National Institute of Allergy and Infectious Diseases

AADCRC

UCSF-01

Allergen Challenge for Evoked Phenotypes in Asthma

Short Title: ACE Study

VERSION 1.0, 8/1/2014

IND# TBD

Study Sponsor(s):

NIAID Funding Mechanism: U19AI077439-06

IND Holder: **Prescott G. Woodruff, MD, MPH**

Study Drug Manufacturer/Provider: Greer Laboratories, Lenoir, NC

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National Institute of Allergy and Infectious Diseases

INVESTIGATOR SIGNATURE PAGE	
Protocol: AADCRC-UCSF-01	**Version/Date: 1.0, 8/1/2014**
Site Principal Investigator: Prescott G. Woodruff, MD, MPH	
Title: Allergen Challenge for Evoked Phenotypes in Asthma (the ACE Study)	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	

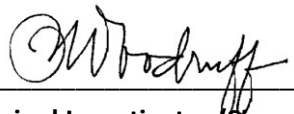
INSTRUCTIONS: *The site Principal Investigator should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent. After signature, please return the original of this form by surface mail to:*

Katherine Thompson
Nurse Consultant, Project Manager
NIAID
PO Box 7
Lecanto, FL 34460-0007

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID.

Prescott Woodruff, MD, MPH
Site Principal Investigator



_____ _____8/1/2014_____
Site Principal Investigator (Signature) **Date**

National Institute of Allergy and Infectious Diseases

Protocol Synopsis

Title	Allergen Challenge for Evoked Phenotypes in Asthma
Short Title	ACE study
Clinical Phase	Mechanistic
Number of Sites	One (1) University of California, San Francisco
IND Sponsor/Number	TBD
Investigational Product(s)/Intervention(s)	Products: From GREER® Laboratories, Inc. (Lenoir, NC) 1. Allergen extract house dust mite (HDM) 2. Allergen extract cat allergen 3. Phenolized diluent Procedures: 1. Methacholine challenge testing; provocholine 2. Skin testing; diluent, dust mite, and cat allergen (Standardized Cat Allergen Extract and Standardized Dust Mite Allergen from Greer Laboratories) 3. Segmental allergen challenge with Bronchoscopy
Study Objectives	To provide a defined allergic stimulus that will promote the cellular and molecular events to increase the observed biological signal and allow time-course analyses.
Study Design	Single site mechanistic study of 26 asthmatics with stable or well-controlled asthma, 6 allergic non-asthmatic controls and 6 non-allergic, non-asthmatic controls. Three visits total with an initial characterization visit, followed by two visits for bronchoscopy before and after segmental challenge with allergen and diluent (both will be done in each subject)
Primary Endpoint(s)	The primary scientific endpoint of this study is the fold-difference in expression of epithelial miRNAs in allergen challenged versus diluent – challenged lung segments.
Secondary Endpoint(s)	Secondary endpoints include: a. Enumeration and sorting of Th1, Th2 and Th17 cells in BAL and

	blood using flow cytometry and mass cytometry b. Analysis of epithelial mRNA markers of Th2 and Th17 inflammation for immune phenotyping of inflammatory responses c. Collection of endobronchial biopsies for immunostaining of immune cell localization, immunoblotting of smooth cell protein phosphorylation, analysis of mucin content and smooth muscle cell subculture Banking of epithelial cells, BAL cells, blood cells and biopsies in an IRB-approved bank for subsequent analyses
Accrual Objective	38 subjects will be enrolled and complete the study 26 asthmatics with stable or well-controlled asthma (not on inhaled corticosteroids) 6 allergic non-asthmatic controls 6 non-allergic non-asthmatic controls
Study Duration	3 years of rolling accrual and follow-up Study visits will be concluded after the bronchoscopy on day 8 or day 14, with approximately 18 hours total participation per subject overall
Treatment Description	Participants will undergo bronchoscopy with segmental allergen administration in one lobe and diluent administration in a corresponding lobe of the contralateral lung. Allergen will be an FDA-approved allergen extract of either house dust mite (HDM) or cat Allergen, from GREER® Laboratories, Inc. (Lenoir, NC), using protocols similar to those previously approved by the NIAID for an AADCRC study (MGH site, "Effects of Standardized Cat or Dust Mite Allergen on Mediators of Asthma").
Inclusion Criteria	<u>Inclusion Criteria for asthmatics:</u> 1. Subject must be able to understand and provide informed consent 2. Prior physician-diagnosed asthma per participant report 3. Age 18 to 50 (inclusive) at the time of consent 4. Pre-BD Baseline (V1) FEV1 >75 % of predicted 5. Skin test reactivity to house dust mite or cat allergen with wheal ≥3mm larger than the diluent control at V1 6. Methacholine PC20 < 8 mg/mL <u>Inclusion Criteria for allergic non-asthmatic controls:</u> 1. Subject must be able to understand and provide informed consent 2. Age 18 to 50 (inclusive) at the time of consent 3. Pre-BD Baseline (V1) FEV1 >90% of predicted 4. Skin test reactivity to house dust mite or cat allergen with wheal ≥3mm larger than the diluent control at V1 5. Methacholine PC20 > 16 mg/mL

	Inclusion Criteria for non-allergic non-asthmatic controls: 1. Subject must be able to understand and provide informed consent 2. Age 18 to 50 (inclusive) at the time of consent 3. Pre-BD Baseline (V1) FEV1 > 90% of predicted 4. Negative skin test reactivity with wheal <3mm than the positive histamine control at V1 5. Methacholine PC20 > 16 mg/mL
Exclusion Criteria	Exclusion Criteria for asthmatics: 1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol 2. Primary language is not English 3. Use of systemic steroids within the previous 6 weeks 4. A history of intubation for asthma exacerbation 5. Use of Xolair (omalizumab - anti-IgE monoclonal antibody) within the last 6 months 6. Immunotherapy with cat or dust mite extract now or in the past 5 years 7. ≥10 pack-years smoking and any in the past year 8. Women of childbearing potential who are documented to be pregnant (based on urine beta-HCG testing), are sexually active and not willing to use contraception during the study, are seeking to become pregnant, or who are nursing 9. Intolerance to albuterol, atropine, lidocaine, fentanyl, or midazolam 10. Presence of a comorbid condition thought to increase the risk of bronchoscopy by the investigator such as diabetes mellitus, congestive heart failure, ventricular arrhythmias, history of a cerebrovascular accident, renal failure, history of anaphylaxis, or cirrhosis 11. Use of systemic steroids or inhaled steroids, beta-blockers, tricyclic anti-depressants and monoamine oxidase (MAO) inhibitors or a visit for an asthma exacerbation within 1 month of the screening visit 12. Other lung diseases, such as sarcoidosis, bronchiectasis or active lung infection 13. History of dermatographia 14. History of anaphylaxis to cat allergen 15. Participation in research study involving a drug or biologic during the 30 days prior to the study 16. Quantitative skin-prick test positive reaction at an allergen concentration of 0.056 BAU or AU/mL 17. Participants with a high possibility of poor compliance with the study 18. Current indoor cat and/or indoor second-hand cigarette smoke

	exposure 19. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study 20. Any of the following hematologic abnormalities, confirmed by repeated tests: White blood count <3,000/µL or >14,000/µL; Lymphocyte count <500/µL; Platelet count <150,000 /µL; Hemoglobin <8.5 g/dL; or Neutrophil count <2,000 cells/µL. 21. Subjects who have a 20% drop in FEV1 with diluent at the beginning of the methacholine challenge will be excluded from the study. <u>Exclusion Criteria for allergic non-asthmatic controls (both allergic and non-allergic):</u> 1. 1. History of asthma or other lung diseases 2. 2. Any of the exclusions #1-21 listed for asthmatics above Exclusion Criteria for non-allergic non-asthmatic controls: 1. History of asthma or other lung diseases 2. Serum specific IgE to cat and house dust mite > 0.35 IU/ml 3. Any of the exclusions #1-21 listed for asthmatics above
Study Stopping Rules	1. If 3 participants meet individual stopping rules 2. If 1 study-related SAE occurs (including emergency room visit, hospitalization, or an unexpected hospitalization or death) 3. If more than 1 participant develops a significant systemic allergic reaction (Grade 2 or more) 4. Dyspnea or cough that prevents daily activity 5. Fever/nausea grade 3 or more 6. Death of any participant

Table of Contents

Glossary of Abbreviations

ACRC	Airway Clinical Research Center
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IEC	Institutional Ethics Committee
IMM	Independent Medical Monitor
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases
PC	Protocol Chair
PI	[Site] Principal Investigator
SAC	Segmental Allergen Challenge
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SPT	Skin Prick Test
SUSAR	Serious Unexpected Suspected Adverse Reaction
UCSF	University of California San Francisco

1. Background and Rationale

1.1. Background and Scientific Rationale

Asthma is a common inflammatory disease with a current prevalence > 11% in The US. The parent grant which this clinical study serves focuses on the mechanisms of initiation and persistence of allergic asthma. In our preliminary work it has become clear that two cytokines, IL-13 and IL-17, make important contributions to the most critical functional endpoints in asthma (airway hyper-responsiveness and mucus metaplasia) through spatially and temporally restricted effects on airway epithelial cells and airway smooth muscle. In the parent grant, three projects will focus on how each cytokine regulates airway epithelial cell differentiation and mucous metaplasia, how these cytokines work alone and in combination to regulate contractility of airway smooth muscle, and the dynamic behavior of the cells that generate these cytokines in the airway wall. Each of these projects will depend on a Clinical Subject and Biospecimen Core to provide basic characterization of airway physiology, evidence for IL-13 and IL-17 bioactivity, and samples of bronchoalveolar lavage fluid, epithelial brushings and airway biopsies for use in each of the three projects. These samples and clinical information will be obtained from participants enrolled in three studies, one of which is the segmental allergen challenge study described here (ACE, or "Allergen Challenge for Evoked Phenotypes in Asthma"). Here we provide a detailed protocol description of the ACE study. The other two studies include a study of asthmatics before and after inhaled corticosteroids (RITA, ClinicalTrials.gov # NCT01484691) and bronchoscopies performed at UCSF as part of the Severe Asthma Research Program (SARP, ClinicalTrials.gov# NCT01718197). They are already in progress and are not discussed further here. We do not propose any analyses that compare the subjects in the current proposed study and those other two studies, because the research questions in those other studies are distinct.

The primary goal of this ACE study is to provide a defined allergic stimulus that will promote the cellular and molecular events to increase the observed biological signal and allow time-course analyses. Prior studies have shown that segmental allergen challenge can provoke the influx of eosinophils and activated T-cells expressing Th2 cytokines at 24 hours [1]. Whether allergen challenge is also associated with an influx of Th17 cells is uncertain, and will be directly studied in this protocol, providing the field with new data on this asthma-relevant cytokine. These prior data and outstanding questions constitute our rationale for studying participants at 24 hours after allergen challenge using multi-color flow-cytometry. Although the influx of Th2 and Th17 cells (if observed) will likely be present at 24 hours after allergen challenge, our preliminary data suggest that the respiratory epithelial response to Th2 and Th17-driven inflammation may require as long as 7 days to fully manifest. Specifically, we have found that IL-13 exposure, in vitro, represses the expression levels of a set of epithelial miRNAs including members of the miR-34/449 family, but that decreased expression levels of the mature miRNAs can take as long as 7 days [2]. Since identifying the response of these and other epithelial miRNAs to allergen-evoked Th2 and Th17 inflammation is one of the goals, we will perform 1/2 of the follow-up bronchoscopies in the ACE study at 7 days rather than 24 hours. Finally, we expect that both time points will provide biopsies valuable for understanding the trafficking of dendritic cells and T-cells. Through the proposed studies we hope to gain new insights into the dynamic effects of IL-13 and IL-17 in asthma, to develop better tools to characterize subsets of patients with asthma, and to improve the prospects for targeted therapy of this disease.

