

University Hospitals Cleveland Medical Center Clinical Research Protocol

Methods to Identify and Treat Severe Asthma Patients Project 1: GSNOR Phenotyping and GSNO Challenge

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STUDY SITES

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1. BACKGROUND AND RATIONALE

S-nitrosylation signaling is relevant to a broad range of diseases, including severe asthma (1,2,4-17). Work in the Severe Asthma Research Program (SARP) and other research networks has established that asthma is remarkably heterogeneous (18, 19). The response to standard asthmatic therapies is sub-optimal in many patients. Targeting expected responders, or personalizing approach to treatment, would lead to improved outcomes and decreased treatment costs. Furthermore, since patients with asthma that is not responsive to standard therapies are highly symptomatic despite standard therapy, this subset may derive particular benefit from a personalized approach that includes clinical phenotyping, directed diagnostic testing to confirm underlying pathophysiology, and treatment directed specifically towards those findings.

The classical conceptualization of asthma as a disease of allergic inflammation is based on findings that many patients with asthma have a “Th2 high” phenotype (22) characterized by high circulating levels of IgE, eosinophils and periostin. Patients with these features are particularly amenable to exciting, new antibody-based therapies (23-25). However, many patients are not in this phenotype, and, within the generalized phenotype, there is a Gaussian distribution of response (26). Further, these antibody treatments tend to be expensive and require parenteral administration. A myriad of alternative potential targets has been identified in patients with asthma who do not respond to standard asthma therapies, ranging from high levels of airway chitinase (20) to low levels of vitamin D (21).

Our early work documenting the presence of S-nitrosothiols (SNOs) in human airways and characterizing the potent bronchodilator activity of S-nitrosoglutathione (GSNO) (2,13,44,45,50) led us to consider that in some patients asthma may represent a disorder in pulmonary SNO homeostasis, shown in Figure 1. The focus of this study is on the subset of

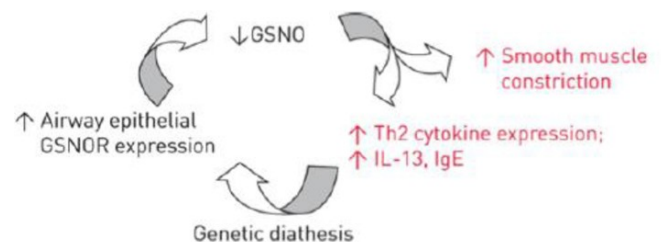


Figure 1. Dysregulated nitrosothiol homeostasis in asthma: role of increased S-nitrosoglutathione reductase (GSNOR) expression and activity

patients with asthma who have increased airway S-nitrosoglutathione reductase (GSNOR) activity (17). Increased activity of GSNOR results in a reduction in the available GSNO, resulting in increased smooth muscle constriction, and increased inflammation, as shown in Figure 1. This is a highly targetable process for which specific therapeutic agents are now becoming available (28-30). This approach can provide a paradigm for other personalized strategies. Identifying alternative approaches for patients with asthma do not respond to standard asthma therapies has important public health implications. We conservatively estimate that formulation of personalized asthma therapies, including the current study, could halve the morbidity and societal costs of asthma. This in turn would result in fewer disease-related deaths and billions of dollars in economic savings per year in the US – the current annual costs of asthma to the American economy are estimated at \$56 billion (31).

Preliminary work from the Severe Asthma Research Program suggests that patients with increased GSNOR activity and increased catabolism of the endogenous bronchodilator GSNO17 have characteristic phenotypic features (younger age, earlier onset of asthma, higher IgE) (16), but this work

needs to be expanded through a combination of mechanistic assessments and clinical testing. We have shown in preliminary work that GSNOR activity in bronchoalveolar lavage (BAL) fluid and in cell lysates from BAL fluid is higher on average in participants with asthma compared with healthy volunteers (Fig 2 A/B). We have further shown the relevance of this finding to asthma in that there is a relationship between GSNOR activity and airway hyper- responsiveness, a hallmark of asthma. Figure 2C shows the significant linear association between GSNOR activity and the concentration of methacholine that provokes a 20% fall in FEV₁ (Forced Expiratory Volume in 1 Second) in participants with asthma but not in healthy volunteers. While we have shown that GSNOR activity is higher on average in participants with asthma compared with healthy volunteers, activity levels are quite variable across the spectrum of asthma severity (Fig 3). Accurately identifying patients with asthma who have elevated GSNOR activity levels for targeted therapies is an essential next step.

In our preliminary data shown here, GSNOR activity was measured directly using bronchoscopic techniques to collect BAL fluid and directly measure activity levels. While our experience in the Severe Asthma Research Program shows that bronchoscopies can be done safely in participants with asthma (3), it will be important to develop non-invasive methodology to identify patients with asthma and elevated GSNOR activity in order to make it more practical and feasible to test and use targeted treatments. The purpose of this protocol is to confirm previous work demonstrating that participants with asthma have higher GSNOR activity levels than healthy volunteers, expand our ability to predict who will have elevated GSNOR activity levels based on clinical phenotype, and to develop non-invasive and point of care testing that can accurately identify those with elevated GSNOR levels.

2. STUDY OBJECTIVES AND HYPOTHESES

2.1 Primary Objectives

- 2.1.1 To validate and characterize the increased S-nitrosogluthathione reductase (GSNOR) phenotype in asthma by measuring enzyme expression and activity in bronchoscopic biopsies from adult asthmatics and healthy volunteers. We will corroborate our previous airway GSNOR activity data in participants with asthma across the spectrum of severity to confirm that the phenotypic features are relevant to asthma, and to have a validated GSNOR measure against which to compare the results of the GSNO challenge test in the same patients.
- 2.1.2 To validate non-invasive methodology to identify participants with asthma who have elevated airway GSNOR activity using inhaled GSNO challenge followed by test the hypothesis that inhaled GSNO challenge followed by Fractional Exhaled Nitric Oxide (FeNO) measurements will confirm the diagnosis of increased GSNOR activity in the airways of patients with SA. We will use FeNO as a measure of inflammatory biochemistry. This will provide a diagnostic test for patients suspected clinically of enhanced GSNOR activity.

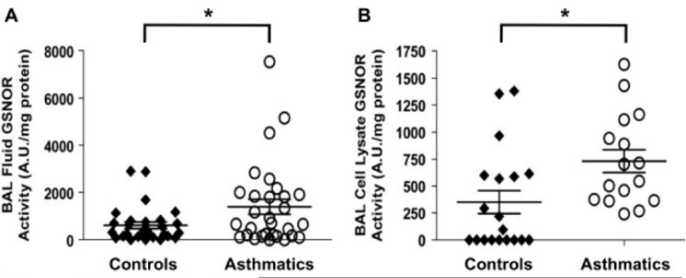


Figure 2. A. Activity of S-nitrosogluthathione reductase (GSNOR) in bronchoalveolar lavage (BAL) fluid was significantly higher in subjects with asthma compared with healthy volunteers (p=0.03). B. GSNOR activity was also higher in BAL cell lysates from subjects with asthma compared with healthy volunteers (p=0.02). C. Regression analysis showed a significant association between the concentration of methacholine that provoked a 20% decrease in FEV₁ (PC₂₀) and BAL cell lysate GSNOR activity in subjects with asthma.

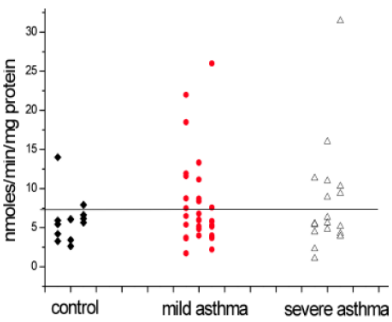


Figure 3. S-nitrosogluthathione reductase (GSNOR) activity levels in the bronchoalveolar lavage (BAL) cell lysate fluid was higher in subjects with non-severe and severe asthma compared with controls, but showed variability that was not associated with asthma severity.

If bronchoscopies cannot be consistently performed due to viral infection crisis regulations, the primary objective will be:

2.1.1.1 Measure the AUC difference in FeNO over time after inhalation of GSNO between patients with disease and those without.

2.2 Hypotheses

2.2.1 Elevated GSNOR activity ($> 7.5 \text{ mmol} \times \text{min}^{-1} \times \text{ug protein}^{-1}$) will be present in approximately 40% of participants with asthma irrespective of asthma severity compared with $<10\%$ of healthy volunteers.

2.2.2 Participants with asthma who have elevated airway GSNOR activity can be reliably identified by their FeNO profile after inhaled GSNO challenge.

3. STUDY DESIGN

This is a three to four visit study, spanning approximately four to twelve weeks, in which participants with asthma and healthy volunteers will undergo screening, baseline characterization, a research bronchoscopy, and a non-invasive challenge test with inhaled GSNO followed by repeated measurements of airway function and inflammation.

All participants will be screened for eligibility (Visit 1). Participants with asthma will undergo extensive disease characterization similar to characterization in the Severe Asthma Research Program (Visit 1) (3). During the research bronchoscopy (Visit 2), endobronchial biopsies (for histology), BAL fluid (for biochemical analysis including measurement of GSNOR activity), and brush biopsies (for airway pH measurements, cell culture and mRNA measurements) will be obtained and processed as described in current SARP protocol.

Participants will return after the research bronchoscopy for a non-invasive test, inhaled GSNO challenge, which we hypothesize will identify those with elevated BAL fluid GSNOR activity levels (defined as $> 7.5 \text{ mmole} \times \text{min}^{-1} \times \text{ug protein}^{-1}$) (16). At this visit (Visit 3), a baseline FeNO measure is obtained then the participants will inhale 2.5 ml of a nebulized 10 mM GSNO solution. Serial measurements are obtained every 10 minutes for 60 minutes post-exposure. Oxygen saturation and heart rate will be continuously monitored using pulse oximetry; additional vital signs will be measured at set intervals of 15 minutes. Baseline FeNO values, rate of rise, and duration of Nitric Oxide (NO) elevation will be related back to GSNOR activity as described in the analysis plan). Investigators and research staff conducting the non-invasive diagnostic inhaled glycine buffer challenge test will be blinded to lab data and analysis from the bronchoscopy and the participant's airway GSNOR phenotype. Data will be blinded by database users having various levels of access.

