JOHN V. FAHY, MD, MSC
Clinical Research Protocol

THE EFFECT OF NAC ON LUNG FUNCTION AND CT MUCUS SCORE (ENACT)

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Development Phase:	4
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Approval:

_____, Professor 11/21/2018

PI or Sponsor Signature (Name and Title) *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants 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: 18-26680

Protocol Title: The Effect of NAC on Lung Function and CT Mucus Score

Protocol Date: 11/20/2018

11/21/2018

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
FEV1	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
NAC	n-acetylcysteine
PI	Principal Investigator
SAE	serious adverse experience

PROTOCOL SYNOPSIS

TITLE	The Effect of NAC on Lung Function and CT Mucus Score
SPONSOR	John V. Fahy, MD, MSc
FUNDING ORGANIZATION	National Institutes of Health, Departmental funds, Division of Pulmonary and Critical Care Medicine, Department of Medicine, UCSF
RESEARCH SITES	University of California, San Francisco and University of California, Davis
RATIONALE	Mucus plugging of the airway is consistently found in fatal and near-fatal asthma. The role of mucus as a cause of airflow obstruction in acute severe asthma suggests that mucus 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. Using a novel approach of scoring mucus occlusion, we have used CT imaging to uncover that a majority 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. Historically, studies of mucolytics, like n-acetylcystine (NAC), have not shown benefit in other obstructive lung diseases, like COPD. However, utilizing CT mucus scores as a biomarker, we believe that mucolytic treatment may prove useful for those with significant mucus impaction.
STUDY DESIGN	This is a multi-center, randomized, double-blind, placebo-controlled 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 PARTICIPANTS	36
PARTICIPANT SELECTION CRITERIA	<u>Inclusion Criteria</u> 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) Historical methacholine data may be allowed. An exception will be made for enrollees whose FEV1 is < 50% predicted (precluding methacholine challenge testing. If

	bronchodilator reversibility is <12% in these participants, a diagnosis of asthma acceptable to the investigator is sufficient for inclusion in ENACT. 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. If the participant has past evidence of mucus impaction as described by a radiologist on a prior chest CT scan, then post-bronchodilator FEV1 needs to be >40% but <95% predicted. 8. Asthma requiring treatment with inhaled corticosteroids (ICS) for 3 months or greater. 9. CT mucus score ≥ 5 10. Ability to tolerate study drug reflected by a post-treatment FEV1 ≥ 80% of pre- treatment, pre-bronchodilator FEV1 <u>Exclusion Criteria</u> 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. Uncontrolled asthma as evidenced by an asthma exacerbation in 6 weeks prior to study participation or albuterol use greater than 8 puffs per day 6. 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 7. Unwillingness to follow study procedures 8. History of allergy or intolerance to study drug 9. 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	10% NAC (3 ml) and 2.5 mg albuterol (0.5 ml) Product will be administered every 8-12 hours (2 times per day) for 14 days. Medication will be administered via a portable nebulizer.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	0.9% saline (3mL) and 2.5 mg albuterol (0.5ml) Product will be administered every 8-12hours (2 times per day) for 14 days. Medication will be administered via a portable nebulizer.

DURATION OF PARTICIPATION AND DURATION OF STUDY	Participants will be enrolled in the study up to 178 days: **Screening:** up to 90 days **Treatment Period 1:** 14 days **Washout:** 60 days **Treatment Period 2:** 14 days The total duration of the study is expected to be 24 months. Participant recruitment will happen on a rolling basis.
CONCOMMITANT MEDICATIONS	Prohibited: • thiols and thiol derivatives • activated charcoal • inhaled insulin
EFFICACY EVALUATIONS	
PRIMARY ENDPOINT	The primary outcome is the % change in FEV1 from the start to the end of the treatment period (either placebo or 10% NAC).
SECONDARY ENDPOINTS	Secondary efficacy endpoints include: • CT mucus score • FVC • PEF (AM and PM) • Air trapping (RV/TLC ratio) • FeNO • Blood eosinophils
OTHER EVALUATIONS	Rheological properties of sputum.
SAFETY EVALUATIONS	Incidence of adverse events
PLANNED INTERIM ANALYSES	No interim analysis is planned Serious adverse events will be monitored by the ACRC data safety monitoring committee on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	The primary outcome is the % change in FEV1 from the start to the end of the treatment period (either placebo or 10% NAC). The first analysis will be to test the assumption of negligible carryover effects from study period 1 to period 2. We will compare the sum of the outcome values measured in the two periods for each participant across the 2 sequence groups (placebo-10% vs. 10%-placebo) using an unpaired t-test. After no or negligible carryover effect has been confirmed, we will test for the difference in treatment effects between placebo and 10% NAC using a paired t-test.
Rationale for Number of Participants	The primary goal of this study is to assess whether N-acetyl cysteine improves FEV1 after a 2-week treatment period in patients with asthma when compared to a two week treatment period of placebo. The study has a cross-over design which reduces the sample size. With help from David Mauger, Ph.D., biostatistician at Penn State, who has access to 2-week FEV1 data in NIH network trials and cohort studies in asthma that UCSF has participated in, we calculate a sample size of 36

	patients. This sample size is obtained when we factor in an effect size of 5% improvement in FEV1, a standard deviation for change in FEV1 of 20%, a within patient correlation for repeated measures of FEV1 of 0.9, and a power of 90%.

