Master Protocol

Phase I Assessment of Hypertonic Saline in Moderate to Severe Asthmatics

NCT number NCT03556683

Document Date 06/08/2023

Protocol:

Complete Title:	Phase I Assessment of Hypertonic Saline in Moderate to Severe Asthmatics
Drug Name:	Sodium Chloride 7% solution for inhalation (Hypertonic Saline); albuterol
Protocol Date:	June 8, 2023

Study Principal Investigator: David B. Peden, MD, MS

UNC Center for Environmental Medicine, Asthma and Lung Biology

104 Mason Farm Road, CB 7310
Chapel Hill NC 27599-7310
Phone 919-966-0768

email: david_peden@med.unc.edu

ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse Events
AED	Automated External Defibrillator
ATS	American Thoracic Society
BP	Blood Pressure
CEMALB	Center for Environmental Medicine, Asthma and Lung Biology
CF	Cystic Fibrosis
Co57	*Cobalt 57*
COPD	Chronic Obstructive Pulmonary Disease
Covid	Sars CoV 2 virus/infection
CTCAE	Common Terminology Criteria for Adverse Events
EMS	Emergency Medical Services
FVC	Forced Vital Capacity
FEF25-75	Maximal Mid-Expiratory Flow Rate
FEV1	Forced Vital Capacity in one second
IUD	Intra Uterine Device
HR	Heart Rate
HS	Hypertonic Saline
KeV	Kilo electron volts
LABA	Long Acting Bronchodilator Agent
LAIV	Live Attenuated Influenza Vaccine
MMAD	Mass Mean Aerodynamic Diameter
MCC	Mucociliary Clearance
mCi	Millicurie
NaCl	Sodium Chloride
NHLBI	National Heart, Lung and Blood Institute
NIH	National Institute of Health
NSAIDS	Non-steroidal anti-inflammatory drugs
PE	Physical Examination
PEF	Peak Expiratory Flow
PHI	Personal Health Information
PPE	Personal Protective Equipment
RR	Respiratory Rate
Tc99m-SC	Sulfur colloid
uCi	Microcurie
UP	Unanticipated Problems

PROTOCOL SYNOPSIS

Study Title	Phase I Assessment of Hypertonic Saline in Moderate to Severe Asthmatics
Funder	NIH
Clinical Phase	Phase I
Study Rationale	Mucociliary clearance (MCC) is the process by which mucus is propelled from the lung toward the upper airway. Studies at our Center and others have demonstrated that **hypertonic saline (HS)** increases the volume of airway surface liquid, improves rheologic properties of mucus, and accelerates mucus transport rates *in vitro*. Inhaled 7% HS enhances MCC in healthy volunteers as well as those with muco-obstructive diseases like cystic fibrosis and chronic bronchitis. Improvements in MCC were also associated with improved lung function in CF. Use of HS as a treatment for asthma has been limited. Airway mucus plugging is a key feature of asthma and is responsible for much of the morbidity and mortality associated with the disease. We will explore the efficacy of albuterol alone and 7% HS with albuterol, compared to baseline for improving MCC in moderate to severe asthmatics.
Study Objective(s)	<u>Primary Objective</u> To determine the efficacy of HS for accelerating MCC acutely (immediately following HS) compared to baseline in well-controlled moderate to severe asthmatics. <u>Secondary Objectives</u> - To compare the efficacy of albuterol alone vs 7% HS with albuteol pretreatment for accelerating MCC compared to baseline - To assess the safety of HS in moderate to severe asthmatics by measuring spirometry, vital signs, and symptom questionnaire scores. - Induced sputum samples after HS to assess for mucins and inflammatory endpoints
Test Article(s)	Sodium Chloride 7% solution for inhalation (Hereafter referred to as Hypertonic Saline or 7% HS) and albuterol for oral inhalation
Study Design	This is an open label proof-of-concept study. Participants will have a baseline MCC assessment, followed by sputum induction. They will return for another MCC assessment which will include treatment with 4 puffs of albuterol, and followed by a sputum induction . For the HS visit, in order to assess for acute effects of HS on MCC, subjects will be treated with 4 puffs of albuterol, and then will receive 7% HS inhalation immediately after start of the MCC measurement. Subjects will undergo sputum induction procedure after completion of MCC. All subjects will return the day following the HS visits for

	medical assessment and to obtain lung images to approximate retention of radiolabeled particles at 24 hours.
Subject Population - Criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Age 18-60. 2. Moderate to severe asthma at baseline (determined by Step 3 therapy or greater or by degree of impairment secondary to asthma). Subjects will be Well Controlled asthma at the time of enrollment, or Not Well Controlled as determined by National Heart, Lung and Blood Insistute (NHLBI) Expert Panel Report 3 guidelines for diagnosis and treatment of asthma. Subjects who meet the Not Well Controlled criteria must be free of wheezing, and must be at their baseline prior to any hypertonic saline inhalation. 3. Subjects of childbearing potential must be non-pregnant, non-lactating, and using an acceptable method of birth control (an IUD with a failure rate of <1%, hormonal contraceptives, barrier method, or abstinence) for the duration of participation study; if practicing abstinence, the subject must agree to use one of the acceptable methods of contraception if she becomes sexually active. **Exclusion Criteria** 1. Current tobacco smokers (or >10 pack years of smoking) or e-cigarette users (including use within 1 year of the study) 2. Other chronic illness, which could impact safety or study results 3. Radiation exposure within the past year that would cause them to exceed Federal radiation safety regulations.
Study Duration	Each subject's participation will last up to 4.5 months. The entire study is expected to last up to 6 years.
Study Phases **Screening** **Study Treatment** **Follow-Up**	(1) Baseline MCC: Informed consent obtained, medical history including radiation exposure, vital signs and lung function assessed with spirometry. FEV1 must be ≥ 70% of predicted. A baseline MCC scan will be performed over 2 hours. (2) Baseline MCC with 4 puffs of albuterol: Subjects will return at least 1 week later for a MCC scan with 4 puffs of albuterol immediately after the start of the MCC measurement. (3) Acute effects of HS on MCC: At least 1 week after albuterol visit, subjects will receive 4 puffs of albuterol and a dose of 7% HS immediately after the start of MCC measurement over a 2 hour period to assess the acute effects of HS on MCC. Subjects will undergo sputum induction after each MCC visit. (4) Follow-up: Medical screening for safety endpoints, spirometry, and lung retention imaging will be performed the day following each treatment visit, and a discontinuation visit will be performed 5 to 10 days after the last study visit.
Efficacy Evaluations	To determine the efficacy of albuterol and HS with albuterol for acutely accelerating MCC in adult patients with moderate to severe asthma. We will prioritize comparison of acute effects of HS on MCC to baseline MCC measurements.