Crossing Over from Airway pH Phenotyping Study (Project 2)

Participants with asthma who have completed Project 2 may be eligible to enter this study:

- Screening data from Project 2 will be applied to this study unless it's been more than 56 days since the last study visit for Project 2. If it's been greater than 56 days, the participant will have a screening visit before the inhaled GSNO visit.
- The bronchoscopy will not be repeated.
- Depending on which study is completed first, visits in the schedule of events may appear to be completed out of numerical order.

This study began before the COVID-19 global pandemic, but is still taking place amid the crisis. Many institutions have implemented viral infection crisis contingency plans for standards and practices which are generally more strict and rigid than pre-crisis practices, for the safety of employees and the public;

these might impact study site availability. While institutions are phasing back into previously-held standards, practices, timelines to return to pre-crisis availabilities are unknown. Therefore, the design of this protocol includes various options to accommodate subjects who enroll while there are any level of viral infection crisis restrictions in place, as well as options for those who enroll during times when there are no viral infection crisis restrictions in place.

All study visits and procedures, no matter where they take place, will be using all the same equipment as originally intended and approved.

The study team has considered a no-touch approach, but have resolved the study cannot produce ample or accurate results exclusively from no-touch interactions with subjects.

4. SELECTION, ENROLLMENT, AND PROCEDURES

We will be recruiting and studying 50 participants with asthma and 10 healthy human control participants with no pre-existing pathologic conditions. For clarity we have split the inclusion criteria for the two cohorts:

4.1 Inclusion Criteria

Participants with Asthma

- a) Adult males or females age ≥ 18 and ≤ 50 years at the time of enrollment
- b) Non-smoker
- c) Physician diagnosis of asthma for at least one year
- d) FEV₁ bronchodilator reversibility $\geq 12\%$ or methacholine PC₂₀ ≤ 16 mg/ml or PD₂₀ ≤ 400 mcg (historical methacholine data from previous NIH trials including SARP and AsthmaNet will be allowed)
- e) Baseline post maximal bronchodilator FEV₁ $>55\%$ predicted

Healthy Volunteers

- a) Adult males or females age ≥ 18 and ≤ 50 years at time of enrollment
- b) Non-smoker
- c) No history of asthma, chronic obstructive pulmonary disease (COPD), or other chronic lung disease
- d) No history of severe allergic/atopic disease requiring immunotherapy or immunomodulators

4.2 Exclusion Criteria

General (applying to all participants)

- 1. > 5 pack year smoking history
- 2. Body mass index (BMI) > 45
- 3. Unable to perform repeatable consistent efforts in pulmonary function testing
- 4. Individuals with prior diagnosis of vocal cord dysfunction or an anatomical anomaly that would increase the risks associated with the bronchoscopy procedure
- 5. Prior diagnosis of cystic fibrosis, COPD, or other additional lung disease that in the investigator's opinion would make participant unsuitable for study participation
- 6. History of premature birth before 35 weeks gestation
- 7. Planning to relocate away from the clinical center (Cleveland, Ohio or central Indiana) area before study completion
- 8. Lack of reliable communications channel (hard-wire phone, cell phone, email for follow-up contacts after bronchoscopy)

9. Allergic to anesthetic medication(s) that would prevent participation in the study's bronchoscopy (e.g., lidocaine)
10. Blood pressure parameters outside the normal range of 90-180 mm Hg systolic and 50-100 mm Hg diastolic at time of screening
11. Individuals with diabetes mellitus (type 1 or type 2)
12. Individuals with renal failure or creatinine > 1.8 mg/dl at time of screening
13. Individuals who are pregnant, breastfeeding, or are unwilling to use a medically acceptable method of birth control (as indicated on the Birth Control Methods Reference Card) from the time of consent until the end of the study to avoid pregnancy
14. Individuals who report additional chronic diseases requiring medication of the heart, lungs, kidney, liver, brain, etc., or afflicted with any acute or chronic pathology that in the opinion of the screening physician makes them unsuitable for study such as coronary artery disease
15. Asthma exacerbation requiring oral corticosteroids within the previous 30 days (can be rescreened)
16. More than 3 exacerbations within the past 6 months
17. Intubated for asthma within the past 12 months
18. Respiratory or other infection requiring systemic antibiotics within the previous 14 days (can be rescreened)
19. Current use of a vitamin K antagonist (warfarin) or other anticoagulant (e.g., heparin, clopidogrel, enoxaparin or dalteparin)
20. Current use of beta-adrenergic blockers, tricyclic antidepressants, meperidine (or related central nervous system (CNS) agents), or nitrates
21. Unable or unwilling to withhold medications prior to certain study procedures (skin test, spirometry, methacholine challenge)
22. Inherited or acquired blood coagulation disorder, congenital methemoglobinemia, or a familial hemoglobinopathy that impacts oxygen delivery (e.g., sickle cell)
23. Any illness, condition or recent surgeries that may increase the risks associated with the study
24. Participation in any investigational drug study other than the *Airway pH Study* within the 4 week period prior to screening.
25. Any acute viral illness, including active COVID-19 infection or acute viral respiratory symptoms; can rescreen 4 weeks after positive test result.

4.3 Vulnerable Populations

The indicated vulnerable populations (patients, students, CWRU/UH employees, IU employees etc.) may become aware of the study (through advertisement, word of mouth, contact with study physicians) and may freely choose to participate as volunteers. It will be made clear that if such individuals express interest in participating, a subsequent decision to either not participate or withdraw consent prior to study completion will not impact their clinical care, class standing, or employment status. There will be no pressure placed on these vulnerable volunteers nor will any of the investigators' staff, students, etc. face any repercussions for not participating.

4.4 Study Enrollment Procedures

University Hospitals of Cleveland Medical Center

Participants with asthma will be recruited from the existing databases at our institutions and the Severe Asthma Research Program (SARP) databases, from patients in our clinics, as well as by public advertisement and ResearchMatch. Flyers are displayed in public spaces throughout the UH Cleveland Medical Center campus, as well as throughout the CASE Western Reserve University

campus. Brochures and flyers can be handed to patients in clinic. A patient will only be approached for study participation after the physician who has a treatment relationship with the patient (which may or may not be the PI) has given permission. Any of the individuals listed on the UHCMC IRB checklist (the PI, sub-investigators, or those listed as obtaining consent) may contact the patient about participation in the study. Patients will be recruited at the time of a regular clinic visit or by telephone/email. Physicians may contact their patients individually or have a designee make the contact. If a patient is interested, CWRU/UH research team members may provide (in person or by mail, fax, or email) the patient with a copy of the consent form to review. Patients will also be referred to us from physician practices outside UHCMC. The PI or research staff may provide information about the study such as a synopsis and/or consent form to outside (non-UH) physicians. Outside physicians may identify patients who may be potential participants and discuss the study with them. Patients interested in the study would be provided with our research team's contact information and the patients would contact our site directly for more information.

Obtaining medical data for outside patients:

Prior to the Screening Visit (Visit 1), research team members would ask for permission to contact the patient's physician to obtain the clinical information needed to verify eligibility. Research team members would send (by mail, e-mail or fax) the patient a Release of Information form to sign and return (by mail, email or fax). Upon receipt of a signed Release, research team members would contact the patient's physician (providing a copy of the signed release) and obtain the necessary information: history/physical, diagnostic test results, lab reports, relevant medication history. In some cases, the patient may provide the Release Form to his/her physician directly. Research team members will request a partial waiver for HIPAA Authorization in order to obtain the necessary medical records from the patient's physician prior to the Screening Visit. This will ensure that there is enough time to retrieve the records so that the participant's eligibility may be verified and enrollment can occur within the study's visit windows. The rationale for the partial waiver is that it would not be practical to have a patient travel here for a screening visit unless research staff could first assess his/her potential eligibility through medical record review. Also, the patient's completion of the Release of Information Form documents the patient's permission for this information to be shared. If, based on the medical record review, the participant does not meet eligibility and the participant will not complete a Screening Visit, the medical records obtained would be confidentially destroyed and no information related to the patient would be recorded anywhere. If the participant does complete a Screening Visit (signs consent and HIPAA Authorization) and is a screen-failure, research team members may maintain all study-related documentation with the other study records in the locked Pediatric Pulmonary Research Program Office.

The healthy volunteers will be recruited through the placement of fliers in approved locations on the campus of University Hospitals Cleveland Medical Center and the campus of Case Western Reserve University and the surrounding/adjacent facilities and neighborhoods. The fliers will be located in parts of the hospital where such individuals could be expected to visit or transit. Fliers may also be placed in the community areas bordering the university and on notice boards in the research buildings. If a healthy volunteer is interested, research team members may provide (in person or by mail, fax, or email) the patient with a copy of the consent form to review.

All participants will complete an initial screening questionnaire and characterization to establish whether the participant qualifies to participate in the study based on his or her medical history. The information obtained during this pre-screening period will be maintained in a study specific database at each site, only accessible to qualified study personnel.

Electronic media may be used to increase visibility of the study and facilitate recruitment efforts. No participants will be excluded based on race, ethnicity, education, or socioeconomic status.

Whenever possible, the default location for study visits to take place will be in the clinical settings provided for research.

Viral Infection Crisis considerations for University Hospitals of Cleveland Medical Center Site:

For times when any level of viral infection crisis restrictions are in place, and if research facilities are not fully operational, University Hospitals of Cleveland Medical Center still plans to use the clinic and hospital setting for research visits as much as possible, with the following alternative options:

1. Study team may offer that the subject can complete a procedure at an alternative outpatient facility if results can be satisfactorily sent to study team in timely manner.
2. Study team may offer that certain activities and procedures be completed virtually if possible

Indiana University

Participants will be recruited in a variety of ways. Subjects will be recruited through the use of posted flyers, social media campaigns, coordinator tabling and participation in community events, and in collaboration with organized groups or platforms, such as clinicaltrials.gov and other research-based outreach platforms that have an existing network of research volunteers or patients who may be interested in volunteering for research (e.g., All IN For Health). Additionally, participants with asthma and CF will be identified by reviewing internal clinic lists (including but not limited to clinic for CF, asthma, ENT and allergy) and daily inpatient censuses, and will be recruited from PI and Sub-I referrals, referrals from other IUH network physicians or from other Indianapolis-based physicians who are part of hospital networks or private practice physicians who have relevant patients and have been educated about the study and invited to share with their patients. Research team members will provide information to CF and asthma patients who are over 18 and to the parents of our pediatric patients who happen to have asthma during their child's clinic visits.