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 suggests that mucus 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 asthma.

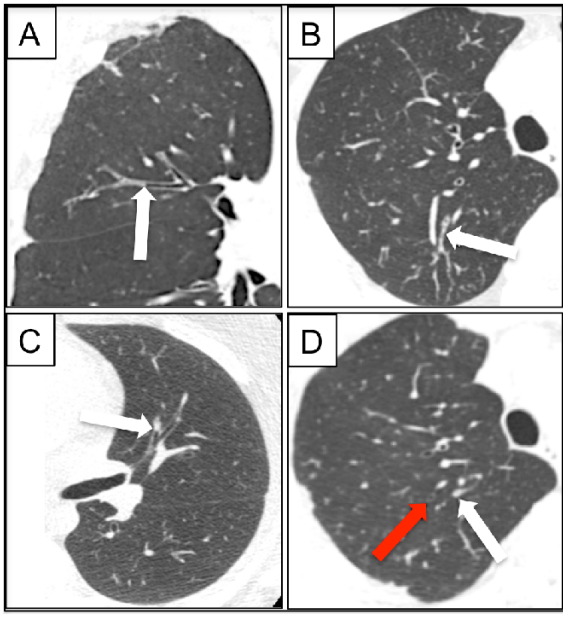

Figure 1: Intraluminal mucus in CT lung images of asthmatics. Mucus plugs in CT scan images (white arrows) were defined as complete occlusion of an airway lumen by mucus and were identified as tubular structures in longitudinal section with branching (A & B) or without branching (C) or as rounded opacities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

1.1 Overview of Clinical Studies

Against this background, the preliminary data we show in Figs 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 scores achieve a post albuterol FEV1 > 80% much less frequently than asthmatics who have a zero mucus score (Fig 3).

Examined another way, we find that all asthmatics with an FEV1 < 60% after albuterol treatment have abnormal mucus scores, whereas the majority of asthmatics

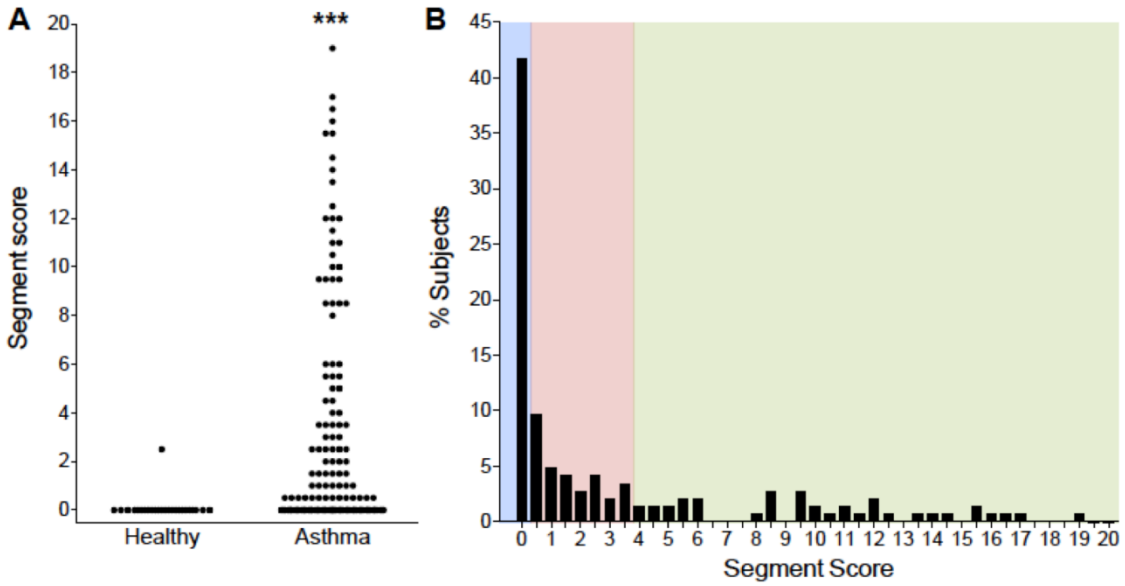

Figure 2. A. The CT mucus segment score is usually 0 in health but many asthmatics enrolled in the NHLBI Severe Asthma Research Program have scores above 0 with some as high as 15-19. B. The frequency distribution of mucus score in asthma shows that 42% of patients have a score of 0 (zero mucus score); 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".

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. This is well illustrated in the table, which presents data for the prevalence of mucus-related symptoms in asthmatics with different mucus score categories. None of the mucus-related symptoms were significantly different in the zero, low or high mucus subgroups, and it is clear that these symptoms have very limited utility to investigate the role of mucus in asthma or to identify asthmatics who might benefit from mucus-directed treatments.

