

Title: In situ analysis of sputum-derived cellular targets after Xolair® (Omalizumab)

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Background and significance - Study purpose and rationale:

Omalizumab improves asthma control and quality of life, and reduces corticosteroid use and asthma exacerbations in severe-persistent allergic asthma¹. Recent studies also suggest efficacy in non-allergic asthma². Early understanding of mechanisms of action of omalizumab centered around its targeting of the IgE-Fc ϵ R1 (high affinity IgE receptor) complex and interruption of the allergic cascade. Recent studies in non-allergic asthma now suggest additional mechanisms involving regulation of innate immune responses^{3,4}. These findings raise the exciting possibility that a larger population might benefit from the addition of omalizumab and reinforce the need to identify a potentially responsive population. Endotyping, or biologic phenotyping of patients with asthma, may help identify individuals with potential to respond to omalizumab and has potential to provide information on mechanisms of action of omalizumab. **There is an unmet need for non-invasive sampling of innate immune pathways in asthma to improve our ability to endotype patients with asthma.**

We have recently developed a novel technique to enrich for rare sputum cells from induced sputum, including bronchial epithelial cells (sputum-derived human bronchial epithelial cells; sHBEC). We have shown increased Th2 cytokine gene expression in sHBEC from subjects with asthma, and now propose to use this technique to test the hypothesis that **treatment with omalizumab in adults with asthma reduces bronchial epithelial cell-derived Th2 cytokine gene expression**. We will test this hypothesis with two specific aims:

Specific Aim 1. Using sHBEC from well-characterized patients with moderate-persistent asthma, we will test the hypothesis that treatment with omalizumab reduces mRNA for sHBEC Th2/Th17 targets.

SA1.a Design and complete a randomized, controlled, 16 week study to obtain sputum-derived human bronchial epithelial cells.

SA1.b. Compare levels of candidate Th2/Th17 supporting cytokines in sHBEC mRNA in patients with moderate persistent asthma treated with omalizumab.

Specific Aim 2. Using sHBEC from well-characterized patients with moderate-persistent asthma, we will test the hypothesis that treatment with omalizumab results in a novel gene “signature” in sHBEC.

SA2.a. Identify omalizumab response genes in sHBEC to identify novel mRNA targets regulated by treatment of patients with moderate-persistent asthma with omalizumab in sHBEC mRNA (gene expression).

SA2.b. Validate sHBEC omalizumab response gene “signature” using nanoString technology to generate gene signature for potential use for patient stratification.

Significance

Current clinical indications for omalizumab include its use in those with elevated allergen-specific IgE to perennial allergens. However, the presence of heterogeneity of inflammatory pathways is increasingly being recognized in asthma and there is a need for biologic phenotyping (endotyping) to identify patients who will respond to targeted therapy. Little is known about downstream biologic targets of omalizumab in key cells associated with asthma. Downregulation of sputum eosinophils, bronchoscopy-obtained tissue eosinophils, cells expressing the high-affinity Fc receptor for IgE and surface IL-4 staining cells, have been shown after omalizumab treatment ⁵. Recent studies suggest that blood-derived plasmacytoid dendritic cells (pDC) express high levels of the FcγR1 and that expression is reduced in both allergic and non-allergic asthmatic patients after treatment with omalizumab ². In refractory atopic dermatitis, treatment with omalizumab reduces plasma levels of thymic stromal lymphopoietin (TSLP), OX40L, and TARC, all involved in Th2 polarization ⁶. The suggestion has also been made that individuals with elevations in FeNO, serum periostin, and blood eosinophils might have improved response to omalizumab, suggesting their use as biomarkers for phenotyping and providing clues for mechanism of action ⁷.

These studies raise the exciting possibility that additional mechanisms of action of omalizumab can be identified that will lead to improved stratification of patients for treatment. Understanding the response of specific innate immune effector cells in the lung can provide clues to these questions. **We now propose to address these questions using a non-invasive technique of rare cell isolation from induced sputum.**

The airway epithelium actively participates in the response to allergens as well as to infections and pollutants. Human bronchial epithelial cell (HBEC)-derived cytokines signal to downstream innate immune and effector cells, including the recently described type 2 innate lymphoid cells (ILC2s) and airway dendritic cells (DC). We, and others have described an airway microenvironment regulated by airway epithelial cells, with potential to promote the activation of downstream innate immune cells^{8,9}. HBEC-derived cytokines that promote a Th2 response include but are not limited to: **thymic stromal lymphopoietin (TSLP), IL-33 and IL-25**. Using *in vitro* studies, we described the human Th2-supporting HBEC-DC nexus in response to pollutants¹⁰⁻¹²; upregulation of HBEC-derived cytokines by other stimuli has also been shown. This nexus is linked to the initiation and maintenance of asthma in animal models. Invasive human studies using bronchoscopy show upregulation of TSLP and other epithelial cell-derived mediators even in patients on high dose inhaled corticosteroids (ICS); **the invasive nature of bronchoscopy limits its applicability for endotyping and therapeutic stratification and outcomes.**

Analysis of whole induced sputum is under development for endotyping for asthma ¹³. Although exciting, analysis of whole induced sputum provides limited information about specific cell responses, **and low level or rare cell-specific alterations, particularly those of airway epithelial cells, can be masked**. The isolation of discrete cell populations from induced sputum allows sampling of rare cells

from conducting airways, repeated sampling, and cell-specific detailed genomic evaluation. We recently developed a novel technique to simultaneously enrich innate immune cells from sputum¹⁴. This technique allows for *in situ* analyses of sputum-derived human bronchial epithelial cells (sHBEC). The non-invasive nature of the technique provides a unique tool for *in vivo* human studies.

Innovation

These studies will be the first to use non-invasive measures of a discrete cell population to examine the downstream effects of omalizumab treatment in the lung. Information derived from these studies will help clarify mechanisms of action of omalizumab and help identify potential tools for patient endotyping and stratification for therapeutic interventions.

SA1.a Design and complete a randomized, parallel-group, double blind 16-week study to obtain sputum-derived human bronchial epithelial cells.

Preliminary data

The PI (Reibman) has extensive experience performing clinical trials as part of the American Lung Association Asthma Clinical Research Center (ALA ACRC), a multi-center consortium, as well as Phase III asthma studies. These studies have resulted in numerous publications, with selected publications noted here¹⁵⁻¹⁸. The current study at the ALA ACRC is to examine step-down therapy in patients with moderate-persistent asthma and was designed by her site. In addition, the PI has developed a registry of asthma and control participants (New York University/Bellevue Asthma Registry), designed to study characteristics of severe asthma¹⁹⁻²².