IU research team members will attend severe asthma clinic meetings and division meeting presentations and will enhance recruitment through our ongoing relationships with the Asthma Program Nurses and the Asthma Program Director at Riley Children's Hospital.

Viral Infection Crisis considerations for Indiana University Site:

For times when any level of viral infection crisis restrictions are in place, and if research facilities are not fully operational, Indiana University plans to use the clinic and hospital setting for research visits as much as possible, with the following alternative options:

1. If approved by Infection Control, the study may take place at any time in the CRC at IU Health University Hospitals in the negative flow rooms.
2. Study procedures may occur in two tents pitched on the grounds of the research building, adjacent to the hospital. The first tent will have a canopy, under which a subject can come for V1, sign the consent and get a COVID-19 test. The next day, the subject can return if asymptomatic and the COVID test is negative. The first tent can be used for the subject's spirometry, MBD, physical exam, blood draw and questionnaire administration. The second tent can be for privacy- for collecting a urine specimen and performing the 12-lead EKG. This two-tent system may be used for all visits, including GSNO challenge, except bronchoscopy. Equipment in both tents will be completely cleaned and disinfected between subjects. A portable air conditioner and heater will be available for the main tent for use when the temperatures are >85 degrees or < 60 degrees. Spirometry calibration will be critical. When the clinical research space is open again for enrollment, the investigators can break camp. If more than one subject is being screened, they will be scheduled so that there is a 60 minute break between subjects to allow for appropriate cleaning to take place. Equipment would be brought inside after each day's studies.

3. Study team may offer that the subject can complete a procedure at an alternative outpatient facility if results can be satisfactorily sent to study team in timely manner.
4. Study team may offer that certain activities and procedures be completed virtually if possible

4.5 Participant Numbering

Each screened participant is assigned a unique screening number in sequence.

If the participant is deemed eligible for the study and will continue to the baseline visit, a protocol specific participant number will be assigned. Once assigned, a participant number will not be reused. The participant number becomes definitive as soon as a participant begins evaluations at the assessment visit.

There should be a source document maintained at the site which links the screening number to the participant number (once assigned). This source document should be provided to all appropriate parties as soon as this is available.

4.6 Schedule of Events

Table 1. GSNOR Phenotyping and GSNO Challenge study visit schedule and procedures

Visit	V1	V1A ^c	V2	PC1	PC2	PC3	V3 ⁱ
Informed Consent / Assign Study ID ^{*+}	x						
Confirm Ongoing Consent ^{*+}		x	x				x
Review eligibility criteria ^{*+}	x	x	x				x
Demographics ^{*+}	x						
Medical History ^{*+}	x						
Viral infection test ^{k+^}	x		x ^l				
Environmental History ^{*+}	x						
Asthma Control Questionnaire (ACQ) Asthma Quality of Life Questionnaire (AQLQ) Asthma Control Test (ACT) (<i>Asthma Participants Only</i>) ^{*+}	x		x				x
Interval History ^{*+}			x				x
Concomitant Medications ^{*+}	x		x				x
Urinalysis ^{+^}	x						
Urine pregnancy test (if applicable) ^{+^}	x	x	x				x
Blood draw ^{+^} CBC with differential Comprehensive chemistry panel	x						
Blood draw- DNA/RNA testing ^{a+}	x						
Allergy Skin Testing ⁺	x ^j						
Vital signs (including height and weight) ⁺	x	x	x				x
Full physical exam ⁺	x						
Abbreviated physical exam ^{*+}			x				x
Pulmonary function testing checklist ⁺	x		x				x
Fractional Exhaled Nitric Oxide (FeNO) ^{h+}	x						x ^g
Spirometry ⁺			x ^d				
Baseline Spirometry with max bronchodilator reversibility testing ⁺	x						x
Review/record historical Methacholine Challenge Test (MCT) if applicable (<i>Asthma Participants Only</i>) ^{*+}	x						
12 lead Electrocardiogram ⁺	x						
Chest X-Ray ^{b^}	x						
Methacholine Checklist ^c		x					
Methacholine Challenge Test (MCT) (<i>Asthma Participants Only</i>) ^c		x					
Schedule bronchoscopy/provide instructions ^{m*}	x	x					
Pre-bronchoscopy checklist [*]			x ⁿ				
Bronchoscopy consent (clinical procedure consent)			x				
Bronchoscopy checklist			x				

Visit	V1	V1A ^c	V2	PC1	PC2	PC3	V3 ⁱ
Bronchoscopy Airway gas samples Airway pH measurement Additional brushings and biopsies Bronchoalveolar lavage			x				
Post bronchoscopy assessment			x ^e	x	x	x	
Inhaled GSNO challenge test ⁺ Administer GSNO Continuously monitor HR and pulse oximetry Measure and record BP and RR q 15 minutes							x
Adverse Events [*]	x	x	x	x	x	x	x

^a Blood draw for DNA/RNA testing is optional for participant.

^b Historical chest x-ray result within the previous year is acceptable. If not available, chest x-ray may be performed and reviewed by investigator by 48 hours prior to bronchoscopy (V2). Subject can opt out of chest x-ray if viral infection crisis restrictions are still in place and subject is uncomfortable with radiology visit.

^c Visit 1A and MCT with checklist done only if: 1) participants with asthma fail to demonstrate 12% reversibility in FEV₁ (max BD testing) and there is no historical MCT available, 2) viral infection crisis restrictions do not inhibit clinic setting accessibility, and 3) Subject is comfortable entering clinical setting even while viral infection crisis restrictions may be in place.

^d Spirometry performed before and after bronchoscopy at V2

^e Post bronchoscopy assessment completed in the post procedure monitoring area prior to discharge and by telephone the evening of the bronchoscopy visit. Assessment completed by phone on days following bronchoscopy.

^g FeNO is measured at baseline and then every 10 minutes after inhaled GSNO nebulization for 60 minutes.

^h FeNO will be measured prior to any other assessments of lung function to be done at the same visit

ⁱ For participants enrolling in this study within 1 to 3 months of completing the Airway pH study, participants will have a modified screening and only participate in Visit 3. Consent will be obtained. The modified screening will consist of a full physical exam and the following Visit 3 assessments to confirm eligibility: questionnaires (if applicable), interval history, concomitant medications, urine pregnancy test (if applicable), vital signs (including height and weight), and adverse event review.

^j Allergy Skin Testing will be initiated after all assessments of lung function have been completed at Visit 1

^k Viral infection testing requirement dictated by each institution's requirement. Each site will comply with institutional requirements for research and procedures. If required, available locations for testing will be provided, or the study team will provide subject with institutional-approved viral infection test.

^l Subject must follow any institutional instructions required prior to bronchoscopy

^m May be done by phone up to 48 hours prior to V2

^{*} Virtual – optional in addition to clinical setting

⁺ Research tent – optional in addition to clinical setting (IU only)

[^] Alternative outpatient facility – optional, if possible; results to be sent to research team, in addition to clinical setting

Viral infection crisis restrictions considerations

If at any time during the study, there are viral infection crisis restrictions at either institution, the following procedures may be optional or unavailable:

Methacholine Methacholine Challenge Test can only be completed in the clinical setting; if a clinical setting is not available during crisis restrictions, subjects may be forced to opt out. If a clinical setting is available, but a subject does not feel comfortable entering a clinical building due to viral infection risks, they may opt out of the Methacholine Challenge Test.

The Methacholine Challenge Test serves as a tool to maximize the inclusion of subjects – if a subject does not have a diagnosis of asthma, they would take the Methacholine Challenge to confirm or rule out eligibility based on Methacholine Challenge Test results. It is possible that a subject may need the Methacholine Challenge Test to be considered eligible, but will not be able to complete the challenge test due to clinical setting availability or due to subjects' level of comfort with the clinical setting, and may therefore be considered ineligible because of not being able to complete it. The study team anticipates this will not be a common occurrence.

Chest X-Ray During viral infection crisis restrictions, a chest x-ray may be completed at an alternative radiology outpatient facility if results can be sufficiently provided to the study team, or it may be completed at the site's radiology facility. If the subject is uncomfortable entering a radiology facility due to viral infection risks, they may choose to opt out of completing the chest x-ray part of the study.

Bronchoscopy Bronchoscopies may only be completed at the hospital. If research bronchoscopies are not permitted at the institution, a subject may choose to defer the bronchoscopy for up to 1 year, or may be forced to opt out due to the availability. If research bronchoscopies are permitted at the institutions, but the subject does not feel comfortable entering the bronchoscopy suite, they may choose to defer the bronchoscopy for up to 1 year or may choose to opt out of the bronchoscopy entirely. If a subject chooses or is forced to opt out of the bronchoscopy, their study data set may be considered complete without bronchoscopy; it is possible that only a subset of subjects may complete bronchoscopies and therefore have bronchoscopy data. Additionally, if a subject is allergic to anesthetic medications (e.g., lidocaine) and they opt out of the bronchoscopy, they may continue in the study.

Outside times of viral infectious crisis restrictions, subjects who do not wish to complete any of these procedures will not be eligible to continue in the study or will be withdrawn.

4.7 Description of Procedures and Evaluations

4.7.1 Description of Study Visits

There are three to four in-person visit opportunities, two possible unscheduled visits, and three phone call assessments (Safety Phone Calls). Details about procedures at each visit are found below.

1. Consent, screening, and characterization (V1)
2. Methacholine Challenge Test (MCT) (V1A) – only for participants with asthma who do not demonstrate bronchodilator reversibility and do not have historical MCT results available
3. Research bronchoscopy (V2)
4. Safety phone calls (PC1, PC2, PC3)
5. Inhaled GSNO challenge testing (V3)
6. Unscheduled visits are being included in case the participant needs to return to complete study activities (e.g., the participant forgot to withhold allergy medications on the day of allergy testing).