1.2. Rationale for Selection of Investigational Product or Intervention

The investigational products are commercially available Standardized Cat Allergen Extract and Standardized Dust Mite Allergen. We are not testing the efficacy of these investigational products in this study. Rather we are using these products (standardized allergens) to evoke a phenotype in our participants to answer scientific questions. We have chosen these products because they have been used in exactly this manner in other clinical studies including an NIAID-funded AADCRC project (http://clinicaltrials.gov/ct2/show/NCT01612936 from the Massachusetts General Hospital). These products are the most suitable for this application because they reliably and safely induce local allergic responses in the lung that are well-tolerated clinically and sufficient to study T-cell mediated inflammation.

1.3. Preclinical Experience

According to the manufacturer's brochure, Standardized Cat Hair is prepared from the hair and dander of cats. Briefly, the material is obtained from hair of healthy cats maintained in a controlled environment. The material is extracted with saline buffer, and the crude extract is dialyzed before standardization. The finished product is tested for potency by *Fel d* 1 protein assay. The final product is supplied in vials containing a glycerosaline solution at a concentration 10,000 Bioequivalency Allergen Units (BAU) per milliliter. The Standardized Mite extract is prepared from whole mite bodies, fecal particles, and containing not more than 1% growth medium components. The mites are grown on a medium that contains no material of human origin but may contain minute amounts of pork-derived materials. Each vial contains a sterile extract of Mite 10,000 AU/ml (*Dermatophagoides farinae* and/or *D. pteronyssinus*), in 0.50% sodium chloride, 0.25% sodium bicarbonate, 50% glycerin by volume, and 0.4% phenol as a preservative. (See manufacturer package insert for more information).

1.4. Clinical Studies

Allergen extracts have been used for bronchoprovocation testing in humans for more than a quarter of a century, after the program directors of the asthma and allergic disease centers led an effort encouraged by the National Institute of Allergic and Infectious Diseases of the National Institutes of Health that published standardized protocols for inhalational challenge studies in humans. The study described here will study the effects of standardized cat and dust mite allergen preparations, that have been licensed by the agency for the treatment of asthma (PLA 90-0556, U.S. License 308) by measuring their effects when they are applied to a subsegment of the lung by injection though a bronchoscope. Immunotherapy with allergen extracts has been used for the treatment of aeroallergen-induced rhinitis and asthma since the turn of the 20th century. Clinical studies demonstrating the effectiveness of immunotherapy for allergic airway disease have appeared in the peer-reviewed literature since the report of Frankland and Augustin more than a half century ago [3]. The effectiveness of immunotherapy with extracts of cat allergen was reported in 1978 [4] and confirmed in subsequent trials [5-7]. Studies using well-characterized extracts of dust mite allergen have demonstrated the effectiveness of these extracts as immunotherapeutic agents in blinded randomized trials [8-11] and their safety when administered to the airways [9-12]. The characterization and availability of standardized extracts, including cat hair [13, 14] and dust mite, have permitted more uniform clinical responses and improved the safety of immunotherapy because the effective amount of antigen delivered is less variable than with non-standardized extracts. Immunotherapy with cat extracts has been shown to be as safe as immunotherapy with ragweed extract given in similar doses. At least four double-blinded randomized control trials have shown that this therapy is associated with decreased sensitivity to inhaled cat extract [4, 6, 7, 15]. In each of these studies, cat-allergic asthmatics underwent inhalational allergen challenge, and no adverse consequences of cat allergen inhalation were reported when cat allergen extract was delivered to the whole lung. Limiting allergen to a single lobar subsegment would be expected to be even safer. Cat-induced bronchoconstriction is known to be

directly related to the quantity of airborne cat allergen, as determined by levels of the major cat dander epitope *Fel d* I in FDA units [16]. The levels of aerosolized *Fel d* I in a standard room containing live cats are significantly and directly associated with the concentration of *Fel d* I that induced bronchoconstriction in participants inhaling standardized cat extract [14]. There were no reported adverse reactions to the inhalation of standardized cat extract in these studies.

2. Study Hypotheses/Objectives

Overarching hypothesis of the AADCRC grant: Allergen exposure enhances airway smooth muscle contractility and epithelial cell mRNA/miRNA production as a consequence of increased T-cell derived cytokine production locally.

Based on this hypothesis the projects proposed in the parent AADCRC grant will address the following aims:

1. To identify critical miRNAs that are differentially expressed in the airway epithelium of patients with asthma at baseline and in response to allergen challenge or corticosteroid treatment, to determine the roles of IL-13 and IL-17 in regulating these miRNAs and to identify miRNAs that mediate cytokine-induced mucous metaplasia.

2. To determine the relative importance of responses of airway smooth muscle and epithelium to IL-17 in the induction of airway hyperresponsiveness and to determine the individual and combined effects of IL-13 and IL-17 on airway smooth muscle contractility and on clinical responses of patients with severe and mild-to moderate asthma and in response to allergen challenge.

3. To determine the temporal and spatial dynamics of the interactions of IL-13 and IL-17 producing cells with antigen-presenting cells and with airway epithelium and airway smooth muscle in lung slices from allergen challenged mice and in human airway biopsies from patients with severe and mild-to-moderate asthma and in response to allergen challenge or treatment with corticosteroids.

The studies above that depend on allergen challenge in human participants will utilize the ACE study as described here. The studies that will test the effects of inhaled corticosteroids will use a separate clinical protocol.

2.1. Hypotheses

Allergen exposure enhances airway smooth muscle contractility and epithelial cell mRNA/miRNA production as a consequence of locally increased T-cell derived cytokine production.

2.2. Primary Objective(s)

The primary objective of the allergen challenge study (ACE) is to provide a defined allergic stimulus that will promote the cellular and molecular events studied to increase the biological signal and allow time-course analyses.

With respect to the specific scientific studies that we will pursue we have posited the following as our primary endpoint: fold-difference in expression of epithelial miRNAs in the allergen-challenged versus diluent challenged lung segments.

2.3. Secondary Objective(s)

Secondary objectives include:

a. Enumeration and sorting of Th1, Th2 and Th17 cells in BAL and blood using flow cytometry and mass cytometry

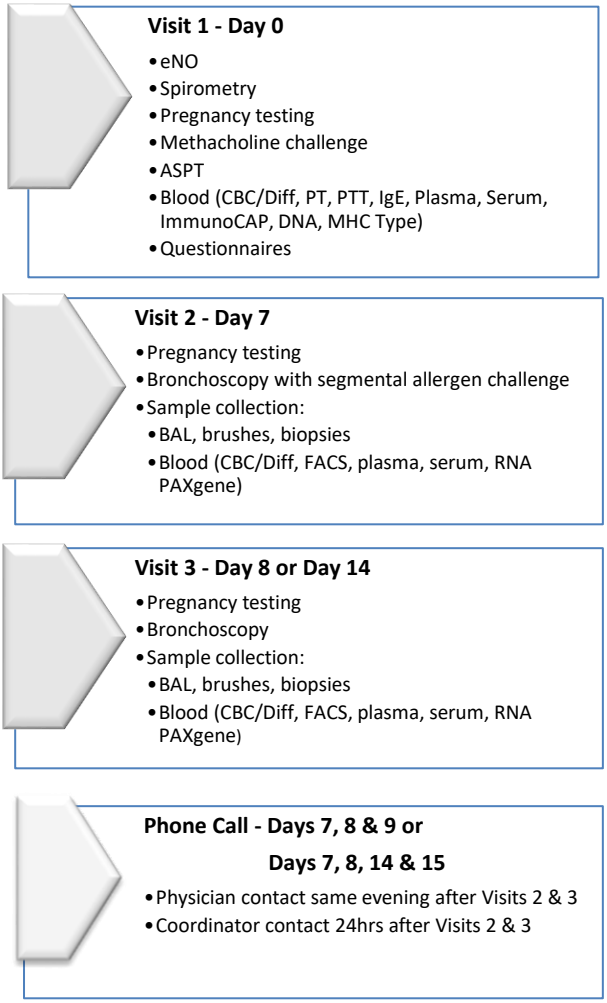
b. Analysis of epithelial mRNA markers of Th2 and Th17 inflammation for immune phenotyping of inflammatory responses
c. Collection of endobronchial biopsies for immunostaining of immune cell localization, immunoblotting of smooth cell protein phosphorylation, analysis of mucin content and smooth muscle cell subculture
d. Banking of epithelial cells, BAL cells, blood cells and biopsies in an IRB-approved bank for subsequent analyses

3. Study Design

3.1. Description of Study Design

This protocol describes a single site mechanistic study to investigate miRNAs that are differentially expressed in the airway epithelium of patients with asthma at baseline and in response to allergen challenge. A three-visit study is proposed. At Visit 1, participants will be characterized in detail (see Section 8). At Visit 2, participants will undergo bronchoscopy with lavage of the left upper lobe (LUL) for baseline studies, then segmental allergen administration in either the right middle lobe (RML) or right upper lobe (RUL, selected randomly) and then diluent administration in either the RML or RUL (the lobe which did not receive allergen). Baseline samples of bronchoalveolar lavage (BAL), epithelial brushings and endobronchial biopsies will be collected. At Visit 3 (either 24 hours later [n=13 asthmatics, 3 allergic non-asthmatics and 3 non-allergic non-asthmatics] or 7 days later [n=13 asthmatics, 3 allergic non-asthmatics and 3 non-allergic non-asthmatics], assigned by blocked randomization) bronchoscopy will be performed for bronchoalveolar lavage (BAL), epithelial brushings and endobronchial biopsies from the sites of allergen and diluent challenge. Blocked randomization will also ensure that equal numbers of subjects who undergo a second bronchoscopy at 24 hours and at 7 days will receive cat allergen. Similarly an equal number of subjects who undergo a second bronchoscopy at 24 hours and at 7 days will receive house dust mite allergen. Sample analysis will include measurement of miRNA and mRNA expression in epithelial brushings (RNAseq and qPCR); analysis of cell surface markers on BAL cells and blood cells; and collection of endobronchial biopsies for immunostaining of immune cells localization, immunoblotting of smooth cell protein phosphorylation, analysis of mucin content and smooth muscle cell subculture. A total of 37 subjects (25 asthmatics with stable or well-controlled asthma, 6 allergic non-asthmatics and 6 non-allergic non-asthmatics) will complete the study. The total time of trial participant in the study is 14 days.

Figure 1. *Study Design*



3.2. Primary Endpoint(s)/Outcome(s)

The primary scientific endpoint of this study is the fold-change in expression of epithelial miRNAs after allergen-challenge as compared to after diluent challenge.

3.3. Secondary Endpoint(s)/Outcome(s)

a. Enumeration and sorting of Th1, Th2 and Th17 cells in BAL and blood using flow cytometry and mass cytometry
b. Analysis of epithelial mRNA markers of Th2 and Th17 inflammation for immune phenotyping of inflammatory responses

c. Collection of endobronchial biopsies for immunostaining of immune cell localization, immunoblotting of smooth cell protein phosphorylation, analysis of mucin content and smooth muscle cell subculture

3.4. Exploratory Endpoint(s)/Outcome(s)

Not applicable

3.5. Stratification, Randomization, and Blinding/Masking

Blocked randomization will be performed to ensure that 13 asthmatics, 3 allergic non-asthmatics and 3 non-allergic non-asthmatics will have their second bronchoscopy at 24 hours, and 13 asthmatics, 3 allergic non-asthmatics and 3 non-allergic non-asthmatics will have their second bronchoscopy at 7 days. Blocked randomization will also ensure that equal numbers of subjects who undergo a second bronchoscopy at 24 hours and at 7 days will receive cat allergen. Similarly an equal number of subjects who undergo a second bronchoscopy at 24 hours and at 7 days will receive house dust mite allergen. None of these randomizations will be blinded.