Pharmacokinetic Evaluations	None
Safety Evaluations	Vital signs, symptom questionnaire scores, spirometry
Statistical And Analytic Plan	Primary Endpoint: difference between MCC measured at baseline and MCC measured with albuterol compared to MCC measured during and immediately after 7%HS inhalation, with MCC reported as the average percent retention of radiolabeled particles over 120 minutes of acquiring images. We will perform descriptive analysis of this difference; all point estimates will be tabulated along with their 95% confidence intervals. As we have assumed normality of MCC measurements, we will test the null hypothesis of no difference between baseline MCC and MCC immediately post-HS, against the alternative hypothesis of some difference, using a paired two-sided one-sample t-test with a 5% type I error rate. If the observed p-value is above 0.05, the test will be interpreted as being inconclusive. Following this test, we will perform sensitivity analyses to assess how realistic the assumption of normality was, and how much it may have impacted our conclusions. In particular, we will examine normal q-q plots and other descriptive tabulations, and we will examine the results of a Wilcoxon Signed Rank test. Should extreme or questionable observations arise in the study, we will also examine regression diagnostics to assess the influence of how those data were handled. Secondary Endpoints: difference between albuterol and 7% HS effect on MCC and mucins/neutrophils in induced sputum post treatment. Finally, we will report the proportion of enrolled patients who do not tolerate HS at baseline, along with the 95% confidence interval of this proportion.
DATA AND SAFETY MONITORING PLAN	A study coordinator will enter data into REDCap, and then a second person will verify the data. Subjects will be monitored in real time for safety by study staff. A study physician will be immediately available during inhalation procedures and will determine the severity and relatedness of any adverse events (AEs). The NC TraCS Data Safety Monitoring Board (DSMB) will be chartered to set data monitoring and reporting requirements, in collaboration with the PI and study biostatistician. The DSMB will address issues regarding safety concerns, efficacy concerns, termination of the trial due to pre-specified stopping criteria, and ethical concerns. The DSMB will be furnished with relevant information by the Principal Investigator to make these decisions. The DSMB will review after 5 subjects have completed study procedures or at 6 months after enrollment begins, whichever is soonest, and then every 6 months thereafter.

1 BACKGROUND AND RATIONALE

1.1 Introduction

Asthma is a chronic, inflammatory disease of the lungs. It is the most commonly encountered respiratory disease in children and adults in the United States, and is a leading cause of morbidity worldwide. Asthma exacerbations account for a significant proportion of healthcare costs associated with asthma. Viral infection, allergen, and pollutant exposures are the most common triggers for asthma exacerbation, which is characterized by airway inflammation, mucosal edema, contraction of airway smooth

muscle, and increased production of airway mucus. Airway dysfunction leads to reduced mucociliary clearance (MCC), which contributes to airway mucus plugging and further deterioration of lung function. Mucus plugging is a feature associated with fatal asthma (1-3).

Pulmonary MCC is dependent on airway secretory cells and submucosal glands that produce a mucin-rich fluid layer on the airway surface and ciliated cells that hydrate and propel mucus out of the lung and into the upper airway. Rates of MCC are dependent on ciliary beat frequency, hydration, and the rheologic properties of mucus. *In vitro* studies have demonstrated that HS, through an osmotic effect on airway surfaces, improved hydration and mucus rheologic properties, and accelerated mucus transport rates. Inhaled HS has also been shown to produce an acute acceleration of MCC (4-6), suggesting potential benefit in the context of increased mucus production and plug formation associated with asthma.

MCC is estimated *in vivo* through the use of gamma scintigraphy. A gamma camera is an imaging technique used to map functions and processes within the body by detecting radiation from a tracer introduced into the volunteer's body. The most commonly used tracer is technetium-99m (Tc99m). Briefly, volunteers inhale aerosolized radiolabeled sulfur colloid (Tc99m-SC) then sit in front of a gamma camera, which detects emitted radiation, for a period of 120 minutes. During this time, technicians measure an average percent retention of radiolabeled particles in the lung. This is used to estimate the proportion of particles that are cleared from the lung per unit time, also referred to as MCC.

1.2 Name and Description of Investigational Product or Intervention

- Albuterol sulfate for oral inhalation
- Sodium Chloride (NaCl) 7% solution for inhalation (Hypersal®, PARI Respiratory Equipment, Inc, Midlothian, VA).

1.3 Non-Clinical and Clinical Study Findings

Studies at our Center and others have demonstrated that hypertonic saline (HS) increases the volume of airway surface liquid, improves rheologic properties of mucus, and accelerates mucus transport rates *in vitro*, and that inhaled HS enhances mucocilliary clearance (MCC) in healthy subjects with and without asthma (4-6) as well as patients with cystic fibrosis (7, 8, 9). The improvement in MCC with HS in CF appears dose-dependent based on the findings of one study, showing increasingly greater improvements in MCC from NaCl 0.9% up to NaCl 12% (10). In a study examining the effect of a single dose of nebulized HS 7% vs amiloride, HS 7% + amiloride, or NaCl 0.9%, HS 7% alone was superior for increasing MCC in cystic fibrosis patients (11).

1.4 Relevant Literature and Data

Much of the published data relevant to this proposal has been generated by investigators in the UNC Center for Environmental Medicine, Asthma and Lung Biology (CEMALB); these are highlighted below.

<u>The following is a short list of articles that provide support for our rationale and objectives</u>.

1. Kuyper LM, Pare PD, Hogg JC, Lambert RK, Ionescu D, Woods R, Bai TR. Characterization of airway plugging in fatal asthma. *Am J Med* 2003; 115: 6-11.
2. Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in

submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* 2002; 57: 677-682.
3. 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: 32-38.
4. Alexis NE, Bennett W, Peden DB. Safety and benefits of inhaled hypertonic saline following airway challenges with endotoxin and allergen in asthmatics. *J Asthma* 2017; 54: 957-960.
5. Bennett WD, Wu J, Fuller F, Balcazar JR, Zeman KL, Duckworth H, Donn KH, O'Riordan TG, Boucher RC, Donaldson SH. Duration of action of hypertonic saline on mucociliary clearance in the normal lung. *J Appl Physiol (1985)* 2015; 118: 1483-1490.
6. Daviskas E, Anderson SD, Gonda I, Eberl S, Meikle S, Seale JP, Bautovich G. Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. *Eur Respir J* 1996; 9: 725-732.
7. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med* 2006; 354: 241-250.
8. Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, Marks GB, Belousova EG, Xuan W, Bye PT, National Hypertonic Saline in Cystic Fibrosis Study G. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006; 354: 229-240.
9. Forouzan A, Masoumi K, Delirrooyfard A, Asgari Darian A, Mokhtar Gandomani L. Effect of Nebulized 3% Hypertonic Saline with Salbutamol on Management of Acute Asthma in Outpatient Adults: A Double-blind, Randomized Clinical Trial in Emergency Department. *Iran J Allergy Asthma Immunol* 2017; 16: 370-377.
10. Robinson M, Hemming AL, Regnis JA, Wong AG, Bailey DL, Bautovich GJ, King M, Bye PT. Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. *Thorax* 1997; 52: 900-903.
11. Robinson M, Regnis JA, Bailey DL, King M, Bautovich GJ, Bye PT. Effect of hypertonic saline, amiloride, and cough on mucociliary clearance in patients with cystic fibrosis. *Am J Respir Crit Care Med* 1996; 153: 1503-1509.
12. Matsui H, Grubb BR, Tarran R, Randell SH, Gatzy JT, Davis CW, Boucher RC. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell 95: 1005–1015, 1998.
13. Alexis NE, Hu SC, Zeman K, Alter T, Bennett WD. Induced sputum derives from the central airways: confirmation using a radiolabeled aerosol bolus delivery technique. Am J Resp Crit Care Med 164: 1964–1970, 2001
14. Sood N, Bennett WD, Zeman K, et al. Increasing concentration of inhaled saline with or without amiloride: effect on mucociliary clearance in normal subjects. Am J Respir Crit Care Med 167: 158–163, 2003.
15. Button B, Cai LH, Ehre C, Kesimer M, Hill DB, Sheehan JK,, Boucher RC, Rubinstein M. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science 24: 937–941, 2012.

2. STUDY OBJECTIVES

2.1 Primary Objective

This study will assess the efficacy of inhaled HS for accelerating MCC acutely in well-controlled moderate to severe asthmatics.

2.2 Secondary Objectives

To determine if albuterol is as effective as 7% HS in accelerating MCC. We will also evaluate the safety of HS in moderate to severe asthmatics by determining the proportion who are 'intolerant' to HS treatment (see Section 5.3).

