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

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Investigational Product:	n-acetylcysteine
Development Phase:	4
Sponsor:	John V. Fahy, MD, MSc 513 Parnassus Avenue, HSE 1307 San Francisco, Ca 94143-0130
Funding Organization:	Stulbarg Endowed Chair and the Sandler Asthma Basic Research Center discretionary funds
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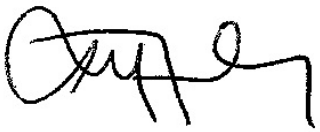

, Professor — 11/11/2015

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: 15-17919

Protocol Title: Clinical Trial of NAC in Asthma

Protocol Date: 11/11/2015

11/11/2015

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-acetylcystine
PI	Principal Investigator
SAE	serious adverse experience

PROTOCOL SYNOPSIS

TITLE	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	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 randomized, double-blind, placebo-controlled phase 4 study.
PRIMARY OBJECTIVE	The primary objective is to assess the clinical efficacy as measured by the change in pulmonary function over the seven day treatment period.
SECONDARY OBJECTIVES	The secondary objectives are to assess the clinical efficacy as measured in a subgroup of participants by the change in CT mucus score from baseline to end of treatment, and identify characteristics of those participants who benefit from mucolytic treatment.
NUMBER OF PARTICIPANTS	30
PARTICIPANT SELECTION CRITERIA	<u>Inclusion Criteria</u>: 1. Male or female between the ages of 18 and 70 years of age at Visit 0. 2. Able to perform reproducible spirometry according to ATS criteria 3. Clinical history consistent with moderate to severe asthma for 1 year or greater.

	4. Post-bronchodilator FEV1 <90% of predicted 5. Prescription and daily use of inhaled corticosteroid (ICS) equivalent to 80mcg of beclomethasone or greater 6. CT mucus score >3 (determined during the initial screening process, provided the prior two conditions are met) 7. Written informed consent obtained from participant and ability for participant to comply with the requirements of the study. 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. History of intolerance to study medications. 4. Current use of carbamazepine 5. Angina which includes a treatment plan with PRN nitroglycerin or nitrites 6. Smoking of tobacco or other recreational inhalants in last year and/or >10 pack-year smoking history 7. Current participation in an investigational drug trial 8. 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.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	20% NAC (5) Product will be administered every 6-8 hours (3 times per day) for 7 days. Medication will be administered via a portable tabletop nebulizer.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	0.9% saline (5mL) Product will be administered every 6-8 hours (3 times per day) for 7 days. Medication will be administered via a portable tabletop nebulizer.
DURATION OF PARTICIPATION AND DURATION OF STUDY	Participants will be enrolled in the study up to 132 days: **Screening:** up to 90 days **Treatment Period 1:** 7 days **Washout:** 28 days **Treatment Period 2:** 7 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 • Carbamazepine • Nitroglycern and nitrates
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 20% NAC).
SECONDARY ENDPOINTS	• Our secondary analysis is to determine the characteristics of participants that respond to NAC.
OTHER EVALUATIONS	Rheological properties of sputum.
SAFETY EVALUATIONS	Incidence of adverse events
PLANNED INTERIM ANALYSES	When approximately 50% of patients have completed the study through Visit 6, an interim analysis for safety will be conducted by the UCSF Airway Clinical Research Study Quality Assurance Committee. Serious adverse events will be monitored by the 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 20% 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-20% vs. 20%-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 20% NAC using a paired t-test.
Rationale for Number of Participants	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 >3.0. 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).

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.

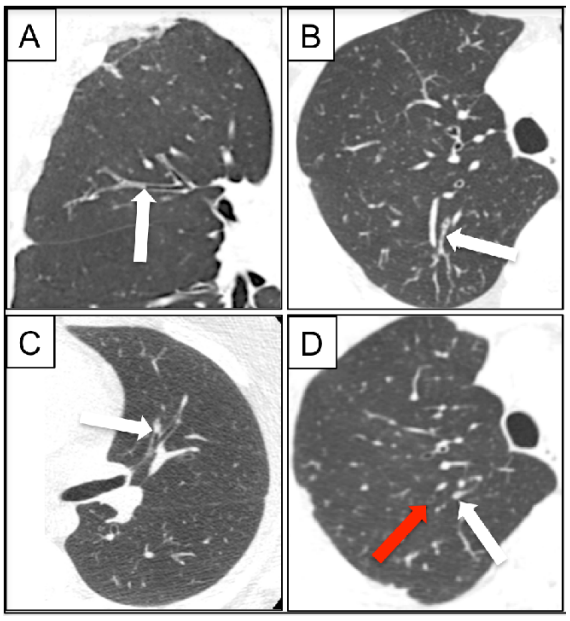


Figure 1: Intraluminal mucus in CT lung images of asthmatics. Mucus plugs in CT scan images (white arrows) were defined as complete occlusion of an airway lumen by mucus and were identified as tubular structures in longitudinal section with branching (A & B) or without branching (C) or as rounded opacities 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