3.5.1 Procedure for Unblinding/Unmasking

Not applicable (no blinding)

3.5.2 Securing Blinding and Randomization Information

Not applicable (no blinding)

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

Participants with stable or well-controlled asthma are suitable for the study because, when properly selected based on responsiveness to the allergens used, they will respond to segmental allergen challenge with T-cell mediated inflammation in the airway. Furthermore, restriction to participants with stable or well-controlled asthma will reduce the risk of clinical significant deterioration in lung function after the segmental allergen challenge. A small number of non-allergic, non-asthmatic healthy controls will be studied because we expect that any changes observed in these participants should reflect the non-specific effects of prior bronchoscopy. They will serve as negative controls for inflammation in the allergen challenge protocol. That is, we expect to see that they do not have allergic inflammation in the lung (eosinophils and Th2 cells) after allergen challenge. If they do have allergic inflammation in the lung after allergen challenge we will have to reconsider aspects of the protocol that might have led to this surprising and confounding result. In addition, a small number of allergic but non-asthmatic healthy controls will be studied because data presented at the 2013 AADCRC meeting by the MGH group showed that this group can get airway inflammation after allergen challenge. There may still be differences in the type and extent of that airway inflammation as compared to subjects with allergic asthma. Therefore, this allergic/non-asthmatic group will serve a control for the responses in allergy that are not necessarily asthma-specific. If this group has allergic inflammation in the lung after allergen challenge, then we will not change aspects of the protocol, but we will be more cautious in attributing the allergic inflammation that we see to asthma as opposed to the presence of allergy (with or without asthma). We propose to avoid studying children as we are adult pulmonologists with bronchoscopic experience limited to adults. We propose to avoid older participants (>50yo) to minimize the risk of research bronchoscopy and because older participants are more likely to have atypical, non-allergic causes of asthma.

4.2. Inclusion Criteria

Inclusion Criteria for asthmatics:

1. Subject must be able to understand and provide informed consent
2. Prior physician-diagnosed asthma per participant report
3. Age 18 to 50 (inclusive) at the time of consent
4. Pre-BD Baseline (V1) FEV1 >75 % of predicted
5. Skin test reactivity to house dust mite or cat allergen with wheal ≥ 3mm larger than the diluent control at V1
6. Methacholine PC20 < 8 mg/mL

Inclusion Criteria for allergic non-asthmatic controls:

1. Subject must be able to understand and provide informed consent
2. Age 18 to 50 (inclusive) at the time of consent
3. Pre-BD Baseline (V1) FEV1 >90% of predicted
4. Skin test reactivity to house dust mite or cat allergen with wheal ≥3mm larger than the diluent control at V1
5. Methacholine PC20 > 16 mg/mL

Inclusion Criteria for non-allergic non-asthmatic controls:

1. Subject must be able to understand and provide informed consent
2. Age 18 to 50 (inclusive) at the time of consent
3. Pre-BD Baseline (V1) FEV1 >90 % of predicted
4. Negative skin test reactivity to all allergens in the testing panel with wheal <3mm than the positive histamine control at V1
5. Methacholine PC20 > 16 mg/mL

4.3. Exclusion Criteria

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

Exclusion Criteria for asthmatics:

1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol
2. Primary language is not English
3. Use of systemic steroids within the previous 6 weeks
4. A history of intubation for asthma exacerbation
5. Use of Xolair (omalizumab - anti-IgE monoclonal antibody) within the last 6 months
6. Immunotherapy with cat or dust mite extract now or in the past 5 years

7. ≥10 pack-years smoking and any in the past year
8. Women of childbearing potential who are documented to be pregnant (based on urine beta-HCG testing), are sexually active and not willing to use contraception during the study, are seeking to become pregnant, or who are nursing
9. Intolerance to albuterol, atropine, lidocaine, fentanyl, or midazolam
10. Presence of a comorbid condition thought to increase the risk of bronchoscopy by the investigator such as diabetes mellitus, congestive heart failure, ventricular arrhythmias, history of a cerebrovascular accident, renal failure, history of anaphylaxis, or cirrhosis
11. Use of systemic steroids or inhaled steroids, beta-blockers, tricyclic antidepressants and monoamine oxidase (MAO) inhibitors or a visit for an asthma exacerbation within 1 month of the screening visit
12. Other lung diseases, such as sarcoidosis, bronchiectasis or active lung infection
13. History of dermatographia
14. History of anaphylaxis to cat allergen
15. Participation in research study involving a drug or biologic during the 30 days prior to the study
16. Quantitative skin-prick test positive reaction at an allergen concentration of 0.056 BAU or AU/mL
17. Participants with a high possibility of poor compliance with the study
18. Current indoor cat and/or indoor second-hand cigarette smoke exposure
19. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study
20. Any of the following hematologic abnormalities, confirmed by repeated tests:

    White blood count <3,000/µL or >14,000/µL;
    Lymphocyte count <500/µL;
    Platelet count <150,000 /µL;
    Hemoglobin <8.5 g/dL; or
    Neutrophil count <2,000 cells/µL.
21. Subjects who have a 20% drop in FEV1 with diluent at the beginning of the methacholine challenge will be excluded from the study.

<u>Exclusion Criteria for allergic non-asthmatic controls</u>

1. History of asthma or other lung diseases
2. Any of the exclusions #1-21 listed for asthmatics above

<u>Exclusion Criteria for non-allergic non-asthmatic controls:</u>

1. History of asthma or other lung diseases
2. Serum specific IgE to cat and house dust mite > 0.35 IU/ml

3. Any of the exclusions #1-21 listed for asthmatics above

4.4. Selection of Clinical Sites/Labs

Single center (University of California, San Francisco, Domestic US)

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product or Intervention as cited in Investigator Brochure or Package Insert

The physician must be prepared to treat anaphylaxis should it occur and have the necessary drugs and equipment on hand to do so.

5.2. Risks of Investigational Product or Intervention cited in Medical Literature and/or those based on the Investigators' experience

The main risk of the interventional product (standardized allergens) when given in a segmental lung challenge is that the subject will experience a worsening of his/her asthma or anaphylaxis. The administration of allergen to a lung segment is expected to provoke an asthmatic response in that segment. The risks of this procedure include coughing, bronchoconstriction, wheezing, chest tightness, hives, hay fever symptoms, lower blood pressure, shortness of breath and a rare chance of anaphylaxis. A physician will perform the procedure and be present to administer appropriate treatment if necessary. On rare occasions (< 2%), a systemic allergic reaction, including hives, coughing, sneezing, bronchospasm and/or lowered blood pressure can occur that requires treatment with an injection of epinephrine. There is a small chance of a more severe asthmatic reaction, and a chance of a late-phase reaction in which symptoms of asthma can occur a few hours after the procedure.

5.3. Risks of Other Protocol Specified Medications

Albuterol is an inhaled beta-agonist used to measure reversibility after methacholine challenge testing and prior to bronchoscopy. Dosage of albuterol administered in these scenarios is 90mcg per actuation or 360mcg total. In clinical studies, at least 3% of patients taking albuterol report headache, tachycardia (increased heart rate), muscle pain, dizziness, pharyngitis (sore throat), and rhinitis (runny nose). Less than 3% of patients taking albuterol report chest pain, infection, diarrhea, glossitis (swelling of the tongue), anxiety, dyspnea (difficulty breathing), ear disorder, ear pain, and urinary tract infection were reported. In small cumulative dose studies, tremor, nervousness, and headache were most frequently reported.

Provocholine is a parasympathomimetic (cholinergic) bronchoconstrictor agent to be administered in solution only, by inhalation, for diagnostic purposes. Provocholine is utilized during methacholine challenge testing, starting at a low dose of 0.0781mg/mL and a maximum dose of 20mg/mL. During testing, 2mL of each dose is loaded into a nebulizer and a five-breath dosimeter protocol is followed. Adverse reactions associated with 153 inhaled methacholine chloride challenges include one occurrence each of headache, throat irritation, lightheadedness and itching. Per protocol, we will not perform methacholine challenge in subjects with an FEV1<75% of predicted. Furthermore subjects who have a 20% drop in FEV1 with diluent at the beginning of the methacholine challenge will be excluded from the study.

Lidocaine is utilized as a topical anesthetic prior to bronchoscopy. Lidocaine has been associated with adverse systemic side effects, including seizures. In the past a bronchoscopy-related death in a research subject at another institution has been attributed to lidocaine toxicity although the nature of the toxicity has not yet been fully described (the volunteer was a small female who received 1200 mg of lidocaine - a dose well above our upper dose limit of (i) 600mg total or (ii) 10mg/kg if under 60kg, whichever is less).

During bronchoscopy, conscious sedation is attained by using intravenous fentanyl and midazolam. Fentanyl is administered 50 mcg/mL injection 25-50mcg IV push, not to exceed a total of 200 mcg Fentanyl. Midazolam is administered 1 mg/mL injection 0.5-1mg IV push, not to exceed a total of 4 mg Midazolam.

Fentanyl is an opioid and it can cause nausea, itching, headache, feeling dizzy, faint or lightheaded, feeling weak or out of breath and drowsiness. Rare instances of allergic reactions have been reported.
Midazolam is a sedative/anxiolytic/amnestic agent, and it can cause transient drowsiness, fatigue, headaches, a loss of coordination, low oxygen level, respiratory depression, variations in blood pressure and pulse and possible short-term memory loss.

Prednisone may be given in some subjects if they have a more generalized allergic reaction after one of the allergen challenges (skin or lung). The prednisone course will consist of ten 20mg tablets to be taken for delayed worsening of asthma symptoms after the instruction of one of the study physicians. Risks of a short course of prednisone include an increase in appetite, hyperglycemia, difficulty sleeping, mood change, headache, changes in blood pressure and an increased risk for oral candidiasis

5.4. Risks of Study Procedures

Withholding Medication
Asthmatic participants will be asked to withhold short-acting beta-agonist medications for 6 hours before each study visit and long acting beta agonists for 48 hours before study visits. This is to allow for lung function measures that are not influenced by beta-agonist treatment. Holding beta-agonists in this way could cause asthma symptoms to worsen and may even cause asthma exacerbations, which in severe cases have the potential to be fatal. For these reasons the participants will be instructed to use their medications if they develop wheezing before the start of their study visit.

Spirometry
Spirometry may cause transient cough or light-headedness and can rarely worsen bronchospasm. This has not caused any serious problem in our experience in clinical studies of asthma. Participants are monitored closely during the procedure, and worsening bronchospasm is obvious from the fall in FEV1 from the first to third required maneuver, and the bronchospasm responds promptly to administration of an inhaled beta-agonist (albuterol) if needed.

Venipuncture
Venipuncture, IV placement and blood draws have minor risks including pain and/or hematoma formation that may occur at an intravenous puncture site. Dizziness or fainting during blood sampling may occur.

Methacholine Challenge

Methacholine challenge testing may be associated with cough, sense of throat irritation, and shortness of breath that may subside without treatment or can be reversed promptly using standard inhaled bronchodilator therapy. To minimize risk, the test will not be performed if the subject is symptomatic with wheezing or if baseline spirometry is < 75% predicted (which would be exclusionary for study participation in any case for both asthmatics and healthy controls). Furthermore, subjects who have a 20% drop in FEV1 with diluent at the beginning of the methacholine challenge will be excluded from the study.

Allergen Skin Prick Test (SPT)

Allergen skin prick testing is a common test for allergy and carries the risk for itching and burning at the site of the test, and the discomfort of the needle scratch. There is a very small risk of anaphylaxis during allergen skin testing. Facilities and medications are available for treatment if anaphylaxis should occur and a physician will be readily available when the skin tests are performed.

Exhaled Nitric Oxide Test

Exhaled nitric oxide testing involves breathing into a machine while wearing a nose clip. No specific adverse effects are expected.