3. INVESTIGATIONAL PLAN (brief overview)

3.1 Study Design

Type of design: Prospective open label study

Provide brief overview of the study phases:

- **Visit 1 Baseline MCC:** Participants will inhale Tc99m-SC, assessment of MCC over a 2-hour period to define baseline MCC. Participants will then perform spirometry, followed by sputum induction.
- **Visit 2 Follow-up Baseline Visit**: Participants will return the following morning for a query of symptoms overnight. Spirometry will be performed. Participants will sit in front of the gamma camera for 30 minutes to estimate retention of Tc99m-SC in the airways.
- **Visit 3 Albuterol Treatment visit:** Between 1-6 weeks of the baseline visit, participants will return to the lab to complete spirometry and the symptom questionnaire. They will then inhale Tc99m-SC and immediately be scanned by gamma scintigraphy to measure deposition of the radiolabeled particles in the airways (4 minute scan). While they remain seated in front of the gamma camera they will then inhale 4 puffs of albuterol. MCC measurement will be performed for a 2-hour period post-radioaerosol inhalation to measure acute effects of albuterol on MCC. Subjects will undergo sputum induction at the completion of the MCC scan.
- **Visit 4 Follow-up Albuterol Treatment Visit:** Participants will return the following morning for a query of symptoms overnight. Spirometry will be performed. Participants will sit in front of the gamma camera for 30 minutes to estimate retention of Tc99m-SC in the airways.
- **Visit 5: 7% HS concentration comparison:** Participants will return to the lab
- Between 1-6 weeks of the albuterol visit, participants will return to the lab to complete spirometry and the symptom questionnaire. They will then inhale Tc99m-SC and immediately be scanned by gamma scintigraphy to measure deposition of the radiolabeled particles in the airways (4 minute scan). While they remain seated in front of the gamma camera they will then inhale 4 puffs of albuterol followed by 4 mL of nebulized 7% HS. MCC measurement will be performed for a 2-hour period post-radioaerosol inhalation (which includes the period of HS inhalation) to measure acute effects of HS on MCC. Subjects will undero sputum induction at the completion of the MCC scan.
- **Visit 6 Follow-up HS Treatment Visit:** Participants will return the following morning for a query of symptoms overnight. Spirometry will be performed. Participants will sit in front of the gamma camera for 30 minutes to estimate retention of Tc99m-SC in the airways.
- **Visit 7 Study Discontinuation:** The subject will return to the research lab 5-10 days after the completion of the treatment visits for AE inquiry, vital signs and spirometry.
- **Unscheduled Visits:** There is no expectation for unplanned visits. Subjects will simply be rescheduled if they have a change in health status.

3.2 Allocation to Treatment Groups and Blinding (if applicable): N/A

3.3 Study Duration, Enrollment and Number of Subjects:

All subjects will be involved in the study for seven study visits. The Treatment Visits will occur within 1-6 weeks of the

screening visit. The total commitment for each subject will be a maximum of 14 weeks; however if a subject becomes ill we will delay visits until they are well. This may extend the total time for an individual. A total of 28 subjects will be enrolled to complete the study. The entire study should be completed in about 4I years.

3.4 Study Population

Inclusion Criteria

1. Age 18-60 of both genders
2. Moderate to severe asthma at baseline (determined by Step 3 therapy or greater or by asthma impairment) and be well-controlled at the time of enrollment, as determined by NHLBI Expert Panel Report 3 guidelines for diagnosis and treatment of asthma. Or
3. Moderate to severe asthma at baseline (determined by Step 3 therapy or greater or by asthma impairment) and not be well-controlled at the time of enrollment, as determined by NHLBI Expert Panel Report 3 guidelines for diagnosis and treatment of asthma
4. Negative pregnancy test for females who are not s/p hysterectomy with oopherectomy
5. FEV_1 of at least 70% of predicted for age, sex, height, and race/ethnicity (without use of bronchodilating medications for 12 hours or long acting beta agonists for 24 hours).
6. Documented Covid 19 vaccination

Exclusion Criteria

Subjects who meet *any* of these criteria are *not* eligible for enrollment as study participants:

1. Clinical Contraindications:

a) Any chronic medical condition considered by the PI as a contraindication to the study including significant cardiovascular disease, diabetes, chronic renal disease, chronic thyroid disease, history of chronic infections/immunodeficiency, or history of tuberculosis
b) Any acute infection requiring antibiotics within 4 weeks of study.
c) Mental illness or history of drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements.
d) Medications which may impact the results of the study treatment, or may interfere with any other medications potentially used in the study (to include systemic steroids, beta antagonists, non-steroidal anti-inflammatory agents)
e) Active smoking to include e-cigarettes within 1 year of the study, or lifetime of > 10 pack years of smoking
f) Allergy/sensitivity to study drugs, or their formulations.
g) History of intubation for asthma
h) Unwillingness to use reliable contraception if sexually active (birth control pills/patch, condoms).
i) Viral upper respiratory tract infection within 4 weeks of challenge.
j) Radiation exposure history in the past year that would cause the participant to exceed Federal radiation safety guidelines.
k) Positive Covid test in the past 90 days

2. Pregnant women and children (< 18 years as this is age of majority in NC) will also be excluded since the risks associated with hypertonic saline inhalation to the fetus or child and the risk of radiation are unknown and cannot be justified. Breastfeeding women will be excluded from this protocol.

3. Use of the following medications:

a. Systemic corticosteroids; subjects with systemic corticosteroid-dependent asthma will be excluded. All use of systemic steroids in the last year will be reviewed by a study physician.
b. Use of daily theophylline within the past month
c. Use of beta blocking medications
e. Receipt of LAIV (Live Attenuated Influenza Vaccine), also known as FluMist®, within the prior 30 days, or any vaccine within the prior 5 days
f. Multivitamins, Vitamin C or E or herbal medications in the 4 days prior to the treatment visit
g. Non-steroidal anti-inflammatory drugs in the 4 days prior to the treatment visit

4. Allergy/sensitivity to study drugs or their formulations:

Known IgE-mediated hypersensitivity to albuterol, diphenhydramine or corticosteroids.

5. Physical/laboratory indications:

a. Abnormalities on lung auscultation
b. Temperature > 37.8
c. Systolic BP >150 mm hg or < 90 mm Hg or diastolic BP> 90 mm Hg or < 50
d. Oxygen saturation of < 93%

6. Inability or unwillingness of a participant to give written informed consent.

4 STUDY PROCEDURES (See Appendix for Table of Study Procedures)

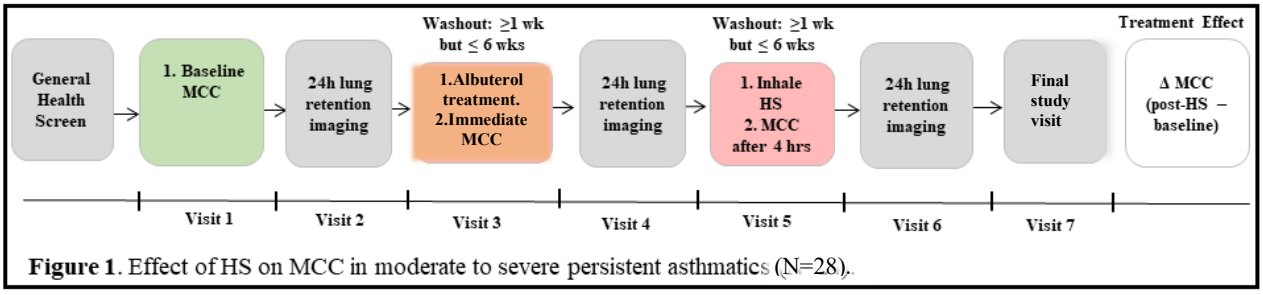

Figure 1. Effect of HS on MCC in moderate to severe persistent asthmatics (N=28).