1.2 Overview of Non-Clinical Studies

To test the hypothesis that airway mucus in asthma has abnormal elasticity, we performed rheology on induced sputum from 40 asthmatic participants recruited to UCSF as part of the SARP. The majority of these asthmatic participants (65%) had severe asthma. We compared the elastic modulus (G') of these participants to induced sputum G' in 11 healthy controls. We found that the G' in the asthmatic group was significantly higher than normal (Fig 4A). Notably, we found a strong inverse correlation between G' and FEV1 % (Fig 4B). This finding suggests that stiff mucus in the airway of asthmatics could be a mechanism of mucus impaction of airways and airflow obstruction. It further suggests that decreasing mucus G' could be a treatment

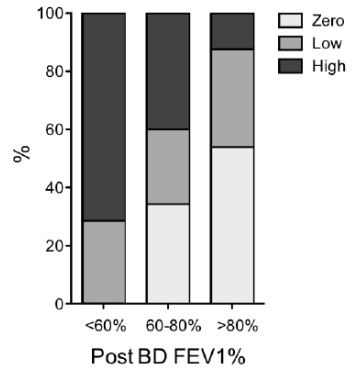

Figure 3. Mucus scores in asthmatics with different FEV1 values (post 6-8 puffs of albuterol). Note that all asthmatics with an FEV1 <60% have abnormal mucus scores.

strategy to improve airflow. To determine if the G' of asthmatic sputum is a result of excessive mucin disulfide bridges (as it is in CF), we measured the G' in a subgroup of 6 asthmatics before and after the addition of n-Acetylcystine (NAC) (61mM). We found that NAC markedly decreased the G' at both 2 minutes and 12 minute time-points after the addition of NAC (Fig 4A & B). This indicates that disulfide bonds are a mechanism of high sputum elasticity and a target for mucolysis in asthma, and it suggests thiol-based compounds as a rational mucolytic strategy.

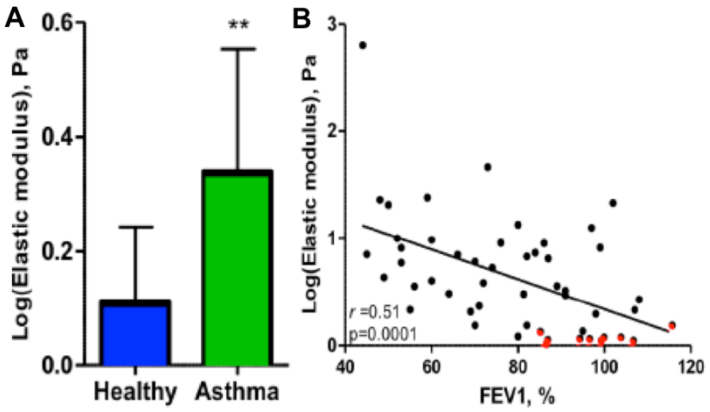

Figure 4. A. Sputum in asthma has an increased elastic modulus (G'). B. The G' of asthma sputum is significantly and inversely related to FEV1 (*ex vivo* data from 40 asthmatics and 11 healthy participants [red dots])

2 STUDY RATIONALE

Because we have shown that the high elasticity of asthmatic mucus can be markedly decreased with NAC *ex vivo* (Fig 4), we propose here an *in vivo* study to treat asthmatics with NAC to improve their lung function. 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 had 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. Although NAC is a suboptimal mucolytic, we are confident that high dose (10%) treatment will effectively lyse airway mucus to improve airflow and pave the way for later clinical trials of a thiol-saccharide.

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 eight 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 administered the first two treatments under the supervision of the study team in the Airway Clinical Research Center and will only be eligible to remain in the study if their post-treatment FEV1s are greater than 80% of their pre-treatment, pre-bronchodilator FEV1.

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 multi-center, double-blind, randomized, 2 period crossover trial in 36 subjects with asthma who demonstrate mucus plugging on a screening CT lung scan. Screening data will be reviewed to determine participant eligibility. Participants who meet all inclusion criteria will self-administer nebulized 10% NAC (3 mL) and 2.5 mg albuterol inhalation solution (0.5 mL) or 0.9% saline (3 mL) and 2.5 mg albuterol inhalation solution (0.5 mL) to be taken 2 times per day for 14 days. Following an 8-week washout period, participants will be switched to the alternate group for 14 days of treatment. Participants will be assigned to the treatments in random order. Evaluations will be taken at baseline, end of first treatment, start of second treatment period, and at the end of the second treatment period.

The following treatment regimens will be used:

- Experimental treatment of 3.0 mL 10% NAC + 2.5mg/0.5 mL albuterol
- Placebo treatment of 0.9% saline + 2.5mg/0.5 mL albuterol

Total duration of participation will be 16 weeks. 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 the post-treatment FEV1s to pre-treatment baseline values. We calculate that with a sample size of 36 we will have 90% power to detect an

effect size of 5% improvement in FEV1 and a within patient correlation for repeated measures of FEV1 of 0.9.

5.2 Secondary Efficacy Endpoints

We intend to examine the effect of the treatment on CT mucus score, however, because the CT mucus score is a novel measurement, we do not have preliminary data to describe the natural history of mucus in the airway over time or with treatment. Therefore, for the purpose of this study, we intend to describe any changes we observe.

