PROTOCOL **NCT03780387**

Title: Identification of allergic asthmatics reactive to *Felis Catus* (cat hair) allergen inhalation

Drug Name: *Felis catus* (cat hair) allergen extract (Stallergenes Greer, Lenoir, NC).

FDA IND: 18411 (Sponsor: ML Hernandez)

Sponsor: NIH National Heart, Lung, and Blood Institute (NHLBI)

Protocol Date: May 13, 2019

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PROTOCOL TITLE: Identification of allergic asthmatics reactive to *Felis Catus* (cat hair) allergen inhalation

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ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse Event
AHR	Airway Hyperreactivity
AUC	Area Under the Curve
BAU	Bioequivalent Allergy Units
CBC	Complete Blood Count
CEMALB	Center for Environmental Medicine, Asthma and Lung Biology
CTRC	Clinical Translational Research Center
ENO	exhaled nitric oxide
EPR	Early phase response
FEV_1	Forced Expiratory Volume in 1 Second
FRC	Forced Residual Capacity
FVC	Forced Vital Capacity
HCG	Human Chorionic Gonadotropin
HRV	Heart Rate Variability
IGE	immunoglobulin E
IL	Interleukin
LAIV	Live Attenuated Influenza Virus
LDMS	Lab Data Monitoring System
LPR	Late phase response
MDI	Metered Dose Inhaler
MMHG	Millimeters of Mercury
NACL	Sodium Chloride
Nasal ELF	Nasal Epithelial Lining Fluid
NO	Nitric Oxide
NSAIDS	Non-steroidal Anti-inflammatory Drugs
NSBR	Non-specific Bronchial Reactivity
PD20	provocative dose of allergen resulting in $\geq$20% fall in FEV_1
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
TH2	T Helper Cell Type 2
TSLP	Thymic Stromal Lymphopoetin

PROTOCOL SYNOPSIS

We will study the use of inhaled cat hair allergen extract as a model of allergen-induced asthma exacerbation in humans. Using this model, we will measure key mediators involved in airway responses to allergen. This will also enable us to study potential asthma therapies and estimate their efficacy for reducing allergen-induced airway inflammation.

Study Title	Identification of allergic asthmatics reactive to Felis Catus (cat hair) allergen inhalation
Funder	NIH/NHLBI
Clinical Phase	Phase II
Study Rationale	Acute exacerbation of asthma is characterized by airway inflammation (recruitment of leukocytes and production of inflammatory cytokines) leading to bronchoconstriction, increased production of airway mucous, and decreased mucociliary clearance with formation of mucous plugs. Humans models of asthma exacerbation are necessary in order to adequately understand the pathophysiology involved and for the development and testing of targeted treatments for asthma-induced airway inflammation. Such work will reduce our field's current dependence on corticosteroids to treat airway inflammation.
Study Objective(s)	Primary • To identify subjects with mild allergic asthma who display a late phase response (LPR) after inhalation of cat hair extract for potential participation in two planned studies of IL-1 receptor antagonist (Anakinra) therapy against features of allergen-induced asthma exacerbation. Secondary • To determine if IL-1β concentrations in the sputum at baseline before challenge are predictive of key asthma outcomes following inhaled allergen challenge, specifically: o maximum drop in %FEV1 during LPR o Change in methacholine reactivity from baseline pre-allergen measurement to 24 hours after allergen challenge. o exhaled nitric oxide (eNO) level o induced sputum: ▪ Granulocyte (neutrophils/eosinophils) numbers and percentages ▪ Mucins (secretion of MUC5AC and MUC5B determined by Western blot and ELISA)

Test Article(s) (If Applicable)	Standardized cat hair (*Felis catus*) allergen extract is at a potency of 10,000 BAU/mL in 50% glycerin, 0.25% Sodium Bicarbonate, and 0.5% Sodium Chloride, provided by Stallergenes Greer, Lenoir, NC.
Study Design	Open-label single-center study
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Age range 18-45 years, inclusive 2. FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of at least 0.7 (without use of bronchodilator medications for 8 hours or long acting beta agonists for 24 hours), consistent with lung function of persons with no more than mild intermittent or mild persistent asthma. 3. Physician diagnosis of asthma 4. Allergic sensitization to cat hair extract as determined by positive immediate skin prick test response or serum cat IgE >0.35 kU/L from screening protocol 98-0799 5. Oxygen saturation of >94% and blood pressure within the following limits: Systolic between 150-90 mmHg and Diastolic between 90-60 mmHg. **Exclusion Criteria** 1. Physician directed emergency treatment for an asthma exacerbation within the preceding 12 months. 2. Symptoms consistent with moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma. 3. Viral upper respiratory tract infection within 4 weeks of challenge. 4. Cigarette smoking >1 pack per month 5. History of intubation for asthma 6. Pregnancy or nursing a baby. 7. Subjects who are prescribed daily inhaled corticosteroids, cromolyn, or leukotriene inhibitors (Montelukast or Zafirlukast) will be required to discontinue these medications at least 2 weeks prior to the screening protocol. 8. Abnormalities on lung auscultation 9. Abnormal vital signs (as defined in the protocol) 10. Inability or unwillingness of a participant to give written informed consent.
Number Of Subjects	We will screen up to 100 people to identify 25 mild asthmatics who experience a LPR to inhaled cat hair allergen extract and are eligible for enrollment into the Anakinra therapy trials.

Study Duration	Each subject's participation will last approximately 1 month. The entire study is expected to last 3 years.
Efficacy Evaluations	Spirometry (lung function measurement) Methacholine challenge Analysis of induced sputum samples Exhaled nitric oxide Venipuncture Heart rate variability measurement by wearable sensors
Pharmacokinetic Evaluations	none
Safety Evaluations	Spirometry Symptom Questionnaires Vital sign measurement Pulse oximetry
Statistical And Analytic Plan	Our analytical plan was developed with Dr. Zhou, the biostatistician for the CEMALB who will oversee all statistical analysis. In all analyses, criterion for significance will be $p \leq 0.05$. Our primary endpoint is identification of subjects who achieve a ≥15% fall in FEV_1 during hours 3-10 following allergen challenge. We will subtract the pre-allergen challenge %FEV1 value from the post-allergen %FEV1 value to make this determination. For analysis of secondary endpoints, we will first use linear regression to determine if there is a linear relationship between baseline sputum IL-1β concentration (pg/mL) and asthma outcomes (maximum fall in %FEV1 during LPR, methacholine reactivity defined by the dose required to produce a ≥20% drop in FEV1, sputum granulocyte content both in terms of % of total cell count and cells/mg of sputum, exhaled nitric oxide concentration) following allergen challenge, adjusting for potential confounders. The point estimate and confidence interval will be reported for the effect of baseline sputum IL-1β on asthma outcomes. If the normality assumption is violated or there exists a non-linear relationship, we will calculate the Spearman correlation coefficient between baseline sputum IL-1β concentration and asthma outcomes.
DATA AND SAFETY MONITORING PLAN	We will use the NC TraCS DSMB to assess for possible changes to the overall risk of the study using inhaled allergen challenge. The DSMB will have full access to research records and source documents. Data regarding unanticipated problems will be provided to them as it is reported to the IRB and to the FDA. They will

review all adverse events, enrollment, and protocol deviations after the first 6 subjects have completed the inhaled allergen protocol, and then every 6 months thereafter. Any serious adverse events or major protocol deviations will be reported immediately to the DSMB via email, which may lead to an earlier review. They will communicate with the PI and the appropriate NIH Medical Officer regarding any safety issues.

Study coordinators will be responsible for efforts and computations for database management. Data will be initially collected by study coordinators on paper documents. Symptom questionnaires will be completed by the subject and collected by the coordinator. Clinical data such as health history and smoking history will be collected by study staff as interview or directly measured, such as vital signs, spirometry, etc. All paper forms (source documentation) are maintained by a study coordinator or PI in a binder, 1 binder per subject. All data will be entered into REDCap by a study coordinator, within 2 weeks of collection and will be verified by a 2nd person within 4 weeks to ensure accuracy and completeness.

Biological samples collected by coordinators will be delivered to the CEMALB lab by the coordinators in properly labeled containers. On receipt in the lab, samples are initially entered into a software program called LDMS (Lab Data Management System) which tracks samples. Processing of these samples is done per the SOP maintained in the CEMALB quality assurance plan, as some samples are processed immediately and others are processed in batches at later time points. This data is entered into REDCap at appropriate intervals, based on sample analysis.

1. BACKGROUND AND RATIONALE

1.1. Introduction

Asthma is an increasingly common chronic illness among children and adults[1], and allergen exposure is among the most common triggers for asthma exacerbations. Exacerbations of allergic asthma are characterized by an early phase response (EPR), mediated by release of preformed mediators like histamine from mast cells, and a late phase response (LPR) 3-10 hours later mediated by chemokines and cytokines that attract neutrophils and eosinophils to the airways, increase mucus production, trigger airway smooth muscle contraction, and result in airway constriction and airway hyperreactivity (AHR). The LPR does not occur in the absence of an EPR. The LPR is thought to be predominantly responsible for the symptoms associated with acute exacerbations of allergic asthma and is often used as the measure of efficacy in trials of asthma therapeutics.

Our lab has taken a particular interest in targeting an inflammatory cytokine, Interleukin-1β, involved in both the early and late phase asthmatic responses to inhaled allergen in allergic asthmatics. In the lung, IL-1β is produced by numerous cell types (including epithelial cells, macrophages, neutrophils, eosinophils, and mast

cells), where it signals through its receptor to induce transcription of pro-inflammatory genes [2-4]. IL-1β is increased in bronchoalveolar lavage fluid from persons with symptomatic asthma vs. those with asymptomatic asthma [5]; likewise, immunohistochemistry of bronchial biopsies of allergic asthmatics reveal increased expression of IL-1β in both bronchial epithelial cells and macrophages[3]. Previous studies in animal and *in vitro* models demonstrate that IL-1β can directly impact three aspects of an airway inflammatory response: 1). granulocyte (neutrophil/eosinophil) recruitment [6, 7]; 2). non-specific [8, 9] and allergen-specific airway reactivity [10, 11]; and 3). production and clearance of airway mucous [12, 13]. Supporting literature and our preliminary studies in human subjects further promote the study of IL-1 blockade for mitigating features of acute allergen-induced asthma exacerbation.