The following guidelines should be used for scheduling participants:

- Visit 1 = Day 0
- Each visit must be completed within 56 days of the previous visit
- Each visit must be at least 1 day apart
 - **Exceptions:** participants who have an MCT must wait at least 2 days before returning for V2 (bronchoscopy); participants who have had a bronchoscopy must wait 7 days before completing the next study visit (whether for the same study or if crossing over)

Significant health findings found during the study will be reported to the participant to further discuss with their regular doctor.

4.7.2 Description of Procedures

Demographics/medical history/asthma history questionnaires will be administered and recorded. Demographics will include gender, race, age, education, insurance status, and socio-economic markers. Relevant medical history, including co-morbid conditions (e.g., cardiovascular, gastroesophageal reflux disease (GERD), sinus and allergic disease, obstructive sleep apnea (OSA), metabolic, inflammatory conditions), will be collected. Asthma history will include age at

onset, health care utilization and exacerbation history, history of previous treatments, and environmental exposures. For asthma participants, medical history data may be obtained from a medical chart and from participant self-report. For healthy volunteers, medical history data will only be obtained from participant self-report.

During regular times, this activity will be completed in the clinical research center. During viral infection crisis restrictions, this activity may be completed virtually or in the clinical research center (or in-tent option for IU).

Interval history questionnaires will assess for intercurrent acute respiratory or other illnesses, health care utilization for asthma and other conditions, and new diagnoses. Any reported event that meets criteria for an adverse event (AE) will also be recorded on the Clinical Adverse Event form as described below.

During regular times, this activity will be completed in the clinical research center. During viral infection crisis restrictions, this activity may be completed virtually or in the clinical research center (or in-tent option for IU).

Viral infection testing may include collection of nasopharyngeal and/or oropharyngeal swabs. Nasopharyngeal Swab: a cotton tipped swab will be inserted into the nares of the subject to the upper part of the nose.

Oropharyngeal Swab: a cotton tipped swab will be inserted into the mouth of the subject to the back of the throat.

During viral infection crisis restrictions, this activity may be completed in the clinical research center (or in-tent option for IU).

Concomitant medications will be documented for 30 days prior to screening and at every study visit, including medication name, dose, route, frequency of administration, and indication.

During regular times, this activity will be completed in the clinical research center. During viral infection crisis restrictions, this activity may be completed virtually or in the clinical research center (or in-tent option for IU). It will take place in the same way as its corresponding visit.

Electrocardiogram (ECG) A 12 lead electrocardiogram will be obtained using standard placement to screen for arrhythmias or other disqualifying cardiac conditions.

During regular times *and* during viral infection crisis restrictions, ECG will be completed only in the clinical research center (or in-tent option for IU).

Chest x-ray: A 2 view chest x-ray may be performed and interpreted in the clinical radiology department to screen for cardiopulmonary conditions that might be disqualifying. If the chest x-ray will be completed, it must be done between V1 and at least 48 hours prior to V2. If the participant has had a chest radiograph in the previous 12 months, the investigator may use that study to determine eligibility. See Schedule of Events for more information about opting out of chest x-ray.

During regular times, chest x-ray will be completed in institutional Radiology Dept. During viral infection crisis restrictions, chest x-ray may be completed only at study's institutional Radiology Dept, or at an alternative Radiology location, with results sent to research team.

Asthma control and quality of life questionnaires will be administered to asthma participants only in accordance with NIH outcomes working group recommendations and with appropriate permissions. Questionnaires will include the Asthma Control Questionnaire (ACQ) (79), the

Asthma Control Test (ACT) (78) and the Asthma Quality of Life Questionnaire (AQLQ) (80).

The ACQ is a validated asthma assessment tool that has been widely used. Six questions are self-assessments (completed by the patient), and 1 item is completed by staff (FEV% predicted). Each item on the ACQ has a possible score ranging from 0 to 6, and the total score is the mean of all responses. A change in score of 0.5 is the minimally important difference.

The ACT is a 5 item self-administered tool with a 4 week recall, scores range from 5 to 25, with scores of >19 indicating well controlled asthma, 15-19 indicating partly controlled asthma, and ≤14 indicating poorly controlled asthma. A change in score of 3 points is the minimally important difference.

The AQLQ is a 32 item tool that assesses physical and emotional impact of asthma in the domains of symptoms, activity limitation, emotional functioning, and environmental exposure. Overall QOL and each domain scores range from 1 to 7 (higher values indicating better QOL) with a minimally important difference of 0.5 (See refs 78-80).

Questionnaires will be administered which assess the clinical, environmental and medication history of the participants. In addition, questions will be asked on work environment and days lost from work/school.

During regular times, this activity will be completed in the clinical research center. During viral infection crisis restrictions, this activity may be completed virtually or in the clinical research center (or in-tent option for IU).

Vital signs and physical examinations will be performed at each study visit. Pulse oximetry, blood pressure (BP), heart rate (HR), respiratory rate (RR), height and weight will be measured. BMI will be calculated. A full physical exam will be performed by a physician at screening including general appearance, HEENT (head, ears, eyes, nose, and throat), cardiovascular, respiratory, gastrointestinal, musculoskeletal, neurologic, and skin. An abbreviated physical exam will be performed at all subsequent visits by a physician or nurse, to include general appearance, HEENT, cardiovascular, respiratory, and skin. Abdominal circumference and neck circumference may be measured as part of a physical exam.

During regular times, these activities will be completed in the clinical research center. During viral infection crisis restrictions, vital signs and full physical examinations must be completed in the clinical research center (or in-tent option for IU), but abbreviated physical examinations may be completed virtually or in clinical research center (or in-tent option for IU).

Lung function testing will include spirometry, maximum bronchodilator reversibility (spirometry post bronchodilator), MCT, and FeNO measurements. All testing will be done according to American Thoracic Society/European Respiratory Society guidelines (66,81,83). Participants will be asked to refrain from taking any short-acting bronchodilator for 12 hours before each visit and any long-acting bronchodilator for 24 hours prior to each study visit.

A. Spirometry (FEV₁, FVC, FEF₂₅₋₇₅, FEV₁/FVC, FET, and peak flow) will be measured using current reference standards (81).

During regular times *and* during viral infection crisis restrictions, spirometry will be completed in the clinical research center (or in-tent option for IU).

B. Maximum bronchodilator reversibility will be performed with spirometry to assess bronchodilator reversibility. The purpose of this test is to establish that participants with asthma have airway obstruction that is reversible by at least 12% increase in FEV₁ with

bronchodilator, and to establish the maximum reversibility by administering increasing doses of bronchodilators until the response has plateaued. Baseline spirometry is performed, then participants will initially receive 4 inhalations of albuterol from a metered dose inhaler (MDI) and valved holding chamber and spirometry will be repeated 15 minutes later. The participant will then be administered an additional 2 puffs of an albuterol MDI and spirometry will be repeated after 15 minutes. The participant will then be administered a final 2 puffs of an albuterol MDI and spirometry will be repeated after 15 minutes. The difference in the FEV₁ after all 8 puffs will be compared to the difference from baseline FEV₁. Subjects with CF will participate in a modified version of bronchodilator reversibility testing.

During regular times *and* during viral infection crisis restrictions, maximum bronchodilator reversibility may only be completed in clinical research center (or in-tent option for IU).

- C. Methacholine Challenge Test (MCT)** will be performed only in participants with asthma who do not demonstrate reversibility and with a pre-diluent FEV₁ of >50% predicted and at least 1.0 liter. A physician will be available during the challenge. Testing will be performed according to each site's standard of care. Participants will not be discharged until their FEV₁ is within 10% of their baseline FEV₁. See Schedule of Events for more information about opting out of methacholine challenge test.

During regular times *and* during viral infection crisis restrictions, methacholine challenge test may only take place in clinical research center (NOT to take place in-tent option for IU).

- D. Fractional Exhaled Nitric Oxide (FeNO)** is a non-invasive procedure that is considered to be an indirect measurement of airway inflammation. Participants will be instructed to take in a deep breath and blow air out at a constant pressure as directed by personnel. One measurement will be taken and recorded. FeNO will be measured prior to any other assessments of lung function to be done at the same visit.

During regular times *and* during viral infection crisis restrictions, FeNO may only be completed in clinical research center (or in-tent option for IU).

Allergy Skin Testing is skin prick testing of standard allergens that will be performed at visit 1 (after all Lung Function testing has been completed) per the Allergy Skin Testing MOP, including grass mix, tree mix, weed mix, mite mix, cockroach mix, mouse, cat, dog with positive and negative controls. All allergens will be applied with the Multi-Test II device using standard preparation from Greer Laboratories (Lenoir, NC). After 20 minutes, the wheal reactions are observed and recorded as being positive or negative. Please refer to the Allergy Skin Testing MOP to determine results and for specific drug withholds. If participants are unable to withhold medications for the skin prick allergy test, a blood test may be used instead.

During regular times *and* during viral infection crisis restrictions, Allergy Skin Testing may only be completed in clinical research center (or in-tent option for IU).

Venipuncture will be performed for complete blood counts and a comprehensive chemistry panel. A total of 15 ml blood will be collected for this testing. An additional (5 ml) blood sample may be collected to isolate and store DNA and RNA to evaluate human genes and how they may be related to the treatment responses (optional to the participant). For participants who are unable to withhold medications for the skin prick allergy test, we will collect an additional 5mL of blood for the blood allergy test.

During regular times *and* during viral infection crisis restrictions, venipuncture may only be completed in clinical research center (or in-tent option for IU).

Urine collection will be completed to perform urinalysis as part of the safety screening. If the participant is a female of child bearing potential, urine pregnancy tests will be conducted as outlined in Table 1.

During regular times, urine collection will be completed in clinical research center. During viral infection crisis restrictions, urine collection may be completed in the clinical research center (or in-tent option for IU), or at an alternative outpatient facility with results sent to study team.

GSNO Challenge Testing GSNO is to be manufactured under Good Manufacturing Practice conditions by an appropriate commercial entity (Ricerca Biosciences) under the IND held by James D. Reynolds, PhD. One dose of 2.5 mL 10 mM GSNO in normal saline, prepared by a qualified sterile compounding pharmacy, will be administered for this protocol as we have previously published (14). Study drug will be stored and dispensed by a local, qualified sterile compounding pharmacy.