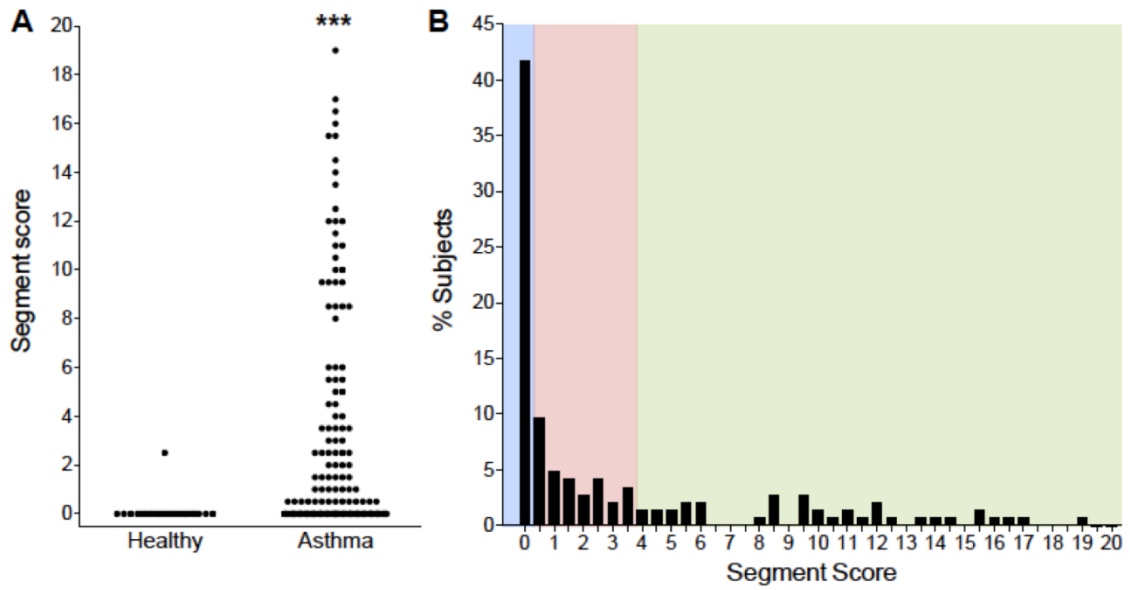


Figure 2. A. The CT mucus segment score is usually 0 in health but many asthmatics enrolled in the NHLBI Severe Asthma Research Program have scores above 0 with some as high as 15-19. B. The frequency distribution of mucus score in asthma shows that 42% of patients have a score of 0 (zero mucus score);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

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. 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

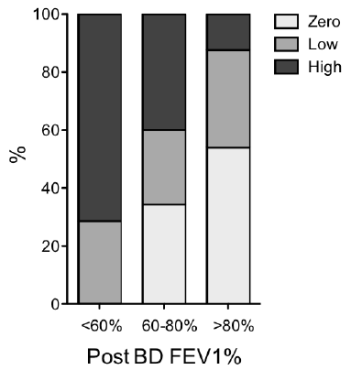


Figure 3. Mucus scores in asthmatics with different FEV1 values (post 6-8 puffs of albuterol). Note that all asthmatics with an FEV1 <60% have abnormal mucus scores.

impaction of airways and airflow obstruction. It further suggests that decreasing mucus G' could be a treatment 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.

