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An Evaluation of Treatment with Omalizumab to Improve the Asthmatic Response to an Experimental Infection with Rhinovirus

VERSION 13.0 (August 1, 2016)

Omalizumab/Rhinovirus IND: BB-IND #10510

IND Sponsor: Peter Heymann, M.D., University of Virginia

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PRINCIPAL INVESTIGATORS

Thomas Platts-Mills, MD, PhD, FRS
Department of Medicine
P.O. Box 801355
University of Virginia
Charlottesville, VA 22908
Tel# 434-924-2209
Fax# 434-924-5779
Email: tap2z@virginia.edu

Peter Heymann, MD
Department of Pediatrics
P.O. Box 800386
University of Virginia
Charlottesville, VA 22908
Tel# 434-982-3654
Fax# 434-924-5778
E-mail: pwh5a@virginia.edu

CO-INVESTIGATOR(S) *(if applicable)*

Ronald Turner, PhD Professor of Pediatrics
Associate Dean for Clinical Research
Department of Pediatrics
University of Virginia School of Medicine
P.O. Box 800386
Barringer Building, Room 4441
Hospital Drive
Charlottesville, VA 22908
Phone: 434-243-9864
Fax: 434-982-4246
Email: rbt2n@virginia.edu

Anne-Marie Irani, MD
Professor of Pediatrics and Medicine
Head, Division of Pediatric Allergy & Immunology
Virginia Commonwealth University
Medical College of Virginia
Tel# 804-828-9620
Fax# 804-828-1751
E-mail : airani@vcu.edux

BIOSTATISTICIAN

James T. Patrie MS
Senior Biostatistician
Division of Biostatistics and Epidemiology
Department of Public Health Sciences
University of Virginia Health Sciences
Charlottesville, VA 22908
Phone: 434-924-8576
Fax: 434-243-5787
email jp4h@virginia.edu

NIAID MEDICAL OFFICER
Lisa M Wheatley MD MPH Division of Allergy, Immunology and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane 6B56Rockville, MD 20892Phone: 240-627-3573lisa.wheatley@nih.gov

INVESTIGATOR SIGNATURE PAGE

Protocol U01-UVA-02	**Version/Date:** **VERSION 13.0 (August 1, 2016)**
IND Number **Omalizumab – BB-IND #10510**	**Principal Investigator:** Peter Heymann, MD

Short Title: Omalizumab to Improve Asthmatic Response to RV-16 Infection

IND Sponsor: Peter Heymann, MD

INSTRUCTIONS: The Principal Investigator will print, sign, and date at the indicated location below. A copy should be kept in the investigator's records and the original signature page sent to the NIAID.
After signature, please return the original of this form by surface mail to:

Project Manager: Steven Sigelman, BSN
DAIT/ NIAID/ AAABB
5601 Fishers Lane 6B57
Rockville, MD 20892

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312 , and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance" dated April 1996 *(this should be reference #1 in Section 13)* and the spirit of the Declaration of Helsinki, Further, I will conduct the study in keeping with local, legal, and regulatory requirements.

As the Principal Investigator, I agree to conduct AADCRC-UVA-02: *An Evaluation of Treatment with Omalizumab to Improve the Asthmatic Response to an Experimental Infection with Rhinovirus.* I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission by the NIAID, the local IRB and the FDA.

_____
Principal Investigator (Print)

_____ _____
Principal Investigator (Signature) Date

Synopsis

Title	An Evaluation of Treatment with Omalizumab to Improve the Asthmatic Response to an Experimental Infection with Rhinovirus
Short Title	Omalizumab to Improve Asthmatic Response to RV 16 Infection
Rationale	Population surveys have shown a positive correlation between increased levels of total serum IgE and bronchial hyperreactivity and it is also clear that exacerbations of asthma are frequently triggered by viral respiratory tract infections, especially human RV. This protocol explores the relationship between rhinovirus and allergen/IgE. Experimental challenges with human (RV) result in significantly higher upper respiratory tract symptom scores in asthmatics than in controls. Asthmatics with high levels of IgE showed greater sensitivity to methacholine and higher levels of eNO than those with low levels of IgE. These data suggest that patients with asthma and high levels of IgE are more likely to have pre-existing inflammation of the airways before virus challenge. This study is being done to determine whether anti-IgE antibody will lead to a significant decline in inflammatory biomarkers prior to virus inoculation, and thus reduce the severity of clinical manifestations after experimental human RV challenge.
Clinical Phase	Phase II
Mechanistic Study	×*Yes* □*No*
IND Sponsor	Peter Heymann, MD
Principal Investigators	Peter Heymann, MD and Thomas Platts-Mills, MD, PhD, FRS
Participating Site(s)	University of Virginia (Charlottesville, VA)
Accrual Objective	42 total subjects: 21 with Omalizumab and RV challenge, 21 with placebo and RV challenge
Study Objective	To test the hypothesis that the reduction of total free IgE in asthmatics treated with omalizumab for 8 weeks prior to and during an experimental RV challenge will lead to a significant decline in lower respiratory tract (chest) symptoms recorded by subjects during the first four days of infection following the challenge compared to lower respiratory tract symptoms recorded during the same period by asthmatic subjects treated with placebo.

