table 4. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.

Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

11.3 Medical Monitoring

John Fahy, MD, MSc should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: (415) 476-9940
Mobile: (415) 317-3259

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

Subject withdrawal of consent

Subject is not compliant with study procedures

Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment

Protocol violation requiring discontinuation of study treatment

Lost to follow-up

Sponsor request for early termination of study

Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Refer to Section 10 for early termination procedures.

12.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to Visit 8) should have an early discontinuation visit. Refer to Section 10 for early termination procedures. Subjects who withdraw after Visit 8 but prior to Visit 11 should be encouraged to come in for a final visit (and the procedures to be followed would include those for their next scheduled visit).

12.3 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.
Subjects who withdraw from the study will be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

Failure to meet inclusion/exclusion criteria

Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 DATA SAFETY MONITORING

Adverse events will be monitored by the Clinical PIs (Drs. Fahy, Woodruff and Lazarus) in real-time. Safety and tolerability data will be summarized quarterly. Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug. All AEs will be reviewed quarterly in the regularly scheduled quality assurance meetings of UCSF Airway Clinical Research Center, which is attended by Drs. Fahy, Woodruff, Lazarus and Boushey.

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible patients who receive at least one dose of the study drug (the Safety Population) will be included in the safety analysis.

15.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized: race, gender, age, height, weight, baseline FEV1, and baseline post-bronchodilator FEV1.

15.3 Analysis of Primary Endpoint

The primary analysis is a paired t-test analysis of the % change in FEV1 from the start to the end of the one week treatment period with four times daily NAC (Mucomyst-20). The secondary analysis will be to determine how the change in FEV1 relates to the change in CT mucus score.

15.4 Analysis of Secondary Endpoints

As a secondary analysis we will explore if NAC treatment decreases levels of NO in exhaled breath or the numbers of eosinophils in peripheral blood.

15.5 Interim Analysis

No interim analysis is planned.

15.6 Sample Size and Randomization

We propose a sample size of 30, which will provide us with the power to examine the effect of NAC in a subgroup of individuals with asthma who have CT evidence of intraluminal mucus and to identify the CT mucus score that performs best as a biomarker of treatment response to NAC. Participants will be enrolled if their CT mucus scores are ≥5.0. To calculate sample size for the one-week treatment period study, we used FEV1 measures from 219 adults with asthma published in Corren et al. NEJM, 2011[1]. The authors found that the standard deviation for the change in FEV1 in liters from day 1 to day 7 was 19%. Using a standard deviation of 19 and a two-sided alpha of 0.05, we calculate a sample size of 30 to have 80% power to detect a change in FEV1 of 10% (a reasonable effect size because early studies of Pulmozyme in CF reported a 10% increase in FEV1 at day 3 of treatment).[7]

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

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

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, subject number and initials. If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the clinical research coordinators for resolution. The

study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All participant interviews/visits are conducted in private testing rooms. Information about study

participants is kept in study binders in a locked storage closet, and in our password protected secure electronic file, and is only accessible to authorized study personnel. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR

50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form and HIPAA authorization for submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. A copy of the signed consent form will be given to the subject and the original will be maintained with the subject's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.

10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

VISIT	1	2	3	4	5	6	7	8	9	10	11	12
DAY	-14[a]	-10[a]	-5[a]	1	2	3	4	5	6	13	48[a]	91[a]
Consent and Eligibility	X											
Physical Exam	X								X[b]			
Vital Signs & Oximetry	X	X	X	X					X	X	X	X
Urine Pregnancy Test	X	X	X						X	X	X	X
FeNO			X	X	X	X	X	X	X	X	X	X
Spirometry	X	X	X	X	X	X	X	X	X	X	X	X
Post-Bronchodilator Reversibility		X[c]	X	X					X	X	X	X
Blood Sample			X[d]						X		X	
Sputum Induction			X								X	
CT Thorax		X[e]							X		X	X
Plethysmography			X						X			
Inpatient Stay				X	X	X	X	X				
Administration of Study Drug				X	X	X	X	X				
AM & PM Peak Flow Monitoring				X	X	X	X	X				
Treatment Peak Flow Monitoring				X	X	X	X	X				

a ±2 days

b abbreviated physical exam

c maximum reversibility

d blood sample to include CBC with 5 part differential and serum IgE

e CT mucus plug score ≥ 5 for eligibility

References

1. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med*. 2011;365(12):1088-1098.

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