Objectives:

We propose a randomized, parallel-group, double blind 16-week study to assess the effect of omalizumab treatment on sHBEC expression of target and novel cytokines.

The primary outcome measure will be:

- The effect of omalizumab on TSLP and IL-33 gene expression in sHBEC in moderate persistent asthma

The secondary outcome measures will be:

- The effect of omalizumab on asthma control defined as symptom control (Asthma control test), spirometry, and measures of small airway dysfunction (Impulse oscillometry)

Exploratory outcomes

- The effect of omalizumab on newly identified sHBEC targets (gene expression) and gene “signature” generation

Study design:

Inclusion Criteria

- Age 18-65 years old

- Physician diagnosed asthma
- Lung function (one or more of the following documented in the 5 years before enrollment or demonstration during screening)
 - Bronchial hyperresponsiveness (BhR) confirmed by $\geq 12\%$ improvement in FEV₁ post bronchodilator within the previous 5 years, or
 - Methacholine PC₂₀ < 16mg/dl within the previous 5 years
- Severity criteria
 - Moderate-persistent asthma defined by ATS²³
- Asthma control
 - Partly or uncontrolled asthma according to GINA 2012 guidelines (at least three of the following features: daytime symptoms more than 2 times/week, limitation of activities, nocturnal symptoms, need for rescue inhaler > 2 times/week, FEV₁ <80% predicted)²⁴
- Stable use of moderate-high dose inhaled corticosteroids in previous 3 months (definition derived from GINA 2012 guidelines: e.g. fluticasone propionate >250 mcg/day, budesonide > 400mcg/day)²⁴
- Ability to perform induced sputum maneuvers
- Presence of elevated allergen IgE to any perennial aeroallergen
- FeNO ≥ 19 ppb at V1 (Hanania et al. Am J Respir Crit Care Med; 187pp 804–811, 2013)

Exclusion Criteria

- Age <18 or >65 years old
- Pulmonary function
 - FEV₁ $\leq 70\%$ predicted
- Any major chronic illness including but not limited to COPD, uncontrolled hypertension, coronary artery disease, bronchiectasis, congestive heart failure, stroke, cystic fibrosis, insulin-dependent diabetes mellitus, renal failure, liver disorders, immunodeficiency state, or other condition that would interfere with participation in the study
- Current or > 10 pack a year pack-year tobacco use
- Any investigational study within previous 1 month
- Inability to perform baseline measurements
- Inability to contact by telephone
- Pregnancy at screening and failure to use double barrier pregnancy protection in woman of childbearing age
- Hypersensitivity reaction to omalizumab in the past
- Exceeds limits of dosing table (IgE <30 or 700 IU/ml) or body weight of <30 or > 150kg
- Systemic corticosteroids within the previous month
- Known malignant neoplasm

Investigational and reference therapy:

The investigational therapy will be omalizumab, dosed according to dosing and U.S. administration guidelines for omalizumab. Omalizumab will be dosed every 2-4 weeks based on the patient's pretreatment serum IgE level (IU/mL) and initial visit body weight (kg). Omalizumab will be delivered as a subcutaneous injection. Standard safety precautions for dosing will be observed, including clinical observation after dosing, and provision of an epinephrine pen. Maintenance asthma treatment will

remain unchanged.

Omalizumab will be supplied by Novartis Pharma as a sterile, freeze-dried preparation.

Reference therapy will consist of sterile saline with a volume and injection frequency that would have been indicated based on patient's serum IgE and body weight. Sterile saline will be delivered as a subcutaneous injection and will be supplied by Novartis Pharma.

Treatment or reference therapy will be delivered for a 16-week period and then terminated. No adjustments will be made for dosing after initial dose determination.

Study duration:

This study will be a 16-week study intervention with a 2-week run-in, including screening for each patient. We would anticipate starting the study in March 2015 (after grant approval, IRB approval, finalization of study) and completion of the study within 12 months.

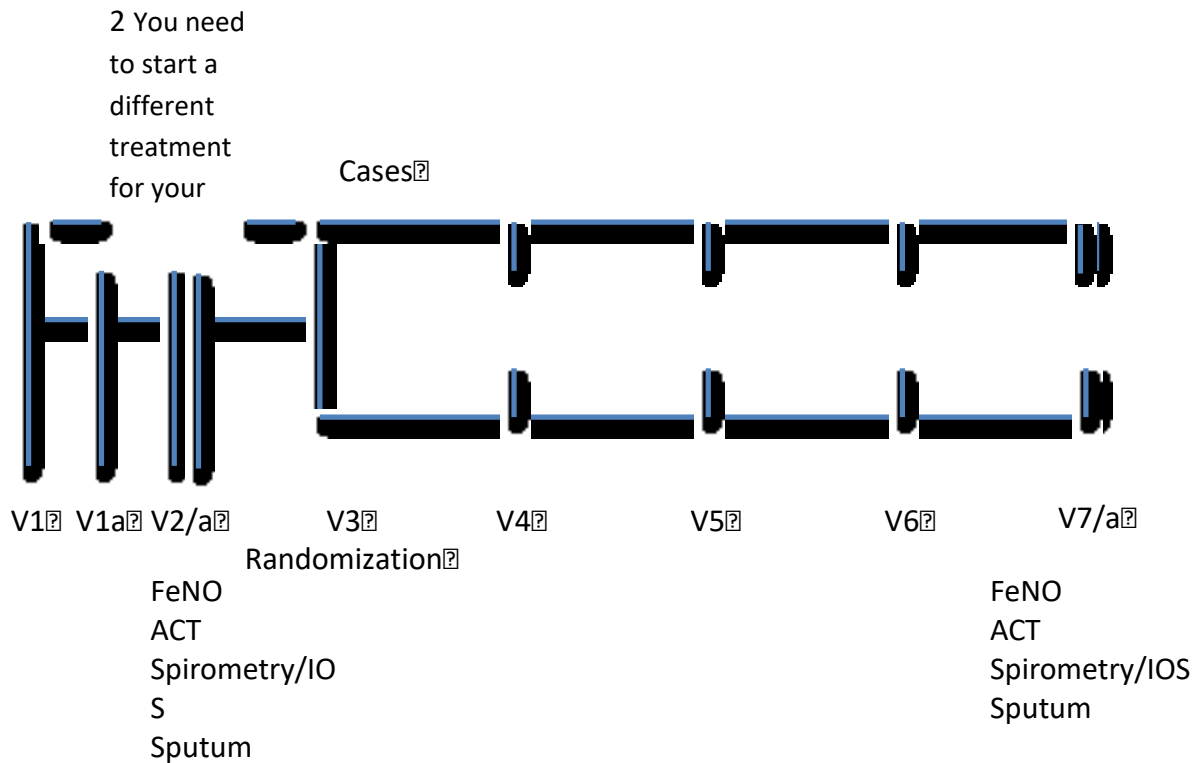
Protocol and Study design:

The study will be a randomized, placebo-controlled, double blind, 16-week intervention study. This will be an exploratory study to show feasibility and suggest proof of concept. Our enrollment goal is 30 subjects.