4.1 Visit 1 Baseline MCC: Subjects will be phone screened for potential eligibly. All subjects will be verbally screened for Covid 19 infection or exposure following current guidelines. The study, including risks and benefits, and what is expected of the subject will be explained and subjects will be given time to ask any questions. Once consent is obtained, a medical history, including all medications, will be collected. Subjects will be queried about any radiation exposure in the past year. A negative urine pregnancy test will be required of all women. Vitals signs, including

temperature, respiratory rate (RR), heart rate (HR), oxygen saturation, blood pressure (BP), and symptom questionnaires will be collected. Fractional exhaled Nitric Oxice (FeNO) will be measured in exhaled breath (used to classify asthma as being predominated by eosinophilic vs non-eosinophilic airway inflammation). Spirometry will be performed to establish that day's baseline values. FEV1 must be ≥ 70% before albuterol. Participants will inhale Tc99m-SC and immediately be scanned by gamma scintigraphy to measure deposition of the radiolabeled particles in the airways (4 minute scan). MCC will be assessed over a 2-hour period to define baseline MCC. A baseline symptom questionnaire will be obtained. Subjects will then undergo a sputum induction procedure

Any subject who experiences a ≥ 10% reduction in FEV1 from that day's baseline at the spirometry measure, or who increases HR, BP or RR more than 30% from baseline, or with audible wheezing on physical exam, or participants who report any symptom as severe (score of 3) on the symptom questionnaire will be considered a intolerant to HS and will not be included in further study. Oxygen saturation will be monitored, and a transient decrease is an expected finding when using mucolytic agents. Sustained (< 90% for more than 1 hour) decrease in oxygen saturation will be considered intolerance. Testing will be stopped at any point the volunteer requests that it stop. Any subject who does not tolerate HS will be monitored until the vital signs return to normal limits, and the symptoms resolve. Subjects will be discharged to home with with contact information for a study physician, an asthma action plan reviewed by the study coordinator, and emergency medications if needed overnight, including an albuterol MDI with spacer and 60 mg of prednisone. Subjects will be instructed to call the physician on call if they require prednisone treatment.

4.2 Visit 2 Follow-up Baseline Visit: Participants will return the following morning for vital signs, symptom questionnaire, 24-hour lung retention imaging, FeNO, and spirometry. Subjects who tolerate the HS dose and do not meet stopping criteria will be invited back for the remaining study visits.

4.3 Visit 3 effect of albuterol inhalation on MCC: At least 1 week but no greater than 6 weeks from Visit 2, subjects will return to the lab. This visit may be delayed longer if the subject has an asthma related exacerbation to ensure good health for a total of 4 weeks before the visit. Vital signs, symptom and radiation exposure questionnaire are collected, and FeNO and spirometry performed. A negative urine pregnancy test will be required of all female participants. Medical history will be updated, including any new medication. Subjects will undergo spirometry and FEV1 must be at least ≥ 70% of predicted for age, height, sex, and race/ethnicity. Participants will then inhale Tc99m-SC and immediately be scanned by gamma scintigraphy to measure deposition of the radiolabeled particles in the airways (4 minute scan). While they remain seated in front of the gamma camera, they will be provided with 4 puffs of albuterol with spacer, followed by a 15-minute wait period. . MCC measurement will be performed for a 2-hour period post-radioaerosol inhalation to measure acute effects of albuterol on MCC. Vital signs, symptom questionnaire, spirometry and discharge procedures will be identical to those described for visit 1.

Subjects will undergo sputum induction following the 2 hour MCC scan.

4.4 Visit 4 Follow-up Albuterol Treatment Visit: Participants will return the following morning for vital signs, symptom questionnaire, 24-hour lung retention imaging, FeNO, and spirometry. Subjects who complete the acute treatment visit and do not meet stopping criteria will be invited back for the remaining study visits.

4.5 Visit 5 Treatment (effect of 7% HS on MCC): Subjects will return at least 1 week, but no more than 6 weeks later to the lab. This visit may be delayed longer if the subject has an asthma related exacerbation to ensure good health for a total of 4 weeks before the visit. Vital signs, symptom and radiation exposure questionnaire are collected, and FeNO and spirometry performed. A negative urine pregnancy test will be required of all female participants. Medical history will be updated, including any new medication. FEV1 must be at least 10% of baseline (established at screening visit) and $\geq$ 70% of predicted for age, height, sex, and race/ethnicity. Subjects will undergo spirometry and FEV1 must be at least > 70% of predicted for age, height, sex, and race/ethnicity. Participants will then inhale. Tc99m-SC and immediately be scanned by gamma scintigraphy to measure deposition of the radiolabeled particles in the airways (4 minute scan). While they remain seated in front of the gamma camera, they will be provided with 4 puffs of albuterol with spacer, followed by a 15-minute wait period They will then inhale 4 mL of nebulized 7% HS. MCC measurement will be performed for a 2-hour period post-radioaerosol inhalation (which includes the period of HS inhalation) to measure acute effects of HS on MCC. Vital signs and symptom questionnaire will be performed at 5 and 30 minutes after HS inhalation while sitting in front of the gamma camera. Vital signs, symptom questionnaire, spirometry and discharge procedures will be identical to those described for visit 1.

Subjects will undergo sputum induction following the MCC scan.

4.6 Visit 6 Follow-up HS treatment Visit: Participants will return the following morning for vital signs, symptom questionnaire, 24-hour lung retention imaging, FeNO, and spirometry.

4.7 Visit 7 Study Discontinuation: The subject will return to the research lab 5-10 days after the completion of the treatment visits for AE inquiry, vital signs and spirometry.

4.8 Unscheduled visits: Any subject who has an unexpected problem as a result of the study treatment will have access to a study physician. Subjects will be invited back to the lab for vital signs collection, physical exam, and spirometry. If the subject has any adverse event not associated with the study, he/she will not complete the study until the AE resolves such that it does not interfere with the study or with safety.

4.9 Concomitant Medication documentation: Concomitant medications are collected at the screening visit and throughout the study visits. Subjects will use previously prescribed medication for their asthma. Subjects will not be asked to withhold any LABA/steroid combination medication on the morning of study visits. The study will provide all subjects with an albuterol MDI with spacer, and with 60 mg of prednisone as part of the asthma action plan at discharge. Subjects will be allowed to keep albuterol MDI with spacers for their personal use. Subjects will be asked to return unused prednisone tablets to the study staff during the follow-up visits.

4.10 Rescue medication administration: As with any testing in a population of subjects with asthma, albuterol (Ventolin® HFA) will always be available for rescue. There is also a code cart immediately available with epinephrine autoinjectors. Oxygen is available for supplementation if required.

4.11 Subject Completion/ Withdrawal procedures: Subjects are considered completed when the final 24-hour imaging, symptom questionnaire, vital signs and spirometry are collected. Subjects may withdraw at any time. Subjects will be asked to return to the lab for a final visit if they withdraw from the study prior to completion of all study procedures to monitor safety.

Subjects may be withdrawn at any time for safety concerns. Subjects who have any unanticipated problems (UPs) with the study procedures will be monitored until resolution of the symptoms.