The role of IL-1 in allergen challenge models has not been fully defined. In a study examining 12 asthmatics allergic to *D. farinae* at our research center, we found that 9 of 12 asthmatics had a greater than 10% reduction in forced expiratory volume in 1 second (FEV_1) after inhaled dust mite challenge [14]. These individuals were considered responders. It was notable that when comparing post-allergen levels of cytokines between responders and non-responders there was a much greater concentration of IL-1β in post-challenge sputum from responders vs. non-responders, Furthermore, within the responders, post challenge IL-1β also significantly correlated with sputum eosinophil concentrations ($r=0.83$, $P<0.05$) and neutrophil concentrations ($r=0.89$, $P<0.05$) 24 hours after allergen challenge. These data suggest that IL-1β may play a role in both immediate airway hyperresponsiveness and the late phase recruitment of inflammatory cells (neutrophils and eosinophils) after inhaled allergen challenge.

Numerous IL-1 blocking agents are FDA-approved for conditions where the IL-1β pathway predominates disease pathophysiology such as in systemic juvenile idiopathic arthritis and the cryopyrin-associated periodic syndromes [15]. Anakinra is a FDA-approved recombinant form of human IL-1 receptor antagonist (IL-1RA), a natural anti-inflammatory cytokine that competes with agonist binding to the IL-1 receptor, suppressing IL-1β and IL-1α signaling. With a fast onset of action, reaching peak concentrations in 3-7 hours, and a short 4-6 hour half-life, anakinra is an ideal candidate to test as a rescue treatment for acute asthma exacerbation.

The purpose of this study is to identify subjects with mild allergic asthma who experience a LPR after inhalation of *Felis catus* (cat hair) extract. Additionally, in order to better understand the role of IL-1β in allergen-induced airway inflammation, we will obtain induced sputum to determine if higher baseline sputum IL-1β concentrations or larger increases in IL-1β following allergen challenge impact non-specific airway hyperresponsiveness, sputum granulocyte recruitment, sputum mucin content (MUC5AC, MUC5B), markers of systemic inflammation, or changes in expression of inflammatory or allergy-related genes. To this last point, little is known about the mechanisms contributing to response patterns in allergic asthmatics undergoing allergen challenge. We are particularly interested in gene expression changes occurring during the window of time between the EPR and LPR, as these expression changes may dictate whether or not a LPR occurs or to what extent it occurs [16, 17].

Once identified, these subjects will be eligible for participation in two randomized, double-blinded, placebo-controlled clinical trials of Anakinra vs placebo administered after allergen challenge for mitigation of features of an asthma exacerbation.

1.2. Name of Investigational Agent and All Active Ingredients

Standardized cat hair (*Felis catus*) allergen extract is at a potency of 10,000 BAU/mL in 50% glycerin, 0.25% Sodium Bicarbonate, and 0.5% Sodium Chloride, and is provided by Stallergenes Greer, Lenoir, NC.

1.3. Non-Clinical and Clinical Study Findings

The study agent is commercially available for use in allergen immunotherapy (allergy shots) for patients with allergic rhinitis triggered by cat exposure. Inhaled cat allergen protocols have been used to reliably, reproducibly, and safely induce mild bronchoconstriction in allergic asthmatics and serve as a model of allergen-induced asthma exacerbation, which can be used to test asthma therapies targeting specific components of this inflammatory response [17-20]. The cat hair extract inhalation protocol was adapted from a published study by Sicherer et al[18] as well as from our existing protocol for house dust mite extract inhalation (IND# 13086).

Our center has experience with inhaled allergen challenge with house dust mite *(D. farinae)* using identical methods for challenge procedures. We currently hold an IND (#13086, PI: Hernandez) for use of inhaled house dust mite extract for allergen challenge in allergic asthmatic adults.

At the doses proposed in this study, the most significant predictable risk to subjects as a result of allergen inhalation is development of immediate airway obstruction (bronchospasm) during the EPR, which would cause shortness of breath, cough and/or wheeze. This drop in FEV_1 during the EPR should be short lived (maximal 10 to 30 minutes after allergen), and reversible with medications such as beta agonists (albuterol). This effect is similar to the effect of methacholine[21], which is an FDA-approved agent employed for the diagnosis of airway reactivity and asthma.

Other possible risks include development of a LPR to allergen inhalation. This occurs in approximately 50% of asthmatics challenged, consisting of similar symptoms described for the EPR. Since a key endpoint of this study is to identify which subjects have a LPR, albuterol for rescue prior to the 10 hour post challenge time point will only be employed if the FEV_1 does not show spontaneous improvement, and/or if the subject has distress necessitating rescue. All subjects will be given 4 puffs of albuterol at 10 hours after the inhaled allergen challenge.

There is an infrequent risk of an asthma exacerbation after the dust mite challenge. Subjects will be monitored until the exacerbation is resolved and will be treated as clinically appropriate.

There are no expected benefits from inhalation of cat allergen extract.

1.4. Relevant Literature and Data

O'Byrne and others have used inhaled allergen challenge to understand the pathophysiology of asthma and screen asthma interventions [1-5]. Their techniques, using a variety of inhaled allergens, have reproducibly resulted in measurable falls in lung function (specifically FEV1) during the EPR and LPR[2, 5, 6]. This technique has been extensively used to screen a number of new asthma agents in Phase IIa studies. This list includes anti-IL5 [7], anti-IgE [8, 9], anti-TSLP [10], and a number of other asthma agents [3, 11-15]. In a recent review, Gauvreau et al [6] have noted that no currently available effective asthma agents failed to demonstrate a physiologically important effect in Phase 2a proof of concept studies using an inhaled allergen/LPR model in mild allergic asthmatics. Overall, allergen-inhalation challenge in mild asthmatic subjects has a very high negative predictive value and a reasonable positive predictive value for screening novel therapeutics for asthma for Phase III pivotal studies.

Inhaled cat allergen protocols have been used to reliably, reproducibly, and safely induce mild bronchoconstriction in allergic asthmatics and serve as a model of allergen-induced asthma exacerbation, which can be used to test asthma therapies targeting specific components of this inflammatory response.

Sicherer et al[16] published a study in which lower airway response to inhalation challenge with cat hair extract (ALK Laboratories Inc) was compared to environmental cat allergen challenge in humans with asthma. In this study, dilutions of cat hair extract were inhaled in increasing increments until a fall of 20% or more in FEV1 was achieved. Each concentration of extract was aerosolized and inhaled 5 times via a nebulizer with dosimeter. The starting inhalation dose was chosen based on the weakest dilution of extract producing a 1 cm wheal when delivered intradermally, then the dose was doubled until a 20% fall in FEV1 was observed or the maximum concentration of 625 BAU/mL was reached. The maximum fall in FEV1 was not reported specifically but was described as being nearly identical to the lower airway response to environmental cat allergen exposure, which was associated with a mean maximum fall in FEV1 of 25% (6-57%).

Arvidsson et al[17] also conducted a study of lower airway responses to inhalation of cat allergen extract (Aquagen® SQ, ALK-Abello). Aqueous dilutions of cat extract were aerosolized and inhaled in increasing concentrations using a dosimeter nebulizer with tidal breathing until a drop in FEV1 of 20% or greater was observed. The starting dose of 13.75 standard quality (SQ) allergen concentration was increased up to a maximum cumulative dose of 7026 SQ. In this study, all 62 participants achieved an EPR with a drop of at least 20% from baseline FEV1 during the first 2 hours, and 56% achieved a LPR with a drop of at least 15% from baseline during the 3 to 24 hours following the EPR. No clinically severe early or late phase reactions were reported during this study.

Lieutier-Colas et al[18] conducted a study of the effects of particle size on early phase response to inhaled cat allergen (Stallergenes Laboratories, Antony, France). Participants inhaled increasing concentrations (0.5 to 100 Indice de Reactivite (IR)) of aerosolized cat allergen solution from different nebulizers producing particles of different sizes (1.4 to 10.3 um). A dosimeter was also used in this study to control for inspiratory flow and puff duration to ensure standardized dosing and deposition in the airways. They found that the provocative dose of cat allergen was significantly lower when inhaling larger particle size (10.3 um compared to 1.4 um) and more comparable to environmental cat exposure. LPR drops in FEV1 did not differ by particle size of inhaled cat allergen.

Brussino et al[19] examined oxidative stress in exhaled breath condensates following inhaled cat allergen (Stallergenes Laboraties, Antony, France) challenge in asthmatic humans. The initial inhalation dose was determined based on a mathematical formula incorporating end point skin prick titration testing and methacholine PC_{20}. Of the 12 study participants, all experienced a 20% or greater drop in FEV1 (mean 26% ± 2.6) during the EPR. During the LPR, the mean drop in FEV1 was 17% ± 1.9.

In Singh et al's study[16], most of the adverse events were mild in nature and the incidence and nature of side effects with inhaled allergen (either dust mite, cat, or grass) were similar to placebo. Two subjects withdrew from the study, one due to asthma exacerbation 4 days after treatment and one due to an allergic reaction to inhaled allergen (causative allergen was not specified) after placebo treatment. The reaction was not further described in the publication. Other studies using inhaled cat allergen previously discussed above[17-19] did not report any significant adverse events.

2. STUDY OBJECTIVE

The primary objective of this work is to use inhaled cat allergen challenge as a model of exacerbation of allergic asthma in humans. Using this model, we will measure key mediators involved in airway responses to allergen. This will also enable us to study potential asthma therapies and estimate their efficacy for reducing allergen-induced airway inflammation.

2.1. Primary Objective

The principal endpoint of this screening inhaled allergen challenge protocol will be to identify those subjects who experience a 15% or greater drop in FEV_1 from baseline within 3-10 hours after the EPR.