Recruitment for the study will be performed with advertisements at Metro NY or AM NY, Craig's list, subjects who have participated in previous studies and have consented to be contacted for future research studies and referral from Bellevue or NYU physicians who are part of the study team. The treating physician, who is part of the study team, will introduce the study to his/her patients and obtain permission for the study team to contact the patient to further discuss the study and see if he/she is interested in participating.

Consenting Process: All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be provided by the PI in a room with privacy. After the subject has read the consent form, the subject will be able to ask any questions to enable their understanding of the protocol.

Visits and procedures are diagramed below:



Briefly, participants will be recruited according to inclusion/exclusion criteria previously defined.

Visit 1. During this visit, subjects will sign consent, be screened for eligibility including severity. Asthma control will be assessed using the Asthma Control Test (ACT), a 5 question survey. We will also assess the ability of the subject to complete the study. A limited physical exam will be performed (a doctor will listen to heart and lungs), and vital signs will be measured. Blood (40ml) will be obtained for baseline allergen-specific IgE measurements if they have not been measured in the previous 5 years, and peripheral eosinophils, and serum will be obtained and blood stored for potential subsequent analysis. We will measure exhaled breath (FeNO). Nitric oxide (NO) is a naturally occurring gas made in the lungs. Measurement of FeNO provides information about the level of inflammation (redness, swelling and irritation) in the lungs. We will ask the participant to seal their lips tightly on the mouthpiece of the FeNO device and inhale (breathe in) as much air as possible. They will then exhale (blow out) the air out of their lungs slowly over a 10-second period of time. This test may have to be done more than once to get a good measurement. Breathing measurements will be performed by spirometry and impulse oscillometry (IOS) before and after albuterol. Spirometry will be performed after a nose clip is placed on the subject and then the subject will be asked to breathe hard and fast through a tube until reproducible measurements are obtained (up to 7 trials). We then administer a bronchodilator via a metered dose inhaler (albuterol sulfate; 360g) and repeat the spirometry measurement. IOS maneuvers will be performed during tidal breathing in the seated position, with a nose clip in place, and with support of the cheeks. A minimum of three trials, lasting 30 seconds, will be performed. This will also be done before and after the bronchodilator.

Visit 1a. This visit will only be necessary if subject does not meet criteria with either a history of bronchodilator reversibility, or a history of a positive methacholine study as defined in inclusion criteria. During this visit, a methacholine challenge test will be performed. Methacholine is an inhaled medication, which is used to diagnose airway hyper-reactivity, a characteristic of asthma. The subject will be asked to inhale a low dose of methacholine and then perform spirometry. We will slowly increase the dose and repeat spirometry until the subject shows a decline in spirometry value of 20% or until we have reached a defined dose of methacholine (16 mg). If spirometry measurement is reduced as expected with asthma, or if subject develops asthma symptoms, the effect is temporary and can be reversed by taking albuterol. At this visit, if the patient is female and of childbearing age, a urine pregnancy test will be done before the methacholine challenge test is done.

Visit 2. During this visit, we will obtain an interim asthma history, obtain vital signs, and perform a limited physical examination. We will measure breathing with a peak flow (a quick exhalation into a peak flow meter). We will perform a sputum induction. To do this, we will first measure baseline breathing with spirometry. We will then ask the subject to inhale albuterol sulfate (360 mcg) and use a standardized protocol, which consists of inhalation of nebulized hypertonic saline solution (3%, NOUVAG 2000 ultrasonic nebulizer; NOUVAG USA, Inc.) for 2- minute intervals for a total of 20 minutes. Volunteers will be encouraged to cough and expectorate throughout the procedure.

Visit 2a. This visit will occur within approximately 1 week after Visit 2. At this visit, we will review symptoms to make sure that there has not been an asthma exacerbation. We will then perform a repeat sputum induction to insure adequate sputum collection. We will also review information about omalizumab and provide education on the use of the epinephrine pen, which we provide to all patients who are getting omalizumab as a standard safety precaution. An epinephrine pen is a device that delivers epinephrine for emergency. This would only be needed to reverse an allergic reaction. We will also provide patients with an "Anaphylaxis" information sheet by the AAIA. At this visit, we will calculate the dose of omalizumab (or placebo) that will be needed based on weight and serum IgE level. Some patients, may require biweekly dosing of omalizumab, to accommodate this dosing (3a, 4a, 5a, 6a & 7a).

Visit 3. Randomization Visit. This visit will be performed at least 1 day after V2a but performed at least 2 weeks after V1 in order to allow confirmation of asthma control as a run-in. **This is a randomization (group assignment) visit and a study drug visit.** At this visit, we will obtain an interim asthma history, measure asthma control (ACT), limited physical examination with a peak flow, and review inclusion/exclusion criteria. The subject will receive the first dose of omalizumab or placebo. The study drug will be injected into the arm with up to 3 subcutaneous injections (just under the skin). The number of injections will be determined by the amount of medication the patient needs. The subject will be observed in the CTSI for 2 hours after the injection of the study drug or placebo. This is the standard protocol for all patients who receive this medication.

Visit 3a. Study drug visit (for subjects who need biweekly doses). If the patient needs to receive omalizumab every two weeks, we will call the subject back for Visit 3a. At this visit we will again measure the vital signs. We will then administer an injection of the study drug or placebo. The subject will again be observed in the CTSI for 2 hours after the injection of the study drug or placebo. This is the

standard protocol for all patients who receive this medication, where a 2 hour observation is routine for the first 2 doses of the drug.

Visit 4. Study drug visit. This visit will occur 4 weeks after V3 and will be a monitoring visit. At this visit, we will review the asthma history or if the patient has had any side effects since the first dose of omalizumab. We will assess asthma control using the ACT. We will perform a limited exam by checking vital signs and listening to the lungs. We will inject the study drug or placebo into the arm with a subcutaneous injection. We will observe the patient in the CTSI for 2 hours for patients on monthly dosing receiving their second dose or for 30 minutes for patients on biweekly dosing receiving their third dose. This is the standard protocol for all patients receiving this medication.

Visit 4a (V4a). Study drug visit (for subjects who need biweekly doses). This visit will occur approximately 2 weeks after V4 if the patient needs omalizumab every 2 weeks. At this visit we will again measure the vital signs and provide the subject with an injection of the study drug or placebo. We will observe the patient in the CTSI for 30 minutes. This is the standard protocol for all patients who receive this medication.