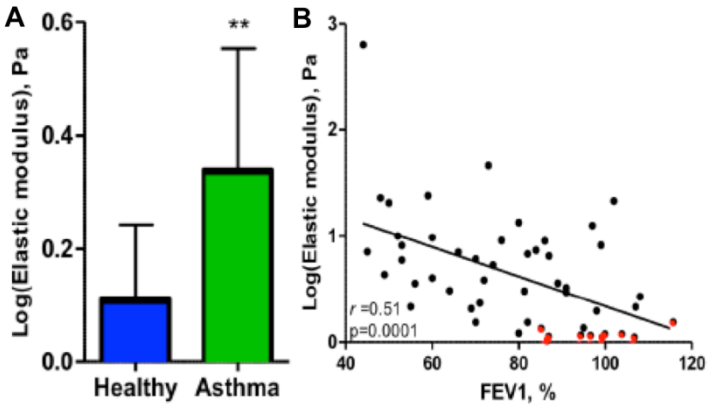


Figure 4. A. Sputum in asthma has an increased elastic modulus (G'). B. The G' of asthma sputum is significantly and inversely related to FEV1 (*ex vivo* data from 40 asthmatics and 11 healthy participants [red dots])

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 (20%) 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

Inhaled NAC carries a relatively low risk profile. The main side effects from inhalational use are nausea, reported in 2-7% of cases, and vomiting, reported in 9-12% of cases. Other reported side effects without documented incidence rates include stomatitis, cough, hemoptysis, rhinorrhea, fever, and chills. Rarely, NAC has been associated with increased intracranial pressure and somnolence. Although these later side effects have been documented in the medical literature, our team of physicians, experienced in using NAC clinically, has not encountered these more serious side effects.

Mucus impaction and the concomitant airway obstruction are associated with severe activity limitation and disease-associated impairment. Currently, there are few effective

mucolytics utilized as part of standard clinical care of patients with severe asthma. This is due in part to the lack of clinical trial data on the effectiveness of NAC, the only nebulized mucolytic currently indicated for asthma. 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, our study team of experienced clinicians will supervise the initial administrations of the study drug in a controlled environment located within a hospital setting.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to assess the clinical efficacy as measured by the change in pulmonary function over the seven day treatment period.

3.2 Secondary Objectives

The secondary objectives are to assess the clinical efficacy as measured in a subgroup of participants by the change in CT mucus score from baseline to end of treatment, and identify characteristics of those participants who benefit from mucolytic treatment.

4 STUDY DESIGN

4.1 Study Overview

This is a single center, double-blind, randomized, 2 period crossover trial. We propose a sample size of 30. Following a five-day run-in period, during which participants will self-administer 0.9% normal saline by nebulizer three times per day, each participant will be randomized to receive a nebulized dose of study drug three times per day for seven days of either 20% NAC (active) or 0.9% saline (placebo). Following a 28-day washout period, participants will be switched to the alternate group for 7 days of treatment. Participants will be assigned to the treatments in random order. Evaluations will be taken at baseline, end of run-in, start of second treatment period, and at the end of each 7-day treatment period.

Screening data will be reviewed to determine participant eligibility. Participants who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used:

- Experimental treatment of NAC 20%
- Placebo treatment of 0.9% saline

Total duration of participation will be nine weeks. Total duration of the study is expected to be 2 years.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

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].

5.2 Secondary Efficacy Endpoints

We intend to examine the effect of the treatment on CT mucus score in a subset of participants. 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.

With respect to drug tolerability, we will first analyze the two treatment periods to assess any negligible carryover effects and then describe frequencies of reported side effects.

5.3 Safety Evaluations

All participants will have their first dose of treatment or normal saline administered in the clinical laboratory environment 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 30 minutes following initiation of each new study period by a study nurse or study clinician.

All participants will be asked to complete a log of study drug administration, concomitant medications, and any subjective side effects (appendix A). 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 triggered 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 70 years of age at Visit 0.
2. Able to perform reproducible spirometry according to ATS criteria
3. Clinical history consistent with moderate to severe asthma for 1 year or greater.
4. Post-bronchodilator FEV1 <90% of predicted
5. Prescription and daily use of inhaled corticosteroid (ICS) equivalent to 80mcg of beclomethasone or greater
6. CT mucus score >3 (determined during the initial screening process, provided the prior two conditions are met)
7. Written informed consent obtained from participant and ability for participant to comply with the requirements of the study.

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. History of intolerance to study medications.
4. Current use of carbamazepine
5. Angina which includes a treatment plan with PRN nitroglycerin or nitrites
6. Smoking of tobacco or other recreational inhalants in last year and/or >10 pack-year smoking history
7. Current participation in an investigational drug trial
8. 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.

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

6.4 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.