2.2. Secondary Objective

In addition to identifying subjects for participation in the planned studies of Anakinra vs placebo for mitigating features of asthma exacerbation, the additional purpose of this study is to determine if IL-1β concentrations in the sputum at baseline before challenge are predictive of key asthma outcomes following inhaled allergen challenge, specifically:

a). maximum drop in %FEV1 during LPR
b). Change in methacholine reactivity from baseline pre-allergen measurement to 24 hours after allergen challenge.
c). exhaled nitric oxide (eNO) level
d). induced sputum:
  i. Granulocyte (neutrophils/eosinophils) numbers and percentages
  ii. Mucins (secretion of MUC5AC and MUC5B determined by Western blot and ELISA)

Additionally, as exploratory endpoints, we will assess the effects of allergen challenge on markers of systemic inflammation and changes in inflammatory gene expression as described below:

a). **venipuncture** for assessment of systemic inflammatory markers (CBC with differential for absolute neutrophil count and absolute eosinophil count), pro-inflammatory cytokine levels such as IL-1β, IL-6, IL-8).
b). **gene array analysis** from blood and induced sputum at the baseline visit, again on the day of the challenge and, finally, at 24 hours post challenge. To identify signaling pathways potentially impacted by allergen challenge, we will use Nanostring® technology to measure differential expression of a panel of 594 immunology-related genes. This will be accomplished by collecting and isolating peripheral blood mononuclear cells from venous blood samples collected before allergen challenge, during allergen challenge, and 24 hours after allergen challenge.
c). **heart rate variability** measurement via commercially-available monitor (Space Labs, Inc). We will identify if specific cardiac changes occur during allergen-induced asthma exacerbation.
d). **environmental sensors** developed through the National Science Foundation-funded Center for Advanced Self-Powered Systems of Integrated Sensors and Technologies (ASSIST) program will be worn by participants during inhaled allergen challenge. These health and exposure tracker devices include an ECG chest patch and a wrist band that will measure Heart Rate, motion and ambient temperature and ozone concentration.

3. INVESTIGATIONAL PLAN

3.1. Study Design

Type of Design: open-label, single arm study

This will be a non-blinded study to identify mild allergic asthmatics with measurable LPR to inhaled cat hair allergen extract. The principal endpoint will be the presence of a LPR, defined as a decline in FEV1 of ≥15% from baseline values 3-10 hours after the onset of the EPR (which typically occurs within 30 minutes of allergen challenge).

Prior to enrollment, subjects will undergo a screening visit to establish eligibility (part of a separate screening protocol IRB#98-0799). Study visits will consist of a baseline visit to determine baseline values for study endpoints (Visit 1), a pre-allergen challenge visit to determine pre-challenge values for study endpoints (Visit 2), an allergen challenge visit (Visit 3), a visit 24 hours post-challenge to evaluate symptoms (Visit 4), and a final study discontinuation visit 5-10 days following allergen challenge.

3.2. Study Duration, Enrollment and Number of Subjects

The duration of each subject's participation is expected to last approximately 1 month.

The entire study is expected to last approximately 3 years.

We will screen up to 100 people to identify 25 mild asthmatics who experience a LPR to inhaled cat hair allergen extract and are eligible for enrollment into the Anakinra therapy trials.

3.3. Study Population

Inclusion Criteria:

1. Age range 18-45 years, inclusive
2. FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of at least 0.7 (without use of bronchodilator medications for 8 hours or long acting beta agonists for 24 hours), consistent with lung function of persons with no more than mild intermittent or mild persistent asthma.
3. Physician diagnosis of asthma
4. Allergic sensitization to cat hair extract as demonstrated by positive immediate skin prick test response or serum cat IgE >0.35 kU/L.
5. Negative pregnancy test for females who are not s/p hysterectomy with oophorectomy or who have been amenorrheic for 12 months or more.
6. Oxygen saturation of >94% and blood pressure within the following limits: Systolic between 150-90 mmHg and Diastolic between 90-60 mmHg.

Exclusion Criteria:

1. Clinical contraindications:
    a. Any chronic medical condition considered by the PI as a contraindication to participation in the study including significant cardiovascular disease, diabetes, chronic renal disease, chronic thyroid disease, history of chronic infections or immunodeficiency.
    b. Physician directed emergency treatment for an asthma exacerbation within the preceding 12 months.
    c. Exacerbation of asthma more than 2x/week which could be characteristic of a person of moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma.
    d. Daily requirements for albuterol due to asthma symptoms (cough, wheeze, chest tightness) which would be characteristic of a person of moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma (not to include prophylactic use of albuterol prior to exercise).
    e. Viral upper respiratory tract infection within 4 weeks of challenge.
    f. Any acute infection requiring antibiotics within 6 weeks of challenge or fever of unknown origin within 6 weeks of challenge.
    g. Severe asthma

h. Mental illness or history of drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements.
i. Cigarette smoking >1 pack per month
j. Nighttime symptoms of cough or wheeze greater than 1x/week at baseline (not during a clearly recognized viral induced asthma exacerbation) which would be characteristic of a person of moderate or severe persistent asthma as outlined in the current NHLBI guidelines for diagnosis and management of asthma.
k. Allergy/sensitivity to study drugs or their formulations, including a history of anaphylaxis following exposure to cat allergen.
l. Known hypersensitivity to methacholine or to other parasympathomimetic agents
m. History of intubation for asthma
n. Unwillingness to limit coffee, tea, cola drinks, chocolate, or other foods containing caffeine after midnight on the days that methacholine challenge testing is to be performed.
o. Unwillingness in females to use reliable contraception if sexually active (IUD, birth control pills/patch, condoms).

2. Pregnancy or nursing a baby. Female volunteers will be asked to use effective birth control (stable regimen of hormonal contraceptive use for at least 3 months, intrauterine device placement, tubal ligation or endometrial ablation for at least 3 months through at least one week after study completion) and will provide a urine sample to test for pregnancy on study days. If the test is positive or the subject has reason to believe she may be pregnant, she will be dismissed from the study. Women who have been amenorrheic for 12 months may participate.

3. Usage of the following medications:
    a. Use of systemic steroid therapy within the preceding 12 months for an asthma exacerbation. All use of systemic steroids in the last year will be reviewed by a study physician.
    b. Subjects who are prescribed daily inhaled corticosteroids, cromolyn, or leukotriene inhibitors (Montelukast or Zafirlukast) will be required to discontinue these medications at least 2 weeks prior to their screening visit.
    c. Use of daily theophylline within the past month.
    d. Use of allergen immunotherapy.
    e. Use of any immunosuppressant therapy within the preceding 12 months will be reviewed by the study physician.
    f. Receipt of LAIV (Live Attenuated Influenza Vaccine), also known as FluMist®, or any other live viral vaccine within the prior 30 days, or any vaccine for at least 5 days
    g. Use of beta blocking medications
    h. Antihistamines in the 5 days prior to allergen challenge
    i. Routine use of NSAIDs, including aspirin.

4. Physical/laboratory indications:
    a. Abnormalities on lung auscultation
    b. Temperature >37.8 C
    c. Oxygen saturation of <94%
    d. Systolic BP>150 mmHg or <90 mmHg or diastolic BP>90 mmHg or <60 mmHg

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

4. STUDY PROCEDURES

4.1. Screening/Baseline Visit Procedures:

Potential participants will be initially screened for eligibility through a separate screening protocol (IRB#98-0799).

Within 12 months of completing the screening protocol, they will return for Visit 1. Sensitization to cat as determined by skin testing or IgE levels is not expected to change over the course of 12 months. Participants will have to meet all eligibility criteria at the time of the Baseline Visit. On Visit 1:

1. Consent will be obtained
2. Review of subject's medical history and current medications. Allergy skin prick test results from screening protocol #98-0799 will be included in the history.
3. Vital sign measurements (temperature, pulse, respiratory rate, blood pressure), oxygen saturation, and symptom scoring
4. Urine pregnancy test for women of child bearing potential
5. eNO
6. Spirometry
7. Venipuncture for CBC with Diff, Total and cat hair IgE, cytokines, and gene expression (approximately 50 cc total)
8. Physical exam of the ears, nose, throat and chest
9. Sputum induction
10. Collect nasal epithelial lining fluid (ELF).

After completion of the baseline visit, and if for any reason that subject cannot return for the challenge session in the required time, a repeat baseline visit will be done. At this visit the subject will repeat the following:

1. review history and meds
2. vitals
3. UPT
4. eNO
5. spirometry
6. PE

If more than 2 months has elapsed after the original baseline visit, or if the visit was rescheduled due to illness in the participant, the blood work will also be repeated. Subjects must continue to meet all other study inclusion criteria.