Other Secondary efficacy endpoints will include:

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

5.3 Safety Evaluations

All participants will have their first two doses of each treatment period administered in the clinic under the supervision of a study nurse or study clinician. Participants will receive instruction on nebulizer use utilizing a teach-back method to ensure comprehension. Participants who are unable to successfully teach back nebulizer setup or drug self-administration will be excluded from continued participation.

Additionally, while acute anaphylactoid reactions have only been observed in the setting of IV administered NAC, each participant will be observed for the first two treatments, lasting approximately 6 hours, by a study nurse or study clinician.

All participants will be asked to complete a log of study drug administration and any subjective side effects.

Additionally, participants will have access via pager to a study nurse or clinician 24 hours a day, 7 days a week.

Interim safety evaluations will be done after 7 days of treatment during each treatment period. Additional safety evaluations will also be done if anticipated adverse events exceed frequencies documented in the literature, unanticipated adverse events occur, or rare but serious adverse events occur. Definitions of these terms and details of our safety evaluation plan are described in section 14.

6 PARTICIPANT SELECTION

6.1 Study Population

Participants 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) Historical methacholine data may be allowed. An exception will be made for enrollees whose FEV1 is < 50% predicted (precluding methacholine challenge testing. If bronchodilator reversibility is <12% in these participants, a diagnosis of asthma acceptable to the investigator is sufficient for inclusion in ENACT.
4. Clinical history of asthma per patient report or medical record
5. Pre-bronchodilator FEV1 > 35% predicted
6. Post-bronchodilator FEV1 > 40% but < 90% predicted. If the participant has past evidence of mucus impaction as described by a radiologist on a prior chest CT scan, then post-bronchodilator FEV1 needs to be >40% but <95% predicted.
7. Asthma requiring treatment with inhaled corticosteroids (ICS) for 3 months or greater.
8. CT mucus score ≥ 5
9. Ability to tolerate study drug reflected by a post-treatment FEV1 ≥ 80% of pre-treatment, pre-bronchodilator FEV1

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. Uncontrolled asthma as evidenced by an asthma exacerbation in 6 weeks prior to study participation or albuterol use greater than 8 puffs per day
6. 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
7. Unwillingness to follow study procedures
8. History of allergy or intolerance to study drug
9. Any other criteria that places the subject at unnecessary risk according to the judgment of the Principal Investigator

6.4 Concurrent Medications

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

6.5 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.

6.6 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

7 STUDY TREATMENTS

7.1 Method of Assigning Participants to Treatment Groups

Up to 36 eligible patients will be randomly assigned to receive either the experimental treatment (10% NAC) or the placebo treatment (0.9% saline) during period 1 in a 1:1 ratio using a SAS-based computer-generated randomization scheme accessed by the investigational pharmacist. The UCSF investigational pharmacist will complete a randomization worksheet and log the group assignment for subjects enrolled at both UCSF and UC Davis. The UCSF investigational pharmacist will contact the UC Davis pharmacist by email or phone to inform them of the randomization. The randomization log will be shared with investigators upon study completion or in the event of a serious safety concern.

7.2 Blinding

Due to the objectives of the study, the identity of experimental and placebo treatments will not be known to investigators, research staff, or patients. The following study procedures will be in place to ensure double-blind administration of study treatments.

Access to the randomization code will be strictly controlled.

Packaging and labeling of experimental and placebo treatments will be identical to maintain the blind.

The study blind will be broken on completion of the clinical study and after the study database has been locked.

During the study, the blind may be broken **only** in emergencies when knowledge of the patient's treatment group is necessary for further patient management. When possible, the Investigator should discuss the emergency with a Quality Assurance officer and a representative from the Committee on Human Research prior to unblinding. Should unblinding become necessary the Investigator will contact the investigational pharmacist directly.

7.3 Formulation of Test and Control Products

NAC (trade name: Mucomyst) is manufactured by American Regent. The active drug studied here is 10% NAC. It will be delivered via nebulizer.

7.3.1 Formulation of Test Product

10% 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. 10% 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 10% NAC, control product and albuterol

	10% NAC	**0.9% Saline**	**Albuterol**
Active Ingredient, mg/mL	Acetylcysteine 100mg/mL		Albuterol sulfate 2.5 mg/0.5mL
Other ingredient, mg/mL	Edetate disodium, sodium hydroxide, purified water	Sodium chloride Purified water	Water, sodium chloride, sulfuric acid
pH	7	5.7	3-5

7.3.2 Packaging and Labeling

Subjects will receive a total of 14 syringes of study drug or placebo and 14-unit dose containers of albuterol (plus 2 extras in the event of breakage) for each treatment period.

Each kit with syringe of study drug or placebo and the albuterol ampule will be labeled with the required FDA warning statement, the study ID, protocol number, the name of the sponsors, and directions for patient use and storage.

7.4 Supply of Study Drug at the Site

Study drug will be supplied to both sites by Mariner Advanced Pharmacy Corp (MAPRx). MAPRx is a professional 503(a) patient-centered compounding pharmacy which prepares sterile and nonsterile drug preparations compliant with United States Pharmacopeia standards and the California State Board of Pharmacy.