Prohibited Medications and Treatments

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

- Thiols and thiol derivatives
- Carbamazepine
- Nitroglycern and nitrates

7 STUDY TREATMENTS

7.1 Method of Assigning Participants to Treatment Groups

Up to 30 eligible patients will be randomly assigned to receive either the experimental treatment (20% NAC) or the placebo treatment (0.9% saline) during period 1 in a 1:1 ratio using a SAS-based computer-generated randomization scheme developed by the study data manager. The investigational pharmacy, who will supply the prepared study medication packets, will complete a randomization worksheet at the end of run-in and log group assignment. This 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 20% NAC delivered via nebulizer. The placebo is 0.9% saline. Both solutions will be delivered by the Sponsor's compounding pharmacy in single-use rubber-topped glass vials.

7.3.1 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.

Table 1: Formulation and Measured pH of 20% NAC and Control Product

	20% NAC	**0.9% Saline**
Active Ingredient, mg/mL	Acetylcysteine 200mg/mL	
Other ingredient	Edetate disodium Sodium hydroxide Purified water	Sodium chloride Purified water
pH	7	5.7

7.3.2 Formulation of Control Product

A placebo solution of 0.9% saline will be prepared by the UCSF Drug Products Services Laboratory in single-use rubber-topped glass vials.

7.3.3 Packaging and Labeling

Study drug is supplied in a package containing 24 single-use rubber-topped glass vials (3 extra vials in the event of breakage).

Each package of study drug will be labeled with the required FDA warning statement, the protocol number, a treatment number, the name of the sponsors, and directions for patient use and storage.

7.4 Supply of Study Drug at the Site

The UCSF Drug Products Services Laboratory will prepare Study Drug and deliver it to the Airway Clinical Research Center. Study drug kits will be delivered upon successful completion of the run-in period on an individual participant basis.

7.4.1 Dosage/Dosage Regimen

During each treatment period (see Appendix B), participants will nebulize one vial of study drug using a tabletop nebulizer. Participants will be instructed to take three doses per day, spaced at approximately 6-8 hour intervals. No adjustments will be made based weight or age. Because NAC can cause transient lung irritation, participants will be instructed to take 2 puffs of supplied albuterol 15 minutes prior to each study treatment.

7.4.2 Dispensing

Study medication will be dispensed by either a study nurse or physician and only after direct observation of the administration of the first dose of each treatment period.

7.4.3 Administration Instructions

Nebulizer and Air Compressor Assembly

a. Place the compressor on a clean flat surface where it can safely reach its power source and where the ON/OFF switch can be reached.
b. Wash hands prior to preparing each treatment.
c. Use a clean nebulizer.

d. Inspect your nebulizer and ensure that the green exhalation valve on the mouthpiece is pointing down.
e. Connect one end of the tubing to the bottom of the nebulizer and the other end to the air outlet on the compressor. Ensure that both ends are securely attached.
f. Make sure the nebulizer top is in breath actuated mode.
g. Remove one vial of study drug from refrigerator and carefully add to the nebulizer.

Prior to Beginning Study Drug Treatment

a. Take 2 puffs of the albuterol inhaler (provided by study) 15 minutes before starting treatment.

Beginning Study Drug Treatment

a. Place the mouthpiece in your mouth, making sure your lips are sealed around the edge but are not covering the exhalation valve.
b. Hold the nebulizer in an upright position. This prevents spilling and promotes nebulization.
c. Breathe in slowly and deeply and then exhale normally through the device.
d. Occasionally tapping the side of the nebulizer helps the solution drop to where it can be misted.
e. Continue taking slow, deep breaths until the noise created by the device changes—this sound is sometimes referred to as a 'sputter'.
f. Switch off compressor when finished with treatment.

Care and Cleaning of Nebulizer Equipment after Each Use

a. Wash hands.
b. Remove tubing from nebulizer.
c. Open nebulizer cup and shake out any remaining liquid.
d. Empty the supplied saline bullet into the nebulizer cup. Swirl in cup and empty out excess.
e. Remove nebulizer top and mouthpiece from the nebulizer cup.
f. Shake the nebulizer parts to remove excess liquid.
g. Thoroughly air dry parts on drying rack before reassembling.

Care and Cleaning of Nebulizer Equipment Every Other Treatment Day

a. Wash hands.
b. Take the nebulizer apart.
c. Put all of the parts, except tubing and compressor, in boiling water for 10 minutes.
d. After boiling the nebulizer shake off any excess water, taking care not to burn skin.
e. Thoroughly air dry parts on drying rack before reassembling.