4.2. Intervention/Treatment procedures

Visit 2: Methacholine Challenge Day (at least 2 days but less than 2 weeks after the baseline visit V1)

1. Review any change in medical status since last visit
2. Vital signs, oxygen saturation, and symptom score
3. Urine pregnancy test
4. Baseline HRV measurement
5. eNO
6. Spirometry
7. Physical exam of the ears, nose, throat and chest

8. Methacholine challenge if inclusion and exclusion criteria are still met (refer to section 5.4 for methacholine challenge procedure)

Visit 3: Allergen challenge day (24-48 hours after V2)

1. Review any change in medical status since last visit
2. Vital signs, oxygen saturation, and symptom score
3. Placement of the HRV device
4. Placement of the ASSIST devices (cardiac electrodes and wrist band)
5. eNO
6. Collect nasal ELF
7. Spirometry
8. If inclusion and exclusion criteria are still met, allergen challenge will be performed as described in section 5.1.
    a. If subject is asymptomatic and FEV1 drops <10% at the completion of allergen challenge, subjects will be monitored for 2 hours post challenge and will be discharged following post-challenge venipuncture with rescue medications (albuterol with aerochamber and oral prednisone 60 mg), instructions for use of these medications if asthma symptoms occur, and contact information for the on-call physician. Subject will resume protocol beginning with Study visit 4 detailed below.
    b. Any subject whose FEV1 drops ≥10% will stay for the 10 hour post-allergen monitoring period as outlined below.
9. Collect nasal ELF after completion of the allergen challenge, either following the dose producing a 20% or greater fall in FEV1 (in early phase responders) or following completion of the highest dose of inhaled allergen (in those who do not exhibit an early phase response).
10. Post-challenge monitoring of vital signs every 10 minutes for the 1st hour, then every 30 minutes until 2 hours post-challenge, then hourly until 10 hours post-challenge
11. Spirometry when vital signs are obtained
12. eNO hourly between 2 and 10 hours post-challenge
13. Collect nasal ELF at 7 hours post-challenge (7 hours after the onset of the early phase response).
14. Venipuncture for CBC with diff, cytokines, and gene expression 10 hours post-challenge (approximately 50 cc total)
15. All subjects will receive 4 puffs of albuterol 10 hours post-challenge. Spirometry will be performed 15 minutes after albuterol if post-challenge FEV1 at 10 hours was not approximately within 10% of baseline.
    a. Albuterol will be repeated every 20 minutes up to three doses if repeat FEV1 is not within approximately 10% of baseline value or if subject remains in distress.
    b. If needed, after three inhaled albuterol treatments, additional medications, including nebulized albuterol, atrovent and oral prednisone 60mg, will be given per physician discretion.
16. Subjects will be discharged 10 hours following allergen challenge when clinically stable and when FEV1 is within approximately 10% of baseline FEV1. HRV monitoring will be discontinued prior to discharge. At discharge, all subjects will be provided with an albuterol inhaler and aerochamber, a single dose of oral prednisone 60 mg, clear instructions for use of rescue medications in the event of symptoms related to bronchoconstriction, and contact information for the on-call physician. Subjects will reside overnight at their home if their residence is within 10 miles of UNC or a local hotel within 10 miles of UNC.

Visit 4: 24 hours post challenge

1. Vital signs, oxygen saturation, and symptom score
2. eNO
3. Spirometry
4. Venipuncture for CBC with Diff, gene expression, cytokines (approximately 50 cc total).
5. Methacholine challenge.
6. Sputum induction.
7. Collect nasal ELF.

4.3. Follow up Procedures

Post Challenge Observations/Reporting

1. Subjects will be contacted for phone call follow-up 24 hours after post-challenge sputum induction (see phone script included in the study worksheets section)
2. Each volunteer will be given a symptom scoring sheet for each day up to 96 hours (4 days) after challenge (see symptom scoring sheet in section 5.8.).
3. Any subject who requires a dose of an oral corticosteroid (prednisone 60 mg) for treatment of asthma symptoms will be contacted by phone within 24 hours of the dosing to assess asthma symptoms. These subjects will return to the research lab within 48-72 hours of the dose for FEV1, symptom scoring and physical exam by a study physician to assess need for continued steroid treatment.

Study discontinuation visit within 10 days of the final challenge dose:

1. Vital signs, oxygen saturation, and symptom score
2. Spirometry
3. eNO
4. If any findings are abnormal, medical evaluation as directed by the study physician will be undertaken.

4.4. Unscheduled Visits

We do not anticipate any unscheduled visits. If the subject is determined to be too ill to participate in the study visit procedures, then their research visit will be rescheduled after resolution of illness.

4.5. Concomitant Medication Documentation

Medication review will be obtained by a study coordinator at each study visit and documented in the participant's paper chart.

4.6. Rescue Medication Administration

Albuterol (via metered dose inhaler) will be available at all times during the allergen challenge and throughout the study visits. Albuterol will be administered any time the participant requests it for respiratory symptoms. Additionally, 4 puffs of albuterol will be administered if the participant experiences a 40% or greater decrease in FEV1 or FVC from pre-challenge values during the allergen challenge.

All subjects will receive 4 puffs of albuterol 10 hours after allergen challenge. If clinically stable and if FEV1 is approximately within 10% of baseline pre-challenge FEV1, subject will be discharged with an albuterol inhaler with aerochamber and a single oral dose of prednisone 60 mg, clear instructions on use of medications in the event of symptoms related to bronchoconstriction, and contact information for the on-call physician. If FEV1 has not recovered to within 10% of baseline pre-challenge values by 10 hours after the allergen challenge, 4 puffs of albuterol will be administered, and spirometry will be repeated 15 minutes later.

If the subject requires albuterol for 48 hours or longer after the inhaled allergen challenge, they will be instructed to begin a single dose of prednisone 60 mg and continue following instructions from the provided asthma action plan for albuterol use. If the subject is instructed to begin prednisone treatment, the team will contact the subject 24 hours after initiation to assess their symptoms and identify if the subject needs to be seen in the research clinic that day. Subjects who require a dose of prednisone will be seen in the research lab 48-72 hours after the dose to assess the need for a full course of oral corticosteroids.

4.7. Subject Completion/Withdrawal Procedures

Participants will be terminated early 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 participant develops a medical condition or is started on a new medication(s) not previously mentioned in the list of prohibited medications that, 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 of the data obtained from the study.
5. The participant no longer meets inclusion or exclusion criteria as defined in Section 3.3 or if the participant does not meet the previously defined safety criteria in section 9.4.
6. The study is terminated for any reason.

4.8. Criteria for Screen Failure (for Anakinra Studies)

Participants who do not fulfill the following safety criteria will be considered screen failures and will not be eligible for enrollment into the Anakinra study protocols:

1. Less than or equal to 40% decrease in FEV_1 or FVC from pre-challenge values following EPR or the LPR to cat hair allergen (the target endpoint is a $\geq$20% decrease in FEV_1 from baseline during the EPR and a $\geq$15% decrease in FEV_1 from baseline during the LPR); 2) If albuterol is employed as rescue, FEV1 must recover to within 90% of baseline value within 20 minutes; 3) Oxygen saturation should remain $\geq$93% throughout the challenge period and must be no lower than 2% of baseline measure;

2. Albuterol for rescue therapy should be required no more than three times within the first hour after challenge

3. Albuterol for rescue therapy should be required no more than three times within 12 hours of discharge.

4. FEV_1 should return to within 90% of baseline value at the study discontinuation visit which occurs within 10 days after allergen challenge.

5. Subjects who require more than 1 dose of oral corticosteroid (prednisone 60 mg) to treat an asthma exacerbation associated with inhalation challenge will be considered screen fails and will not continue.

Subjects who are withdrawn for medical concerns related to the study, such as but not limited to prolonged decreased FEV_1 or persistent wheezing, will be followed by study physicians until resolution of these events. As this is a screening protocol, these subjects will not be replaced.

If following all doses of inhaled allergen challenge a participant remains asymptomatic and FEV1 drops <10% at the completion of the challenge, that participant will be considered a screen failure and will be monitored for 2 hours post challenge. The participant will be discharged following post-challenge venipuncture with rescue medications, including albuterol with aerochamber and oral prednisone 60 mg, instructions for use of these medications in the event of symptoms related to bronchoconstriction, and contact information for the on-call physician. Subject will resume the protocol beginning with Study visit 4 detailed above.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1. Allergen challenge procedure:

Immediately prior to allergen challenge, determination of adequate lung function without use of bronchodilating medications for 8 hours will be made via spirometry with FEV_1 of at least 80% of predicted and FEV_1/FVC ratio of at least .70, consistent with lung function of persons with mild intermittent or mild persistent asthma being considered minimal acceptable lung function.

Doses will be administered from a DeVilbiss 646 nebulizer. Each volunteer will inhale 5 breaths from the nebulizer at each concentration starting with diluent control. Each breath is started from resting end-expiratory lung volume (Functional Residual Capacity) and continued until a maximum inhalation is reached. After administration of the control diluent, concentrations of allergen will be administered starting with solutions of 0.31, 0.61, 1.22, 2.44, 4.89, 9.77, 19.5, 39, 78.13, 156.25, 312.5, and 625 BAU/ml via nebulizer. During the inhalation challenge the subject will be under direct observation of a physician experienced in treating asthma. Epinephrine for intramuscular administration and albuterol for inhalation will be immediately available. The challenges will be performed in the Clinical and Translational Research Center (CTRC) of the University of North Carolina at Chapel Hill. Full emergency equipment and nursing personnel are immediately available at this location.

The FEV_1 will be measured prior to and 10 minutes after each aerosol inhalation. Starting with the control (saline diluent) solution, increasing concentrations of allergen will be inhaled at 10-minute intervals starting with 0.31 BAU/ml. The FEV_1 measurement obtained after inhalation of the control solution (saline) will be considered the baseline value. If the FEV1 has declined by less than 10% of baseline after a given concentration of allergen is inhaled, the next higher concentration of allergen will be given. If after inhalation of an allergen dose, the FEV_1 declines by ≥10% but <20% from the baseline FEV1 measurement, spirometry will be repeated every 5 minutes for 15 minutes or until a clear nadir in the decline has been reached. If the nadir after 15 minutes is a decline in FEV1 of less than 10% of baseline, the next higher concentration of allergen will be given. If, however, the nadir after 15 minutes is a decline in FEV_1 ≥ 10% but < 20% from the baseline measurement, the previous allergen dose will be repeated. To prioritize the safety of subjects and to prevent severe or precipitous decline in lung function, decisions to repeat doses of allergen (rather than escalate dosing) or perform additional spirometry measurements will be made in real time at the discretion of the supervising physician, who will be physically present throughout the allergen challenge procedure. Once a decline in FEV of ≥ 20% is reached, the challenge will be stopped. These activities are summarized in **Figure 1**. Once the challenge is stopped, FEV_1 will be monitored every 10 minutes for the first hour, then every 30 minutes until 2 hours post-challenge, and then hourly until 10 hours after the last allergen dose.