Electrocardiogram

Electrocardiogram (ECG) will be performed prior to the bronchoscopy to rule out any potential cardiac issues. ECG does not involve any risks or significant discomforts, except for a possible minor skin itching or irritation at the site the electrodes are placed.

Bronchoscopy

The passage of the bronchoscope through the vocal cords can cause laryngospasm. This can be prevented by lidocaine pretreatment. The presence of a bronchoscope in the large airways can also cause bronchospasm, but this can be prevented by albuterol pretreatment which is standard of care in our laboratory. Participants occasionally complain of a mild sore throat after bronchoscopy and a temperature elevation (100-101°F) occurs within 12 hours about 5-10% of the time. Bronchitis or pneumonia can occur within the first week after bronchoscopy, and would be treated with antibiotics. Bronchoscopy may also cause increased shortness of breath following the procedure, requiring increased use of bronchodilators. The risk of infection or asthma exacerbation following research bronchoscopy in patients with asthma is not available in the current literature; in our experience from our clinical work, we estimate these risks to be rare, probably less than 5%. However, if these complications occur, they would require treatment with antibiotics or oral steroids.

Bronchoalveolar Lavage, Brushing and Biopsy

Bronchoalveolar lavage is ordinarily associated with little sensation, but has caused coughing in some patients. Bronchial brushing may cause cough and may also cause some bleeding from the airway wall that is usually minor but may be noticed as blood streaking of sputum by the subject. Bronchial biopsies are usually accompanied by a mild tugging sensation with each biopsy, and may also cause some bleeding from the airway wall that is usually minor but may be noticed as blood streaking of sputum by the subject. Biopsies carry approximately a 5% risk of a visible scar at the biopsy site, but such scarring is not known to lead to any health consequence in our experience or in the experience of other investigators who do these research procedures whom we have specifically queried. The risk of pneumothorax is lower with endobronchial biopsy than with transbronchial biopsy, however pneumothorax remains a rare but possible complication of the bronchoscopy that we propose. Should any

complications of bronchoscopy occur, we are prepared to treat them with the emergency equipment available in our Bronchoscopy Suite.

Segmental Allergen Challenge

As indicated above, the study described here will study the effects of standardized cat and dust mite allergen preparations, that have been approved by the FDA for the treatment of allergies (PLA 90-0556, U.S. License 308) by measuring their effects when they are applied to a subsegment of the lung by injection though a bronchoscope. The characterization and availability of standardized extracts, including cat hair [13, 14] and dust mite, have permitted more uniform clinical responses and improved the safety of immunotherapy because the effective amount of antigen delivered is less variable than with non-standardized extracts. Adverse reactions that are possible include anaphylaxis, asthma exacerbation, and exacerbation of rhinitis. As an additional safety precaution and in attempts to limit the influence of endotoxin on the allergic response being studied, endotoxin levels will be measured in all allergen extracts prior to use. Endotoxin levels must be $\leq$ 100 EU/ ml in the concentrated extract to meet standards for subsequent study use.

5.5. Potential Benefits

There are no direct benefits to the participants enrolled in this study outside of any new knowledge that a subject might gain about the severity of his or her asthma based on the spirometry that is performed and subsequent discussions with the study physician. Subjects should not expect health improvement as a result of participation. Potential benefits to society relate to the acquisition of knowledge concerning the cellular sources and roles of IL-13 and IL-17 in the lung in asthma. These studies have the potential to inform blockade of these interleukin pathways as a therapeutic approach in asthma and to inform the proper selection of participants most likely to respond to these therapeutic approaches.

6. Investigational Agent /Device/Intervention

6.1. Investigational Agents/Devices/Interventions

6.1.1. Investigational Agent #1

Standardized Cat Hair allergen, Greer Laboratories, Inc

6.1.1.1. Formulation, Packaging, and Labeling

Standardized Cat Hair is prepared from the hair and dander of cats. The final product is delivered in a glycerin-containing solution at a concentration of 5,000 or 10,000 Bioequivalency Allergen Units (BAU) per milliliter. Detailed information regarding the formulation of this product can be found in the manufacturer's package insert. We will use as few lots as necessary based on the constraints of available expiration dates and the length of the study.

6.1.1.2. Dosage, Preparation, and Administration

Endotoxin levels will be measured in the concentrated extracts to confirm endotoxin levels < 100 EU/ml. For this investigation, the route of administration will be topical application of the titrated allergen to a bronchoscopically isolated subsegment of one lobe of one lung. The dose of the biologic product will be determined from the skin-prick titration testing done at the screening visit. The allergen solution will be serially diluted (see Section 6.2), and the lowest concentration that gives a positive skin-prick test will be determined, called the threshold allergen concentration. A "test dose" will be administered initially in

the isolated subsegment, which will consist of 2 mL of allergen at 1/10th of the threshold allergen concentration. If on visual inspection through the bronchoscope after at least 5 minutes after administration, there is no evidence of mucosal inflammation, a second segmental allergen challenge is instilled through a bronchoscope maintained in the "wedge" position in the right middle lobe using 2 mL of the full-dose allergen. This dose will consist of 2 mL of allergen at the threshold allergen concentration. Stock allergen vials will be stored at 4 degrees C in the UCSF Investigational Drug Service (IDS), as described in Section 6.2.

6.1.2. Investigational Agent #2

Standardized Mite extract, Greer Laboratories, Inc

6.1.2.1. Formulation, Packaging, and Labeling

The Standardized Mite extract is prepared from whole mite bodies, fecal particles, and not more than 1% growth medium components. Each vial contains a sterile extract of Mite (*Dermatophagoides farinae* or *D. pteronyssinus*), 0.50% sodium chloride, 0.25% sodium bicarbonate, 50% glycerin by volume, and 0.4% phenol as a preservative. Detailed information regarding the formulation of this product can be found in the manufacturer's package insert. We will use as few lots as necessary based on the constraints of available expiration dates and the length of the study.

6.1.2.2. Dosage, Preparation, and Administration

Endotoxin levels will be measured in the concentrated extracts to confirm endotoxin levels < 100 EU/ml. For this investigation, the route of administration will be topical application of the titrated allergen to a bronchoscopically isolated subsegment of one lobe of one lung. The dose of the biologic product will be determined from the skin-prick titration testing done at the screening visit. The allergen solution will be serially diluted (see Section 6.2), and the lowest concentration that gives a positive skin-prick test will be determined, called the threshold allergen concentration. A "test dose" will be administered initially in the isolated subsegment, which will consist of 2 mL of allergen at 1/10th of 1/3rd of the threshold allergen concentration. If on visual inspection through the bronchoscope at least 5 minutes after administration, there is no evidence of mucosal inflammation, a second segmental allergen challenge is instilled through a bronchoscope maintained in the "wedge" position in the right middle lobe using 2 mL of full-dose allergen. This dose will consist of 2 mL of the allergen at 1/3rd of the threshold allergen concentration. Stock allergen vials will be stored at 4 degrees C in the UCSF Investigational Drug Service (IDS), as described in Section 6.2.

6.1.3. Investigational Agent #3

Phenolized Diluent, Greer Laboratories, Inc

6.1.3.1. Formulation, Packaging, and Labeling

Sterile Diluent is a 10% Saline-Glycerin mixture with a 0.4% phenol preservative.

6.1.3.2. Dosage, Preparation, and Administration

For this investigation, the route of administration will be topical application of the diluent to a bronchoscopically isolated subsegment of one lobe of one lung. A 2mL volume of diluent will be instilled

through a bronchoscope maintained in the "wedge" position in the right upper lobe. Diluent will be administered prior to administration of either allergen (Investigational Agents #1-2).

6.2 Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding the disposition of the investigational product will be available for inspection.

The UCSF Investigational Drug Service (IDS) is an inpatient pharmacy located in the UCSF Medical Center whose services will be utilized to handle the Investigational Agents mentioned in Section 6.1. The UCSF IDS will take the stock solutions ordered from Greer Laboratories and will aliquot single-use vials of each Investigational Agent at the appropriate dosages to be used during the quantitative skin prick test and segmental allergen challenge. These dilutions will be prepared fresh on the day of study visits using sterile techniques by the IDS pharmacist. These vials will be labeled appropriately with allergen dosages and dispensed to the research laboratory for use. A dispensation log will be kept to track vials used during study procedures. The UCSF IDS will also send off aliquots from each stock solution from every unique manufacturer's lot number in order to obtain endotoxin measurements. All Investigational Agents will be stored at 4 degrees C in the IDS facility. Any unused or expired vials will be discarded via the Medical Center Inpatient Pharmacy.

6.3 Assessment of Participant Compliance with Investigational Agent

The investigational agent will only be administered by study personnel and will never be administered by the subject.

6.4 Toxicity Prevention and Management

Bronchoscopy: The bronchoscopy is an invasive medical procedure that is accompanied with inherent risks; the study physicians at our site have performed research bronchoscopies for over 25 years, have anticipated the risks and have identified methods to minimize these risks as much as possible. The bronchoscopy is performed in the dedicated ACRC Bronchoscopy Suite. This suite is dedicated for research bronchoscopy only, is equipped with a wall oxygen source, wall suction, blood pressure monitoring equipment, oximeter, ECG, emergency medications (Pharmacy Pyxis in place with naloxone, flumazenil, epinephrine, prednisone, albuterol, methylene blue), and has emergency airway adjuncts (nasal airways, oral airways, bag and mask, endotracheal tubes, laryngoscopes) and a telephone in the room. The Bronchoscopy Suite has a code cart accessible and is located within 100 feet of an intensive care unit of the Medical Center. Our center reinforces emergency procedures with "mock codes" on a regular basis. The passage of the bronchoscope through the vocal cords can cause laryngospasm. This can be prevented by lidocaine pretreatment. The presence of a bronchoscope in the large airways can also cause bronchospasm, but this can be prevented by albuterol pretreatment which is standard in our

laboratory. Bronchoscopy may also cause increased shortness of breath following the procedure, requiring increased use of bronchodilators. The risk of infection or asthma exacerbation following research bronchoscopy in patients with asthma is not available in the current literature; in our experience from our clinical work, we estimate these risks to be rare, probably less than 5%. However, if these complications occur, they would require treatment with antibiotics or oral steroids.

In addition, established lidocaine and other medication guidelines, included in the MOP, are in place to prevent excess administration. These include careful monitoring of total dose of lidocaine administered and post-procedure monitoring will be adjusted based on lidocaine dose. Participants will be observed for at least 4 hours after bronchoscopy. Limiting the risks of conscious sedation is done by following the conscious sedation guideline (included in the MOP) established for the UCSF Medical Center. Participants have careful hemodynamic monitoring, continuous measurement of oxygen saturation and experienced physicians and nurses are in charge of the procedure.

Before discharge the participants will be examined and have their vital signs checked by one of the physicians. Only participants who have fully recovered from bronchoscopy and the effects of anesthesia will be discharged from the Bronchoscopy Suite. Participants not fully recovered at 4 hours after bronchoscopy will be kept in the Bronchoscopy Suite, under observation, until the physician present considers that the subject is medically fit for discharge. Participants who have received fentanyl or midazolam will not be allowed to drive themselves home. All participants will be given the contact numbers of the physician investigators so that complications can be reported and the subject seen if necessary. We will also contact all participants by telephone the same evening of the bronchoscopy procedure (physician contact) and 24 hours after bronchoscopy (coordinator contact) to inquire if complications have developed.

To minimize the risk of significant allergic reactions, the lowest necessary doses of allergen will be used in segmental allergen challenge by basing dosing on the results of quantitative skin prick testing in the same subject and using a test dose during the bronchoscopy, as described here.