Criteria for safety prior to HS inhalation:
(Subjects not meeting these criteria will not proceed with HS inhalation):

1. FEV_1 of at least 70% of predicted
2. FEV1 within 10% of baseline value established at the screening visit.
2. Baseline oxygen saturation of at least 93%.
3. No history of viral respiratory tract symptoms within 4 weeks of challenge.
4. Vital signs within the previously defined normal parameters.
5. Symptom questionnaire score ≤ 2, , and no more than mild symptoms (ranked as "1") reported for any category (see Section 5.3).

Criteria for safety of a given individual following HS inhalation which would suspend the individual from further participation in the study will include:

1. A 10% or greater decline in FEV1 (from that day's baseline) at the 30-minute spirometry assessment.
2. *Continued* Symptoms of asthma exacerbation such as wheezing or respiratory distress.
3. Heart rate, blood pressure, or respiratory rate increase of more than 30% from baseline values (established on the same day).
4. Oxygen saturation < 90% for > 1 hour.
5. ANY reported shortness of breath, and any symptom ranked as severe ("3").
6. Requirement of oral corticosteroid treatment for exacerbation of asthma associated with the HS inhalation.

Safety criteria for iniating suspension of further study until consultation with the DSMB and IRB will include the following:

1. The occurrence of any Serious Adverse Event.

2. If 3 of the first 10 participants fail the individual safety criteria outlined above.

Subjects will be under direct supervision of a study physician throughout the HS inhalation and MCC measurement, and will have contact information for after-hours access to a study physician. An asthma action plan with emergency medications will be provided to each subject after the HS visits in the event of an unexpected event, such as wheezing or bronchospasm overnight.

4.12 Screen failure procedures. For subjects who do not meet enrollment criteria, all study related activity will stop once it is determined that the subject does not meet criteria.

5. STUDY EVALUATIONS AND MEASUREMENTS

List variables that will be abstracted from medical charts: none
Describe screening evaluation: Vital signs, including heart rate, respiratory rate, temperature and blood pressure will be collected. Oxygen saturation levels will be noted, as well as breath sounds. A brief physical examination, including but not limited to the cervical lymph nodes, eyes, ears, nose, throat, cardiovascular and respiratory systems.

Describe how measurements will be taken:

Covid:
All University, National and State guidelines will be followed during the Covid 19 pandemic, and the protocol will make changes as required to be in compliance. There will only be 1 subject at any given time in the study area, and areas will be cleaned between subjects. As many of the procedures for this study are considered to be aerosol generating, all subjects will be verbally screened for signs or symptoms of Covid, or recent potential exposure. Staff will be encouraged to receive vaccination. Study particiants will be masked when it does not interfere with measurements. Study staff will wear appropriate PPE during visits. Spirometry and aerosol treatments will be done in rooms with either > 6 air exchanges per hour or with running HEPA filters. Any subject will be deferred for at least 90 days after a positive Covid test, and volunteers will be required to show proof of up to date vaccination.

Spirometry:
This test measures the volume of air that can be exhaled and the rate of airflow during exhalation after a maximal inhalation, and is performed to American Thoracic Society (ATS) guidelines. Subjects will inhale as deeply as possible, then exhale as rapidly and completely as possible into the spirometer. Measurements obtained from each maneuver include the forced vital capacity (FVC), the forced expiratory volume in the first second (FEV1), the maximal mid-expiratory flow rate (FEF 25-75%) and the peak expiratory flow (PEF). The largest FVC and FEV1, from at least 3 acceptable trials, are selected for analysis; the flow rates are selected from the trial with the largest sum of FVC and FEV1.

FeNO:
Fractional Exhaled nitric oxide (FeNO) is measured using the Niox MINO (Aerocrine Inc) device. The subject inhales through the mouth to total lung capacity (TLC) and then exhales immediately. The NO analyzer samples the expirate continuously. Since exhaled NO plateau values can vary with exhalation flow rate, subjects must achieve a standardized expiratory flow rate for the results to be acceptable. eNO is measured in parts per billion (ppb). The procedure is performed in accordance with ATS guidelines.

HS Inhalation:
The subject will be instructed on the proper use of the PARI LC Star Nebulizer System, an approved device for aerosol inhalation. The subject will use the nebulizer until the HS dose is completed. All subjects in this study will be pretreated with albuterol prior to HS inhalation.

MCC procedures:
Prior to each MCC study, a transmission Co57 scan will be performed to define the lung boundaries, to assign regions of interest, and to normalize these regions for lung volume differences (**Fig 2**). A rectangular phantom containing the radioisotope Co57 (< 25 mCi) will be placed in front (5cm) of the subject sitting with his/her back to the gamma camera for 30 seconds. The transmission scan has been used by us (e.g. 05-2358, 08-0795, 06-1016, 13-1605, 15-938) and others to provide a delineation of lung boundaries for assessing regional deposition /clearance of the inhaled radioaerosol. Prior to the transmission scan on each study day we will place 2 spot markers of Americium241 (0.9 microcurie (uCi) each, gamma 66 KeV) on the upper and lower back of each subject during scanning (both Tc99m-SC deposition/retention and Co57 transmission). With dual isotope imaging, these spot markers will allow alignment of images for more accurate determination of regional deposition/retention. These very low radiation sources have been obtained from commercially available home smoke alarms. The placement of these markers will be determined to be outside the lung field during the transmission scan. Their location

will be marked in semi-permanent ink for later placement during Tc99m deposition/retention scans. The shielded side of this source will be placed/taped onto the subject's skin.

Radiolabeled Tc99m-sulfur colloid will be delivered using a modified Pari-LL nebulizer (MMAD 9.5 um). This is a closed delivery system that produces 80 ml/sec air flow, and therefore limits the inspiratory flow rate to this value. While seated in front of a gamma camera subjects will perform single inhalations lasting ~10 seconds each from the delivery system, and will exhale at 500 ml/sec (using feedback from a flow meter in the breathing circuit). Approximately 5 of these inhalation maneuvers will be required to deposit an adequate isotope dose to the lung. Subjects will be allowed to breathe normally (off the nebulizer) in between each inspiratory maneuver. Each volunteer will practice these maneuvers prior to the actual radioaerosol inhalation to guarantee his/her proficiency. The activity of Tc99m-SC loaded in the nebulizer will be adjusted to provide an estimated 40 uCi deposited in the lung for each MCC scan. A single crystal detector will be placed at the subject's back during inhalation to monitor dose to the lung. Total inhalation time should be less than 5 minutes in all cases. Immediately following isotope inhalation, the subject will gargle and drink water to clear activity that deposited in the mouth into the stomach. The subject will then (within a minute of final inhalation maneuver) be seated in front of a large-field-of-view gamma camera to begin acquiring particle retention images.

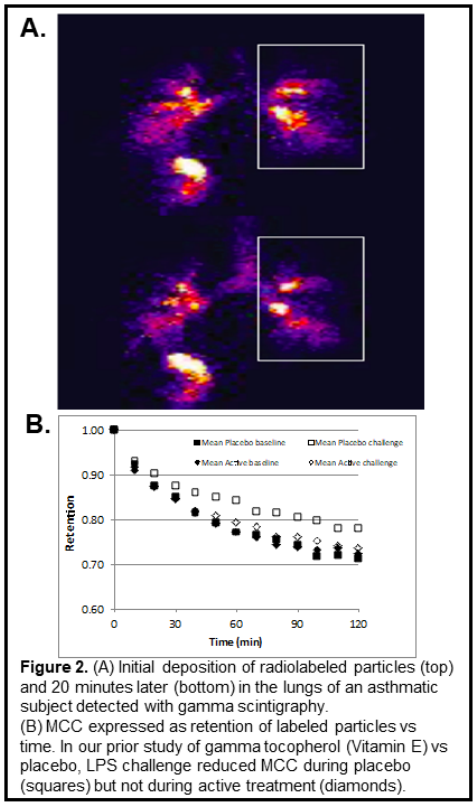

Figure 2. (A) Initial deposition of radiolabeled particles (top) and 20 minutes later (bottom) in the lungs of an asthmatic subject detected with gamma scintigraphy. (B) MCC expressed as retention of labeled particles vs time. In our prior study of gamma tocopherol (Vitamin E) vs placebo, LPS challenge reduced MCC during placebo (squares) but not during active treatment (diamonds).