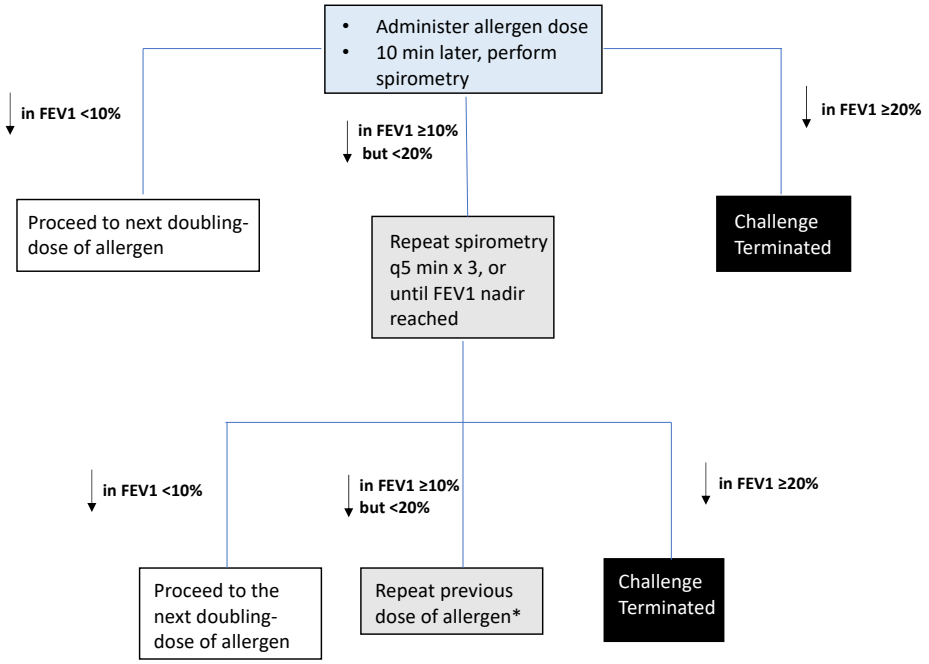

Figure 1. Inhaled allergen challenge schematic. *At discretion of supervising physician

Oxygen saturation by pulse oximeter will be measured during the challenge and with each spirometric evaluation. The physician responsible for the study will determine if any medication is necessary based on the subject's lung function and symptoms. Vital sign measurements including pulse oximetry evaluation will continue to be performed at hourly intervals during and for 10 hours after the exposure. Oxygen saturation by pulse oximeter will be measured during the challenge and with each spirometric evaluation. All subjects will receive 4 puffs of albuterol 10 hours after allergen challenge. If clinically stable and if FEV1 is approximately within 10% of baseline prechallenge FEV1, subject will be discharged with an albuterol inhaler with aerochamber and a single oral dose of prednisone 60 mg, clear instructions on use of medications in the event of symptoms related to bronchoconstriction, and contact information for the on-call physician. The subject will reside in either a local hotel within 10 miles of UNC or their home if their residence is within a 10 mile radius of UNC.

5.2. Sputum induction procedure:

Prior to sputum induction, subjects will receive 4 puffs of albuterol from an MDI attached to a spacer device. FEV1 and FVC will be measured after this step to determine the post-bronchodilator baseline FEV1 and FVC values. Next, an ultrasonic nebulizer filled with 20 cc of 3% hypertonic saline (inhalation grade for respiratory use only, 3% NaCl) will be set to the maximum output setting and turned on. The subject will be instructed to latch his/her mouth onto the nebulizer mouthpiece and breathe normally (i.e., tidal breaths) for 7 minutes as the saline is nebulized through the mouthpiece in a jet stream and inhaled. The nose will not be occluded for this procedure. The subject will be encouraged to come off the mouthpiece at any time to cough if a sputum sample from the lower airways (i.e. not from the back of the throat) is ready for expectoration. Prior to expectoration, subjects will be asked to blow their nose, rinse their mouth with water, and clear their throat to avoid the inclusion of non-airway fluid samples. The sample will be expectorated into a sterile specimen jar and capped.

Following the measurement of FEV_1 after the first 7 minute inhalation period, the concentration of saline will be increased from 3% to 4%, provided the FEV_1 decrement is < 10% from the post-bronchodilator value. If the FEV_1 falls between 10-20% of the post bronchodilator value, the test will proceed but the concentration of saline will remain the same. If the FEV_1 falls by > 20% or if troublesome symptoms occur, the nebulization will be discontinued, and albuterol will be immediately available if necessary to relieve symptoms. The same procedure will be followed for the final 7 minute inhalation period using 5% hypertonic saline provided the FEV_1 safety parameters described above have been met. The nebulization is stopped after a total of 21 minutes or earlier if a sputum sample of good quality is obtained (i.e. visible sputum plugs).

5.3. Spirometry:

Standard methodology conforming to the American Thoracic Society/European Respiratory Society guidelines for measurement of spirometry will be used[22]

5.4. Methacholine Challenge:

This test measures the responsiveness of the airways to a standard cholinergic bronchoconstriction agent. Methacholine challenge testing is a standard clinical procedure to determine airway reactivity in patients with known or suspected airway disease. Subjects will be asked to limit caffeine on the day of testing. Subjects will inhale 5 breaths from the nebulizer at each concentration starting with saline control. Each breath is started from resting end-expiratory lung volume (Forced Residual Capacity) and continued until a maximum inhalation is reached. Increasing (doubling) concentrations are inhaled until the FEV_1 falls by at least 20% from the post saline value, the highest concentration has been inhaled, or the subject experiences discomfort or anxiety sufficient for the investigator or subject to consider further testing unacceptable. Methacholine concentrations will be 0.075, 0.156, 0.312, 0.625, 1.25, 2.5, 5.0, and 10.0 mg/ml. If subjects experience symptoms that do not rapidly and spontaneously remit at the end of the procedure, he/she will receive 4 puffs of an inhaled bronchodilator (albuterol) MDI. If needed an additional 4 puffs or a nebulizer treatment with standard dose albuterol (0.083mg) will be given. The subject will not be discharged until spirometry levels return to baseline.

5.5. Venipuncture:

Blood will be drawn to evaluate a complete blood count with differential and also to determine what genes may play a role in a person's response to allergen challenge. At the time of each venipuncture, up to 50 cc will be drawn, and the total for the study will be 150 cc or less.

5.6. Exhaled nitric oxide measurement:

We will measure the amount of nitric oxide (or exhaled NO, eNO) present in orally expired air. An increased concentration of eNO in exhaled air may be found in normal persons with acute inflammation. Thus, measurement of the concentration of eNO may be useful as an indirect assessment of airway inflammation. Lung production of eNO will be measured by asking subjects to exhale briefly into a mouthpiece.

5.7. Allergy Skin Testing

A Greer® Pick® device that has been immersed in a solution of allergen extract purchased from Stallergenes Greer is used to apply allergen epicutaneously by placing the device at a 45 degree angle to the skin and gently lifting up to create a skin prick. All testing instruments are single patient use. Each patient will undergo skin prick testing with the following allergen preparations: House Dust Mite (*D. farinae*), House dust mite p (*D. pteronyssinus*), Cockroach, Tree mix, Grass Mix, Weed Mix, Mold Mix 1, Mold Mix 2, Rat, Mouse, Guinea Pig, Rabbit, Cat and Dog. The allergens will be placed on the forearm. After 15 minutes, a research coordinator will compare the wheal size (a raised bump surrounded by red itchy skin) for each allergen to that of the negative and positive control (histamine). A positive skin test is defined by a 3 mm wheal greater than the

saline control. For a volunteer to be considered non-allergic, the histamine control must be reactive and all others must be negative.

5.9. Symptom Score (administered in person at study visits and by telephone for post-challenge observation):
Allergen Challenge Phone Follow Up Questionnaire: (24 hours post challenge)

When speaking with the subject, use the following script:

On a scale of 0 to 3, with 0=no symptoms, 1=mild symptoms, 2=moderate symptoms and 3=severe symptoms, please rate the severity of each of the following symptoms:

Waking up at night due to cough (0-3): _____
Shortness of Breath (0-3): _____
Cough at rest (0-3): _____
Cough with Exercise (0-3): _____
Wheeze at Rest (0-3): _____
Wheeze with Exercise (0-3): _____

Total_____

2. Do you have any health concerns since we saw you on the day of the challenge?

3. Have you needed to see a doctor for any reason since your last visit?

4. Have you need to use any over the counter medications?

If the symptom score is 6 (out of a possible 18), or if any single symptom is a 3, or if the answers to questions 2, 3, 4, or 5 are "yes", then the subject will be offered the opportunity to come back to the laboratory for follow up assessment by a study physician.

5.10. Nasal Epithelial Lining Fluid (ELF) Collection:
ELF is obtained by spraying the nostril with 0.9% sterile, normal saline irrigation solution (about 100 µl per nostril) one time per nostril. One 10x55mm strip of filter paper, cut from Leukosorb paper (Pall Scientific, Port Washington, NY) on a laser cutter, is inserted into the anterior portion of each nostril. The nostrils are clamped shut using a padded nose clip for two minutes. Strips are then removed from the nostril and collected in 1.5ml tubes. They are then stored in strips in a -20°C freezer until elution. ELF strips will be analyzed for cytokines and other chemical mediators.

6 STATISTICAL CONSIDERATION

6.1. Primary Endpoint
The principal endpoint of this screening inhaled allergen challenge protocol will be to identify those subjects who experience a 15% or greater drop in FEV_1 from baseline within 3-10 hours after the EPR.