Quantitative skin-prick testing: Standardized Cat hair extract or Standardized mite extract (either *Dermatophagoides farinae* or *Dermatophagoides pteronyssinus*) prick testing reagents from Greer will be used, beginning with a 3-fold dilution of the full-strength reagent for prick testing (10,000 BAU or AU/mL). This beginning dose will be 3,333 BAU or AU/mL and range down to 0.056 BAU or AU/mL, in addition to a histamine positive control and diluent negative control. Subsequent skin prick testing will be done with serial 3-fold dilutions of each extract. The “threshold” level of sensitivity will be the lowest concentration of extract that elicits a wheal sum of 3 mm greater than the diluent control. A positive reaction at 0.056 BAU/mL will disqualify the participant due to the extreme level of sensitivity. Testing will be done on the right or left arm. The “threshold” level of sensitivity will be confirmed on the opposite arm by repeating the threshold concentration and one dilution above and below this level.

Segmental Allergen Challenge: After successful determination of the threshold allergen concentration, a separate visit will be scheduled for segmental allergen challenge. Prior to segmental allergen challenge bronchoscopy, the participant’s lung function will be measured and participants with an FEV1 that is not at least 75% of the predicted normal value are excluded. At bronchoscopy, a "test dose" concentration of allergen is administered first. For cat

allergen, the “test dose” will consist of 2 mL of allergen at 1/10th the threshold allergen concentration. For house dust mite allergen, the “test dose” will consist of 2 mL of allergen at 1/10th of 1/3rd the threshold allergen concentration, which is equivalent to 1/30th the threshold allergen. If on visual inspection through the bronchoscope, there is no evidence of mucosal inflammation after at least 5 minutes after administration, a second segmental allergen challenge is done in the right middle lobe using 2 mL of full-dose allergen. For cat allergen, the “full dose” will consist of 2 mL of allergen at the threshold allergen concentration, with the maximum dose available as 1,000 BAU (500 AU/mL x 2mL). For house dust mite, the “full dose” will consist of 2 mL of allergen at 1/3rd the threshold allergen concentration, with the maximum dose available as 740 BAU (370 AU/mL x 2mL).

Each subject will be given an albuterol rescue inhaler and ten 20 mg pills of prednisone to take home in case they experience a delayed worsening of their asthma following the segmental allergen challenge (SAC) procedure. They will also be instructed to contact Dr. Woodruff or one of the study physicians in case they experience worsening of symptoms.

Many details in the protocol have been developed to protect against this risk. From the literature, there is significant evidence that lung challenge with allergens can be done safely. Published studies using well-characterized extracts of dust mite allergen have demonstrated the effectiveness of these extracts as immunotherapeutic agents in blinded randomized trials [8-11] and their safety when administered to the airways [9-12]. The characterization and availability of standardized extracts, including cat hair [13, 14] and dust mite, have permitted more uniform clinical responses and improved the safety of immunotherapy because the effective amount of antigen delivered is less variable than with nonstandardized extracts. Immunotherapy with cat extracts has been shown to be as safe as immunotherapy with similar doses of ragweed extract given in similar doses. At least four double-blinded randomized control trials have shown that this therapy is associated with decreased sensitivity to inhaled cat extract [4, 6, 7, 15]. In each of these studies, cat-allergic asthmatics underwent inhalational allergen challenge, and no adverse consequences of cat allergen inhalation were reported when cat allergen extract was delivered to the whole lung. Limiting allergen to a single lobar subsegment would be expected to be even safer.

6.5 Discontinuation of Investigational Agent

In accordance with the Declaration of Helsinki, patients have the right to withdraw from the study at any time for any reason. Participants may withdraw with or without medical advice. The investigator also has the right to withdraw participants from the study.

Study therapy may be prematurely discontinued for any participant for any of the following reasons: Participants will be removed from the study for the following reasons: adverse experience; intercurrent illness or medication that in the judgment of the investigator may place the subject at risk; request of the investigator or participant for administrative or other reasons; protocol violation; determination that the participant is non-compliant or has unreliable behavior. Withdrawal from this study will have no impact on the future care of the participant at UCSF. Since this is not a clinical trial of a specific agent, no “intention-to-treat” issues apply.

Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

7. Other Medications

7.1 Concomitant Medications

7.1.1 Protocol-mandated

- Albuterol MDI 4 puffs (360 mcg) after Visit 1 methacholine challenge testing (if needed); and at Visits 2 and 3 prior to bronchoscopy
- Provocholine to be utilized during methacholine challenge test

7.1.2 Other permitted concomitant medications

- Any other medications not listed in Section 7.3 *Prohibited Medications*, after review and clearance from study physician

7.2 Prophylactic Medications

Not applicable.

7.3 Prohibited Medications

Medications to be discontinued prior to enrollment and for the duration of the study are listed below. The risks of withholding of short-acting bronchodilators can be discussed with subjects by telephone prior to the screening visit. Withholding of any other medications will only be done after informed consent is obtained and after in-person discussion of the risks. This may require that Visit 1 be split across more than one day.

- Astemizole: 12 weeks prior
- Steroids (oral, inhaled, or nasal): 6 weeks prior
- Nedocromil sodium, sodium cromoglycate (inhaled, nasal or ocular): 4 weeks prior
- Long-acting methylxantines: 2 days prior
- Short-acting methylxantines: 12 hours prior
- Montelukast: 7 days prior
- Zafirlukast: 7 days prior
- Salmeterol: 2 days prior
- Anti-histamines (both oral and nasal): 7 days prior
- Omalizumab: 6 months prior

7.4 Rescue Medications

- Albuterol MDI 2 puffs prn for shortness of breath or increased asthma symptoms post-bronchoscopy
- Prednisone 40mg PO x 5 days, course to be determined by study physician depending on increased asthma symptoms post-bronchoscopy

8 Study Procedures

Blood Draw: At the screening visit (V1), approximately 70mL of blood will be collected for clinical labs (CBC/Diff, IgE), PT/PTT, plasma, serum (for ImmunoCAP testing for IgE specific for cat and house dust mite), DNA and for HLA DRA/DRB1 typing. All abnormal CBC and PT/PTT values will be repeated. ImmunoCAP specific IgE testing will be used for characterization of asthmatic and allergic subjects but will also be used to determine which allergen to give to non-allergic non-asthmatics. Since non-allergic non-asthmatics are a negative control group, we will require that they have a specific IgE < 0.35 IU/ml to either cat or house dust mite allergen. If their specific IgE is ≥ 0.35 IU/ml to one of the allergens, we will use the other allergen. If their specific IgE is ≥ 0.35 IU/ml to both of the allergens, then we will withdraw them from the study. HLA DRA/DRB1 typing for use of antigen:HLA tetramers to track antigen-specific T

lymphocytes in blood and BAL. Our goal is to study the movement of inflammatory cells into the lungs in response to allergen exposure (either cat dander or dust mite antigen). Each subject is exposed to only one allergen, based on the results of the allergy skin testing. The allergic inflammatory response includes the recruitment of T lymphocytes expressing allergen-specific T cell receptors (TCR) as well as many other T lymphocytes and other inflammatory cells that do not express allergen-specific TCR. Allergen-specific tetramers are synthetically created molecular complexes containing allergen:HLA molecules complexed together with a fluorescent protein. Whereas each TCR recognizes a single allergen:HLA molecular complex, the binding avidity of a soluble allergen:HLA complex to the TCR is too low to form a stable interaction. A tetramer overcomes this problem by linking 4 identical allergen:HLA complexes with a fluorescent protein. The result is a macromolecular complex that forms a stable interaction with TCR on allergen-specific T lymphocytes. Bound tetramers are usually detected in a flow cytometer. In order for a subject's T cells to recognize and bind antigen-specific tetramers, the tetramer must contain the correct HLA molecule. There are currently only a limited number of synthetic tetramers. We will use any available tetramers that recognize the cat allergen *Fel d* 1 or dust mite *Dermatophagoides pteronyssinus Der p* 1 allergen in association with the relevant alleles at HLA-DR and -A. These tetramers will allow us to track allergen-specific T lymphocytes in those cat and dust mite allergic participants who have HLA-alleles for which tetramers are available. If the subject is negative for any available HLA alleles, the subject will still participate in the study. However, we will only be able to track allergen-specific T lymphocyte responses in the participants expressing one of the appropriate HLA alleles. Since tetramer staining is a secondary analysis, there will be no minimum on the number of subjects we will require with specific HLA alleles.

Urine Collection: Urine will be collected at all study visits for Urine Pregnancy Testing for participants with childbearing potential. Urine beta-HCG determination will be checked immediately before methacholine challenge testing and skin testing, and immediately before each bronchoscopy.

Allergen Skin Testing: Routine allergen prick skin testing is performed at Screening Visit (V1) using a battery of 12 standardized allergen extracts. The skin prick testing panel includes histamine positive control; diluent negative control; mite (*Dermatophagoides farinae*); mite (*D. pteronyssinus*); cockroach mix; mouse; rat; *Penicillium* mix; *Aspergillus* mix; *Alternaria alternata*; cat; and dog. For asthmatics and allergic non-asthmatic controls, those with demonstrated positive reactions to either cat or dust mite allergen, quantitative allergen extract skin-prick testing will be performed with one of these extracts from the specified lot numbers. For those with a positive reaction to both cat or dust mite allergen, the cat allergen will be used for quantitative allergen extract skin-prick testing. For those with a positive reaction to only house dust mite, then house dust mite allergen will be used. We will perform a blocked randomization so that equal numbers of subjects who undergo a second bronchoscopy at 24 hours and at 7 days will receive house dust mite. Similarly, blocked randomization will ensure that equal numbers of subjects who undergo a second bronchoscopy at 24 hours and at 7 days will receive cat allergen. For non-allergic non-asthmatic controls, no quantitative skin-prick test will be performed. One half of non-allergic non-asthmatic controls will receive cat allergen challenge and one half will receive house dust mite challenge.

Quantitative Skin Prick Test: Standardized Cat hair extract or Standardized mite extract (either *Dermatophagoides farinae* or *Dermatophagoides pteronyssinus*) prick testing reagents will be used, beginning with 3,333 BAU or AU/mL and range down to 0.056 BAU or AU/mL, in addition to a histamine positive control and diluent negative control. Subsequent skin prick testing will be done with serial 3-fold dilutions of each extract. The "threshold" level of sensitivity will be the lowest concentration of extract that elicits a wheal sum of 3 mm greater than the diluent control. A positive reaction at 0.056 BAU/mL will disqualify the participant due to the extreme level of sensitivity. Testing will be done on

the right or left arm. The "threshold" level of sensitivity will be confirmed on the opposite arm by repeating the threshold concentration and one dilution above and below this level.

Spirometry: Lung function is determined on a calibrated instrument that meets the standards set by the American Thoracic Society.

Interview: Asthma and respiratory disease history is obtained, and a validated respiratory symptom questionnaire is administered (Asthma Control Test, ACT).

Routine Physical Examination: Blood pressure and heart rate are measured with a mercury manometer that meets the specifications of the American Heart Association. Weight, height, body temperature, and respiratory rate are determined. Evaluation of the head, eyes, ears, nose, throat, neck, heart, chest, lungs, abdomen, extremities, peripheral pulses, neurological status, skin, and other physical signs of note assess general physical well-being.

8.1 Enrollment (V1)

The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Once the informed consent has been signed, the participant is considered enrolled in the study and will be assigned a unique participant number.

8.2 Screening/Baseline Visit

The purpose of the Baseline visit is to confirm eligibility to continue in the study.