Results to date from clinical testing of GSNO have been positive with no identified toxicities or drug-related adverse events (14). Nonetheless, out of an abundance of caution, participants scheduled for GSNO treatment will be required to have normal vital signs, pulse oximetry >90%. All participants must be free from an intercurrent illness within the previous 14 days. Participants with asthma must be free from an exacerbation of asthma in the previous 14 days, defined as increased asthma symptoms requiring an increase in short acting bronchodilator use above that established at the baseline visit for more than 24 hours or use of systemic corticosteroids.

Baseline FeNO and spirometry measurement will be recorded. One unit dose of GSNO is administered via standard nebulization. Following administration of inhaled GSNO, participants will be monitored for signs of bronchospasm (e.g., wheeze, cough, dyspnea, etc.). Serial FeNO measurements will be made every 10 minutes for 60 minutes. Heart rate and pulse oximetry will be monitored continuously. Other vital signs will be monitored every 15 minutes. Spirometry will be performed at the conclusion of the FeNO measurements. A study physician will be available to evaluate participants complaining of cough, wheezing, dyspnea, or with a decline in FEV₁ of 10% or greater from pre-challenge baseline. Inhaled beta-agonist may be given at the study physician's discretion.

During regular times and during viral infection crisis restrictions, GSNO challenge testing may only be completed in clinical research center (or in-tent option for IU).

Bronchoscopy Informed consent documentation will be reviewed and the volunteer will be reminded that their decision to participate or not in the research study is voluntary. Any decision to withdraw will not affect the volunteer's care at UH or Riley Hospital for Children at Indiana University Health now or in the future nor for CWRU/Indiana University students and CWRU/UH or Indiana University personnel will such a decision affect their class standing or employment status.

Clinical informed consent to proceed with the procedure will be obtained using standard hospital procedures and documentation. Bronchoscopies will be performed by a qualified physician in a bronchoscopy suite at both University Hospitals Cleveland Medical Center and IU Health University Hospitals once the study physician has determined the participant meets the following criteria.

A. Exclusion from Bronchoscopy for Periods from 2 days to 6 weeks

The presence of any of the following characteristics will exclude a participant from participating as a bronchoscopy volunteer. The pre bronchoscopy checklist (completed 48

hours prior to test) and bronchoscopy checklist should be used to document appropriateness for participation:

1. Events occurring within 6 weeks of bronchoscopy

- a. Hospitalization for asthma within the past 6 weeks
- b. Hospitalization for any other acute illness within the previous 6 weeks (all participants)

2. Events occurring within 4 weeks of bronchoscopy

- a. Use of antibiotics (oral, inhaled, or intravenous) for treatment of an acute respiratory condition (e.g., pulmonary exacerbation, sinusitis, pneumonia)

3. Events occurring within 2 weeks of bronchoscopy

- a. Increased oral corticosteroid use in the past 14 days, recognized as a dose which is both numerically at least twice that of baseline, and which is at least 20 mg/day greater than the baseline dose
- b. Acute respiratory illness

4. Events occurring within 48 hours before bronchoscopy

- a. Pulse oximetry demonstrating oxygen saturation <90% on room air
- b. Use of more than 16 puffs of a short acting beta-agonist per day in the past 48 hours (1 nebulizer treatment = 2 puffs)
- c. Significant increase in asthma symptoms in the past 48 hours, recognized as an increased use of short acting beta-agonists of more than 8 puffs/day (more than 8 puffs/day over baseline) (1 nebulizer treatment = 2 puffs)
- d. Significant decline in pre-bronchodilator FEV₁ from baseline obtained at screening (more than 5%).
- e. BMI > 45 kg/m² (height and weight must be measured and BMI must be calculated no more than 48 hours prior to proceeding with research bronchoscopy).
- f. Positive screening results for any active viral infection, or additional criteria according to hospital regulations.

B. Sedation and Monitoring

Conscious sedation may be used at the discretion of the bronchoscopist and participant, but maximum doses outlined in Table 2 will not be exceeded. If adequate sedation cannot be attained within these dosing limits and within the dosing limits for topical anesthesia (see section E, below), the procedure will be aborted. Monitoring includes oxygen saturation, heart rate, blood pressure, and ECG. All hospital conscious sedation policies relevant to conscious sedation will be observed.

To ensure participant safety, and to facilitate processing of samples, the following minimum in suite personnel are needed for all bronchoscopies:

- Bronchoscopist
- Bronchoscopy Assist (nurse, respiratory therapist, or trained technician): to assist the bronchoscopist in monitoring the participant
- Sample processor

C. Topical Anesthesia

Lidocaine dose to the participant should be minimized. For purposes of calculation, all lidocaine administered is to be included, whether delivery is via gargle, nebulization, spray, or instillation (above and below the vocal cords). For participant safety, the total lidocaine limit will be 300 mg or 5 mg/kg whichever is the lesser amount. The amount of lidocaine at hand in the room during a bronchoscopy procedure should be no greater than this amount. This limit

focuses on optimizing safety. The amount of lidocaine used should be recorded in the procedure record sheet.

Table 2. Bronchoscopy medications and maximum doses

Class	Drug	Maximum Dose
Anxiolytic	Midazolam (IV)	10 mg
Bronchodilator	Albuterol (nebulized)	10 mg
Anticholinergic	Atropine (IV) Glycopyrrolate (IV)	0.6 mg 0.4 mg
Narcotic (one, but not both)	Fentanyl (IV) Morphine (IV)	250 mcg 8 mg
Topical anesthetic	Lidocaine (nebulized, spray Instilled)	5 mg/kg, max of 300 mg

D. Approach

A trans-nasal or trans-oral approach will be the route documented in the procedure record. Samples will be obtained in the following order to ensure standardization, at the discretion of the bronchoscopist: airway pH and NOx samples from the left and from the right, using a new brush for each sample, right airway gas samples, left airway gas samples, right airway bronchial brushings, left airway bronchial brushings, right bronchoalveolar lavage (BAL), in middle lobe or lingula, and right bronchial biopsies. Sample collection will include samples collected for shared use and samples collected for site specific purposes. Airway pH measurements may be collected as brushings and/or using a ZepHr pH Probe and Recorder.

A summary of shared biospecimens from bronchoscopy is as follows:

1. Airway pH and NOx samples: up to 14 measurements or until adequate pH readings are obtained (up to 6 for pH on left, 1 for NOx on left, then up to 6 for pH on right, 1 for NOx on right)
2. Airway gas samples: 4 (2 on right then 2 on left)
3. Bronchial brushings: up to 6 (3 on right then 3 on left)
4. Bronchoalveolar lavage: 150 mL instilled and recovered (on right)
5. Bronchial biopsies: 4 (on right)

Collection of any sample may be aborted if the bronchoscopist deems that the participant is not tolerating the bronchoscopy, prioritize pH measurements and BAL.

E. Details of Bronchoscopy Procedures

Airway pH and NOx Measurement

This procedure will be done first on both the left and right sides, prior to subglottic lidocaine instillation which could result in inaccurate pH measurement. Measurements may be collected by a brushing or by a ZepHr pH probe or both. When a brush is used, the channel will be flushed with air after it is advanced to a 4th generation bronchus. The channel tip will be “wiped” on the mucosal surface. The brush of a protected brush will be withdrawn 2 cm from the sheath tip, and the sheath advanced to the mucosa. The sheath will be advanced at a narrow angle along the mucosa for ~ 3cm, then withdrawn and wiped using a sterilized Kimwipe, which has been autoclaved; the moistened Kimwipe is then placed against the probe tip of the (Hanna Instruments HI99181 Portable Waterproof Skin) pH Meter until the primary LCD screen displays the pH value of the sample. The procedure will be repeated until up to 6 samples from each side, adequate for a pH reading have been obtained and two for

NOx, cleaning the probe tip in between sample measurements using the appropriate cleaning solution. Please refer to the manufacturer's guide for additional details related to instrument calibration and probe tip cleaning specifications.

When the pH probe is used, it will be inserted through the bronchoscope channel similar to the brush and gently laid on the airway wall. Unlike the brush, which removes airway surface liquid for measurement outside of the patient, the probe will measure pH of the airway surface liquid directly without requiring removal of the liquid.

Airway Gas Samples

This procedure will be done on the right then left sides, after the pH and NOx measurements, while the participant is on room air if possible. The bronchoscopist can administer lidocaine as needed prior to airway gas sample collection. If the participant is being administered continuous O₂, flow rate at the time of sample retrieval should be documented. While the channel, of the bronchoscopy, is wedged in the lingula or other 3rd to 4th generation airway on the right side, an empty sterile blood gas glass syringe is attached to the bronchoscope and 20cc of air is withdrawn. The syringe is disconnected, capped, and placed on wet ice. This is repeated one more time on the right side and then two times on the left side. On the right side the channel is wedged in the right middle lobe or other 3rd or 4th generation airway. These (4; 2 right, 2 left) glass syringes will be analyzed using the (1 right, 1 left) Oxigraf CPXMax and the (1 right, 1 left) Sievers 280i NOA series NO analyzer. Standard protocols for both machines are followed when analyzing the airway gas samples.

Bronchial Brushings

Up to six brushings will be obtained, from different subsegmental bronchi in the lower lobes from both sides. A cytology brush (Endoscopy Cytology Brush, Diameter & Length - 1.9mm x 150cm) is introduced under direct visualization with gentle back-and-forth strokes (typically 2 to 5 strokes) to dislodge epithelial cells. Care should be taken to avoid trauma from overly aggressive brushing strokes, friction of the wire against the airway wall, or brushings taken too distal in the airway (and beyond direct visualization). Brushings will be processed for RNA expression of proteins of interest, and to generate epithelial cell line cultures.

Bronchoalveolar Lavage (BAL)

Collection of bronchoalveolar lavage (BAL) fluid will be performed before biopsies are obtained. Care must be taken to clear the working channel of the bronchoscope of contaminating blood and other debris only using 5 ml sterile saline to clear the bronchoscope before the lavage is initiated if the field is too bloody to visualize. Unless there is a clinical indication to perform the BAL in a different location, BAL should be performed preferentially in the right middle lobe (RML). If the RML cannot be accessed easily or if there is too much blood present, then the BAL could be performed in the lingula or upper lobes. Fluid to be used for BAL will be at 37° C placed through the suction channel into syringe using a syringe (push-pull method), and the fluid will be recovered into the same syringe using hand suction.