6.2. Secondary Endpoint

In addition to identifying subjects for participation in the planned studies of Anakinra vs placebo for mitigating features of asthma exacerbation, the additional purpose of this study is to determine if IL-1β concentrations in the sputum at baseline before challenge are predictive of key asthma outcomes following inhaled allergen challenge

1) Baseline sputum IL-1β concentration (pg/mL)
2) Asthma outcomes following allergen challenge as measured by:
   a). maximum drop in %FEV1 during LPR
   b). Change in methacholine reactivity from baseline pre-allergen measurement to 24 hours after allergen challenge.
   c). exhaled nitric oxide (eNO) level
   d). induced sputum:
      i. Granulocyte (neutrophils/eosinophils) numbers and percentages
      ii. Mucins (secretion of MUC5AC and MUC5B determined by Western blot and ELISA)

Exploratory Endpoints

Additionally, as exploratory endpoints, we will assess the effects of allergen challenge on markers of systemic inflammation and changes in inflammatory gene expression as described below:

a). **venipuncture** for assessment of systemic inflammatory markers (CBC with differential for absolute neutrophil count and absolute eosinophil count), pro-inflammatory cytokine levels such as IL-1β, IL-6, IL-8).

b). **nasal ELF** for assessment of inflammatory mediators associated with allergen-induced airway inflammation.

c). **gene array analysis** from blood and induced sputum at the baseline visit, again on the day of the challenge and, finally, at 24 hours post challenge. To identify signaling pathways potentially impacted by allergen challenge, we will use Nanostring® technology to measure differential expression of a panel of 594 immunology-related genes. This will be accomplished by collecting and isolating peripheral blood mononuclear cells from venous blood samples collected before allergen challenge, during allergen challenge, and 24 hours after allergen challenge.

d). **heart rate variability** measurement via commercially-available monitor (Space Labs, Inc). We will identify if specific cardiac changes occur during allergen-induced asthma exacerbation.

e). **environmental sensors** developed through the National Science Foundation-funded Center for Advanced Self-Powered Systems of Integrated Sensors and Technologies (ASSIST) program will be worn by participants during inhaled allergen challenge. These health and exposure tracker devices include an ECG chest patch and a wrist band that will measure Heart Rate, motion and ambient temperature and ozone concentration.

6.3. Statistical Methods

Our analytical plan was developed with Dr. Zhou, the biostatistician for the CEMALB who will oversee all statistical analysis. In all analyses, criterion for significance will be $p \leq 0.05$. All statistical estimates will be tabulated along with the corresponding confidence intervals.

Our primary endpoint is identification of subjects who achieve a ≥15% fall in FEV_1 during hours 3-10 following allergen challenge. We will subtract the pre-allergen challenge %FEV1 value from the post-allergen %FEV1 value to make this determination.

For analysis of secondary endpoints, we will first use linear regression to determine if there is a linear relationship between baseline sputum IL-1β concentration (pg/mL) and asthma outcomes (maximum fall in %FEV1 during LPR, methacholine reactivity defined by the dose required to produce a ≥20% drop in FEV1, sputum granulocyte content both in terms of % of total cell count and cells/mg of sputum, exhaled nitric oxide concentration) following allergen challenge, adjusting for potential confounders. The point estimate and confidence interval will be reported for the effect of baseline sputum IL-1β on asthma outcomes. If the normality assumption is violated or there exists a non-linear relationship, we will calculate the Spearman correlation coefficient between baseline sputum IL-1β concentration and asthma outcomes.

For analysis of exploratory endpoints, we will use two sample comparison tests to compare systemic inflammatory markers and gene expression levels before and after allergen challenge. We will use two-sample t tests or Mann Whitney U tests depending on whether the normality assumption is met. The point estimate and confidence interval will be reported for mean changes. We will transform the data to achieve normality if needed. HRV endpoints are exploratory, as we do not know if there will be changes during bronchospasm.

As we have considered additional individuals in our sample size calculation, we do not anticipate missing data will be a major issue affecting our study power.

6.4. Sample Size and Power

Sample size was calculated based on the sample required for our upcoming randomized placebo-controlled crossover studies of anakinra for mitigating features of allergen-induced asthma exacerbation. We hypothesize that anakinra treatment reduces the LPR, as assessed by the change in maximum percentage fall in %FEV1 during hours 3-10 following allergen challenge. We have developed protocols for two anakinra treatment clinical trials, each of which will incorporate a crossover study design: one will provide anakinra treatment (v. placebo) immediately after the EPR, and one will provide anakinra treatment (v. placebo) at the onset of the LPR (i.e. 3 hours after the EPR).

We calculated the sample size for the anakinra treatment protocols using a model of inhaled allergen challenge similar to what we propose in this project (69) based on a paired t test. The maximal drop in %FEV1 during the LPR was 25.6% $\pm$ 9.8%(SD). To detect a 50% attenuation of the drop in %FEV1 during the LPR (12.8%) by anakinra v. placebo treatment, assuming that group-wise standard deviations are similar (10.28% for anakinra; 9.8% for placebo), with β=0.9 & α=0.05, a sample size of 6.4 would be required for each anakinra protocol. We estimate that 25% of subjects will require rescue short-acting beta agonist treatment during the EPR or the LPR, and will additionally account for a 25% dropout rate for a crossover study, inflating our required sample size to at least **N=12** for each anakinra treatment protocol.

Previous studies of inhaled allergen challenge models in allergic asthmatics have found that 50-70% of subjects who undergo challenge will experience an EPR [19, 20]. House dust mite is one of the most commonly utilized allergens for inhaled challenge. Gauvreau et al [21] reported that >75% of house dust mite allergen challenged volunteers who display an EPR will also have a LPR. Due to the lack of data on the frequency of LPR with cat hair allergen inhalation challenge and given that both are perennial allergens, we will extrapolate the results from house dust mite challenge to estimate the number of participants needed to screen. Using a conservative estimate of 50% of our screened volunteers with a EPR and 50% of those with a LPR, we will screen up to 100 subjects to identify at least **25** LPR subjects eligible to enroll into the anakinra treatment studies. Screening will stop following identification of 25 LPR subjects.

6.5. Interim Analysis
There are no planned interim analyses.

7 STUDY INTERVENTION

- **Description:** Standardized cat hair (*Felis catus*) allergenic extract, 10,000 BAU/mL
- **Receipt/Storage:** Product will be shipped from the manufacturer (Stallergenes Greer, 639 Nuway Circle NE, Lenoir, NC 28645) to the UNC Investigational Drug Pharmacy.
- **Packaging/labeling:** The UNC Investigational Drug Service (IDS) will prepare allergen dilutions, and each dose vial will be labeled with the dilution (for example: 39 BAU/mL).
- **Dosing:** The University of North Carolina Investigational Drug Pharmacy (IDS) will use commercial vials of preservative-free, sterile 0.9% sodium chloride for injection manufactured by Abbott or Hospira. Prepared dilutions will be stored in the University of North Carolina Investigational Drug Pharmacy refrigerator which is temperature controlled and which will alarm if the refrigerator exceeds a specified range. Doses will be made up no more than 24 hours prior to administration. Doses of allergen will include concentrations of zero (diluent), 0.31, 0.61, 1.22, 2.44, 4.89, 9.77, 19.5, 39, 78.13, 156.25, 312.5, and 625 BAU/mL. Prior to study initiation, the IDS will create a formulation sheet with detailed instructions for the serial dilutions for the investigational agent used in this protocol. An information sheet will also be created, and trained staff members will be in-serviced regarding the preparation of study drug. The dilutions are prepared in a laminar air flow hood by a pharmacy technician. Each step of the dilution process is checked by a pharmacist, and dose vials are labeled as each dilution is complete.
- **Treatment compliance and adherence:** Given the nature of this protocol, all doses of study product will be administered directly by study personnel.
- **Drug return/destruction:** Unused dilutions of allergen will be discarded at the end of the allergen challenge procedure.
- **Drug Accountability**: Drug storage, handling and dispensing will be the responsibility of the IDS. Documentation of administration and destruction of unused product will be the responsibility of the study personnel.

8 STUDY INTERVENTION ADMINISTRATION

As this is an open-label single arm intervention study, all participants will receive the same treatment without blinding.

9 SAFETY MANAGEMENT

9.1. Definition of Adverse Event (AE) and Serious Adverse Event (SAE):
Adverse events will be recorded and reported according to 21 CFR 312.32. An adverse event for a given volunteer will be defined as any untoward medical occurrence associated with use of the investigational agent whether or not considered related and will also include failure of any of the safety criteria outlined above. Additionally, minor upper respiratory tract infections occurring within 10 days of the exposure will be considered adverse events. Other, non-specified clinical illnesses, which occur within 10 days of each challenge will also be reported as an AE. Specific potential adverse events which may occur with allergen challenge include the following: Allergic Reaction, Cough, Dyspnea, Urticaria, Syncope, and Voice Changes.

Also, any decrease in lung function or increase in symptom score, as outlined in section 4.8. will be considered an adverse event. Failure of a total symptom score to return to no greater than 6 above baseline within 10 days will be considered an adverse event. Any symptoms that induce a volunteer to seek medical attention from any provider within 10 days of challenge will be considered an adverse event.

9.2. Grading Criteria

Adverse events will be graded using Common Terminology Criteria for Adverse Events v3.0, (CTCAE website at http://ctep.cancer.gov/reporting/ctcnew.html and http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx). Symptom scores will be graded as outlined elsewhere in this application.

The severity grades of AE's are defined as follows:
Grade 1: Mild AE
Grade 2: Moderate AE
Grade 3: Severe AE

A serious adverse event will be defined as any event that requires hospitalization or results in, life threatening illness or injury, permanent (or likely to be permanent) illness or injury (CTCAE Grade 4), or death, (CTCAE Grade 5), if these events occur within 10 days of challenge (or if the clinical scenario leading up to hospitalization, illness, injury or death begins within 10 days of a challenge).

9.3. Risks to Subjects

At the doses proposed in this study, the most significant predictable risk to subjects as a result of allergen inhalation is development of immediate airway obstruction (bronchospasm) during the EPR, which would cause shortness of breath, cough and/or wheeze. This drop in FEV_1 should be short lived (maximal 10 to 30 minutes after allergen) and reversible with medications such as beta agonists (albuterol). This effect is similar to the effect of methacholine, which is an FDA-approved agent employed for the diagnosis of airway reactivity and asthma.