Visit 5. This visit will occur 4 weeks after V4 (week 8 of study drug) and will be a monitoring visit. At this visit, we will again review the asthma history and whether the patient has had any side effects since the last dose of omalizumab. We will assess asthma control (ACT) and we will auscultate the lungs and check vital signs. Study drug or placebo will be injected subcutaneously. We will observe the patient in the CTSI for 30 minutes. This is the standard protocol for all patients who receive this medication.

Visit 5a (V5a). Study drug visit (for subjects who need biweekly doses). This visit will occur approximately 2 weeks after V5 if their need omalizumab every 2 weeks. At this visit we will again measure vital signs and administer an injection of the study drug or placebo. We will observe the patient in the CTSI for 30 minutes.

Visit 6. This visit will occur 4 weeks after V5 (week 12 of study drug) and will be a monitoring visit. At this visit, we will again review the interim asthma history and whether the patient has had any side effects since the last dose of omalizumab. We will assess asthma control (ACT) and we will auscultate the lungs and check vital signs. Study drug or placebo will be injected subcutaneously. We will observe the patient in the CTSI for 30 minutes.

Visit 6a (V6a). Study drug visit (for subjects who need biweekly doses). This visit will occur approximately 2 weeks after V6. We will again review the asthma history and whether the patient has had any side effects since the last dose of omalizumab. We will assess asthma control (ACT) and we will auscultate the lungs and check vital signs. Study drug or placebo will be injected subcutaneously. We will observe the patient in the CTSI for 30 minutes. This is the standard protocol for all patients who receive this medication.

Visit 7. This visit will occur 1-3 weeks after the last dose of medication (V6 or V6a). At this time, subjects will be assessed for safety indices and asthma control. In addition, subjects will undergo measurement of FeNO, Spirometry and IOS. There will be no more administration of study drug. Sputum induction will be performed as previously described. Blood will be obtained from a vein from their arm or hand for laboratory tests (approximately 40 ml or 3 tablespoons).

Visit 7a (V7a). Final sputum induction visit. This visit will occur approximately 1 week after Visit 7. At this visit, we will again review whether there have been any side effects. We will assess asthma control and we will auscultate the lungs and check vital signs. Sputum induction will be performed as previously described.

Once the patient has completed that study, they will receive a letter containing their treatment assignment.

Because of differences in reconstitution time and viscosity between omalizumab and placebo, the research pharmacy will reconstitute and the nurses will inject the drugs independent of the investigator.

Initial comparison will be performed between the omalizumab group compared to control group with mean of the individual difference (i.e. change, expressed as the % of baseline) and the values observed at V7 of the two groups.

We have elected to include patients with moderate or uncontrolled asthma as opposed to severe asthma. The goal of this study is based on sputum induction, and so for our pilot study, for reasons of safety and experience, we have elected to include patients with moderate or partly/uncontrolled asthma. In addition, we have included the ability to successfully undergo the induced sputum maneuver (ability to tolerate the procedure, ability to expectorate good quality sputum) as an inclusion criteria. We will keep track of the number of patients that we have excluded because of this criteria.

Subjects will be stratified on a 1:1 basis. There are no requirements for assignment as a treatment or control subject. Subjects will remain on current therapy (inhaled corticosteroid, with or without long acting beta-agonist). This treatment will remain stable during the study period. Use of a short acting beta-agonist will be allowed. Use of rescue prednisone during the study period will not be allowed, and subject will be discontinued from the study. Subjects who fail initial screening can be re-screened.

Study Treatment

Description of Study Treatments: treatments used in this study will include

- Omalizumab will be dosed according to dosing and U.S. administration guidelines for omalizumab. Omalizumab will be dosed every 2-4 weeks based on the patient's pretreatment serum IgE level (IU/mL) and initial visit body weight (kg). Omalizumab will be delivered as a subcutaneous injection. Standard safety precautions for dosing will be observed, including clinical observation after dosing, and provision of an epinephrine pen. Maintenance asthma treatment will remain unchanged.
- Placebo reference therapy will consist of sterile saline with a volume and injection

frequency that would have been indicated based on patient's serum IgE and body weight. Sterile saline will be delivered as a subcutaneous injection and will be supplied by Novartis Pharma.

Pre-randomization Study Treatment:

- There will be no specific pre-randomization treatment

Randomization Procedure

- Randomization will occur at visit 3 (V3). This visit will be performed at least 1 day after V2 but performed at least 2 weeks after V1 in order to allow confirmation of asthma control as a run-in. At this visit, inclusion/exclusion criteria will be reviewed and baseline spirometry will be performed. Subject will receive first dose of omalizumab or placebo. Patients will be randomized in a 1:1 ratio to either omalizumab or placebo. Omalizumab will be supplied by Novartis Pharma as a sterile, freeze-dried preparation. Randomization will be generated by a computer randomization program. The study team will be blind with the exception of one coordinator. The research pharmacy will as well as one coordinator of the study team will be unblind and will maintain the randomization code linked to the subjects.
- Maintenance asthma treatment will remain unchanged throughout the sixteen week study

Side Effects of Drugs:

- Omalizumab (Xolair®) is an anti-IgE antibody indicated for moderate to severe persistent asthma in patients with positive skin test or in vitro reactivity to a perennial aeroallergen and symptoms that are inadequately controlled with inhaled corticosteroids

Potential serious side effect

- Anaphylaxis
- Malignancy
- Cardiovascular side effects. A recent 5 year study of omalizumab revealed a higher incidence rate of overall cardiovascular and cerebrovascular serious adverse events in patients treated with omalizumab. This was an observational study and could not take into account possible baseline imbalances in cardiovascular risk factors and inability to adjust for possible risk factors. An additional analysis of shorter studies with a younger age population failed to show this increased risk. The FDA has required a black box warning.

Common side effects

- Injection site reaction
- Viral infections
- Upper respiratory tract infections
- Sinusitis
- Headache
- Pharyngitis

- Methacholine. Methacholine is delivered during a methacholine challenge test. Some people have no reaction to this at all while for others, this test can reproduce some asthma symptoms. Some people have coughing or a tight sensation in their chest from breathing the methacholine, but it is usually mild. However, the reaction to methacholine can include severe bronchoconstriction (such as a severe asthma attack) and a reduction in breathing function. If a subject develops an asthma episode during this procedure or if lung function drops, we will stop the procedure and reverse the symptoms and lung function reduction with albuterol to reverse the effects of the methacholine. A physician involved in the study will provide this and the subject will be monitored until symptoms and breathing has returned to its baseline. If a subject is female and of childbearing age, a pregnancy test will be performed before the methacholine test.
- Albuterol sulfate (bronchodilator). We will ask you to inhale albuterol sulfate, a bronchodilator, during some breathing tests and before your sputum induction. Albuterol sulfate is a medicine that is commonly used for all people with asthma to relax the muscles in the airways and open up the lungs. We will do this as a screening test to see if you have reversible airflow obstruction or to reverse any reduction in airflow that you may have experienced with methacholine or with the sputum induction. Inhalation of albuterol sulfate can make you feel jittery or make your heart beat faster than usual.