For the baseline MCC measurement, the gamma image capture will begin immediately and will capture continuously for the first 34 minutes. Thereafter, 2 consecutive 2-minute images will be obtained at the start of every 10-minute period until 2 hours have passed. For visit 3/5, participants will first inhale Tc99m-SC as described above then sit in front of the gamma camera for 4 minutes during which images of the lung will be continuously captured. While still sitting in front of the gamma camera, they will inhale HS. Image capture will be continuous during HS inhalation and for the next 34 minutes, after which 2 consecutive 2-minute images will be obtained at the start of every 10-minute period until 2 hours have passed. The subject will also return the day (visit 4/6) following each MCC measurement to sit in front of the gamma camera for 30 minutes to obtain images of the lungs to visualize retention of Tc99m-SC particles. No Tc99m-SC will be administered at this visit. Spirometry, symptom questionnaire, and vital signs will be collected prior to discharge on the follow up day.

Hypertonic Saline Induced Sputum procedure:
Prior to sputum induction, subjects will have spirometry measured for FEV1 and FVC values. Next, an ultrasonic nebulizer filled with 20 cc of 3% hypertonic saline (inhalation grade for respiratory use only, 3% NaCl) will be set to the maximum output setting and turned on. The subject will be instructed to latch his/her mouth onto the nebulizer mouthpiece and breathe normally (i.e., tidal breaths) for 7 minutes as the saline is nebulized through the mouthpiece in a jet stream and inhaled. The nose will not be occluded for this procedure. The subject will be encouraged to come off the mouthpiece at any time to cough if a sputum sample from the lower

airways (i.e. not from the back of the throat) is ready for expectoration. Prior to expectoration, subjects will be asked to blow their nose, rinse their mouth with water, and clear their throat to avoid the inclusion of non airway fluid samples. The sample will be expectorated into a sterile specimen jar and capped.

Following the measurement of FEV1 after the first 7 minute inhalation period, the concentration of saline will be increased from 3% to 4%, provided the FEV1 decrement is < 10% from the post bronchodilator value. If the FEV1 falls between 10 20% of the post bronchodilator value, the test will proceed but the concentration of saline will remain the same. If the FEV1 falls by > 20% or if troublesome symptoms occur, the nebulization will be discontinued, and albuterol will be immediately available if necessary to relieve symptoms. The same procedure will be followed for the final 7 minute inhalation period using 5% hypertonic saline provided the FEVl safety parameters described above have been met. The nebulization is stopped after a total of 21 minutes or earlier if a sputum sample of good quality is obtained (i.e. visible sputum plugs). Subjects may request early termination at any time the procedure becomes intolerable.

5.1 Efficacy Evaluation (if applicable)
To determine the efficacy of HS for accelerating MCC associated with 3% vs. 7% HS treatment as compared to baseline MCC in adult patients with moderate to severe asthma. MCC is measured as the average % retention of radiolabeled particles in the lung over a 120 minute measurement period.

5.2 Pharmacokinetic Evaluation (if applicable)
None

5.3 Safety Evaluations: The primary safety criteria will be change in FEV1 from baseline measures obtained on the same day. A drop in FEV1 by 10% at the 30-minute mark after HS inhalation will be considered an adverse event for patient safety and will result in withdrawal of the subject from further participation in the study.

During visit 1, subjects will be monitored for 1 hour after HS inhalation. If a subject's FEV1 is reduced by ≥ 10% from that day's baseline at the 5-minute mark, spirometry will be repeated every 5 minutes up to 30 minutes. If at the 30-minute mark FEV1 is still reduced by ≥ 10%, 4 puffs of albuterol will be administered. Albuterol 4 puffs with spacer will be repeated if the participant has symptoms, or if FEV_1 does not spontaneously return to within 5% of baseline values at the 1-hour mark.

Oxygen saturation will be monitored during the HS inhalation through the rest of the day's study procedures. Several studies have shown that the oxygen saturation drops transiently in patients with CF and COPD when they inhale a mucolytic agent. It is anticipated that asthmatic subjects will have a similar response.

Vital signs are monitored, including HR, RR, and BP. Auscultation is performed to determine if wheezing is present.

We will assess symptoms at baseline, prior to HS, and following HS whenever vital signs are measured. We will use the following symptom score questionnaire:
Symptom score (scale 0-none, 1-mild, 2-moderate, 3-severe):

Malaise____, Fatigue____, Shortness of Breath_____, Cough_____, Myalgia_____, Chills_____, Fever_____, Headache_____

Mild: Symptom minimally noticeable but would not ordinarily cause you to stop normal activities such as going to work or school and would not keep you from undertaking physical exercise.

Moderate: Symptom present but would not ordinarily cause you to stop normal activities such as going to work or school, although it might stop you from doing physical exercise ("working out")

Severe: Symptom clearly present and would cause you to consider not going to work or school or seek medical attention (such as calling a health care provider, going to student health, going to the emergency room, or taking medications for these symptoms).

HS treatment will be deferred if baseline total symptom score > 2, OR any shortness of breath or fever are reported, OR if any symptom rated as moderate (2) or severe (3). For symptom scores obtained after HS treatment, subjects will be assessed by a study physician if total symptom score >2, if ANY shortness of breath is reported, or if any symptom is rated as moderate (2) or severe (3). If following HS treatment, the participant ranks any symptom as severe (3), that participant will be excluded from further study.

6 STATISTICAL CONSIDERATION

6.1. Primary Endpoint:

change in MCC from baseline immediately after 7% HS inhalation

6.2 Secondary Endpoints

a. change in MCC from baseline immediately after albuterol inhalation. This endpoint is of exploratory interest.

b. Safety measures for HS treatment in moderate to severe asthmatics will include change in the following from baseline:

- Vital signs (HR, RR, BP and oxygen saturation)
- Spirometry (FEV1, FVC)
- Symptom Questionnaires
- Physical exam (wheezing on chest auscultation, for example)

We will also assess:

- Adverse events and Serious Adverse events
- Proportion of participants who are deemed 'intolerant' to HS (i.e. those who experience a persistent reduction in FEV1 of ≥ 10% from that day's baseline at 30 minutes post-HS, those who report shortness of breath, those who report a score of "3" for any individual symptom criterion, or those who experience oxygen saturation levels below 90% for > 1 hour).

Safety endpoints will be assessed continuously during the study period for monitoring of adverse events and individual stopping criteria. All adverse events whether or not listed in the NCI-CTCAE (see Section 9) or whether they are or are not related to study participation will be graded on a scale from 1 to 5 according to the standards in the NCI-CTCAE manual. Adverse events will be reported to the UNC IRB, the UNC DSMB, and the NHLBI project officer. The DSMB will review the protocol after 5 subjects have completed study procedures or at 6 months after enrollment begins, whichever is soonest, and then every 6 months thereafter.

Exploratory endpoints will also be assessed. These will focus on sputum cellularity, mediators in sputum (e.g. T1 cytokines: IL-1β, IL-6, IL-8, TNFα, T2 cytokines, IL-4, IL-5, IL-13), eicosanoids,

and mucins (total mucins, MUC5AC, MUC5B).