Other possible risks include development of a LPR to allergen inhalation. This occurs in approximately 50% of asthmatics challenged, consisting of similar symptoms described for the EPR. Since an important secondary endpoint of this study is to identify which subjects have a late asthmatic response, albuterol for rescue prior to the 10 hour post challenge time point will only be employed if the FEV_1 does not show spontaneous improvement, and/or if the subject has distress and requests rescue. All subjects will be given 4 puffs of albuterol 10 hours post-challenge with additional doses given based on FEV1 and subject distress.

Sputum induction may induce cough, chest tightness or bronchospasm, and albuterol will be immediately available.

Venipuncture carries a risk of hematoma, and this procedure will be performed only by trained staff members.

There is no risk to measurement of eNO since subjects are simply asked to exhale into a mouthpiece.

Nasal ELF collection is associated with a rare risk of sneezing.

There is no known risk associated with HRV monitors or ASSIST devices. The ASSIST devices are under development and are not in clinical use, therefore no data collected from them will impact subject management

or care in this study. Heart rate, respiratory rate, blood pressure, and oxygen saturation will be monitored during the visits using standardized equipment approved for clinical use.

There is a potential risk for breach of confidentiality and privacy.

9.4. Measures to Minimize Risk

Only clinically healthy volunteers will be recruited for this project. Further, subjects will be deferred for challenge until 4 weeks after complete resolution of each of the following acute illnesses: viral respiratory tract infection, pneumonia or bronchitis requiring antibiotic therapy (must be off antibiotics and well for 4 weeks after the last dose of antibiotics), or acute illness resulting in fever. Also, unspecified illnesses, which in the judgment of the investigator increase the risks associated with allergen inhalation challenge, will be a basis for exclusion.

All subjects will need to fulfill objective lung function and symptom criteria prior to initiating the challenge study. Subjects not meeting these criteria on the morning of the challenge will not proceed with the allergen challenge:

1. FEV_1 of at least 80% of predicted and a ratio of FEV_1 to Forced Vital Capacity (FVC) of at least .70 (without use of bronchodilating medications for 8 hours, anticholinergic therapy (such as ipratropium) within 2 hours, or antihistamines within 5 days prior to challenge).

2. Baseline oxygen saturation of at least 95%.

3. No history of asthma exacerbation requiring systemic corticosteroid treatment, visit to an emergency room or hospitalization within 12 months of inhalation challenge.

4. No current history of requiring short-acting beta agonists more frequently than every 8 hours for asthma symptoms. Any use of albuterol in the week prior to allergen challenge will be reviewed by the study physician.

5. No history of viral respiratory tract symptoms within 4 weeks of challenge.

Female subjects of child-bearing potential will undergo urine HCG testing for pregnancy within 48 hours prior to administration of any challenge.

A physician familiar with the protocol will be available for all challenge procedures. Emergency treatment with albuterol (via MDI or nebulizer), oxygen, oral corticosteroids, oral cetirizine, and epinephrine (subcutaneously or intramuscularly) will be available to those subjects who require such therapy. An emergency “crash cart” with standard emergency medications, IV fluids and a defibrillator are is also readily available in the UNC CTRC as well as at CEMALB in the unlikely event of a medical emergency during any challenge or study visit.

If the covering physician feels that the subject’s respiratory status is such that providing an induced sputum sample or performing methacholine challenge would place them at increased risk for significant bronchospasm, we will prioritize spirometry assessments (as part of routine clinical care) and not obtain induced sputum samples or perform methacholine challenge for that subject.

All subjects will be monitored in the CTRC for 10 hours following allergen challenge as outlined in previous sections. Upon discharge to either the subject's home or local hotel, subjects will be provided with an albuterol inhaler with aerochamber and a single dose of oral prednisone 60 mg, clear instructions for use of these medications in the event of bronchoconstriction, and the contact information for the on-call physician. In the event of post-challenge bronchoconstriction that does not improve with albuterol, subject will be instructed to present to the UNC emergency department for further evaluation and management.

Subjects will also be assessed the morning after the allergen challenge in the CEMALB. If the subject has wheezing on physical examination the morning after the inhaled allergen and/or has an FEV1 that is decreased $\geq$15% from baseline measures (indicating that they are still experiencing a late phase response), they will be given a single dose of prednisone 60 mg and continue following instructions from the provided asthma action plan for albuterol use. These subjects will be contacted by phone within 24 hours of receiving the dose to assess asthma symptoms and will be evaluated in person within 48-72 hours of the dose to monitor FEV1 and to assess the need for continued therapy.

In addition, all subjects will receive a follow-up call 48 hours after challenge (24 hours after the follow-up sputum induction) to ensure that subjects are well, and all subjects will be provided with a contact telephone number for access to a study physician who is on call 24 hours/day. If it is discovered during the 48 hour follow up phone call that the subject has continued to require albuterol for 48 hours after the inhaled allergen challenge, they will be given a single dose of prednisone 60 mg and continue following instructions from the provided asthma action plan for albuterol use. These subjects will be contacted by phone within 24 hours of receiving the dose to assess asthma symptoms and will be evaluated in person within 48-72 hours of the dose to monitor FEV1 and to assess the need for continued therapy.

Subjects who are withdrawn for medical concerns related to the study, such as but not limited to prolonged decreased FEV_1 or persistent wheezing, will be followed by study physicians until resolution of these events. As this is a screening protocol, these subjects will not be replaced.

Multiple actions will be taken to minimize the risk of breach of privacy or confidentiality, including conducting study visits in private clinic rooms, keeping all paper case report forms in a locked office in a secured building, using study identification numbers instead of personal identifiers whenever possible, etc. Please see section 10.2. for a detailed description of these procedures.

9.5. Reporting of AEs and SAEs

UNC-Chapel Hill Human Research Protection Program Standard Operating Procedures will be followed for reporting adverse events/unanticipated problems to the Biomedical IRB and NC TraCS DSMB. All SAEs will be reported to the CBER of the FDA as well as to the UNC OHRE, DSMB, and the NIH sponsor within 24 hours of recognition of the event, including the filing of an IND safety report. Adverse events will be reported to both the FDA, UNC IRB, and DSMB on no less than a quarterly basis, or when the protocol is completed. If criteria for suspension of the protocol are met, then the FDA and UNC IRB will be notified within 24 hours.

All subjects with a non-fatal SAE will be evaluated medically by a study physician, in concert with their own physician as appropriate. Likewise, all subjects with an adverse event will be examined and evaluated by a study physician. All assessments will include the same lung function and vital sign assessments outlined for challenge observation. Other assessments will be undertaken as needed. Any unspecified event, which in the judgment of the PI of the study, constitutes an unusual, unexpected or prolonged event (greater than 96 hours) will be reported to both the FDA, the UNC IRB, and the NC TraCS DSMB.

Failure to meet the following safety criteria would result in suspension of the study pending discussion with the DSMB and UNC IRB.

1. No occurrence of any Serious Adverse Event

2. Each SAE and the events listed in 2 a-g would be reported to the FDA, IRB, and DSMB and discussed before performing subsequent challenges or enrolling further participants. In addition, no more than 20% of subjects will fail the individual safety criteria outlined below. As it is anticipated that 100 subjects will be recruited for this screening protocol, then 20 or more subjects who fail individual safety measures will result in suspension of further study until consultation with the CBER of the FDA. Additionally, in order to further monitor safety, we will assess this after completion of 20 subjects, and annually.

    a. Less than or equal to 40% decrease in FEV_1 or FVC from pre-challenge values following EPR or the LPR to cat hair allergen (the target endpoint is a $\geq$20% decrease in FEV_1 from baseline during the EPR and a $\geq$15% decrease in FEV_1 from baseline during the LPR).

    b. If albuterol is employed as rescue, FEV1 must recover to within 90% of baseline value within 20 minutes.

    c. Oxygen saturation should remain $\geq$93% throughout the challenge period and must be no lower than 2% of baseline measure.

    d. Albuterol for rescue therapy should be required no more than three times within the first hour after challenge

    e. Albuterol for rescue therapy should be required no more than three times within 12 hours of discharge.

    f. FEV_1 should return to within 90% of baseline value at the study discontinuation visit which occurs within 10 days after allergen challenge.

    g. No occurrence of an asthma exacerbation associated with inhalation challenge requiring more than a single dose of prednisone (60 mg).

A Data Safety Monitoring Board (DSMB) through the NC TraCS Institute will independently review all research activities outlined for the study protocol.

To ensure correct reporting, with each subject binder (in the Case Report Form), we have added a discharge worksheet to capture the above information to ensure availability in real time and to guarantee that we do not overlook a violation of safety criteria.

9.6. Data and Safety Monitoring Plan

The PI will be responsible for data quality management and ongoing assessment of safety. Safety be monitored throughout the course of the study by the investigators.

We will use the NC TraCS DSMB to assess for possible changes to the overall risk of the study using inhaled allergen challenge. The DSMB will have full access to research records and source documents. Data regarding unanticipated problems will be provided to them as it is reported to the IRB and to the FDA. They will review all adverse events, enrollment, and protocol deviations after the first 6 subjects have completed the inhaled allergen protocol, and then every 6 months thereafter. Any serious adverse events or major protocol deviations will be reported immediately to the DSMB via email, which may lead to an earlier review. They will communicate with the PI and the appropriate NIH Medical Officer regarding any safety issues.

10 DATA COLLECTION AND MANAGEMENT

10.1. Data Management

Study coordinators responsible for efforts and computations for database management will include Carole Robinette, MS; Martha Almond, RRT; Brian Ring, RRT; and Katherine Mills, BA. Data will be initially collected by study coordinators on paper documents. Symptom questionnaires will be completed by the subject and collected by the coordinator. Clinical data such as health history and smoking history will be collected by study staff as interview or directly measured, such as vital signs, spirometry, etc. All paper forms (source documentation) is maintained by a study coordinator or PI in a binder, 1 binder per subject. All data will be entered into REDCap by a study coordinator, within 2 weeks of collection and will be verified by a 2nd person within 4 weeks to ensure accuracy and completeness. REDCap is a secure web application developed at Vanderbilt University and can be used to collect virtually any type of data (including 21 CFR Part 11, FISMA, and HIPAA-compliant environments). It is specifically geared to support online or offline data capture for research studies and operations. Missing data will be noted and a comment made in the database to explain the missing data. Time stamps will be used when appropriate. At minimum, the visit number will be indicated.