7.5 Supply of Study Drug at the Site

Study drug supply will be maintained by the UCSF Investigational Pharmacy and will be supplied on a per participant basis. 7-day study drug supplies will be delivered to the clinical laboratory within 1 hour of study visit corresponding to the start of each treatment

period. Replacement kits will be available for up to 8 participants, should replacement participants be needed in order to obtain the final enrolled cohort of 30 participants.

7.5.1 Storage

Study drug should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). 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 at room temperature 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. The number of study drug dispensed and returned by the participant will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

7.7 Measures of Treatment Compliance

Participants will be asked to keep a patient diary noting the day and date they take their study drug 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 (Appendix A).

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 B.

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 visits 4, 5, 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 Visits 2, 5, and 8. 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	24h
Ultra-long-acting bronchodilators (indacaterol, tiotropium)	24h
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 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 (2,5, and 8) and 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 who is a physician 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.

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 1-8 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

Spirometry will be repeated 15 minutes following the administration of four puffs of albuterol to assess bronchodilator reversibility. This procedure will be performed at visits 2-8.

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 (Appendix C). 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 and at least one liter 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, 5, and 8. The procedure will be carried out according to the UCSF Airway Clinical Research Center Sputum Induction Manual of Procedures (Appendix C).

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 B).

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 baseline (visit 2) and following both treatment periods (visits 5 and 8).

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 4 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 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

Blood will be obtained and sent to the clinical hematology lab for a complete blood count (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 inflammation.

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

8.3.1 Sputum Cell Count and Differential

Sputum for determination of total and differential cell counts will be collected at visits 2, 5, and 8. 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, 5, and 8. Specimens will be collected and processed according

to the UCSF Airway Clinical Research Center Sputum Induction Manual of Procedures (Appendix C).

8.3.3 Sputum Rheology

Sputum for the determination of rheological properties will be collected at visits 2, 5, and 8. The rheological measurements may be made using a portion of the sample collected at this time.

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 in 7 days.

9.2 Visit 2a

1. Concomitant medications review.
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 post-bronchodilator spirometry.
7. Collect blood for clinical laboratory tests.
8. Obtain a CT scan of the thorax.
9. Collect induced sputum.
10. Schedule participant for Visit 3.

8.2.1 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 blood for clinical laboratory tests.
7. Obtain a CT scan of the thorax.
8. Collect induced sputum.
9. Schedule participant for Visit 3.

9.3 Visit 3 (Day 0)

1. Record changes to concomitant medications.
2. Perform abbreviated physical examination.
3. Perform and record vital signs.
4. Perform and record oximetry.
5. Perform and record urine pregnancy test.
6. Perform and record spirometry.
7. Perform and record post-bronchodilator spirometry.
8. Provide education on use of personal nebulizer and administration of study medication.
9. Dispense study drug and initiate study diary.
10. Perform RN/MD supervised administration of first run-in dose of nebulized 0.9% normal saline.
11. Schedule participant for Visit 4 in 5 days.

9.4 Visit 4 (Day 5)

1. Record any adverse experiences and/or review participant diary for adverse experiences and exclusionary medication use.
2. 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 diary card.
11. Randomize if adherence is greater than or equal to 80% during run-in.
12. Provide education on use of personal nebulizer and administration of study medication.
13. Dispense study drug and initiate study diary.
14. Perform RN/MD supervised administration of first dose of nebulized study drug.
15. Schedule participant for Visit 5 in 7 days.

9.5 Visit 5 (Day 14)

1. Record any adverse experiences and/or review participant diary for adverse experiences and exclusionary medication use.
2. 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 spirometry.
7. Perform and record post-bronchodilator spirometry.
8. Collect any unused study drug.
9. Review diary card.
10. Obtain a CT scan of the thorax.
11. Collect induced sputum.
12. Schedule participant for Visit 6 in 14 days.

9.6 Visit 6 (Day 28)

1. Record changes to concomitant medications.
2. Perform and record vital signs.
3. Perform and record oximetry.
4. Perform and record spirometry.
5. Perform and record post-bronchodilator spirometry.
6. Schedule participant for Visit 7 in 14 days.