Potential Benefits: This study will provide a comprehensive assessment of response to omalizumab that will may improve the health of a subject and provide guidance about subsequent treatment. . The providers will select appropriate patients who may benefit from treatment. The results from this study should add to the guidance health care providers with regard to measuring response to omalizumab treatment more objectively.

Handing of Drug:

- CTSI pharmacy as per their standard protocol will conduct drug accountability, storage, diluting and destruction.

Unmasking:

Participants and clinical center investigators will be double masked as to treatment assignment during the randomization phase. The investigators believe that under most circumstances, if there is a potential adverse reaction to study drug, the drug can be discontinued without unmasking of participant or staff. In the event of an emergency, unmasking can occur by reviewing pharmacy notes with drug assignment number on the outside and treatment. The study PI will have a 24-hour contact number. Instances of unmasking prior to the termination of the trial data collection will be reported to the Data Safety Monitoring Board (led by Dr. John Hay) Before the first subject is entered into the study, a member of the study team will review and discuss the requirements of the clinical study protocol and related documents with the investigational staff and also train them in any study-specific procedures and system(s) utilized. The principal investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The principal investigator will maintain a record of all individuals involved in the study.

- Once a participant completes the study, the participant will receive a letter containing the treatment assignment.

Safety and Asthma Control

Routine asthma treatment

- All participants must have partially or uncontrolled asthma according to GINA 2012 guidelines (at least three of the following features: daytime symptoms more than 2 times/week, limitation of activities, nocturnal symptoms, need for rescue inhaler > 2 times/week, FEV₁ <80% predicted)²⁴. Participants cannot have received treatment with Xolair within the 6 months prior to enrollment. At the time of enrollment, if the participant has been on a stable treatment regimen using other asthma and allergy treatments, including SABAs, LABAs, ICS, combination ICS/LABA, leukotriene modifiers, theophylline preparations, antihistamines, immunotherapy (allergy shots), and or nasal steroids, the participant may continue these treatments during the course of the study. If a participant experiences an asthma exacerbation or other signs of significant worsening of their asthma, the participant will be referred to the primary care physician or asthma provider for re-evaluation of their asthma regimen. If the asthma care provider is not available in a timely fashion, the clinical center physician will evaluate the participant's regimen and make recommendations for treatment.

Asthma Action Plan

- Participants will receive an asthma action plan summarizing the procedures for home care in the event of increase in asthma symptoms or a drop in peak flow. At visit 1 the study physician or his/her designee will give the participant a temporary asthma action plan sheet with instructions for its use. The "red", "yellow" and "green" zones will be based on peak flow readings and symptoms at Visit 1. At Visit 3, upon randomization the PEF values from the diary cards will be reviewed and symptoms reviewed. Zones will be defined as "green" (> 85% of baseline PEF), "yellow" (65—85% of baseline PEF), and "red" (less than 65% of baseline PEF). These personal cut-offs will be entered onto an Asthma Action Plan Card. Both the temporary asthma action plan given at visit 1 and the Asthma Action Plan Card given at visit 3 will instruct participants to seek medical attention immediately if they are experiencing a serious asthma attack. Participants who experience less serious asthma symptoms or a drop in peak flow into the yellow zone or red zone are instructed to take 2 puffs of their rescue inhaler and wait 15-20 minutes. If symptoms persist or peak flow does not return to the green zone, participants are instructed to take 2 more puffs of the inhaler and wait 15-20 minutes up to 2 more times. If after a total of 3 administrations of the rescue inhaler, symptoms persist or peak flow is not in the green zone participants are instructed to seek medical care immediately.

Response to Treatment Failures

- This study is not designed to evaluate clinical treatment effects. Participants who experience worsening asthma, defined as an increase in use of albuterol, will be treated according to best medical judgment and continue follow-up in their same treatment group. However, should a participant require a rescue course of prednisone (systemic corticosteroids), emergency room visit, or hospitalization during the study period (V2 – 7), the participant will be withdrawn from the study.

This will not be considered an adverse event due to study drug, because both EPR3 and GINA guidelines, consider one exacerbation per patient per year an indication in and of itself of lack of asthma control.

Serious Adverse Events

- A serious adverse event (SAE) is an adverse event that results in one of the following outcomes: anaphylaxis, death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or congenital anomaly/birth defect. Also, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention (treatment) to prevent any of the outcomes previously listed in this definition. When an investigator or clinical center staff member becomes aware of an SAE, it will be reported to the study PI within 72 hours with follow up reporting until the event is terminated.

Adverse events

Definition of an AE: Any untoward medical occurrence in a subject administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the investigational medicinal product.

Investigational Medicinal Product (IMP) includes the drug under evaluation and the comparator drug(s) if specified as part of the research objective, given at any time during the study. Medical conditions/diseases present before starting the drug of interest are only considered adverse events if they worsen after starting the drug of interest.

The occurrence of adverse events will be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. All adverse events will be recorded in the study database including the following information:

1. the severity grade (mild, moderate, severe)
2. its relationship to the drug(s) of interest (suspected/not suspected)
3. its duration (start and end dates or if continuing at final exam)
4. whether it constitutes a serious adverse event (SAE)

Serious adverse event reporting

A SAE is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,

- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is otherwise a significant medical event.

This includes any SAEs likely to arise from the trial indication or progression of underlying/concomitant illness (es) (e.g. progression of cancer in oncology trials), unless specified in the protocol as study specific exemptions.

Any SAE, irrespective of causality, occurring after the subject has provided informed consent and until four weeks after the subject has stopped study participation must be reported unless otherwise stated in the protocol. SAEs occurring after four weeks from ending study participation should only be reported if considered by the Investigator attributable to the exposure to the investigational drug(s) during the trial period. This includes the period in which the study protocol interferes with the standard medical treatment given to a subject, even if study treatment has not yet started (e.g. withdrawal of previous treatment during washout period, change in treatment to a fixed dose of concomitant medication).

Timelines: All serious adverse events (SAEs) from interventional clinical trials must be reported by the sites to Sponsor within 24 hours of occurrence of the SAE. The timelines for investigator initiated trials reporting to Novartis will be done as per Third Party Study/Investigator Initiated Trial Agreement.