The study medication will be supplied by MAPRx and delivered as needed per prescription. MAPRx can guarantee medication delivery to the study site within 1-2 business days of submitting a prescription via MAPRx's secured dashboard webportal.

7.4.1 Dosage/Dosage Regimen

During each treatment period participants will nebulize one syringe of study drug and one unit dose container of albuterol using a portable nebulizer. Participants will be instructed to take two doses per day, spaced at approximately 8-12 hour intervals. No adjustments will be made based on weight or age.

7.4.2 Dispensing

Study medication will be dispensed by either, the study coordinator, study nurse or physician and only after observation of the administration of the first two doses of each treatment period.

7.4.3 Administration Instructions

Charge Check

1. Wash hands prior to preparing each treatment.
2. Use a clean nebulizer.
3. Check the battery level by pressing the on/off button on the handset
   a. If the LED is **SOLID GREEN** the battery is charged
   b. If the LED is **SOLID AMBER**, there is enough charge for at least one more treatment
      *Please charge your nebulizer after your treatment
   c. If the LED **FLASHES AMBER** and then switches off there is not enough charge to take a treatment
      *Please charge your nebulizer before treatment
   d. If the LED does not illuminate, please refer to the troubleshooting sections of the manual and call the study coordinator

Nebulizer Assembly

1. Empty the contents of one-unit dose container labeled “albuterol” into the medication chamber with the green lid
2. Empty the contents of one syringe into the medication chamber
3. Close the lid to the medication chamber

Treatment

1. Hold the handset in your hand and place the mouthpiece between your teeth with your lips sealed around it.
2. Press the on/off button on the handset to switch the nebulizer on and begin nebulization and breathe normally through your mouth
3. Ensure aerosol is coming out of the mouthpiece assembly
4. Keep the nebulizer in an upright position during the course of the treatment. This prevents spilling and promotes nebulization
5. If you need to take a rest, press the on/off button to stop your treatment. To continue your treatment press the on/off button again
6. Your treatment is finished when the nebulizer beeps and the LED flashes. The device will turn off automatically
7. Check the medication chamber for residual medication. If there are more than a few drops remaining, press the on/off button again to continue your treatment

After each use

1. Press the mouthpiece assembly release button to separate the mouthpiece assembly from the handset.
2. Rinse the mouthpiece assembly thoroughly under running tap water
3. Shake off excess water and allow to air dry fully before storing

Daily cleaning

1. Once per day, wash the mouthpiece assembly by hand in a bowl of warm soapy water (liquid dish soap) for 2 minutes
2. Rinse the mouthpiece assembly thoroughly under running tap water
3. Shake off excess water and allow to air dry fully before storing

Weekly disinfection

1. Prior to disinfection, ensure all parts are visibly clean and free from dirt/debris
2. Ensure the medication chamber lid is open and boil the mouthpiece assembly in water deep enough to prevent mouthpiece from touching the bottom for 10 minutes
3. Shake off excess water and allow to air dry fully before storing

7.5 Supply of Study Drug at the Site

Study drug supply will be maintained by MAPRx and will be supplied on a per participant basis. 7-day study drug supplies will be delivered to the clinical site within 48 hours of study visit corresponding to the start of week one of the treatment period and week two of the treatment period.

7.5.1 Storage

Study drug should will be shipped and stored by the study site at refrigeration temperature, 2 to 8ºC (36 to 46ºF). If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee and captured as a deviation. Participants will be instructed to store the medication in original packaging in the refrigerator according to the instructions outlined on the Drug Administration Instructions.

7.6 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each participant will be maintained on an ongoing basis by a member of the study site staff.

Measures of Treatment Compliance:

Participants will be asked to keep a patient diary noting the day and date they take their study drug, whether they cotreat with albuterol and any adverse events. They will be asked to bring their patient diary to each study visit along with all used and unused study drug containers. After the first 7 days of treatment, subjects will be asked to return to the research center for a follow-up visit to measure adherence and to obtain the last 7 days of study drug. If the subject's adherence is less than 70%, during the first treatment period subjects will be terminated and will not receive any additional intervention or follow-up.

8 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 A.

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 participant.

8.1 Clinical Assessments

8.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Baseline/Screening and at early termination when applicable. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

8.1.2 Medication Withholding

Bronchodilator reversibility will be used to establish eligibility at Visit 2a and will also be performed at visits 3-10. Since some asthma medications can blunt the response to bronchodilators, we may ask that participants withhold these medications according to the guidance provided in Table 2.

Table 2. Medication Withholding Parameters

Medication	**Withholding period**
Leukotriene modifiers	24h
Ultra-long-acting bronchodilators (indacaterol)	24h
Long-acting muscarinic antagonists (*tiotropium, glycopyrronium, umeclidinium)*	24h
Long-acting muscarinic antagonists *(aclidinium)*	12h
Long-acting beta-agonists	12h
Theophylline	12h
Short-acting anticholinergic	6h
Short-acting beta-agonists	4h

Participants will be evaluated at visit 1, without any medication holds, to establish the safety of withholding asthma medications. If a study clinician 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 or email 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 instruction to resume asthma medications if symptoms arise.