BAL will consist of up to 150 mL total instillate, split into three 50 mL aliquots. The standard technique will be instillation and recovery of one aliquot through suction channel into syringe using push-pull method, followed by instillation and recovery of the second aliquot, and then instillation and recovery of the third aliquot. The return from these three syringes will be pooled and distributed accordingly (See PACT Bronchoscopy Standards MOP for details).

Endobronchial Biopsy

Up to four endobronchial biopsies (from 2 different sites) will be collected. 1-2 should be taken from a right segmental carina and 1-2 from the main carina as tolerated. Opposite the site of the prior bronchial brushing procedure, endobronchial biopsies will be performed under direct

visualization rather than blindly, using a biopsy forceps (Single-Use Pulmonary Biopsy Forceps, Working Diameter & Working Length 2.0mm x 100cm). The forceps will be rinsed in sterile saline between each biopsy obtained. The biopsy location will be recorded in the procedure record.

F. Post procedure Follow-up and Monitoring

One hour minimum recovery time is required; two hours minimum recovery time is required if conscious sedation is used. Participants may be observed up to 3 to 4 hours in the procedure area.

For participants with asthma, albuterol 2.5 mg may be administered by nebulizer or metered dose inhaler as soon as practical following the procedure at investigator discretion. For healthy volunteers, the administration of inhaled beta-agonist following the procedure will be guided by clinical need, and the presence and degree of airflow limitation.

For participants with asthma, methylprednisolone IV may be administered following the procedure at the investigator's discretion. Antipyretics may also be administered to all participants at standard doses per hospital procedures. Clinical documentation of all medications administered (including conscious sedation medications) will serve as source documentation.

Assessment for the presence of hospitalization indicators should be made (Table 3). If any are present, the participant should be hospitalized overnight. The development of hospitalization indicators and subsequent hospitalization, *does* constitute a serious adverse event.

Post bronchoscopy spirometry should be measured a minimum of 1 hour after the procedure is ended. The participant may be discharged following the minimum recovery time if FEV₁ reaches $\geq 85\%$ of that observed in the pre albuterol spirometry obtained prior to bronchoscopy.

Post bronchoscopy assessment should be administered after the procedure, by telephone the evening of the procedure, and by telephone daily for the following 3 days.

Table 3. Indications for overnight hospitalization following bronchoscopy

Failure of FEV ₁ after bronchodilator administration to return to within 15% of pre-bronchodilation FEV ₁ at end of the monitoring time
Persistent hypoxia <90% at end of monitoring time
Persistent tachycardia (HR 130 bpm) at end of monitoring time
Unexpected mental status changes during or after the procedure
Significant hemoptysis (> 50 mL)
Requirement for bronchodilator every 2 hours on more than 3 occasions
Significant distressing cough beyond 2 hours after completion of the procedure
Investigator discretion

Prior to the procedure, the participant must identify a responsible adult to whom the participant will be discharged following the procedure. Research and bronchoscopy staff will review standard post bronchoscopy instructions with the participant and the responsible adult.

Despite appropriate selection, preparation, procedural technique, post bronchoscopy monitoring, and physiologic assessment prior to discharge, it is possible that participants occasionally will require urgent care following discharge from the procedure unit.

Infrastructure should be in place to recognize the need for, and to facilitate, such care should the need arise.

Research coordinator and investigator contact numbers will be provided to the participant. Contact numbers of two responsible adults must be obtained from the participant. A follow-up call will be made to each participant daily for three days (PC1, PC2, and PC3) following the bronchoscopy. If unable to contact participant on any of the follow-up phone calls (PC1, PC2, and PC3) then the alternative contacts identified by the participant will be contacted. If unable to reach the participant or back up contacts on any of these follow-up phone calls, then the PI should be contacted immediately and plan devised as appropriate. In the unlikely event that post bronchoscopy concerns or issues have not resolved at the end of the scheduled contacts, ongoing contact and clinical care should be provided until the matter is appropriately resolved.

Any non-serious and serious adverse events should be documented whether anticipated or non-anticipated. Following documentation, all adverse events will follow the outlined reporting guidelines in the Safety Assessment Section (Section 5) of this protocol.

See below Schedule of Events for more information about bronchoscopy options.

During regular times *and* during viral infection crisis restrictions, bronchoscopy may only take place at the study hospital (no in-tent option for IU).

4.8 Evaluations by Visit

Visit 1: Screening Visit

- A. Review the study with the participant and obtain written informed consent and HIPAA authorization
- B. Assign a study ID
- C. Record demographic data
- D. Record medical history, including asthma questionnaires and history as appropriate
- E. Active viral infection screening per institution
- F. Record environmental history
- G. Record concomitant medications
- H. Collect urine for urinalysis, perform pregnancy testing (if applicable)
- I. Collect blood for CBC with differential, comprehensive chemistry panel (total of 15 ml) and optional (5 ml) for DNA / RNA testing
- J. Perform 12 lead electrocardiogram
- K. Review chest x-ray results if obtained, or provide requisition for chest x-ray if no historical is available within the last 12 months, and if subject opts in.
- L. Record vital signs, height, weight, and perform full physical examination
- M. Complete pulmonary function testing checklist
- N. Perform and record FeNO measurements
- O. Perform baseline spirometry with maximum bronchodilator reversibility testing.
- P. For participants with asthma, if reversibility criteria are not demonstrated, record historical MCT results, if obtained (acceptable test is one done per an NIH study protocol in the previous 5 years). If an acceptable historical MCT is not available, the participant will need to return for MCT (Visit 1A) prior to proceeding with the remainder of the procedures.
- Q. Perform Allergy Skin Testing
- R. Confirm eligibility criteria
- S. Schedule bronchoscopy, if possible
- T. Provide pre-bronchoscopy instructions, if proceeding with bronchoscopy
- U. Record adverse events

Visit 1A: Methacholine Challenge Testing (MCT)

For participants with asthma who did not meet bronchodilator reversibility criteria at Visit 1, this visit serves to confirm the diagnosis of asthma prior to proceeding with other study procedures. See below Schedule of Events for more details about accessibility of and opting out of methacholine challenge test.

- A. Confirm ongoing consent to participate
- B. Collect urine for pregnancy test if applicable
- C. Record vital signs, height and weight
- D. Complete MCT checklist
- E. Perform and record MCT
- F. Confirm eligibility criteria
- G. Complete any remaining procedures and forms from Visit 1
- H. Record adverse events

Visit 2: Research Bronchoscopy

See Schedule of Events for more details about accessibility of and opting out of bronchoscopy. Prior to Bronchoscopy, institutions may have subjects do a screening viral test. If active viral infection is present, bronchoscopy may be rescheduled.

- A. Confirm ongoing consent to participate
- B. Complete asthma questionnaires (if applicable)
- C. Obtain and record interval history
- D. Confirm eligibility criteria
- E. Record concomitant medications
- F. Perform urine pregnancy test if applicable.
- G. Record vital signs, height and weight, and perform abbreviated physical examination
- H. Complete pulmonary function testing checklist
- I. Perform pre-bronchoscopy spirometry
- J. Complete pre-bronchoscopy checklist to determine eligibility to proceed (may be done by phone up to 48 hours prior to visit)
- K. Obtain consent for bronchoscopy (clinical procedural consent)
- L. Complete bronchoscopy checklist
- M. Perform bronchoscopy with collection of airway pH and NOx measurements, airway gas samples, brushings, BAL and biopsies
- N. Perform post bronchoscopy spirometry
- O. Perform post bronchoscopy monitoring and assessment
- P. Provide post bronchoscopy instructions if participant meets discharge criteria
- Q. Record adverse events
- R. Perform telephone call in the evening to administer post bronchoscopy assessment

PC1, PC2, and PC3: Safety Phone Call (Day 1, 2, 3 after Visit 2)

- A. Record post bronchoscopy assessment
- B. Record adverse events

Visit 3: Inhaled GSNO Challenge Testing

- A. Confirm ongoing consent to participate
- B. Complete asthma questionnaires (if applicable)
- C. Obtain and record interval history
- D. Confirm eligibility criteria
- E. Record concomitant medications
- F. Record vital signs, height and weight, and perform abbreviated physical examination

- G. Perform urine pregnancy test if applicable
- H. Complete pulmonary function test procedure checklist
- I. Measure and record baseline FeNO
- J. Perform baseline spirometry (before GSNO administration)
- K. Administer 2.5 ml GSNO (10 mM) by inhalation
- L. Record FeNO measurements every 10 minutes after GSNO inhalation for 60 minutes. Monitor HR and pulse oximetry continuously, recording every 5 minutes. Measure BP and RR every 15 minutes.
- M. Perform final GSNO Challenge spirometry
- N. Perform maximum bronchodilator reversibility test
- O. Record Adverse Events
- P. Exit Review

4.9 Participating Laboratories and Pharmacies

Blood and urine samples will be analyzed by:

University Hospitals Cleveland Medical Center Laboratories 11100 Euclid Avenue Cleveland, OH 44106	Indiana University Health Pathology Laboratory 340 West 11 th Street Indianapolis, IN 46202
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Possibly others during viral infection crisis restrictions

All other samples and testing performed for this study will be analyzed by:

Case Western Reserve University Laboratories 10900 Euclid Avenue Cleveland, OH 44106	Wells Center for Pediatric Research 1044 W. Walnut Street, R4-132 Indianapolis, IN 46202
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Possibly others during viral infection crisis restrictions

GSNO will be sterilely compounded and provided by the following pharmacies:

Arena District Pharmacy 262 Neil Ave #130 Columbus, OH 43215	Custom Med Apothecary 6005 W 71 st St Indianapolis, IN 46278
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5. SAFETY ASSESSMENTS

The monitoring in place for assessing the participant's responses will ensure that any untoward change in physiologic status is immediately known. As a result of the study design, individuals will be monitored on a case-by-case basis by the clinical research staff. The experimental design has each volunteer extensively monitored; any manner of medical intervention is immediately available and the appearance of any suspected untoward effect will result in cessation of protocol procedures if decided by the principal investigator.