Temporary exclusion criteria will be reviewed. If any of the following are true, the participant will need to be re-screened at a later date:

- Asthma exacerbation or upper respiratory infection within the previous 6 weeks
- Antihistamines within the previous 7 days
- Systemic or inhaled antibiotics use within the previous 4 weeks
- Any eye, chest or abdominal surgery within the previous 6 weeks
- Any other change in health status within the previous 6 weeks which is deemed by a study physician to require re-screening because it could alter the risk of bronchoscopy or could alter the results of the study

The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility:

Visit 1 – Baseline at Day 0 (approximately 5 hours)

- Consent form
- Vital signs
- Urine pregnancy test (females only)
- Medical history and physical exam
- Questionnaires about general health and/or asthma symptoms
- Skin prick test ("qualitative") - For asthmatics and allergic non-asthmatic controls, need (+) SPT to cat and/or dust mite; for non-allergic non-asthmatic controls, need (-) SPT to all allergens

- Quantitative skin prick test – For asthmatics and allergic-non-asthmatics controls only
- Exhaled nitric oxide (eNO)
- Spirometry – to confirm FEV1 > 75% predicted for asthmatics or FEV1 > 80% predicted for healthy controls
- Methacholine challenge test
- Bronchodilator treatment, if needed
- Blood draw (approximately 70 mL) including ImmunoCAP testing for IgE specific for cat and house dust mite
- Review pre-bronchoscopy instructions

Data from a completed Baseline visit (Visit 1) will be considered valid for 12 weeks. If a subject does not proceed to the second visit during that period of time, the visit must be repeated. If a subject fails screening due to failure of an inclusion or exclusion criterion, the screening visit will not be repeated.

8.3 Study Visits or Study Assessments

Visit 2 – First Bronchoscopy at Day 7 (approximately 7 hours)

- Vital signs
- Urine pregnancy test (females only)
- Electrocardiogram
- Spirometry, pre- and post-bronchodilator
- Short H&P and pre-bronchoscopy assessment
- IV placement and blood draw (approximately 67 mL)
- Bronchoscopy with segmental allergen challenge
- Post-bronchoscopy spirometry
- Dispensation of albuterol and/or prednisone, if needed
- Review second bronchoscopy instructions

Visit 3 – Second Bronchoscopy at either Day 8 or Day 14 (approximately 6 hours)

- Vital signs
- Urine pregnancy test (females only)
- Electrocardiogram
- Spirometry, pre- and post-bronchodilator
- Short H&P and pre-bronchoscopy assessment
- IV placement and blood draw (approximately 67 mL)
- Bronchoscopy
- Post-bronchoscopy spirometry

Schedule of Events

Visit number	1 In-person	2 In-person	3 In-person	4 Phone calls
Time of Visit	Day 0	Day 7	Day 8 or Day 14	Days 7, 8 & 9 or Days 7,8,14 &15
Inclusion/Exclusion criteria	X			
Informed consent	X			
Vital signs	X	X	X	
Medical history and physical examination	X	X	X	
Concomitant medication evaluation	X			
Skin prick test qualitative	X			
Skin prick test quantitative	X			
Urine pregnancy test	X	X	X	
Exhaled nitric oxide	X			
Spirometry	X	X	X	
Methacholine challenge	X			
Bronchodilator treatment	X	X	X	
Blood draw (incl. CBC/Diff, PT/PTT, and ImmunoCAP at V1)	X	X	X	
Review instructions for next visit	X	X		
Electrocardiogram		X	X	
Bronchoscopy		X	X	
Segmental allergen challenge		X		
Post-bronchoscopy spirometry		X	X	
Dispensation of albuterol/prednisone, if needed		X		
Safety monitoring	X	X	X	X

8.4 Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, participants are instructed to contact study personnel and may be asked to return to the study site for an "unscheduled" visit. At these visits, the first priority will be subject health and safety. If, for any reason, the safest path is withdrawal from the study (for example, to allow treatment with prednisone due to a worsening of asthma) withdrawal will be undertaken at this unscheduled visit.

8.5 Visit Windows

Study visits should take place within the time limits specified below:

Visit 1 to Visit 2		1 week minimum; 12 weeks maximum
Visit 2 to Visit 3	(Option 1)	24 hours +/- 4 hours
Visit 2 to Visit 3	(Option 2)	7 days +/- 1 day

If any of the study procedures cannot be performed or samples cannot be obtained at the scheduled visit, the procedure can be conducted (or repeated) at the next trial visit.

9 Mechanistic Assays

a. Measurement of Th2- driven inflammation using epithelial brush gene expression – the "Three Gene Mean" metric**:** We will perform qPCR for three previously validated Th2 signature genes in epithelial brushings (POSTN, CLCA1, and SERPINB2). The log (base-2) transformed relative expression value for each subject will be normalized using the geometric mean of 5 housekeeping genes. We further use a stock solution of cDNA (target and housekeeping), to calibrate the normalized expression values across runs. These values are centered and scaled and then, for each subject, the arithmetic mean of the three centered & scaled genes is calculated, producing the "three-gene-mean" metric which reflects the degree of Th2-inflammation. One long-term goal of this project is to develop a similar metric for IL-17 driven inflammation.

b. Measurement of BAL T-cell production of IL-13 and IL-17 using flow cytometry. We have applied intracellular cytokine staining to directly quantify the production of IL-13 and IL-17 in CD3+/CD4+ cells in BAL. We have also developed protocols for surface staining for CCR6, CCR4, CXCR3 as an alternate strategy to identify and quantify Th2 (CCR6-/CCR4+/CXCR3-) and Th17 (CCR6+/CCR4+/CCR3-) subsets in BAL, confirming intracellular cytokine expression of the appropriate cytokines in these subsets in preliminary experiments.

c. Immunocytochemistry (IHC) for IL-17 and IL-13 producing cells in bronchial biopsies. The enumeration of IL-17 producing cells in bronchial biopsies by immunohistochemistry is feasible and provides quantitative data.

10 Biospecimen Storage

The types of biospecimens collected and processed include: blood, bronchoalveolar lavage (BAL), airway epithelial brushings, and bronchial biopsies. Sample preparation types that are processed from these biological samples and either stored in the UCSF Airway Tissue Bank or provided to project leaders for further analyses in real-time include: sorted PBMCs, serum, plasma, BAL supernatant, whole BAL cell pellets/RNA, FACS sorted cell pellets/RNA, cytospin preps, airway epithelial brush cytospin preps, RNA, dispersed cell preparations, and bronchial biopsies fixed (for quantitative morphometry), homogenized for RNA, and dispersed for flow cytometry. The UCSF Airway Tissue Bank is the sample repository for samples that must be stored before provision to other project leaders and for biospecimens (e.g, serum, tissue and lavage fluid) remaining once the specified analyses are conducted. All subjects must sign the additional UCSF Airway Tissue Bank consent in order to participate in this study. This consent will confer permission for long-term storage and secondary research uses using this IRB-approved tissue banking protocol.

- Protocols: We maintain written Standard Operating Procedures (SOPs) to ensure quality and consistency. All RNA samples are quality-checked using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara CA), graded using RIN numbers and assessed by Nanodrop for accurate quantification of RNA concentration.

- Blood: PBMCs will be isolated by Lymphoprep gradient and then stained with fluorescent conjugated antibodies or tetramers for analysis.
- BAL: BAL fluid is centrifuged at 300 x g for 5 min at 4 degrees C, and the supernatant decanted for aliquot samples. The cell pellet is reserved cell pellet for the production of cytospin slides, flow cytometric analyses, RNA preparation (QIAzol) or protein analysis as desired. In addition cell counts and differentials are performed using a hemocytometer on a reserved fraction and from a cytospin preparation respectively.
- Airway epithelial brushings: Each brush is placed immediately in RNase-Free PBS on ice and gently vortexed. Total and differential cell counts are performed and cytospin slides made. A portion of the cells are used for RNA preparation (QIAzol).
- Bronchial biopsies: Biopsies for immunohistochemistry are fixed overnight in 10% paraformaldehyde and then processed by our laboratory into paraffin with re-embedding in a isotropic uniform random orientation using an isector (spherical) mold. Biopsies for flow cytometry are immediately dispersed with the use of collagenase I (Sigma) reconstituted in RPMI 1640 without L-glutamine at 1 mg per milliliter for a 1-hour period at 37°C prior to staining and flow cytometric sorting.
- Sample Inventory and Database Management: The UCSF Airway Tissue Bank inventory is managed with an Access database which records the location, quality, and quantity of samples and tracks users and is housed on a secure server. The server is protected with a firewall and raided drives, and is backed up daily to tapes. The following procedures ensure database security: 1) management of unique User ID and passwords, and 2) data access control (tracking and monitoring access).

11 Criteria for Participant and Study Completion and Premature Study Termination

11.1 Participant Completion

Completion of screening visit and both bronchoscopy visits

11.2 Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.
5. Participants will be terminated from the study at the point that investigational product (s)/intervention (s) are discontinued for safety reasons.
6. Mild or moderate adverse events such as nausea, vomiting, increased asthma symptoms, allergic dermal reactions, or fever will cause the procedure on the individual patient to be discontinued, will be reported in the required timely manner to the Partners IRB and FDA, but will not automatically cause the study to be halted.

Individual Stopping Rules

7. Any study issue that changes the risk to benefit ratio.
8. Systemic allergic response to study procedure requiring treatment with systemic steroids or epinephrine.
9. Asthma exacerbation in response to study procedure which requires initiation of ICS or systemic steroids. Acute treatment following allergen challenge is excluded.
10. Significant and sustained drop in lung function (FEV1) of >30% from pre-procedure baseline, in response to study procedure. Acute measurements following allergen inhalation challenge are excluded. Significant is defined as >30% from pre-procedure baseline. Sustained is defined as lasting > 24 hours.
11. Significant hypoxia (<85% oxygen saturation) during bronchoscopy.

The following describes Individual Stopping Rules based on the adverse events outlined in Section 12.2.1. If an individual meets any one of the stopping rules, the individual's participation in the study will be stopped. (See Section 12.3.1. for Grading Criteria.)

Adverse event	**Criteria for stopping**
Allergic reaction	Grade 3
Anaphylaxis	Grade 3
Cardiac arrhythmia (Conduction disorder)	Grade 2
Bronchospasm	Grade 3
Cough	Grade 3
Fever	≥ 101°F for ≥ 24 hours after bronchoscopy
Nausea	Grade 3
Pneumonitis	Grade 2
Pneumothorax	Grade 1

11.3 Participant Replacement

Participants who withdraw or are withdrawn will be replaced with a newly enrolled subject until 26 asthmatics, 6 allergic non-asthmatics and 6 non-allergic non-asthmatics have competed the study.

11.4 Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant will be asked to complete a final visit for spirometry and a physical examination.

11.5 Study Stopping Rules

Study enrollment and study procedures will be suspended pending expedited review of all pertinent data by the IRB, the SMC, and the NIAID, if a subject at any time or in any group develops a study-related severe or life threatening adverse event such that requires an emergency room visit, hospitalization, or an unexpected (non-allergic-, or non-asthmatic-related) hospitalization or death. The event will be reported immediately by the PI to the IRB and to the FDA, the SMC, and the NIAID. The study will not resume until approval is given by the FDA and IRB, the SMC, and the NIAID.

In the event that 3 participants meet individual stopping rules (in Section 11.2), or more than 1 participant develops a significant systemic allergic reaction (Grade 2 or more), dyspnea or cough that prevents daily activity, or fever/nausea Grade 3 or more, or a participant dies, the study will be halted until the events are reviewed by the NIAID MM and the local independent safety monitor, with the FDA and IRB. The study will not resume until approval is given by the FDA, IRB and NIAID.

12 Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, *Reporting of Serious Adverse Events and Adverse Events*) to *[DAIT/NIAID]*. Appropriate notifications will also be made to Institutional Review Boards (IRBs), and health authorities.

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version *4.0*: http://ctep.cancer.gov/reporting/ctc.html.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with:

- **Study therapy regimen**: Any event occurring within one week of use of a standardized allergen, or within 24 hours of use of albuterol or methacholine will be considered a possible adverse event of these medications and evaluated further for any relationship.

- **Study mandated procedures**: Any event occurring within one week of a mandated study procedure will be considered a possible adverse event of these procedures and will be evaluated further for any relationship. These procedures include: phlebotomy, spirometry, methacholine challenge testing, allergen skin prick testing, quantitative skin prick test, segmental allergen challenge and bronchoscopy.