9.7 Visit 7 (Day 42)

1. Record changes to concomitant medications.
2. Perform abbreviated physical examination.
3. Perform and record vital signs.
4. Perform and record oximetry.
5. Perform and record urine pregnancy test.
6. Perform and record spirometry.
7. Perform and record post-bronchodilator spirometry.
8. Provide education on use of personal nebulizer and administration of study medication.
9. Dispense study drug and initiate study diary.
10. Perform RN/MD supervised administration of first dose of nebulized study drug.
11. Obtain a CT scan of the thorax.
12. Collect induced sputum.
13. Schedule participant for Visit 8 in 7 days.

9.8 Visit 8 (Day 47)

1. Record any adverse experiences and/or review participant diary for adverse experiences and exclusionary medication use.
2. 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 spirometry.
7. Perform and record post-bronchodilator spirometry.
8. Collect any unused study drug.
9. Review diary card.
10. Obtain a CT scan of the thorax.
11. Collect induced sputum.

9.9 Early Withdrawal Visit

1. Record any Adverse Experiences and/or Review participant diary for adverse experiences and exclusionary medication use.
2. 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.

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.

12.3 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 9) should have an early discontinuation visit. Refer to Section 10 for early termination procedures.

12.4 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 participant or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, participant 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, with the exception of the use of unanticipated nitroglycerin and/or nitrates as part of treatment for cardiovascular emergency.

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 participant.

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 and Lazarus) 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, Lazarus and Boushey.

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 outcome is the % change in FEV1 from the start to the end of the treatment period (either placebo or 20% 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-20% vs. 20%-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 20% NAC using a paired t-test.

14.4 Analysis of Secondary Endpoints

As a secondary outcome, we will include an analysis that examines the change associated with the 7-day treatment with 0.9% saline. Our secondary analysis is to determine the characteristics of participants that respond to NAC. We will classify participants in four groups based on their post-bronchodilator FEV1 in response to NAC. FEV1 of 0-4% change will define non-responders and FEV1 of 5-9%, 10-14%, or > 15% will define the 3 categories of responders. We will examine NAC responders and non-responders to determine if baseline CT mucus scores, lung function measures, or sputum G' measures predict treatment response. We will construct ROC curves to determine the CT score that predicts NAC response thereby identifying a CT biomarker for future thiol saccharide trials. In addition, we will examine how the CT mucus score changes with NAC treatment using a subset of the participants following second period treatment. Finding a decrease in CT score in the 20% NAC group compared to the placebo group will provide support for

our hypothesis that NAC improves lung function by decreasing intraluminal mucus. Other analyses will be to examine the effects of treatment on sputum elasticity (G').

Safety and tolerability data will be summarized by treatment group.

Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug.

14.5 Interim Analysis

When approximately 50% of patients have completed the study through Visit 6, an interim analysis for safety will be conducted by the UCSF Airway Clinical Research Study Quality Assurance Committee. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.

14.6 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 >3.0. 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]

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 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 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.

15.2 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.

15.3 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.

15.4 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.

15.5 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 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.

15.6 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.

15.7 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.

16 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).

16.1 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.

16.2 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.

16.3 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.

16.4 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.

16.5 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. DRUG DIARY

CONA Study Participant Self-Administration Study Drug Diary

Participant ID: _____

Protocol #: 15-17919

Your MD: John Fahy Phone: 415-476-9940

Your RN: Kelly McGrath Phone: 415-476-8494

Your CRC: Ariana Baum Phone: 415-514-1539

STUDY DRUG INSTRUCTIONS:

How Much: Your dose is 5mL.

How Often: You will take one dose three times per day.

When: You should take a dose every 6-8 hours.

SPECIAL INSTRUCTIONS:

Store vials in your refrigerator. Use an entire vial at once; do not store any remaining medication. Remember to take two puffs of albuterol 15 minutes before taking your study medication.

If you forget to take one or more doses, and it is almost time for the next dose, skip the missed dose and take the next dose when it is due. Do not take a double dose to make up for the dose that you missed.

Please bring any unused study drug, all empty vials, and this diary to the next clinic visit.

OTHER MEDICATIONS TAKEN

If you take a daily medication (prescribed or otherwise), please use one line per drug and indicate the start and stop dates under the "Date(s) Taken" section (i.e. 6/2/15-6/5/15).