Any member of the research team will report any unexpected event or problem and any adverse event, either serious or non-serious, to the principal investigator immediately upon discovery. The principal investigator will then proceed to report the event within the specified timeframes mandated by the UHMC IRB and the Data and Safety Monitoring Board (DSMB).

There is a DSMB providing oversight to this study. It functions as an independent medical monitor and adverse event (AE) review adjudication board; it will have the responsibility to review all AEs

and any other duties that the UHCMC IRB requires. In addition, the DSMB will provide feedback and expertise in determining study continuance as it reviews any serious AE.

5.1 Adverse Events and Severity Grading

The UHCMC IRB and the FDA have well-established policies (and means) for prompt reporting of AEs and unanticipated problems involving risk to participants or others and these policies will be strictly followed.

Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. For this study, AEs will be recorded from the time of consent through Study Visit 3. Unexpected events are defined as any adverse event, the specificity or severity of which is not consistent with the risk information described in the general investigative plan (i.e., research plan) or elsewhere in the current application including the consent form. The Investigator or Research Coordinator 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. AEs will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to investigational product, or if unrelated, the cause.

AE Severity

The National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, summarized in Table 4 below, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 4. AE Severity Grading (based on NCI's CTCAE Version 4.0)

Severity (Toxicity Grade)	Description
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*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

A Semi-colon indicates 'or' within the description of the grade. Activities of Daily Living (ADL)

* Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

** Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

5.2 AE Relationship to Investigational Product

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 5.

Table 5. AE Relationship to Investigational Product

Relationship to Drug	Comment
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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 investigational product.

5.3 Serious Adverse Events (SAEs) and Reporting

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.

SAE Reporting

All SAEs will be documented (whether or not related to investigational product) on a SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained and end at Study Visit 3.

All SAEs will be reported and reviewed by the investigator and sent to the DSMB, within 24 hours of learning of the event.

Study staff will notify the DSMB of additional information or follow-up to an initial SAE Report as soon as relevant information is available. Follow-up information is reported on an SAE Report Form.

DSMB recommendations that are voted on and passed are transmitted in writing to the study PI and the Program Director, within three (3) working days, or less if considered urgent, of the meeting/call at which the recommendation was formulated and passed.

The investigator will report all SAEs to the UHCMC IRB, in accordance with the UHCMC standard operating procedures and policies. SAEs will be reported to the FDA in accordance with 21 CFR 312.32.

5.4 Data and Safety Monitoring Board

The appointed DSMB will plan to meet twice annually. Additional meetings will occur anytime the Board has concerns, or when requested by investigators, NIH, or regulatory authorities.

All SAEs will be reported to William Calhoun within 24 hours of learning of the event, or Daniel Sessler in the event that William Calhoun is unavailable. Each SAE will be evaluated, and it will be determined whether it needs to be considered by the full Board.

Data will be reported using appropriate statistical techniques and tools. The reports will be generated in aggregate and split into treatment groups as defined by each protocol. All safety and efficacy data supplied for review may be blinded. Some examples of elements of a report for a protocol:

1. Patients enrolled
2. Protocol deviations
3. Selected demographic/baseline factors to include gender, race and age
4. Unexpected adverse events with resolution
5. Serious adverse events with resolution
6. Narrative reports for each serious adverse event

5.5 Follow-up for Adverse Events

Participant follow-up will be on a case-by-case basis as directed by the study physician and/or the UHCMC IRB and the DSMB as appropriate.

5.6 Specific Risks for Each Procedure

GSNO Challenge Test

We have performed GSNO challenge using this protocol in the past with no AEs (14). Theoretical risks could include hypotension, dizzy, nausea, fatigue, blurred vision, as different inhaled nitrites (such as nitroglycerine) have previously been shown to have this effect in some patients, and hemoptysis, as NO evolve from GSNO can inhibit platelet aggregation.

However, the biology of GSNO, and experience with inhaled NO and ethyl nitrite, suggests that these will not be significant complications (2,4,44,53,55). We are prepared for these if they do occur.

Bronchoscopy

Bronchoalveolar lavage and bronchoscopic brushing via flexible bronchoscopy is widely accepted as a safe procedure and is frequently utilized as a research tool in patients and healthy control volunteers (3). There is no additional risk when obtaining airway gas samples, since air is only being withdrawn. There is a slight risk of pneumothorax the vast majority of which can be treated with local manipulations but some cases may require surgical intervention.

Other risks are hemoptysis (10-25% incidence, very mild, less than 2 tablespoons in 24 h), epistaxis, local discomfort (25% incidence), fever (10-25% incidence), and sore throat. Non-specific adverse effects include acute dyspnea, cough, chest pain, and transient desaturation. There is only one report of a death resulting from a research bronchoscopy. Among those with asthma, there is a risk of transient worsening of asthma and asthma exacerbations. In the Severe Asthma Research Program experience, among the 436 participants who underwent research bronchoscopy, 5 required either unplanned hospitalization or extension of planned hospital stays for asthma exacerbations (3). Three additional participants were seen in an Emergency Room within three days of the procedure but were not treated for worsening asthma (rash, pleuritic chest pain, and panic attack). Four of the 8 participants had very severe asthma (not eligible for participation in that study).

There are specific risks and side effects from any sedative or analgesic administered as part of the bronchoscopy procedure. In addition to somnolence and reduced alertness, the participant may feel nauseous and could vomit. The drugs can induce coughing or headache. Risk of side effects is reduced by appropriately controlling rate of administration and reviewing the participant's medical history to determine if he/she has previously experienced an adverse drug event.

Fluid Collection (including Venipuncture and Urine Collection)

The risks for any type of blood sampling are bleeding, hematoma, and infection. The risk of infection is significantly reduced by the use of sterile technique. The participant may feel a stinging sensation anytime a needle is placed in the vein or artery. Also some pre- and post-procedural lightheadedness and rarely fainting may be experienced.

The genetic testing (optional) may involve social and psychological risks. Although accepted controls are in place to protect this PHI, there is always a risk that information may be unintentionally released or illegally accessed. If such an event occurred it could negatively impact the participant and their family and could also affect insurability.

Urine is self-collected using a clean catch procedure; there are no risks with this methodology.

Allergy skin testing

Possible risks include itching at the site of the skin tests, and systemic allergic reaction to the allergens tested. The allergy reactions range from mild (urticaria) to moderate (wheezing) to severe (upper airway angioedema, anaphylaxis). Medical and nursing personnel, medications and equipment will be available at the study sites to treat and manage any allergic reactions.

Standard Spirometry, maximum bronchodilator reversibility (spirometry post bronchodilator) and Fractional Exhaled Nitric Oxide (FeNO)

Such testing can cause a participant (independent of asthma status) to become light-headed, dizzy or tired. In asthmatic participants testing may also induce or worsen wheeze, shortness of breath, and/or chest tightness. Rescue therapy with Albuterol will be administered as needed. The chance of these symptoms occurring is low and treatment will be readily available. As part of the preparation for testing, a participant will be asked to withhold certain medications so an increase in allergy or asthma symptoms may occur. In such cases participants will be directed to resume their medications and the care staff may administer additional treatments and supportive therapy as warranted.

Methacholine Challenge Test (MCT)

The volunteer may experience coughing, chest tightness, shortness of breath, and/or wheezing during this procedure. These symptoms typically resolve spontaneously 10-15 minutes after testing without active intervention – recovery can be hastened as needed with administration of albuterol.

Chest x-ray

This test involves a small amount of radiation. The radiation exposure from this research is about 200 microsievert. There is radiation that naturally occurs from space and from rocks in the soil. This natural radiation is greater at higher altitudes. This research gives the same amount of radiation an individual would get from living in a high altitude city such as Denver for 12 days, or taking 4 airplane flights from New York to Los Angeles.

A possible health problem seen with radiation exposure is the development of cancer later in life. This extra cancer risk is higher at younger ages and for girls and women. The extra lifetime risk of dying of a fatal cancer due to the radiation exposure from this research may range from about one in 200,000 to about one in 70,000. At such low radiation exposures, scientists disagree about the amount of risk. These estimates are very uncertain, and there may be no extra risk at all. (These radiation estimates are from the Duke University Medical Center Radiation Safety website.)

Withholding medications

Withholding medications such as antihistamines, short-acting/long-acting bronchodilators,

antidepressants, H2 antagonists, or herbal supplements prior to study visits may cause subjects to experience increased symptoms of the condition for which the medication is taken. For example, withholding short-acting/long-acting bronchodilators might result in shortness of breath or breathing difficulty. Participants will be advised that if they experience any worsening symptoms during the pre-visit withholding period, they should use their usual medication(s) as needed and call the study team to cancel or reschedule the study visit. Participants will also receive – in the form of an addendum to the consent – a list of the medications that must be withheld, for how long prior to the study visit/testing, and possible risks associated with doing so.

Albuterol

Albuterol administration can cause nausea, vomiting, heartburn, shakiness, nervousness, dizziness, headaches, hyperactivity, and a non-harmful increase in heart rate. If such symptoms occur, they usually stop in a short period and do not require additional medications.

Viral infection testing

Viral infection testing may include collection of nasopharyngeal and/or oropharyngeal swabs. Nasopharyngeal swabs may cause mild nasal discomfort and may cause temporary mild nosebleed. Oropharyngeal. Oropharyngeal swabbing may cause mild discomfort, gagging or coughing.

6. INTERVENTION/DISCONTINUATION

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

- Participant withdrawal of consent
- Participant is not compliant with or tolerating study procedures.
- Adverse event
- Lost to follow-up
- Physician request for early termination of study
- Participant becomes pregnant

Being a part of this study while pregnant may expose the unborn child to significant risks. Therefore, pregnant women will be excluded from the study. A pregnancy test will be done at the beginning at every study visit for any women of childbearing potential, and it must be negative before they can enter and continue in this study. All Participants must agree to use appropriate contraceptive measures during the study. Medically acceptable contraceptives include: (1) surgical sterilization, (2) approved hormonal contraceptives such as birth control pills, (3) barrier methods (such as a condom or diaphragm) used with a spermicide, or (4) an intrauterine device (IUD). If they become pregnant during this study, they must inform a member of the research team immediately.