For the procedures below, clinical situations are listed that are considered to be outside the normal range of outcomes and will be recorded as Adverse Events. These situations do not limit an investigator from recording and reporting any other events, associated or not with these procedures as AEs.

Blood Draws

- Fainting /vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 5 minutes
- Swelling at puncture site larger than 2 cm

Allergen Skin Prick Testing

- Prolonged (>24 hours) itching at test site
- Swelling (> 10 cm) at site of test lasting more than 24 hours
- Nasal allergic symptoms within 30 minutes from the procedure
- Fainting /vasovagal event within 30 minutes from the procedure
- Systemic reaction

Pulmonary Function Testing

- Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes from the procedure
- Coughing requiring treatment with bronchodilators within 30 minutes from the procedure

Methacholine Challenge

- FEV1 has not returned to at least 90% of the baseline (pre-diluent) value with 4 puffs of albuterol
- FEV1 drops by more than 50% during the procedure

Bronchoscopy

- Asthma exacerbation requiring prednisone in response to bronchoscopy
- Significant decrease in lung function
  a) FEV1 decreases by >60% from baseline
  or
  b) FEV1 decreases by 40-59% from baseline AND does not begin to recover spontaneously during the first 60 minutes post immediate decline in FEV1, OR does not respond to albuterol therapy employed to reverse bronchoconstriction
  or

c) Failure to recover to within 80% of baseline FEV1 at discharge. Albuterol therapy may be used to assist in recovery

- Significant hypoxemia during bronchoscopy - the bronchoscopy procedure results in hypoxemia of <85% oxygen saturation for greater than 30 seconds despite supplemental O2

Segmental Allergen Challenge

- Systemic Reaction
- Asthma exacerbation requiring prednisone
- Significant decrease in lung function
  a) FEV1 decreases by >60% from baseline
  or
  b) FEV1 decreases by 40-59% from baseline AND does not begin to recover spontaneously during the first 60 minutes post immediate decline in FEV1, OR does not respond to albuterol therapy employed to reverse bronchoconstriction
  or
  c) Failure to recover to within 80% of baseline FEV1 at discharge. Albuterol therapy may be used to assist in recovery
- Significant hypoxemia during bronchoscopy - the bronchoscopy procedure results in hypoxemia of <85% oxygen saturation for greater than 30 seconds despite supplemental O2

12.2.1.1 Suspected Adverse Reaction (SAR)

Not Applicable

12.2.2 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the package insert or is not listed at the specificity, severity or rate of occurrence that has been observed; or is not consistent with the risk information described in the general investigational plan or elsewhere in the IND.

12.2.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator and DAIT/NIAID*]*, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study participants according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE, Version 4.0) This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The NCI-CTCAE has been reviewed by the Principal Investigator and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

Events of grade 2 or higher will be recorded on the appropriate electronic AE case report form for this study. These electronic forms generate a paper copy that can be shared and reviewed easily. In addition, an existing DSMP utilized at the UCSF Airway Clinical Research Center will be used to identify events associated with pulmonary-specific procedures such as bronchoscopy and ASPT that meet internal criteria for adverse events (see Appendices A, B).

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

12.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate electronic AE report form. Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Unrelated	There is insufficient evidence to suggest a causal relationship
RELATED CATEGORIES		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

Adverse events will be collected from the time of consent until a subject completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Interviewing the subject [e.g., using a checklist, structured questioning, diary, etc.] .
- Receiving an unsolicited complaint from the subject.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, *Grading and Attribution of Adverse Events.*

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 12.2, Definitions*)* on the appropriate electronic case report form regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

The site investigator will report to the NIAID, the IRB and FDA as follows:

12.5.1 Reporting of Serious Adverse Events to NIAID

The site investigator will report to the NIAID Medical Officer and NIAID Project Manager all serious adverse events (see Section 12.2.3, *Serious Adverse Event)*, regardless of relationship or expectedness within 24 hours of discovering the event. Notification is by phone and e-mail.

Contact information for NIAID Medical Monitor:

Lisa M Wheatley, M.D., MPH
Medical Officer
NIH/DAIT/AAABB
5601 Fishers Lane Rm 6B56
Rockville, MD 20852
Phone:240-627-3573
Cell : 301-641-1301
E-mail: lisa.wheatley@nih.gov

For serious adverse events, all requested information on the AE/SAE paper CRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE paper CRF will be updated and submitted. When an SAE report is sent it will include the PIs assessment of the adverse event and assignment of a relationship to any specific aspect of the study (e.g. a cat extract causing a severe asthma attack).

12.5.2 Reporting to Health Authority

After an adverse event requiring 24 hour reporting (per Section 12.5.1, *Reporting of Serious Adverse Events*) is submitted by the site investigator and assessed by the DAIT/NIAID.

12.5.2.1 Annual Reporting

The investigator will include in the annual study report to health authorities all adverse events classified as:

- Serious, expected, suspected adverse reactions (see Section 12.2.1.1, *Suspected Adverse Reaction,* and Section 12.2.2, *Unexpected Adverse Event*).
- Serious and not a suspected adverse reaction (see Section 12.2.2, *Suspected Adverse Reaction*).
- Pregnancies not reported as serious adverse events.

  Note that all adverse events (not just those requiring 24-hour reporting) will be reported in the Annual IND Report.

The DAIT/NIAID will receive a copy of the annual study report submitted by the investigator to the health authorities.

The investigator shall summarize safety data at the end of the study and periodically throughout the study for IND regulatory filings, the safety monitoring committee (SMC) and for the medical officer (MO). These reports will meet the needs of the SMC and the MO and may include (but are not limited to) masked summaries and listings of AEs, SAEs, changes in vital signs, lab results, events requiring discontinuation of a study-mandated procedure for individual subjects, protocol deviations, and/or events listed as study stopping rules.

12.5.2.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the adverse event is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction** [**SUSAR]** (see Section 12.2.1.1, *Suspected Adverse Reaction* and Section 12.2, *Unexpected Adverse Event* and 21 CFR 312.32(c)(1)i).

The investigator shall report any suspected adverse reaction that is both serious and unexpected. The investigator shall report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
2. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Category 2: **Any findings from studies that suggests a significant human risk**

The investigator shall report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

The investigator shall notify the FDA and all participating investigators of expedited Safety Reports within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.3 Reporting of Adverse Events to IRBs/IECs

The investigator shall report adverse events, including expedited reports, in a timely fashion to their respective IRBs/IECs in accordance with applicable regulations and guidelines.

12.6 Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study subject. A pregnant subject will no longer undergo any study procedures. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report to the DAIT/NIAID all pregnancies within 1 business day of becoming aware of the event using the SAE form. All pregnancies identified during the study shall be followed to conclusion and the outcome of the pregnancy will be submitted to the NIAID Medical Officer and Project Manager. An update using the SAE form will be submitted to the NIAID Medical Officer and Project Manager when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

Should the pregnancy result in a congenital abnormality or birth defect, an SAE shall be submitted to the NIAID Medical Officer and Project Manager using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as the DAIT/NIAID when an "unanticipated problem involving risks to participants or others" is identified, which is not otherwise reportable as an adverse event.

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive annual reports from the protocol investigator compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site on appropriate paper copies of CRFs.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the protocol investigator (See Sections 12.5.1, *Reporting of Serious Adverse Events*, and 12.6, *Pregnancy Reporting*).

12.8.2 SMC Review

12.8.2.1 Planned SMC Reviews

The Safety Monitoring Committee (SMC) shall review safety data at least yearly during planned SMC Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs. The SMC will be informed of an Expedited Safety Report within 10 working days.

12.8.2.2 *Ad hoc* SMC Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the SMC may be called upon for *ad hoc* reviews. The SMC will review any event that potentially impacts safety at the request of the protocol investigator or DAIT/NIAID. In addition, the following events will trigger an *ad hoc* comprehensive SMC Safety Review:

- Any death that occurs in the study, which is possibly or definitely related to study treatment regimen.
- The occurrence of a Grade 3 or higher related and unexpected SAE in any study participants who have received a study treatment.
- The occurrence of Grade 2 or higher events related to segmental allergen challenge in 2 or more of the study participants who have received study treatment.

After review of the data, the SMC will make recommendations regarding study conduct and/or continuation.

12.8.2.2.1 Temporary Suspension of standardized allergen challenge for *ad hoc* SMC Safety Review

A temporary halt in segmental allergen challenge will be implemented if an *ad hoc* SMC safety review is required. No new participants will be enrolled and no bronchoscopies will be performed until the SMC has met and discussed the CRFs.

13 Statistical Considerations and Analytical Plan

13.1 Overview

The scientific objective is to provide a defined allergic stimulus that will promote the cellular and molecular events studied across the three projects of the parent AADCRC grant to increase the observed biological signal and allow time-course analyses. The study design consists of a three-visit study of 25 asthmatics who are proven to be allergic to one of the two allergens chosen and 6 healthy controls. The first visit will be characterization visit at which quantitative allergen skin prick testing will establish safe doses for segmental allergen challenge. The second visit will be for bronchoscopy to deliver segmental challenge with allergen and diluents in two different locations in the lung. The third visit will be for bronchoscopy to collect BAL, brush and biopsy samples from challenged areas.

13.2 Endpoints/Outcomes

The primary scientific endpoint of this study is the fold-change in expression of epithelial miRNAs before and after allergen-challenge

Secondary endpoints include:

a. Enumeration and sorting of Th1, Th2 and Th17 cells in BAL and blood using flow cytometry and mass cytometry
b. Analysis of epithelial mRNA markers of Th2 and Th17 inflammation for immune phenotyping of inflammatory responses
c. Collection of endobronchial biopsies for immunostaining of immune cell localization, immunoblotting of smooth cell protein phosphorylation, analysis of mucin content and smooth muscle cell subculture
d. Banking of epithelial cells, BAL cells, blood cells and biopsies in an IRB-approved bank for subsequent analyses

13.3 Measures to Minimize Bias

Every subject will receive both allergen and diluent as a control.

13.4 Analysis Plan

For the primary analysis, epithelial brushing RNA from the participants with asthma will be analyzed for the difference in expression after allergen challenge as compared to after diluent challenge at two time-points (13 participants with asthma will be studied at 24 hours and 13 subjects with asthma at 7 days). miRNA will first be measured by miRNAseq followed by qPCR validation of specific findings.

13.4.1 Analysis Populations.

All participants with paired samples (the allergen challenge segmental brush and diluent challenge segmental brush) will be analyzed.

13.4.2 Primary Analysis of Primary Endpoint(s)/Outcome(s)

After processing of the sequencing data to read counts, the difference in read count in the allergen challenged as compared to the diluent challenged segment at the given follow-up time point will be calculated for each subject. The change in read count for each miRNA in the allergen samples will be compared to the change in read count in the diluent samples and statistical significance will be assessed using the Benjamini Hochberg false discovery rate method (FDR) to account for multiple comparisons. By convention we have used an FDR <5% as the cut-off for calling statistical significance. Findings will be validated by qPCR. These analyses will be performed using the LIMMA package, in Bioconductor, in paired t-test mode to effect the differential expression (Allergen challenge vs. Diluent challenge) analyses. LIMMA enables use of moderation / penalization in paired (or more general blocked) settings and also allows model fitting in which other covariates can be included. In general, we will analyze whether the change in normalized transcript number (Allergen challenge – Diluent challenge)=0. We will also introduce a term for time period (24 hours versus 7 days) into the model to understand whether time has any additional influence on miRNA transcript number and a term for the allergen used (cat versus house dust mite).

13.4.3 Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

Additional analyses will correlate change in miRNA expression with the degree of increase in Th2 and Th17 cells in the same participants. These will be performed using all miRNAs (~800) using either Pearson or Spearman correlation, as appropriate, controlling for a false discovery rate (FDR) <0.05.