8.1.3 Food & Beverage Withholding

Participants are asked to withhold eating for our hour prior to sputum induction (visits 2, 6, 7 and 10) because 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.

8.1.4 Demographics

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

8.1.5 Medical History

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

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.

8.1.6 Physical Examination

A complete physical examination will be performed by either the investigator or a study clinician (MD, NP, RN, and PA) at visit 1. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit. Abbreviated physical exams may be performed as needed at visits 2-10.

8.1.7 Vital Signs

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

8.1.8 Oximetry

Oximetry will be measured on room air with the participant at rest at all study visits.

8.1.9 Spirometry

Spirometry will be performed at visits 1-10 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 part.

- Less Likely (<5%)
    - Wheezing and chest tightness.
- Rare but serious (<1%)
    - Syncope

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

8.1.10 Post-Bronchodilator Reversibility/Maximal 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 2a. Post-bronchodilator reversibility will be performed at Visits 3-10.

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.

8.1.11 Methacholine Challenge Testing

Methacholine challenge testing will be done according to the steps laid out in the UCSF Airway Clinical Research Center Methacholine Challenge Test Manual of Procedures or the University of California, Davis Pulmonary Services Laboratory standard protocol. Only participants that are unable to achieve 12% improvement in FEV1% following administration of 4 puffs of albuterol and who have a baseline FEV1 >50% predicted will undergo methacholine challenge testing.
Associated risks include the following:

- Likely (>20%)
    - Shortness of breath.
- Less Likely (< 20%)
    - Wheezing, 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 pregnant laboratory animals; there are no human studies to verify this effect during human 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 will undergo the methacholine 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 prediluent FEV1. Medications and personnel will be available to manage and treat bronchoconstriction.

8.1.12 Sputum Induction

A 12-minute sputum induction using 3% hypertonic saline will be performed at visits 2, 6, 7 and 10. The procedure will be carried out according to the UCSF Airway Clinical Research Center Sputum Induction Manual of Procedures. The sputum induction procedure will only be performed at the UCSF site.

Associated risks include the following:

- Likely (>25%)
    - 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.

8.1.13 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 A).

8.1.14 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 visits 3, 6, 7, and 10.

The risks associated with CT scanning are that of the additional amount of radiation exposure. At the UCSF, 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 3mSv. 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 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/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 will 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.

8.1.15 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 4, 6, 8 and 10. 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.

8.1.16 Measurement of Fractional Exhaled Nitric Oxide

Fractional exhaled nitric oxide measurement test will be performed at Visits 3-10. 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.

8.1.17 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).

8.2 Clinical Laboratory Measurements

8.2.1 Hematology

UCSF: Up to 100 mL (about 7 tablespoons) of blood will be obtained by standard venipuncture, for the collection of DNA, plasma, serum, and complete blood count with differential (CBC-D). Blood collection for DNA will be obtained at visit 3 and blood collection for plasma, serum and CBC-D will be collected at visits 3, 6, 7 and 10.

UC Davis: Up to 15 mL (about 1 tablespoon) of blood will be obtained by standard venipuncture for the collection of complete blood count with differential (CBC-D). Blood collection for CBC-D will be collected at visits 3, 6, 7, and 10.

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.

8.3 Research Laboratory Measurements

Induced sputum will be collected at visits 2, 6, 7, and 10.

8.3.1 Sputum Cell Count and Differential

Sputum for determination of total and differential cell counts will be collected at visits 2, 6, 7 and 10. Specimens will be collected and processed according to the UCSF Airway Clinical Research Center Sputum Induction Manual of Procedures (Appendix C).

8.3.2 Sputum Cytokine Gene Expression Measurements

Sputum for determination of RNA gene expression of a number of airway-specific genes will be collected at visits 2. Specimens will be collected and processed according to the UCSF Airway Clinical Research Center Sputum Induction Manual of Procedures.

8.3.3 Sputum Rheology

Sputum for the determination of rheological properties will be collected at visits 2, 6, 7, and 10. The rheological measurements may be made using a portion of the sample collected at this time. Sputum will only be collected for rheology at the UCSF site.

9 EVALUATIONS BY VISIT

9.1 Visit 1

1. Review the study with the participant and obtain written informed consent and HIPAA authorization.
2. Assign the participant a unique screening number.
3. Record demographics data.
4. Record medical history, including history of asthma, diagnosis date, and asthma-related healthcare utilization & 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.

9.2 Visit 2a

1. Review and 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 maximum reversibility and record post-bronchodilator spirometry.
7. If FEV1 bronchodilator reversibility of ≥ 12% collect induced sputum and schedule for Visit 3.
8. If FEV1 bronchodilator reversibility of ≤12% schedule participant for Visit 2b.

9.3 Visit 2b **Optional

1. Perform and record vital signs.
2. Perform and record oximetry.
3. Perform and record urine pregnancy test.
4. Perform and record spirometry.
5. Perform methacholine challenge testing.
6. Collect induced sputum.
7. Schedule participant for Visit 3.