Drug Name	Dose	Dates Taken	Reason Taken

Participant Initials: _____ Date: _____

FOR STUDY TEAM USE ONLY

Staff Initials: _____ Date Dispensed: _____ Date Returned: _____

Vials Dispensed: _____ #Vials Returned: _____

Vials that should have been taken: _____

Discrepancy Notes: _____

DOSING AND SYMPTOM LOG

Please indicate the date and time each dose of study medication is taken. Please record any asthma-related side effects in the appropriate column. Other symptoms can be recorded in the "Other" column. Please evaluate the severity of the symptom according to the following scale:

None (0): No symptom noted

Mild (1): Awareness of symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require treatment.

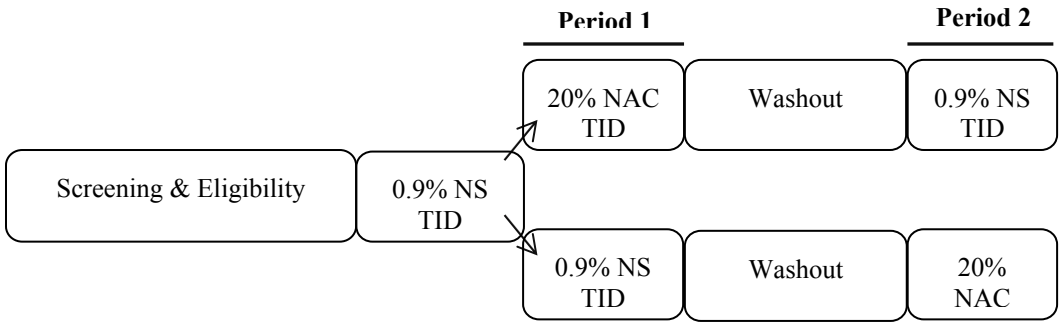
Moderate (2): Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe (3): Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during this time period.

Day	Dose	Date	Time	Wheeze	Cough	Chest Tightness	Shortness of Breath	Other
Example	*1*	*6/2/2015*	*8:32AM*	*0*	*1*	*0*	*0*	*Runny Nose (1)*
1	1							
	2							
	3							
2	1							
	2							
	3							
3	1							
	2							
	3							
7	1							
	2							
	3							
5	1							
	2							
	3							
6	1							
	2							
	3							
7	1							
	2							
	3							

Appendix B. CONA Schedule of Events



Visit	1	2a	2b*	3	4	5[1]	6	7	8[1]
Consent and eligibility	x								
Questionnaires	x			x	x	x	x	x	x
Physical Exam	x			x[3]	x[3]	x[3]		x[3]	x[3]
Vital signs & oximetry	x	x	x	x	x	x	x	x	x
Spirometry	x	x	x	x	x	x	x	x	x
Post-bronchodilator reversibility		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
Blood sample[2]		x	x						
CT thorax		x	x			x			x
Nebulizer & diary card education & teach-back				x	x			x	
RN/MD supervised medication administration				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, blood sample and CT thorax will only be obtained at visit 2a or 2b.

1. Visits have a ±5 day grace period; however, visits 5 and 8 must happen 7 days following the initiation of treatment.
2. Blood sample to include CBC with 5 part differential, serum IgE, and serum & plasma collection
3. Abbreviated physical exam.

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.

2. Hays SR, Fahy JV. The role of mucus in fatal asthma. *Am J Med*. 2003;115(1):68-69.

3. DUNNILL MS. The pathology of asthma, with special reference to changes in the bronchial mucosa. *J Clin Pathol*. 1960;13:27-33.

4. Lang DM, Simon RA, Mathison DA, Timms RM, Stevenson DD. Safety and possible efficacy of fiberoptic bronchoscopy with lavage in the management of refractory asthma with mucous impaction. *Ann Allergy*. 1991;67(3):324-330.

5. Green FH, Williams DJ, James A, McPhee LJ, Mitchell I, Mauad T. Increased myoepithelial cells of bronchial submucosal glands in fatal asthma. *Thorax*. 2010;65(1):32-38.

6. Cotgreave IA, Eklund A, Larsson K, Moldeus PW. No penetration of orally administered N-acetylcysteine into bronchoalveolar lavage fluid. *Eur J Respir Dis*. 1987;70(2):73-77.

7. McCoy K, Hamilton S, Johnson C. Effects of 12-week administration of dornase alfa in patients with advanced cystic fibrosis lung disease. pulmozyme study group. *Chest*. 1996;110(4):889-895.