6.3 Statistical Methods

Our analytical plan was developed in collaboration with Dr. Haibo Zhou, the biostatistician for the CEMALB who will oversee all statistical analyses. The primary analysis of interest pertains to the difference between MCC measured at baseline and MCC measured during and after 7% HS challenge. The MCC measurements that define this difference are defined in section 5, and this difference has a unit of absolute percentage points. We will perform descriptive analysis of this difference; all point estimates will be tabulated along with their 95% confidence intervals. As we have assumed the sufficient underlying normality of MCC measurements, we will test the null hypothesis of no difference between baseline MCC and MCC immediately during and after 7% HS, against the alternative hypothesis of some difference, using a paired two-sided one-sample t-test with a 5% type I error rate. That is, if we let $\mu_{\Delta 3\%}$ denote the mean difference between MCC measured at baseline and MCC measured during and immediately after the albuterol treatment, i.e. we will test the following hypothesis:
H_0: $\mu_{\Delta albuterol}=0$ vs. H_1: $\mu_{\Delta albuterol}\neq 0$.

If the observed p-value is above 0.05, the test will be interpreted as being inconclusive. Following this test, we will perform sensitivity analyses to assess how realistic the assumption of normality was, and how much it may have impacted our conclusions. In particular, we will examine normal q-q plots and other descriptive tabulations, and we will examine the results of a Wilcoxon Signed Rank test. Should extreme or questionable observations arise in the study, we will also examine regression diagnostics to assess the influence of how those data were handled. The secondary analysis, which is of exploratory interest, pertains to the difference between MCC measured at baseline and MCC measured during and immediately after the 7% HS challenge. We will analyze this difference in a similar manner, i.e we will test the hypothesis
H_0: $\mu_{\Delta 7\%}=0$ vs. H_1: $\mu_{\Delta 7\%}\neq 0$.
We will report the proportion of enrolled patients who do not tolerate HS at baseline, along with the 95% confidence interval of this proportion.

The effect of albuterol treatment on MCC will be assessed as a secondary endpoint, using the same techniques as described above for studies of 7% HS. We will also compare results following treatment with albuterol to those observed after 7% saline as an exploratory outcome. To do this analysis, we will assess the difference of the outcomes of these treatments within each individual and conduct appropriate paired t-tests. If these differences are not normally distributed, we will do proper transformation of the data to achieve a normal distribution. We will also conduct Wilcoxon signed rank tests in when data are not normally distributed.

6.4 Sample Size and Power

The primary endpoint of this study is the difference between baseline MCC and MCC immediately after HS administration. In reviewing MCC data under baseline conditions in patients who would fit criteria for moderate asthma, Burbank *et al.* found a mean average % retention of 21% with a standard deviation of 9% (16). We wish to detect a difference of mean MCC equal to 7%. Given the paired nature of the study, we believe 10% to be a reasonable upper bound of the standard deviation of this difference in means. Assuming $\beta=0.9$ and $\alpha=0.05$, a sample size of n=21 would be adequate. We will inflate this by 30% to account for

attrition with multiple study visits, taking the recruited sample to n=28.

6.5 Interim Analysis
Interim analyses of efficacy of 7% HS will be performed.

7. STUDY INTERVENTION (drug, device or other intervention details)

- **Description:** Sodium chloride 7% solution, which is commercially available as Hypersal® (PARI Respiratory Equipment, Inc, Midlothian, VA).
- **Receipt/Storage:** HS will be delivered and stored within the CEMALB.
- **Packaging/Labeling:** This is an open label study.
- **Dosing:** 4mL vial of HS is nebulized and inhaled until the dose is complete, typically 15 minutes.
- **Treatment compliance and Adherence**: Subjects are observed during all dosing periods.
- **Drug Return/Destruction**: NA
- **Drug Accountability:** NA

8. STUDY INTERVENTION ADMINISTRATION (if applicable)

- **Randomization procedures: N/A**
- **Blinding procedures:** This is an open label study.

9. SAFETY MANAGEMENT

- **Definition of Adverse Event (AE) and Serious Adverse Event (SAE)**
  - An adverse event for a given volunteer will be defined as failure of any of the safety criteria outlined above. Any decrease in lung function as outlined above will be considered an adverse event. Any symptoms that induce a volunteer to seek medical attention from any provider within 96 hours of a study visit will be considered an adverse event. A serious adverse event will be defined as any event that requires hospitalization or results in life threatening illness or injury, permanent (or likely to be permanent) illness or injury, or death if these events occur within 96 hours of a study visit (or if the clinical scenario leading up to hospitalization, illness, injury or death begins within 96 hours of a study treatment visit).

Grading criteria

- In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.
- All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates 'or' within the description of the grade.):
- Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
- Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using he toilet, taking medications, and

not bedridden).
  - Grade 4 = Life-threatening consequences; or urgent intervention indicated.
  - Grade 5 = Death related to AE.
- **Adverse Event/Serious Adverse Event reporting procedures:** AE's and UP's will be reported to the IRB. The IRB, DSMB, and funding agency will be notified of any SAEs. The DSMB will review progress after the first 5 participants have completed the protocol or at 6 months after enrollment begins, whichever is soonest, and then every 6 months thereafter.
- **Medical Emergency procedures:** There is a study physician available for all inhalation procedures. A code cart and Automated External Defibrillator (AED) are available in the event of a cardiopulmonary event. Albuterol, both inhalers and nebulizers, are available in the event of bronchospasm. If a subject has an event that is does not immediately respond to care in the research lab, he/she will be transported via EMS to UNC Healthcare for treatment.
- **Data Safety Monitoring Plan:** Data is initially recorded on paper documents, and the documents are maintained in a binder which is kept in a locked office within the EPA Human Studies facility. The EPA HSF is a secure facility with a guarded entrance and requires identification for entry. This data is then entered into REDCap by an initial data entry person, and confirmed by a second user.
- **Potential Risks:**

Potential Risks of Hypertonic Saline Inhalation:

Inhalation of hypertonic saline carries a small risk of bronchospasm, which may lead to the onset of asthma symptoms.

Potential Risks of Spirometry:

Potential risks include possible lightheadedness or wheezing.

Potential Risks of Mucociliary clearance scan:

The radiation risks for the MCC scan, including the Cobalt 57 transmission scan and the Americium 241 disks, which are used as fiducial markers is approximately 133 mRems. Adults in the New York City area receive about 300mRems per year in natural radiation exposure.

Potenial Risks of Sputum Induction:

- Sputum induction may induce cough, chest tightness or bronchospasm **Protections Against Risk**:

Protections to Minimize Risk of Hypertonic Saline Inhalation:

A CEMALB physician is present in the building at all times during HS inhalation. All asthmatic subjects are pretreated with albuterol inhalation prior to hypertonic saline administration. Additional albuterol will be given after HS inhalation if FEV declines by ≥ 10% from that day's baseline at the 30 minute spirometry measurement, if the participant develops symptoms of an asthma attack such as wheezing, or if the participant requests albuterol treatment.

Protections to Minimize Risk of Spirometry:

Subjects will be seated in a non-rolling chair when spirometry is performed and standard methodology conforming to the American Thoracic Society guidelines for measurement of spirometry will be used. Subjects will be instructed to notify the study staff if they feel lightheaded, and albuterol will be available in the event the subject experiences any unanticipated bronchoconstriction.

Protections to Minimize Risk of Mucociliary clearance scan:

Radiation history is collected. Any subject who will exceed safe annual exposure limits will not be enrolled.

Protections to minimize Risk of Sputum Induction:

Subjects will have been given 4 puffs of albuerol in the 2-3 hour time period prior to sputum induction. In the event of bronchospasm associated with the induction, albuterol will be

immediately available for rescue.