Follow-up reports:

SAEs will be followed until resolution or until it is judged to be permanent, and an assessment will be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the drug of interest, the interventions required to treat it, and the outcome.

The Sponsor shall support Novartis in the following-up of all SAEs so that complete information is available to maintain patient safety and also as part of any commitments by Novartis to any Health authority OR specific Health authority follow-up requests for the product under investigation.

Pregnancies

To ensure patient safety, each pregnancy in a patient (or a patients partner) on study drug must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy will be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Specific Procedures

Spirometry

To perform spirometry, a nose clip will be placed on the subject and then the subject will be asked to breathe hard and fast through a tube until reproducible measurements are obtained (up to 7 trials). We then administer a bronchodilator via a metered dose inhaler (albuterol sulfate; 360g) and repeat the spirometry measurement. Pre and post-bronchodilator (albuterol sulfate; 360g) spirometry will be

performed with a Vitalograph spirometer according to American Thoracic Society standards.⁹² Normal values for spirometry will be obtained from Hankinson et al.⁹³

Impulse oscillometry (IOS).

IOS maneuvers will be performed during tidal breathing in the seated position, with a nose clip in place, and with support of the cheeks. A minimum of three trials, lasting 30 seconds, will be performed. This will also be done before and after the bronchodilator. IOS maneuvers (Jaeger Impulse Oscillation System; Jaeger USA; Yorba Linda, CA) will be performed in accordance with published recommendations⁹⁴ and as we have previously described⁹⁵⁻⁹⁷. Maneuvers will be performed during tidal breathing in the seated position, with a nose clip in place, and with support of the cheeks. A minimum of three trials, lasting 30 seconds, will be performed. Only data from trials with constant tidal volume and coherence >0.70 at 5 Hertz (Hz) and 0.85 at 10Hz will be analyzed. Data for analysis will include resistance measured at an oscillating frequency of 5Hz (R5) and frequency dependence of resistance (FDR) calculated as the difference between resistance at 5Hz and 20Hz (R5-20). FDR provides a measure of non-uniformity of airflow distribution, which may reflect regional functional abnormalities in the small airways or distal lung⁹⁸⁻¹⁰⁰.

Mechacholine challenge test.

Methacholine is an inhaled medication, which is used to diagnose airway hyper-reactivity, which is a characteristic of asthma. The subject will be asked to inhale a low dose of this medication and then perform spirometry. We will slowly increase the dose and repeat spirometry until the subject shows a decline in spirometry value or until we have reached a defined dose. If spirometry measurement is reduced as expected with asthma, or if subject develops asthma symptoms, the effect is temporary and can be reversed by taking albuterol.

Sputum Induction and processing

Sputum induction will be performed according to standard methods²⁵. Briefly, volunteers will inhale albuterol sulfate (360 mcg) and sputum will be induced by inhalation of nebulized hypertonic saline solution (3%, NOUVAG 2000 ultrasonic nebulizer; NOUVAG USA, Inc.) for 2- minute intervals for a total of 20 minutes. Volunteers will be encouraged to cough and expectorate throughout the procedure. Sputum will be weighed and treated (37°C, 15 minutes) with freshly prepared dithiothreitol (1% DTT; Sputolysin Reagent; Calbiochem, Germany)²⁵. The sample will be filtered (70 micrometer nylon cell strainer; Becton Dickinson, USA) to remove mucous and debris, centrifuged (300 x g, 10 minutes) and the pellet resuspended in cell dissociation buffer (CDB; Sigma #C5914) with 10% fetal calf serum to achieve a concentration of 10⁶ cells/ml. A total cell count (TCC) will be performed (hematocytometer), cell viability determined (trypan blue exclusion) and slides prepared (cytospin). Quality specimens (less than 50% squamous epithelial cells and > 90% cell viability) will be used and cell counts performed on 400 non-squamous cells. Results of cell counts will be expressed as a percentage of the total non-squamous cell count.

Flow Cytometry

Washed cells will be labeled with pre-titered, monoclonal antibodies (mAbs; 4°C, 30 min). Fresh cells

will be sorted without fixation immediately after antibody labeling and cells will be sorted on a FACSAria II (BD Biosciences) on the same day. Compensation for spillover and spectral overlap will be performed using CompBeads plus (anti-mouse IgG and anti-rat IgG; BD Biosciences) as well as the ArC Amine Reactive Compensation Bead Kit (Invitrogen). Cellular debris/dead cells will be excluded by LIVE/DEAD staining (LIVE/DEAD Fixable Blue Dead Cell Stain Kit; L23105; Invitrogen). Squamous epithelial cells (sqEPI), DC (plasmacytoid and myeloid DC), monocytes, macrophages, and B cells, will be differentiated using size discrimination and fluorescent labeling.

RNA

Total RNA will be isolated using the miRVana (Life Technology) and RNA will be transcribed using the RT² First Strand Kit (SABiosciences, Frederick, MD) and quantitative PCR (qPCR) will be performed using a SYBR Green/ROX qPCR Master Mix and RT² qPCR Primer Assay using specific primers. Levels of respective transcripts will be normalized to GAPDH transcript level as an internal control $\Delta Ct(\text{target}) = Ct(\text{target}) - Ct(\text{GAP-DH})$. TSLP primers will be designed to detect both the long and short splice variants of TSLP that have been described⁵⁰.

FeNO

Measurement of FeNO will be performed with a handheld NIOX MINO (Aerocrine; Stockholm, Sweden) according to a standard protocol.¹⁰¹ FeNO measurements, expressed in parts per billion (ppb), will be reported for an exhalation flow rate of 50 mL/s.

Peripheral Eosinophils

Complete blood counts with cell differential will be performed by an automated laboratory procedure.

Allergen-specific IgE

Measurements of total and allergen-specific serum IgE for allergens common to the Northeastern United States will be performed in a commercial laboratory (Pharmacia ImmunoCAP assay; Quest Diagnostics; Teterboro, NJ). Atopy will be defined as the presence of at least one elevated allergen-specific IgE (≥ 0.35 kilo-international units (kU)/L). Perennial allergy will be defined as an elevated allergen-specific indoor allergen common to the Northeastern U.S.: cat dander, dog dander, *blatella germanica* (cockroach) or *dermatophagoides pteronyssinus* (house dust mite). Outdoor allergy will be defined as an elevated allergen-specific IgE to any of the common outdoor allergens: maple, ragweed, birch, elm, oak and ash. Molds include *aspergillus fumigatus* and *alternaria alternata*.