13.4.4 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

Analyses of the secondary outcomes related to epithelial miRNA will in general take the same form as for the primary analysis. However in these analyses a) the allergen challenged segment (after 24 hours or 7 days) will be compared to the baseline naïve sample (LUL) and b) the diluent challenged segment (after 24 hours or 7 days) will be compared to the baseline naïve sample (LUL). For other secondary analyses we have pre-specified outcomes of interest and will not need to make recourse to FDR adjustments.

13.4.5 Analyses of Exploratory Endpoint(s)/Outcome(s)

Not applicable.

13.4.6 Descriptive Analyses

Descriptive analyses will be performed of the age, gender, race/ethnicity of participants as well as important clinical factors such as lung function (by spirometry), methacholine reactivity and clinically useful measures of atopy (serum IgE and blood eosinophilia). Use of asthma medications will be tabulated. Study completion will be

tabulated. In addition, the Allergic-Non Asthmatics (ANA) and Non-Allergic Non-Asthmatics (NANA) controls are proposed so that we can determine whether allergen challenge causes cellular inflammation (by routine cell counts and flow cytometry) in these two groups at 24 hours and at 7 days. This information will be helpful in understanding whether our protocol will cause allergic airway inflammation in ANA (we suspect that it may) and NANA (we suspect that it will not). These data will help us interpret the generalizability of what we find in asthma and help us understand the protocol we have designed for this study and future studies. Thus we will perform descriptive analyses of total and differential cell counts in the BAL for eosinophils in these subjects as well as the % of T effector cells that are TH2 or Th17 cells by flow cytometry.

13.5 Interim Analyses

None

13.5.1 Interim Analysis of Efficacy Data

Not applicable

13.5.2 Interim Analysis of Safety Data

Safety data analysis will be ongoing and will occur after each bronchoscopy

13.5.3 Futility Analysis

Not applicable

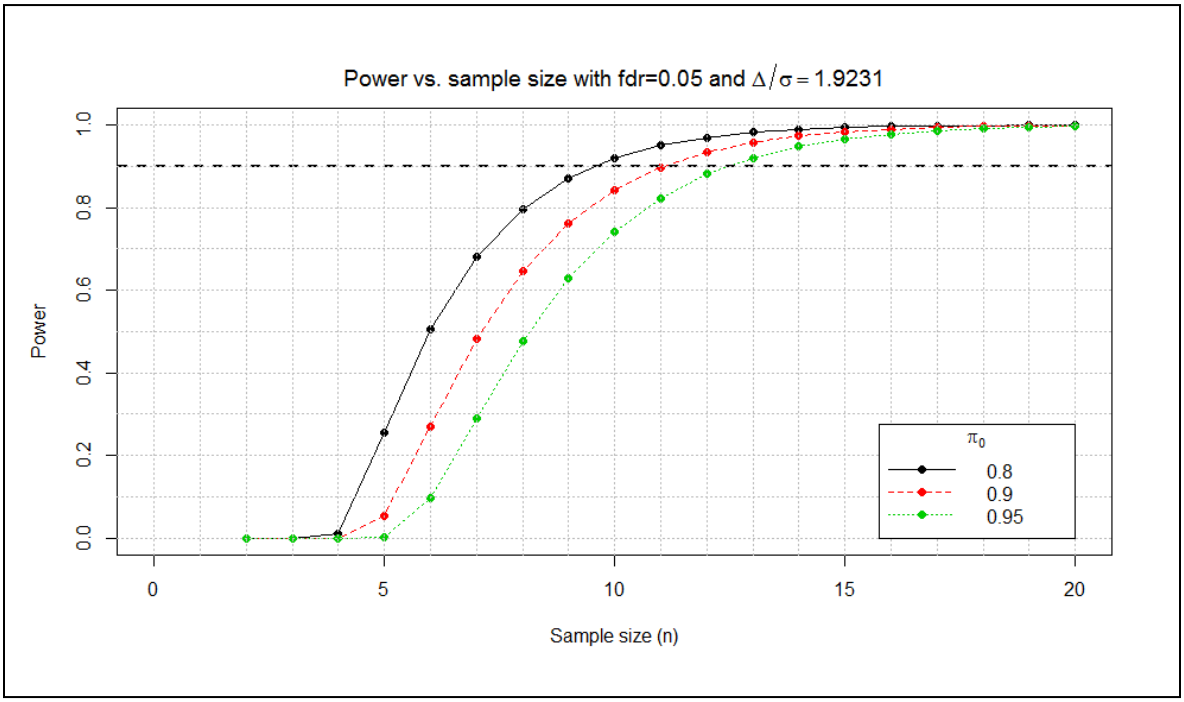
13.6 Statistical Hypotheses

The null hypothesis is that allergen challenge will not result in any changes in epithelial miRNA expression as compared to diluent challenge. The testable hypothesis is that allergen challenge will result in a measurable change in epithelial miRNA expression. The comparison will be between allergen challenge samples and diluent challenge samples and will be superiority in nature.

13.7 Sample Size Considerations

We have published prior cross-sectional data compares subjects with asthma to healthy controls which does not exactly mimic the design of the current pre-post allergen challenge design study but is useful for sample size estimation [2]. Since our prior data is two-group, and we do not have SDs for a within-person change after allergen challenge, we performed power calculations for a two group study and then suggest that these calculations may be extrapolated to a paired pre-post within-subject analysis. We consider this a conservative approach given that within-person SDs should be smaller than between group SDs. Another conservative measure that we adopted was to use the 90th percentile of SDs from our prior data in our estimates, $\sigma=0.52$, rather than the median SD. In part, we chose these conservative approaches because it's possible that changes with allergen challenge will not be of the same magnitude as differences between asthma and healthy. We then assumed an FDR of 0.05 and an effect size of 2-fold change (yielding a $\Delta/\sigma=1.9231$), which are in the conservative range of generally accepted values. Finally we plotted scenarios which correspond with a percentage of miRNAs being truly 2-fold increased with allergen challenge ranging from 20% to 5% (Figure). In brief, we found that studying 13 subjects in each arm (analogous to 13 subjects after allergen challenge versus diluent challenge at each time point), will provide >90% power to detect increases or decreases in epithelial miRNA expression that are >2-fold at an FDR=0.05 even if that occurs only 5% of the time. For comparison, in our cross-sectional data we found that ~25% or miRNAs were differentially expressed. In our final analysis we will build a model using LIMMA that uses data from both time points with a term for "time",

yielding a total n=26. However, these sample size calculations suggest that 13 subjects at each time point will conservatively provide sufficient power for the overall analysis even if the miRNAs expressed at these two time pints are not perfectly congruent. These analyses used the ssize.fdr package in R, using the method of Liu and Hwang [17].



14 Identification and Access to Source Data

14.1 Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial. The source data here will include pulmonary function test print outs from a spirometer, clinical lab reports with CBC/Diff, PT, PTT and serum IgE data, as well as CRFs recording the quantitative skin prick test data.

14.2 Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID as well as to relevant health authorities, such as the FDA. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15 Protocol Deviations

15.1 Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or

procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

15.2 Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations to the NIAID. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation has occurred, the study staff will a) notify the site Principal Investigator, b) notify the NIAID Project Manager and c) will complete a Protocol Deviation form. The DAIT/NIAID Medical Monitor will make the decision as to whether the Deviation is major or not and what the impact of the Deviation on the study participant or the entire study may be. Protocol Deviation reports will also be submitted to the IRB, SMC, FDA and the DAIT/NIAID Medical Monitor and NIAID Project Manager, who will review and approve any action plan that is implemented as a result of the Protocol Deviation.

16 Ethical Considerations and Compliance with Good Clinical Practice

16.1 Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the IRB. Any amendments to the protocol or to the consent materials will also be approved by the IRB before they are implemented.

16.2 Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the Investigator of Record form, will review the consent and answer questions. Consent designees must be experienced clinical coordinators who have undergone training within the UCSF Airway Clinical Research Center in consent procedures and have completed all UCSF clinical research training modules. The participant will have the option to speak to the principal investigator for any questions regarding the study and consent. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any

reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in English and a copy of the signed consent form will be given to the participant.

The consent process will be ongoing, the participant will be made aware at each visit that they can withdraw consent, and the adequacy of consent will be checked before each bronchoscopy. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study. Privacy and Confidentiality

16.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing protected health information (PHI) to the study sponsor or their representatives.

17 Publication Policy

Every possible effort will be made for the primary outcome of the trial to be published in a peer-reviewed journal within 12 months after the database is locked. DAIT/NIAID will review and comment on any manuscript derived from this trial prior to submission (http://grants.nih.gov/grants/policy/data_sharing/data_sharing_guidance.htm#goals) and that data from this trial will be shared in accordance to the specific plan that was included in the grant application.]

18 References

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APPENDIX A: UCSF Airway Clinical Research Center Study Procedures Monthly Totals Form

(Data collected for quarterly Quality Assurance Meetings)

UCSF Airway Clinical Research Center

Rooms: M-1329, M-1333

Monthly Totals: Safety Monitoring and Quality Assurance

Month: **Year:** **Protocol:**

	Total # done	Events	# Adverse events	Comments
Methacholine challenge:		☐>20%fall after diluent ☐fall in FEV1 >40% ☐>360µg or >1 neb BD ☐failed to reach ≥90% BL FEV1 ☐other	☐**None** #	
Sputum induction:		☐>20% fall in post BD FEV1 ☐tolerated < 4 min ☐>360µg or >1 neb BD ☐failed to reach ≥90% post BD FEV1 ☐other	☐**None** #	
Allergen skin prick test:		☐satellite rash/hives ☐ anaphylaxis ☐ other	☐**None** #	
6 minute walk test		☐SaO2 < 80% and no recovery after 3 min rest ☐test stopped due to: chest pain, fall, leg cramps, diaphoresis ☐other	☐ **None** #	
Bronchodilator hold:		☐ FEV1 <50% pred ☐ increased symptoms ☐ ER visit ☐ steroids required ☐ other	☐**None** #	
Use of study medication:		☐ Side effects causing discontinuation of drug ☐ other	☐**None** #	
Other procedures: **Blood draw** **IV line** **Nasal/throat swab** **12 hour fast** **Other**			☐ **None** ☐ **None** ☐ **None** ☐ **None** ☐ **None**	
Bronchoscopy: **Refer to Bronch Tool**			☐ **None**	

QA monitoring tool version 4.1 1 01/13/2011

APPENDIX B: UCSF Airway Clinical Research Center Bronchoscopy Data Collection Tool

UCSF MEDICAL CENTER
BRONCHOSCOPY DATA COLLECTION TOOL
2014

DEPT/LAB: M1333 ACRC	DATA COLLECTOR: Christine Nguyen	Phone #: 476-3824
STANDARDS: UCSF Airway Clinical Research Center guidelines for safe monitoring during and following research bronchoscopy procedures.		**DATA COLLECTION PERIOD:** Jan-Dec 2014

INDICATORS: 30 cases or 100% if case number <30
Instructions: In the columns corresponding to the indicators below, indicate (+) if present or (-) if absent.

1. Laryngospasm
2. Severe bronchospasm
3. Pneumothorax
4. Bleeding > 10 ml during procedure
5. Hypotension: SBP <90 during or 1 hour post bronchoscopy
6. SaO2 <90% during or 1 hour post bronchoscopy
7. Fever >100° F lasting more than 24 hours post bronchoscopy
8. Flu-like syndrome within 48 hours of bronchoscopy
9. Increase in asthma symptoms lasting 2 days post bronch
10. Chest pain
11. Cough over next 3 days without improvement
12. Coughing bright red blood >1-2 TBS within 3 days of bronchscopy
13. Sore throat lasting longer than 3 days post bronch
14. Date of resolution of problem
15. AE/SAE report sent to CHR
16. AE discussed in QA meeting
17. Other

STUDY	ID#	SUBJECT INITIALS	PROC. DATE/MD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.																				
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