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. If the participant withdraws from the study and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected.

The Investigator may retain and continue to use any data collected before such withdrawal of consent. Any samples already being analyzed will be completed and any stored samples will be destroyed if still linkable.

7. STATISTICAL CONSIDERATIONS

7.1 Statistical Analysis of Outcomes and Power Calculations

Based on our preliminary data, we project 44% (n=22) of the 50 participants we enroll with asthma will have elevated GSNOR activity in BAL fluid ($> 7.5 \text{ mmole} \times \text{min}^{-1} \times \text{ug protein}^{-1}$) compared with approximately 15% of healthy volunteers (16). Levels of tissue GSNOR expression will be log-transformed to improve normality assumptions. Values will be compared between those with and without elevated GSNOR in BAL fluid using a two-sample t-test or Wilcoxon rank sum test if normality assumptions are not met. Inter-relationships will be assessed using Pearson or Spearman's correlation. Assuming lognormal distributions and a coefficient of variation of 50%, this study will have $\geq 83\%$ power to detect a 1.5-fold or larger differences between groups. Correlations between BAL enzyme activity and GSNOR expression levels of 0.39 or higher can be detected with 80% power. These and other power and sample size calculations assume two-sided tests with significance taken at $p < 0.05$.

To test the hypothesis that the GSNO challenge test can reliably identify those with elevated GSNOR activity levels, the primary outcome will be the amount that FeNO declines from its observed maximum to its value 15 minutes post-exposure. There will be three cohorts: asthma participants with abnormal GSNOR ($> 7.5 \text{ mmole} \times \text{min}^{-1} \times \text{ug protein}$), asthma participants with normal GSNOR, and ten non-asthmatic healthy controls. One-way ANOVA or Kruskal-Wallis tests be used to primary outcome among cohorts, possibly after log-transformation. If approximate normality of the outcome cannot be achieved with or without transformation, Kruskal-Wallis/Wilcoxon rank sum tests will be used. In addition, a linear mixed repeated-measures model will be used to estimate and compare FeNO vs. time profiles of the three cohorts. Assuming lognormal distributions, a coefficient of variation of 50%, and sample sizes of 22 asthma participants with elevated GSNOR, 28 asthma participants with normal GSNOR, and 10 healthy normal participants, the study will have $\geq 80\%$ power to detect 1.5-fold or larger differences between the increased GSNOR phenotype and non-increased phenotype asthmatics, or to detect a 1.7 fold difference between the increased GSNOR phenotype and the non-asthmatic control participants. Correlations between amount of FeNO decline from the maximum in 15 min, or maximal increase in FeNO and BAL GSNOR levels of 0.39 or higher can be detected with 80% power.

7.2 Stopping Rules

The risks associated with this trial are low but not zero. Investigators will report all serious adverse events within 24 hours of learning of them to the DSMB as described in Section 5.3. The DSMB will review SAEs within 3 business days. All study procedures for the affected participant will be stopped until the DSMB is able to review the event and recommend changes to the protocol if needed. Any SAE deemed by the investigator to be probably or definitely related to study drug or bronchoscopy will result in stopping of all study procedures other than safety monitoring for all active participants until DSMB review is complete. A DSMB review will also be triggered if two or more patients have a greater than or equal to 15% reduction in FEV₁ following inhaled glycine administration that does not return to baseline.

DSMB recommendations that are voted on and passed are transmitted in writing to the study PI and the Program Director, within three (3) working days, or less if considered urgent, of the meeting/call at which the recommendation was formulated and passed.

8. DATA COLLECTION AND QUALITY ASSURANCE

8.1 Data Collection

Study personnel will enter data from source documents corresponding to a participant's visit into the protocol-specific CRF when the information corresponding to that visit is available. Participants will not be identified by name in the study database but will be identified by a unique global ID number that is assigned consecutively in numeric order (never reusing a number) to a participant upon successful enrollment. Study data will be stored in an electronic, certified 21 CFR Part 11 compliant

database. The database vendor is DataTrak (Mayfield Heights, Ohio). Corrections to CRFs will have an audit trail. In conjunction with using DataTrak, measurements and results are entered into a secured, access-only REDCap database for ease of data analysis.

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. All study records will remain at the Investigator's site until the completion of the study.

Protocol deviations that occur throughout the study will be maintained in a study specific UH REDCap database only accessible to qualified study personnel or as paper forms with electronic copies saved.

8.2 Data Management

All procedures for the handling and analysis of data will be conducted using good clinical practice (GCP) meeting FDA guidelines for the handling and analysis of data for clinical trials.

8.3 Quality Assurance

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.

8.4 Archival of Data

Appropriate archiving of the database will be completed according to DataTrak's procedures.

8.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to authorized personnel, IRB, 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 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 (participant files, signed informed consent forms, CRFs, etc.) must be kept secured for a period of 2 years following marketing of the investigational product or for 2 years after centers have been notified that the IND has been discontinued.

8.6 Study Monitoring

This is a multi-site investigator initiated study, taking place at two sites – (1) University Hospitals Cleveland Medical Center and Rainbow Babies and Children's Hospital, and (2) University Hospital on the Indiana University Indianapolis campus. Each site will be monitored by their respective institutional monitoring teams – for the Cleveland site, this will be representatives from the office of the University Hospitals Cleveland Medical Center Clinical Research Center; for the Indiana site, it will be representatives from the Indiana Clinical and Translational Sciences Institute. The Investigator grants permission to each site's monitoring offices, and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

8.7 Participant Confidentiality

In order to maintain participant confidentiality, only a participant number and participant initials will identify all study participants on CRFs. Laboratory reports, flow sheets, and physician order sheets will contain the participant's name and medical registration number.

9. PARTICIPANT RIGHTS AND CONFIDENTIALITY

The following language (9.1-9.11) models that contained the Informed Consent Form used to explain the risks and benefits of the procedures involved.

9.1 Potential Benefits

There is no direct benefit to the participant for participating in this study. Their voluntary participation will help us determine the relationship between GSNOR activity in the lung and the response to inhalation of GSNO (i.e., FeNO levels).

9.2 Potential Risks

The language used in Section 5 to delineate the procedural and experimental risks has been simplified in the informed consent form to insure volunteer understanding.

9.3 Alternatives to Study Participation

This is a voluntary study. The alternative is to choose to not participate. A participant's decision will in no way influence any care a participant might receive at the respective hospital nor will it affect interactions with any of the study personnel.

9.4 Financial Information

The participant will be paid for their participation in this study. Details are provided in the informed consent form.

9.5 Research Related Injury

If injury occurs as a result of their involvement in this research, medical treatment is available from the respective hospital or another medical facility but the participant or their medical insurance will be responsible for the cost of this treatment. A research injury is an injury that happens as a result of taking part in this research study. There are no plans for payment of medical expenses or other payments, including lost wages, for any research related injury.

9.6 Use of Specimens

Test results will not be used to guide clinical care, nor will they be used for purposes outside the scope of this research study.

9.7 Confidentiality

All study data will be treated as confidential information. Similarly, the participant's medical history will also be treated as confidential with no identifiable information released or shared with individuals outside the study team. If the study results are published participant names will not be used. Once all the results are collected any identifiers will be removed and the information assigned a code. The data will be identified by a study number and not by name or identifying information. The code assignment key will be maintained by the study staff on a password-secured computer kept in a locked office. Access to the code key will be limited to a need-only basis.

9.8 Student/Employee Rights

Choosing not to participate or withdrawing from this study will not affect anyone's employment or class standing, nor will a participant's decision or study results be shared with their supervisor unless supervisor is part of the research team.

9.9 Data Safety and Monitoring Board

There is a DSMB providing oversight to this study as outlined in the DSMB charter. The DSMB will review all serious adverse events. All other adverse events will be reviewed at the DSMB's next

meeting date. The time frame for evaluation of SAEs will be –3 working days from the time the event is reported to the DSMB. The DSMB has the authority to request a modification of the study. These modifications are only meant to be a guideline for the DSMB to review the study and make a recommendation to continue, or modify, not necessarily a mandate for terminating the study.

The formal DSMB consists of:

William J. Calhoun, MD (Chair)

Research bronchoscopist

Professor of Internal Medicine, Pulmonary,
Allergy, and Clinical Immunology
University of Texas Medical Branch
4.116 John Sealy Annex
301 University Blvd
Galveston, TX 77550
Email: William.Calhoun@utmb.edu

Daniel I. Sessler, MD

Clinical trials and outcomes researcher

Michael J. Cudahy Professor and
Chair, Department of Outcomes Research
The Cleveland Clinic
9500 Euclid Avenue, P77
Cleveland, OH 44106
Email: ds@or.org

Barry P. Katz, PhD

Statistician

Professor Emeritus of Biostatistics & Health
Data Science
IU School of Medicine
HS 3000 BIOS
1050 Wishard Blvd
Indianapolis, IN 46202
Email: bkatz@iu.edu

Noah Lechtzin, MD

**Adult CF specialist and
research bronchoscopist**

Associate Professor of Medicine
Johns Hopkins University
1830 E. Monument Street, 5th Floor Pulmonary
Baltimore, MD 21287
Email: nlechtz1@jhmi.edu

The members have no involvement in the planned study but do have the requisite clinical expertise to oversee participant safety.

9.10 IRB

It is the Investigator's responsibility to submit the clinical protocol and any Amendments to the UHCMC IRB, if required, for approval prior to any participant being enrolled at the investigational site and to obtain renewals at periods determined by the UHCMC IRB for the duration of the study.

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Protocol Signature Page

Protocol Title:	Methods to Identify and Treat Severe Asthma Patients Project 1: GSNOR Phenotyping and GSNO Challenge
IRB #:	
IND:	137035
Site:	
Protocol Version/Date:	27Jun2023-01

The signature below constitutes approval of this study in full accordance with the provisions of this protocol and the attachments. I agree to conduct this study in compliance with the protocol, in-country and local regulatory requirements, applicable United States (US) Code of Federal Regulations (CFR) and ICH Good Clinical Practices (E6).

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining Sponsor and IRB approval, except when necessary to protect the safety, rights, or welfare of participants.

Printed Name of the Principal Investigator

Date

Signature of the Principal Investigator