Statistical Design

Variables to be analyzed:

The primary outcome is the change in TSLP, IL-33 and IL-25 gene expression in sHBEC in mild-moderate persistent asthma after 16 weeks of treatment of omalizumab or placebo. We will estimate the difference in the gene expression of the treatment group of omalizumab compared with the

placebo group and report the results of estimated mean and 95% confidence interval. The difference between two randomized groups will be compared using the two-group t-test if data are normally distributed, and the nonparametric Wilcoxon test will be used for skewed data. The secondary outcomes include the change sHBEC targets (gene expression array), asthma control defined as symptom control (Asthma control test), spirometry, and measures of small airway dysfunction (Impulse oscillometry). The comparisons of secondary outcomes will be conducted in similar way as the primary outcome. We will account for the issue of false positives due to multiple testing hypotheses by using a conservative alpha level of 0.01. The effect of omalizumab on newly identified sHBEC targets (gene expression array). This is an exploratory goal and analysis will be performed initially with cufflinks and subsequent analyses will be performed with the NYU Genome Technology Center and the NYU Bioinformatics Center.

Sample size and power: This is an exploratory study with a treatment group and control group and the goal is to provide an estimate of the mean difference between two groups and proper confidence interval. Sample size of 15 each group can achieve a margin of error of 0.73 assuming that outcomes are standardized with standard deviation of 1.

Safety:

Before the first subject is entered into the study, a member of the study team will review and discuss the requirements of the clinical study protocol and related documents with the investigational staff and also train them in any study-specific procedures and system(s) utilized. The principal investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The principal investigator will maintain a record of all individuals involved in the study
During the study:

Confirm that facilities remain acceptable

Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the data base, that biological samples are handled in accordance with the protocol and that study drug accountability checks are being performed

Perform source data verification (a comparison of the data in the data base with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating subjects.

Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject. A representative will be available between visits if the investigator(s) or other staff at the center needs information and advice about the study conduct.

We have chosen to include subjects with moderate asthma to reduce the potential for adverse events with sputum induction in patients with severe asthma. The main complication from sputum induction is the potential for bronchospasm following inhalation of hypertonic saline. Standard safety procedures

will be used for sputum induction, which include clinical assessment and spirometry prior to initiation of sputum induction. In addition, peak flow measurements are performed throughout the procedure and the procedure is terminated if there is a reduction in peak flow, or spirometry of >20% baseline. Albuterol sulfate is administered should the subject experience a drop in their peak flow or Spirometry during the sputum induction procedure and the patient is monitored to insure a return to 90% of baseline spirometry.

We will review adverse events at each visit including presence of an asthma exacerbation between visits. An exacerbation will be defined as the use of rescue systemic corticosteroid (po, IM, IV) or an urgent care visit or an emergency room visit. Should this occur, the subject will be discontinued from the study.

Omalizumab will be injected at the CTSI and subjects will be monitored at the CTSI for 2 hours for the first 2 doses and then will be 30 minutes subsequent doses. Subjects will be provided a pamphlet describing symptoms of anaphylaxis and instructions for procedures should they experience these symptoms. Subjects will also be provided an epinephrine auto-injector for the possibility of a rare occurrence of anaphylaxis to omalizumab. Subjects will be monitored for adverse reactions during each visit while at the CTSI as per recommended drug delivery protocol. We will also monitor for infection or skin reaction at injection site.

Urine pregnancy testing for women of childbearing age will be performed prior to methacholine challenge and randomization and before each injection of omalizumab. Should a subject become pregnant during the study, they will be terminated from the study. We will ask for information about the outcome of the pregnancy and the health of the baby.

Adverse events will be collected at each visit after the screening visit and severe adverse events will be reported to the NYU IRB. Adverse events that are not considered severe will be noted in the subject's study binder.

A DSMB (Data Safety and Monitoring Board) will be created to review this study on a semiannual basis. The individuals in this board will include physicians, some of whom will be pulmonary specialists with experience in clinical trials. Information reviewed by the DSMB will include number of subjects screened and enrolled, any adverse events, and any study deviations. The DSMB will meet twice each year. The DSMP will write a short summary of their findings and elaborate on any AE or study deviations, and will make suggestion of improving study safety and efficacy. This summary will be communicated to the IRB. The DSMB may recommend that a study be stopped if analysis of the data suggests that the experimental intervention appears harmful to the trial participants. This recommendation could be made on the basis of serious adverse events or side effects that are cropping up in those getting the experimental intervention.

Confidentiality:

To protect every individual against the loss of confidentiality, the information will be kept in locked

files and in secured databases. Paper source documents and consent forms will be stored in a locked filing cabinet in a locked room on the 6th floor of Bellevue hospital Room 9-13. This room has limited access. Data files will be stored in Red Cap on the NYU secure server.

Samples will be kept and processed in Dr. Reibman's laboratory in the Rom Environmental Lab.

Only members who are IRB approved as investigators on this study or with IRB-approved protocols will be able to access these data and samples. Use of de-identified data will be allowed after review by the PI and coPIs who will act as a Research Oversight Committee and after a data-sharing plan has been approved. If any member requests this information, they will need to follow protocol and be added properly via IRB Application for Study Personnel change form.

PHI information will be stripped from all patient documents and samples and replaced with a unique study subject identifier. There will be one master list that will be under the protection of Dr. Reibman, which will code the patient name to the assigned study number. The linking key will be kept by the PI or her designate. No genetic testing will be done on samples during the study to identify risk for other conditions.

The ICF will incorporate wording that complies with relevant data protection and privacy legislation.

The PI of the study will not provide individual genotype results to subjects, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, a ROC or an investigator might know a subject's identity and also have access to his or her genetic data. Also regulatory authorities may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate.

Sample Storage for Future Use:

We will save the blood and sputum samples in a freezer for up to 10 years for potential testing of additional markers of inflammation, which may include testing of RNA (part of a cell that builds proteins and contains genetic information). Since RNA is a product of DNA (is part of the cell that contains the instructions for how you look and function), measurements of RNA can be considered a type of genetic testing. However, these studies will not provide us with information about their risks for other genetic conditions or the ability to do genetic counseling. Storage for future research is not an optional component to this study as these analyses are an integral part of the study for testing of additional markers of inflammation. Samples will be stored in a freezer for future research in Dr. Reibman's Laboratory (with Rom Environmental Lab). Samples will be labeled as described above with the study number and the unique subject identifier. Only the PI, IRB approved designates will have access to the

stored samples. The linking key will be kept by the PI or her designate. Subjects can contact the PI to withdraw samples from storage and future use.

Costs to Subjects:

The patient will not have to pay for any tests that are in this study or any procedures that are in this study. These include blood tests and breathing tests. These funds are provided to help support with time and travel associated with their participation.

The cost of the study drug, the medical visits and laboratory tests related to the research part of the study will be paid by NovartisPharma.