Biological samples collected by coordinators will be delivered to the CEMALB lab by the coordinators in properly labeled containers. On receipt in the lab, samples are initially entered into a software program called LDMS (Lab Data Management System) which tracks samples. Processing of these samples is done per the SOP maintained in the CEMALB quality assurance plan, as some samples are processed immediately and others are processed in batches at later time points. This data is entered into REDCap at appropriate intervals, based on sample analysis.

We will use the data dictionary or REDCap codebook as our course for data codes. All data will be collected by qualified staff using CEMALB SOPs and will be entered into REDCap. Once the data is entered and confirmed by a 2nd person, it will be considered “locked” and will not be changed without documentation with the reason for the change. REDCap limits and monitors who can change data, a feature developed to ensure data integrity.

10.2. Confidentiality

Risks to subject confidentiality will be minimized by storing records with personal identifiers in an office in CEMALB which is locked when unattended by the study coordinators. Records will be kept for 2 years after completion of data collection. The CEMALB is located in the US Environmental Protection Agency’s Human Studies Facility on the UNC campus which has a security guard and limited access 24 hours/day, 7 days/week.

Clinical labs will be sent to Labcorp, Inc, coded with the study number not the name, and with a dummy birthdate (so that we may get reference values). The spirometry program requires date of birth for values, those will be entered correctly. Other identifying data will only be on listed on the consent form and coordinator worksheets.

All other samples will have just the subject number. All samples will be stored with codes only (no personal identifiers). The samples will not be stored long term or used for analyses other than specified. On receipt in the lab, samples are initially entered into a software program called LDMS (Lab Data Management System) which tracks samples. Processing of these samples is done per the SOP maintained in the CEMALB quality assurance plan, as some samples are processed immediately and others are processed in batches at later time points. This data is entered into REDCap at appropriate intervals, based on sample analysis.

11 RECRUITMENT STRATEGY

Participants will be identified from the screening and database protocol of the CEMALB (98-0799), as well as website (CEMALB and Join the Conquest) and informational email advertising. Subjects who have agreed to participate in the CEMALB database will be contacted by study staff, and others will make the initial call to a CEMALB staff member's office in response to advertisements.

We will also identify potential participants using the Carolina Data Warehouse and will contact them by phone using a prepared phone script.

Subjects will be recruited from the local area (Chapel Hill, including UNC, Durham, Raleigh and even Greensboro). Recent census data suggest that there are sufficient individuals with asthma living in this area. We anticipate that we should be able to recruit the number of subjects required to complete the study.

12 CONSENT PROCESS

Investigators and study staff will explain all study procedures and the benefits and risks of the study to potential participants as part of obtaining informed consent. The consent process will take place in a private clinic room within the EPA Human Studies Facility on UNC Chapel Hill campus. Subjects may withdraw their consent at any time during the study. If they withdraw from the study it will not impact the care they receive at UNC or its affiliated hospitals and clinics. Withdrawal will also not impact the subject's status as a UNC student or employee. Subjects may be withdrawn if the study physician feels it is in the best interest of the subject.

13 PLANS FOR PUBLICATION

Data analysis will be performed once the final subject has completed all study visits. We plan to write and publish a manuscript in a peer-reviewed journal documenting the results of the analysis.

14 REFERENCES

1. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet* 2007;370:1422-1431.
2. Cockcroft DW, Hargreave FE, O'Byrne PM, Boulet LP. Understanding allergic asthma from allergen inhalation tests. *Can Respir J* 2007;14:414-418.
3. Diamant Z, Gauvreau GM, Cockcroft DW, et al. Inhaled allergen bronchoprovocation tests. *J Allergy Clin Immunol* 2013;132:1045-1055 e1046.
4. Gauvreau GM, Boulet LP, Leigh R, et al. A nonsteroidal glucocorticoid receptor agonist inhibits allergen-induced late asthmatic responses. *Am J Respir Crit Care Med* 2015;191:161-167.
5. O'Byrne PM. Allergen-induced airway inflammation and its therapeutic intervention. *Allergy Asthma Immunol Res* 2009;1:3-9.

6. O'Byrne PM, Gauvreau GM, Brannan JD. Provoked models of asthma: what have we learnt? *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 2009;39:181-192.
7. Gauvreau GM, El-Gammal AI, O'Byrne PM. Allergen-induced airway responses. *Eur Respir J* 2015;46:819-831.
8. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-2148.
9. Fahy JV, Cockcroft DW, Boulet LP, et al. Effect of aerosolized anti-IgE (E25) on airway responses to inhaled allergen in asthmatic subjects. *Am J Respir Crit Care Med* 1999;160:1023-1027.
10. Fahy JV, Fleming HE, Wong HH, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997;155:1828-1834.
11. Gauvreau GM, O'Byrne PM, Boulet LP, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *The New England journal of medicine* 2014;370:2102-2110.
12. Cahn A, Boyce M, Mistry S, et al. Randomized trial of allergen-induced asthmatic response in smokers and non-smokers: effects of inhaled corticosteroids. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 2015;45:1531-1541.
13. Davis BE, Illamperuma C, Gauvreau GM, et al. Single-dose desloratadine and montelukast and allergen-induced late airway responses. *Eur Respir J* 2009;33:1302-1308.
14. Kelly MM, O'Connor TM, Leigh R, et al. Effects of budesonide and formoterol on allergen-induced airway responses, inflammation, and airway remodeling in asthma. *J Allergy Clin Immunol* 2010;125:349-356 e313.
15. Oliver A, Bjermer L, Quinn D, et al. Modulation of allergen-induced bronchoconstriction by fluticasone furoate and vilanterol alone or in combination. *Allergy* 2013;68:1136-1142.
16. Singh D, Leaker B, Boyce M, et al. A novel inhaled phosphodiesterase 4 inhibitor (CHF6001) reduces the allergen challenge response in asthmatic patients. *Pulmonary pharmacology & therapeutics* 2016;40:1-6.
17. Arvidsson MB, Lowhagen O, Rak S. Early and late phase asthmatic response in lower airways of cat-allergic asthmatic patients--a comparison between experimental and environmental allergen challenge. *Allergy* 2007;62:488-494.
18. Sicherer SH, Wood RA, Eggleston PA. Determinants of airway responses to cat allergen: comparison of environmental challenge to quantitative nasal and bronchial allergen challenge. *J Allergy Clin Immunol* 1997;99:798-805.
19. Brussino L, Badiu I, Sciascia S, et al. Oxidative stress and airway inflammation after allergen challenge evaluated by exhaled breath condensate analysis. *Clin Exp Allergy* 2010;40:1642-1647.
20. Lieutier-Colas F, Purohit A, Meyer P, et al. Bronchial challenge tests in patients with asthma sensitized to cats: the importance of large particles in the immediate response. *Am J Respir Crit Care Med* 2003;167:1077-1082.
21. Barnig C, Purohit A, Casset A, Sohy C, Lieutier CF, Sauleau E, de Blay F. Nonallergic airway hyperresponsivenss and allergen-specific IgE levels are the main determinants of the early and late asthmatic response to allergen. J Investig Allergol Clin Immunol. 2013;23:267–74.
22. M. R. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, "Standardisation of spirometry," European Respiratory Journal, vol. 26 no. 2, pp. 319-338, 2005.

15 APPENDIX

15.1. Table of Study Procedures

Procedures to be performed at various time points are listed in the following table.

	Visit 1: baseline	Visit 2: 24-48 hrs pre-challenge	Visit 3: allergen challenge day	Visit 4: 24 hrs post-challenge	Visit 5: discontinuation visit
consent	X				
review history/AE's	X	X	X	X	X
Concomitant meds	X	X	X	X	X
urine HCG	X	X	X		
vital signs	X	X	X	X	X
spirometry	X	X	X	X	X
physical exam	X	X			
Methacholine challenge		X		X	
sputum induction	X			X	
symptom score	X	X	X	X	X
allergen challenge			X		
Venipuncture	X		X	X	
Exhaled nitric oxide	X	X	X	X	X
Nasal ELF collection	X		X	X	
HRV measurement		X	X		

15.2. Investigators and Facilities

Role in project	*Name and Address*	*Title*
Principal Investigator	Michelle L. Hernandez, MD CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Associate Professor of Pediatrics Chief Medical Officer, Center for Environmental Medicine, Asthma and Lung Biology
Co-Investigator	Allison J. Burbank, MD CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Assistant Professor of Pediatrics UNC School of Medicine Department of Pediatrics Division of Allergy, Immunology, and Rheumatology

Co-Investigator	Haibo Zhou, PhD 3104C McGavran-Greenberg Hall The University of North Carolina CB #7420 Chapel Hill, NC 27599	Professor of Biostatistics Director of the Biostatistics Core, CEMALB
Co-Investigator	Chanchaldeep (Amika) K Sood, M.D. CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Post-doctoral fellow UNC School of Medicine Department of Pediatrics Division of Allergy, Immunology, and Rheumatology

Facilities. Volunteers for these studies will undergo allergen challenge procedures at the Clinical and Translational Research Center (CTRC) at the University of North Carolina in Chapel Hill, North Carolina, 27599-7310. Additional subject visits and test procedures will be performed at the University of North Carolina Center for Environmental Medicine, Asthma and Lung Biology (CEMALB). All necessary clinical research equipment, medical equipment, and laboratory equipment is located at the CTRC and within the CEMALB.

The Principal Investigator, Co-investigators, and study coordinators will have access to subjects and data. Study coordinators responsible for efforts and computations for database management will include Carole Robinette, MS; Martha Almond, RRT, RCP; Brian Ring, BSRT, RCP; and Katherine Mills, BA. Please see data management section (10.1) for details