9.4 Visit 3 (Day 0)

1. Review and 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. Perform and record post-bronchodilator spirometry.
8. Obtain a CT scan of the thorax.
9. Collect blood for clinical laboratory tests.
10. Schedule participant for Visit 4 within 7 days.

9.5 Visit 4 (Day 7)

1. Review and 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. Perform and record post-bronchodilator reversibility.
8. Perform and record plethysmography with bronchodilation.
9. Randomize
10. Provide education on use of personal nebulizer and administration of study medication.
11. Administer first two treatments of study drug treatment, spaced 4-6 hours. FEV1 measurements should be taken before and after each treatment.
12. Dispense study drug and initiate study diary if subject's post-treatment FEV1 is greater than 80% of pre-treatment FEV1.
13. Schedule participant for phone follow-up in 3-5 days and visit 5 in 7 days.

9.6 Phone follow-up (Day 9, 10, or 11)

1. Administer questionnaire to monitor for treatment related side effects.

9.7 Visit 5 (Day 14)

1. Review and record any adverse experiences and/or review participant diary for adverse experiences and exclusionary medication use
2. Review and record concomitant medications.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. Perform and record urine pregnancy test
7. Perform and record spirometry.
8. Perform and record post-bronchodilator spirometry.
9. Collect any unused study drug.
10. Review diary card.
11. If subject's adherence is greater than 70%, dispense 7 days of study drug.
12. Schedule participant for Visit 6 in 7 days.

9.8 Visit 6 (Day 21)

1. Review and 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. Perform and record post-bronchodilator reversibility.
8. Perform and record plethysmography with bronchodilation.
9. Obtain a CT scan of the thorax
10. Collect induced sputum sample
11. Collect blood for clinical laboratory tests.
12. Schedule Visit 7 after 8 weeks.

9.9 Visit 7 (Day 77)

1. Review and 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. Perform and record post-bronchodilator spirometry.
8. Obtain a CT scan of the thorax.
9. Collect induced sputum sample.
10. Collect blood for clinical laboratory tests.
11. Schedule Visit 8 within 1 week.

9.10 Visit 8 (Day 84)

1. Review and 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. Perform and record post-bronchodilator reversibility.
8. Perform and record plethysmography with bronchodilation.
9. Provide education on use of personal nebulizer and administration of study medication.
10. Administer first two treatments of study drug treatment, spaced 4-6 hours. FEV1 measurements should be taken before and after each treatment.
11. Dispense study drug and initiate study diary if subject's post-treatment FEV1 is greater than 80% of pre-treatment FEV1
12. Schedule participant for phone follow-up in 3-5 days and visit 5 in 7 days.

9.11 Phone follow-up (Day 86, 87, or 88)

1. Administer questionnaire to monitor for treatment related side effects.

9.12 Visit 9 (Day 91)

1. Record any adverse experiences and/or review participant diary for adverse experiences.
2. Perform abbreviated physical examination.
3. Review and record changes to concomitant medications.
4. Record vital signs.
5. Perform and record oximetry.
6. Perform and record urine pregnancy test
7. Perform and record spirometry.
8. Perform and record post-bronchodilator spirometry.
9. Review diary card.
10. Dispense remaining 7 days of study drug.
11. Schedule participant for Visit 10 in 7 days.

9.13 Visit 10 (Day 98)

1. Review and 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. Perform and record post-bronchodilator reversibility.
8. Perform and record plethysmography with bronchodilation.
9. Obtain a CT scan of the thorax
10. Collect induced sputum sample
11. Collect blood for clinical laboratory tests.

9.14 Early Withdrawal Visit

1. Record any Adverse Experiences and/or Review participant diary for adverse experiences and exclusionary medication use.
2. Review and record changes to concomitant medications.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. Perform and record urine pregnancy test.
7. Perform and record spirometry.
8. Perform and record post-bronchodilator spirometry.
9. Collect any unused study drug.
10. Review dairy card.
11. Perform and record plethysmography with bronchodilation*
12. Obtain a CT scan of the thorax*
13. Collect induced sputum sample*

14. Collect blood for clinical laboratory tests*

*Subjects will only be eligible to complete post-treatment plethysmography, CT scan, sputum induction and blood collection if they completed a minimum of five treatment days or 10 treatments.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 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 participant, for the occurrence of AEs during each participant visit and record the information in the site's source documents. Adverse events will be recorded in the participant 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 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 participant 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 participant 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 .

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 participant'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.

10.2 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 participant or require intervention to prevent one of the outcomes listed.

10.2.1 Serious Adverse Experience Reporting

The study site 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.

10.3 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

11 DISCONTINUATION AND REPLACEMENT OF PARTICIPANTS

11.1 Early Discontinuation of Study Drug

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

Participant withdrawal of consent

Participant is not compliant with study procedures

Adverse event that in the opinion of the investigator would be in the best interest of the participant 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 participant is withdrawn from treatment due to an adverse event, the participant will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All participants who discontinue study treatment should come in for an early discontinuation visit as soon as possible.

All participants 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 participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents. Refer to Section 10 for early termination procedures.