Please note that Novartis will not pay for medication such as bronchodilators or other asthma controllers that it may be taking for the asthma outside of this study

Payments to Subjects:

We will reimburse subjects 75 dollars for each visit that includes sputum induction (V2, 2a, 7 7a) and 50 dollar compensation per visit for all other visits

References:

1. Grimaldi-Bensouda L, Zureik M, Aubier M, Humbert M, Levy J, Benichou J, Molimard M, Abenham L, Pharmacoepidemiology of A, Xolair Study G. Does omalizumab make a difference to the real-life treatment of asthma exacerbations?: Results from a large cohort of patients with severe uncontrolled asthma. *Chest*. 2013;143(2):398-405.
2. Garcia G, Magnan A, Chiron R, Contin-Bordes C, Berger P, Taille C, Devouassoux G, de Blay F, Couderc LJ, Didier A, O'Callaghan DS, Girodet PO, Bourdeix I, Le Gros V, Humbert M. A Proof-of-Concept, Randomized, Controlled Trial of Omalizumab in Patients With Severe, Difficult-to-Control, Nonatopic Asthma. *Chest*. 2013;144(2):411-9.
3. Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol*. 2005;115(3):459-65.
4. Lommatzsch M, Korn S, Buhl R, Virchow JC. Against all odds: anti-IgE for intrinsic asthma? *Thorax*. 2013.
5. Djukanovic R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF, Bao W, Fowler-Taylor A, Matthews J, Busse WW, Holgate ST, Fahy JV. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med*. 2004;170(6):583-93.
6. Iyengar SR, Hoyte EG, Loza A, Bonaccorso S, Chiang D, Umetsu DT, Nadeau KC. Immunologic effects of omalizumab in children with severe refractory atopic dermatitis: a randomized, placebo-controlled clinical trial. *Int Arch Allergy Immunol*. 2013;162:89-93.
7. Hanania NA, Wenzel S, Rosen K, Hsieh HJ, Mosesova S, Choy DF, Lal P, Arron JR, Harris JM, Busse W. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med*. 2013;187(8):804-11.

8. Reibman J, Hsu Y, Chen LC, Kumar A, Su WC, Choy W, Talbot A, Gordon T. Size fractions of ambient particulate matter induce granulocyte macrophage colony-stimulating factor in human bronchial epithelial cells by mitogen-activated protein kinase pathways. *Am J Respir Cell Mol Biol*. 2002;27(4):455-62.
9. Reibman J, Hsu Y, Chen LC, Bleck B, Gordon T. Airway epithelial cells release MIP-3alpha/CCL20 in response to cytokines and ambient particulate matter. *Am J Respir Cell Mol Biol*. 2003;28(6):648-54.
10. Bleck B, Tse DB, Jaspers I, Curotto de Lafaille MA, Reibman J. Diesel exhaust particle-exposed human bronchial epithelial cells induce dendritic cell maturation. *J Immunol*. 2006;176(12):7431-7.
11. Bleck B, Tse DB, Curotto de Lafaille MA, Zhang F, Reibman J. Diesel exhaust particle-exposed human bronchial epithelial cells induce dendritic cell maturation and polarization via thymic stromal lymphopoietin. *J Clin Immunol*. 2008;28(2):147-56.
12. Bleck B, Tse DB, Gordon T, Ahsan MR, Reibman J. Diesel exhaust particle-treated human bronchial epithelial cells upregulate Jagged-1 and OX40 ligand in myeloid dendritic cells via thymic stromal lymphopoietin. *J Immunol*. 2010;185(11):6636-45.
13. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify T2-high and T2-low subtypes of asthma. *J Allergy Clin Immunol*. 2013.
14. Kazeros A BB, Lee R, Tse D, Liu M, Chiu A, Chung S, Reibman J. In Situ Upregulation Of Thymic Stromal Lymphopoietin In Sputum Epithelial Cells In Asthma. *Am J Respir Crit Care Med*. 2012:A2782.
15. The safety of inactivated influenza vaccine in adults and children with asthma. *N Engl J Med*. 2001;345(21):1529-36.
16. DiMango E, Holbrook JT, Simpson E, Reibman J, Richter J, Narula S, Prusakowski N, Mastronarde JG, Wise RA, American Lung Association Asthma Clinical Research C. Effects of asymptomatic proximal and distal gastroesophageal reflux on asthma severity. *Am J Respir Crit Care Med*. 2009;180(9):809-16.
17. American Lung Association Asthma Clinical Research C, Mastronarde JG, Anthonisen NR, Castro M, Holbrook JT, Leone FT, Teague WG, Wise RA. Efficacy of esomeprazole for treatment of poorly controlled asthma. *N Engl J Med*. 2009;360(15):1487-99.
18. American Lung Association Asthma Clinical Research C, Peters SP, Anthonisen N, Castro M, Holbrook JT, Irvin CG, Smith LJ, Wise RA. Randomized comparison of strategies for reducing treatment in mild persistent asthma. *N Engl J Med*. 2007;356(20):2027-39.
19. Reibman J, Marmor M, Filner J, Fernandez-Beros ME, Rogers L, Perez-Perez GI, Blaser MJ. Asthma is inversely associated with *Helicobacter pylori* status in an urban population. *PLoS One*. 2008;3(12):e4060.
20. Blaser MJ, Chen Y, Reibman J. Does *Helicobacter pylori* protect against asthma and allergy? *Gut*. 2008;57(5):561-7.
21. Liu M, Rogers L, Cheng Q, Shao Y, Fernandez-Beros ME, Hirschhorn JN, Lyon HN, Gajdos ZK, Vedantam S, Gregersen P, Seldin MF, Bleck B, Ramasamy A, Hartikainen AL, Jarvelin MR, Kuokkanen M, Laitinen T, Eriksson J, Lehtimaki T, Raitakari OT, Reibman J. Genetic variants of TSLP and asthma in an admixed urban population. *PLoS One*. 2011;6(9):e25099.
22. Patrawalla P RL, Ma M-T, Shao Y, Liu M, Cheng Q, Fernandez-Beros M, Reibman J. Replication of the Severe Asthma Research Program Cluster Analysis in an urban population. *American Journal of Respiratory and Care Medicine*. 2011;183:A3718.
23. Fahy JV, Boushey HA, Lazarus SC, Mauger EA, Cherniack RM, Chinchilli VM, Craig TJ, Drazen JM, Ford JG, Fish JE, Israel E, Kraft M, Lemanske RF, Martin RJ, McLean D, Peters SP, Sorkness C, Szeffler SJ.

Safety and reproducibility of sputum induction in asthmatic subjects in a multicenter study. *Am J Respir Crit Care Med.* 2001;163(6):1470-5.