Study Design	Randomized, double-blind placebo controlled study in a group of 42 mild asthmatics. Subjects will be randomized 1:1 to omalizumab (a humanized monoclonal anti-IgE antibody) or placebo for 8 weeks and then inoculated with GMP RV 16 with a subsequent clinical and laboratory (mechanistic) follow up for 4 weeks.
Study Duration	45 months
Primary Endpoint	The primary endpoint will be based on the comparison of cumulative lower respiratory tract symptoms scores (CLRTS) in the asthmatic subjects treated with omalizumab compared to those treated with placebo over the first 4 days of acute infection. The lower respiratory tract symptoms evaluated will include shortness of breath, chest discomfort, and wheezing.
Secondary Endpoints	Secondary endpoints will include a comparison of CLTRS scores in the asthmatic and non-asthmatic subjects over the first 4 days of the acute infection, and will include symptoms of shortness of breath, chest discomfort, wheezing, as well as cough. Secondary outcomes will also include a comparison of changes from baseline in 1) methacholine sensitivity (PC20), 2) expired nitric oxide (eNO), 3) impulse oscillometry, 4) immune cell analyses including frequency of circulating eosinophils and RV and allergen specific regulatory and effector T-cells, 5) concentrations of nasal wash mediators and cytokines, 6) weights of nasal mucous and secretions collected during the first 4 days of the infection, and 7)cumulative upper respiratory tract symptoms scores (CURTS) detected during peak cold symptoms over the first 4 day s of the infection among subjects enrolled in the omalizumab and placebo treated groups. Diary cards will be scored daily for runny nose, sneezing, nasal congestion, sore throat, headache, chills/fever, fatigue, itchy eyes, watery eyes and red eyes using a modification of the Jackson criteria as previously described (16). An additional secondary endpoint will be rate of subjects whose FEV1 drops by more than 20% from their baseline value at the time of enrollment. We will also compare changes in the same parameters and in the CLTRS scores between the treatment groups from baseline, using the same statistical methods described for the primary endpoint, over the first 7 and 21 days of the infection, in parallel with results from the validated ACT questionnaire, to detect differences stimulated by RV, and we will compare changes in markers of inflammation and bronchial hyperreactivity between the omalizumab and placebo groups during the 8 weeks of drug administration before RV challenge in order to evaluate the effect of these changes on the response to RV.
Inclusion Criteria	• Subject must be able to understand and provide written informed consent • Age 18 to $\leq$ 40 years of age, any gender, any racial/ethnic origin

	• Physician-diagnosed asthma • ACT score > 19 • Short-acting beta-agonist use < daily in last 4 weeks • FEV1 > 70%, or FEV1/FVC ratio > 75% for subjects with FVC values between 80 and 87% predicted whose FEV1 values fall below 70%. • Positive Methacholine challenge test (i.e. at least 20% fall in FEV_1 at a Methacholine concentration of ≤16 mg/ml) at screening protocol before enrollment (20). • Total serum IgE level ≥ 125 IU/ml documented during screening protocol. • Positive test for allergen specific IgE antibody by prick skin testing documented during screening protocol. to allergens associated with current allergen exposure at the time of the RV challenge: e.g., dust mite, *Alternaria*, and/or ragweed for subjects challenged with RV in the fall, or positive tests to tree and/or grass allergens for those challenged with RV in the spring. In keeping with the study design goals of inoculating subjects during periods of allergen exposure, sensitization to other allergens (e.g., cat or dog) will also qualify for enrollment if subjects are currently exposed to these allergens at home. Participant must be willing to comply with study procedures and requirements. Participant must be considered eligible for participation based on results of screening procedures conducted by protocol number 20100686 at VCU and IRB# 12656 at UVA). Refer to Appendix 4.
Exclusion Criteria	• Inability or unwillingness of a participant *or subject's legal representative* to give written informed consent *and HIPPA authorization* • Positive test for serum neutralizing antibody to RV-16 at screening within 6 weeks (i.e., subjects with a neutralizing antibody titer ≥ 1:4 will be excluded). • To avoid RV-16 inoculations in subjects with more restrictive lung volumes, those whose FVC is < 80% predicted will also be excluded. • Total IgE levels measured at screening protocol that are too elevated based on a subjects weight, to meet the recommendations proposed by Novartis for treatment with Omalizumab (21). • Chronic heart disease, lung diseases other than asthma, or other chronic illnesses, including primary and/or secondary immunodeficiency. • Hospitalization or treatment in the ER for asthma (unless the treatment involved the use of a bronchodilator only) during the last three years. • Subjects who have had one or more night time awakenings caused by asthma symptoms and/or who have needed their SABA (albuterol) inhaler for asthma symptoms ≥ 4 days during the week before enrollment, or during the week before the inoculation with RV-16. • Intubation or management in the intensive care unit for an asthma