If the participant completes five treatment days or 10 treatments they will be eligible to complete post-treatment plethysmography, CT scan, sputum induction and blood collection during a post-treatment termination visit. If they complete less than five treatment days or 10 treatments. Refer to Section 10 for early termination procedures.

11.2 Withdrawal of Participants from the Study

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

All participants 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 participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents. As noted above, participants who discontinue study

treatment early (i.e., they withdraw prior to Visit 10) should have an early discontinuation visit. Refer to Section 10 for early termination procedures.

11.3 Replacement of Participants

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

12 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.

13 DATA SAFETY MONITORING

Adverse events will be monitored by the Clinical PIs (Drs. Fahy, Woodruff, Peters, Lazarus and Zeki) in real-time. In addition, 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, Peters, and Lazarus and a UCSF patient safety representative.

14 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.

14.1 Data Sets Analyzed

All eligible participants who are randomized into the study and receive at least one dose of the study drug (the Safety Population) will be included in the safety analysis.

14.2 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.

14.3 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 two-week treatment period with two times daily NAC (Mucomyst-10). The secondary analysis will be to determine how the change in FEV1 relates to the change in CT mucus score.

14.4 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.

14.5 Interim Analysis

No interim analysis is planned.

14.6 Sample Size

We propose a sample size of 36, 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. The sample size is obtained when we factor in an effect size of 5% improvement in FEV1, a standard deviation for change in FEV1 of 20%, a within patient correlation for repeated measures of FEV1 of 0.9, and a power of 90%.

DATA COLLECTION, RETENTION AND MONITORING

14.7 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 participant treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a participant's visit into the protocol-specific paper CRF when the information corresponding to that visit is available. Participants 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, participant number and initials.

The Investigator is responsible for all information collected on participants 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.

14.8 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.

14.9 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.

14.10 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.

14.11 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 participant must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that participant. 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.

14.12 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.

14.13 Participant Confidentiality

In order to maintain participant confidentiality, only a site number, participant number and participant initials will identify all study participants on CRFs and other documentation.

Additional participant confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

15 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 binders in a locked storage closet, and in our password protected secure electronic files, and is only accessible to authorized study personnel. Clinical information will not be released without written permission of the participant, 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).

15.1 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.

15.2 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 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.

15.3 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, assent and HIPAA authorization and provide the documents to the IRB/IEC for approval prior. The consent form generated by the Investigator must 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 participant prior to entering the participant into the trial. Information should be given in both oral and written form and participants must be given ample opportunity to inquire about details of the study. A copy of the signed consent form will be given to the participant and the original will be maintained with the participant's records.

15.4 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.

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

Appendix A. Schedule of Study Visits (UCSF)

Visit	**1**	**2a**	**2b***	**3**	**4**	**5**	**6[1]**	**7**	**8**	**9**	**10**
Time Commitment (hours)**	2	2	3	2	8	2	4	4	8	2	4
Consent and eligibility	x										
Questionnaires	x				x	x	x	x	x	x	x
Physical Exam	x				x[2]	x[2]	x[2]		x[2]	x[2]	x[2]
Vital signs & oximetry	x	x	x	x	x	x	x	x	x	x	x
FENO					x	x	x		x	x	x
Spirometry	x	x	x	x	x	x	x	x	x	x	x

Post-bronchodilator reversibility		x		x	x	x	x	x	x	x	x
Methacholine challenge			x								
Urine pregnancy test	x	x	x		x	x			x	x	
Sputum induction		x	x				x	x			x
Blood sample					x[1]		x	x			x
Plethysmography					x		x		x		x
CT thorax				x			x	x			x
Nebulizer & diary card education & teach-back					x				x		
Medication administration					x	x			x	x	
Randomization					x						

* Participation in visit 2b is dependent on post-bronchodilator reversibility test results obtained at visit 2a. The visit 2 sputum induction will only be obtained at visit 2a or 2b.

**Duration of visit is an estimation

1. Blood sample to include DNA, plasma, serum and CBC with 5-part differential
2. Abbreviated physical exam.

Appendix B. Schedule of Study Visits (UC Davis)

Visit	**1**	**2a**	**2b***	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Time Commitment (hours)**	2	2	3	2	8	2	4	4	8	2	4
Consent and eligibility	x										
Questionnaires	x				x	x	x	x	x	x	x
Physical Exam	x				x[1]	x[1]	x[1]		x[1]	x[1]	x[1]
Vital signs & oximetry	x	x	x	x	x	x	x	x	x	x	x
FENO					x	x	x		x	x	x
Spirometry	x	x	x	x	x	x	x	x	x	x	x
Post-bronchodilator reversibility		x		x	x	x	x	x	x	x	x
Methacholine challenge			x								
Urine pregnancy test	x	x	x		x	x			x	x	
Blood sample					x		x	x			x
Plethysmography					x		x		x		x
CT thorax				x			x	x			x
Nebulizer & diary card education & teach-back					x				x		
Medication administration					x	x			x	x	
Randomization					x						

* Participation in visit 2b is dependent on post-bronchodilator reversibility test results obtained at visit 2a. The visit 2 sputum induction will only be obtained at visit 2a or 2b.

**Duration of visit is an estimation

1. Abbreviated physical exam.

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