10. DATA COLLECTION AND MANAGMENT

- **Monitoring Plan:** We will use RedCap for data management. The data will be entered by one person (typically the coordinator or, for lab analysis, a lab staff member) and then checked by a second person. The data is not marked "complete" until the 2nd person verifies the entry. REDCap creates a data dictionary when the database is established, this is used as the codebook.
- **Database documentation**: All databases using equipment generated have a date and user attached to the electronic file. Data will be linked with a codebook (i.e. sample ID) using Excel. A second entry is done manually in a lab notebook, which includes date, samples assayed, assay used, any issues (like missing samples/data), reference to where data is stored, and a printout of the data (all as hardcopies in a lab notebook). Adherence to the codebook is ensured by having a second individual to check the entries. The PI takes responsibility for data management computations.
- **Case report forms:** Case report forms will be developed by the study team, using templates from previous studies. These forms are maintained by study coordinators, and reviewed by investigators as needed.
- **How will confidentiality be maintained:** Subjects will be issued a subject number when they enroll into the study, and this number will only be used to label samples. All PHI will be maintained by the study coordinator or the investigators and will be kept in locked areas when not in use. The identifiers that go into REDCap will only be accessible to those who need them for their job, specifically study physicians, investigators and coordinators. Subjects sometimes undergo procedures – such as sitting in front of the gamma camera – at the same time, however other than basic introductions, nothing about one subject is disclosed to the other subject.

11. RECRUITMENT STRATEGY

Subjects will be recruited by posters and informational emails at UNC. The study will be posted on the JOIN THE CONQUEST website, designed for recruiting subjects into research studies. “Dear Doctor” letters will be sent to pulmonary and family practices doctors who may have patients who are interested in volunteer to participate in the study.

12. CONSENT PROCESS

- **Describe the procedure that will be used to obtain informed consent/HIPAA authorization and assent (if applicable):** The study will be described in detail to the subject, including why the study is being conducted, the medication being studies, the risks and benefits, and what is expected of the subject. The subject will be given adequate time to read the consent, and consent will be obtained prior to any study procedures. Consent may take place on a separate day from the baseline procedures.
- **Who will obtain consent:** Consent will be obtained by a study coordinator or a study physician.
- **Where will consent process take place:** The consent will take place at the UNC CEMALB, located in the Human Studies Facility of the US EPA on Mason Farm Road in Chapel Hill.
- **How will investigator assure that subjects comprehend the nature of the study, procedures, the risks and benefits:** The subject will be encouraged to ask questions

regarding the study and procedures. Open ended questions will be asked of the subject to solicit correct responses, to help ensure that the subject understands the study commitment, procedures and risks and benefits

13. References

1. Kuyper LM, Pare PD, Hogg JC, Lambert RK, Ionescu D, Woods R, Bai TR. Characterization of airway plugging in fatal asthma. *Am J Med* 2003; 115: 6-11.
2. Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* 2002; 57: 677-682.
3. 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: 32-38.
4. Alexis NE, Bennett W, Peden DB. Safety and benefits of inhaled hypertonic saline following airway challenges with endotoxin and allergen in asthmatics. *J Asthma* 2017; 54: 957-960.
5. Bennett WD, Wu J, Fuller F, Balcazar JR, Zeman KL, Duckworth H, Donn KH, O'Riordan TG, Boucher RC, Donaldson SH. Duration of action of hypertonic saline on mucociliary clearance in the normal lung. *J Appl Physiol (1985)* 2015; 118: 1483-1490.
6. Daviskas E, Anderson SD, Gonda I, Eberl S, Meikle S, Seale JP, Bautovich G. Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. *Eur Respir J* 1996; 9: 725-732.
7. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med* 2006; 354: 241-250.
8. Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, Marks GB, Belousova EG, Xuan W, Bye PT, National Hypertonic Saline in Cystic Fibrosis Study G. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006; 354: 229-240.
9. Forouzan A, Masoumi K, Delirrooyfard A, Asgari Darian A, Mokhtar Gandomani L. Effect of Nebulized 3% Hypertonic Saline with Salbutamol on Management of Acute Asthma in Outpatient Adults: A Double-blind, Randomized Clinical Trial in Emergency Department. *Iran J Allergy Asthma Immunol* 2017; 16: 370-377.
10. Robinson M, Hemming AL, Regnis JA, Wong AG, Bailey DL, Bautovich GJ, King M, Bye PT. Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. *Thorax* 1997; 52: 900-903.
11. Robinson M, Regnis JA, Bailey DL, King M, Bautovich GJ, Bye PT. Effect of hypertonic saline, amiloride, and cough on mucociliary clearance in patients with cystic fibrosis. *Am J Respir Crit Care Med* 1996; 153: 1503-1509.
12. O'Riordan TG, Zwang J, Smaldone GC. Mucociliary clearance in adult asthma. The American review of respiratory disease. 1992;146(3):598-603. doi: 10.1164/ajrccm/146.3.598. PubMed PMID: 1519834
13. Shah RV, Amin M, Sangwan S, Smaldone GC. Steroid effects on mucociliary clearance in outpatient asthma. J Aerosol Med. 2006;19(2):208-20. doi: 10.1089/jam.2006.19.208. PubMed PMID: 16796545
14. Sood N, Bennett WD, Zeman K, et al. Increasing concentration of inhaled saline with or without amiloride: effect on mucociliary clearance in normal subjects. Am J Respir Crit Care Med 167: 158–163, 2003.
15. Button B, Cai LH, Ehre C, Kesimer M, Hill DB, Sheehan JK,, Boucher RC, Rubinstein M. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science 24: 937–941, 2012.
16. Burbank AJ, Duran CG, Pan Y, Burns P, Jones S, Jiang Q, Yang C, Jenkins S, Wells H, Alexis

N, Kesimer M, Bennett WD, Zhou H, Peden DB, Hernandez ML. Gamma tocopherol-enriched supplement reduces sputum eosinophilia and endotoxin-induced sputum neutrophilia in volunteers with asthma. J Allergy Clin Immunol 2017 Jun 20 (article in press).

14. APPENDIX

Study Personnel:

Role in Project	Name and Address	Title
Principal Investigator	David B. Peden, MD, MS CEMALB, 104 Mason Farm Rd CB# 7310 Chapel Hill, NC 27599-7310	Professor of Pediatrics & Director, Center for Environmental Medicine, Asthma and Lung Biology
Co-investigator	Richard Boucher, MD 7008 Marsico Hall Chapel Hill, NC 27599-7248	James C. Moeser Eminent Professor of Medicine & Director of the Marsico Lung Institute
Co-investigator	William Bennett, PhD CEMALB, 104 Mason Farm Rd CB# 7310 Chapel Hill, NC 27599-7310	Professor of Medicine, Director of Mucociliary Clearance and Aerosol Research Lab, CEMALB

Summary of Study Procedures:

Study Procedures	Visit 1: Baseline MCC Visit	Visit 2: (24h f/u)	Visit 3: Albuterol and MCC	Visit 4: (24h f/u)	Visit 5: Acute 7% HS	Visit 6 (24h f/u)	Study Completion visit
Time between visits	*1-6 weeks*		*1-6 weeks*		*5-10 days*		
Informed Consent	X						
History, med review	X		X		X		X
Review history, AEs	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Symptom Questionnaire	X	X	X	X	X	X	X
Physical exam	X		X		X		
Urine pregnancy test	X		X		X		
Spirometry	X	X	X	X	X	X	X
Albuterol	X		X		X		
Nebulized HS					X		
MCC Measurement	X		X		X		
24h Lung Retention Images		X		X		X	
Induced Sputum Collection	X		X		X		