	exacerbation • An upper or lower respiratory tract infection within six weeks prior to enrollment • Previous nasal or sinus surgery within the last 12 months. • Who have a 5 pack/year history of smoking, or any smoking within the last 6 months. • Female subjects who are, or who plan to become, pregnant during the study, or who are nursing a baby. Additionally, to be included in this study, a woman of child-bearing potential must have a negative urine pregnancy test at screening, during the run, and prior to viral inoculation and agree to use an effective method of birth control such as, but not limited to, birth control pills, contraceptive foam, diaphragm, IUD, abstinence, or condoms. • Subjects who have used omalizumab within 6 months prior to enrollment, or inhaled corticosteroids,inhaled ipratropium bromide, an inhaled long acting beta agonist, inhaled cromolyn or nedocromil or systemic leukotriene modifiers for their asthma on a daily basis within 4 weeks prior to enrollment or subjects using nasal corticosteroids on a daily basis within 4 weeks prior to enrollment. Subjects who are currently receiving beta-adrenergic blocking agents. • Subjects who are currently receiving allergen immunotherapy (IT), or who have received allergen IT within the last 3 years. • Hemoglobin <11.5 g/dL for non-African American subjects or hemoglobin < 11.0 g/dL for African American subjects detected during screening within 6 weeks of enrollment. • Absolute neutrophil count (ANC) < 1500 cells/mm3 (or 1.5 K/uL) detected during screening within 6 weeks of enrollment or Day -2.
Investigational Product(s)/Intervention(s)	This study will use 2 interventions: 1. All subjects will receive Rhinovirus 16 challenge 2. Half of the subjects will receive omalizumab and the other half placebo instead of Omalizumab
Study Procedures	The following procedures will be performed according to the schedules in Appendix 5 • Medical and allergy history • Limited physical exam • Urine pregnancy test/Urine LTE4 • Nasal wash • Monitoring of nasal mucous and secretion weights during the first 4 days of the infection • Skin testing, including tests for Alternaria, dust mite, and ragweed, grass and tree pollen allergens • Spirometry

	• Exhaled Nitric Oxide • CBC/differential • Serum IgE levels (total and allergen specific) • T-cells blood test • RV 16 antibody titres by neutralization assay • Methacholine challenge • Impulse oscillometry • RV 16 inoculation • Completion of diary cards • Added to this study will be a nasal specimen obtained by using an ASI Rhino-Pro® nasal mucosal curette to do a light scraping along the inferior turbinate of each nostril to collect epithelial cells at 3 time points during the study. • A small piece of gauze will be applied to the inferior turbinate of each nostril at 3 different time points during the study to sample nasal lining fluid to measure albumin, mediators and cytokines.
Statistical considerations	Using Monte Carlo simulation to estimate statistical power, 42 asthmatic subjects will be enrolled. For 8 weeks prior to inoculation with RV-16 and during the infection, 21 subjects will be randomized to a treatment group receiving Omalizumab and 21 subjects will be randomized to a group receiving placebo. Accounting for a 20% drop out rate, 17 subjects in each treatment arm are expected to complete the study which. Based on the power calculations, this will be sufficient to detect a 2.4 unit difference in the CLTRS score during the first 4 days after RV inoculation between the 2 treatment groups. The power of the test was determined to be 0.83. Statistical analyses of the data will use methods published in our previous experimental challenges of asthmatic and non-asthmatic subjects challenged with RV-16 (16).

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15. Addendum: Added Protocol for "Evaluating the Asthmatic Response to an Experimental Infection with Rhinovirus in the Atopic Host 124-142Glossary of Abbreviations/Definitions

AADCRC	Asthma and Allergic Diseases Cooperative Research Center
AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CLRTS	Cumulative lower respiratory tract symptoms
CTCAE	Common Terminology Criteria for Adverse Events
(CURTS)	Cumulative upper respiratory tract symptoms
DAIT	Division of Allergy, Immunology, and Transplantation
cGCP	current Good Clinical Practice
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
PI	Principal Investigator
RV	Rhinovirus
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SUSARs	A Serious, Unexpected Suspected Adverse Reaction

1. BACKGROUND AND RATIONALE

1.1 Background

The investigations described in this proposal are designed to examine alterations in the immune response to rhinovirus (RV) in the atopic host and to provide insight into mechanisms which contribute to exacerbations of asthma caused by this virus. All subjects enrolled will have physician diagnosed asthma and positive methacholine challenge tests characteristic for asthmatic individuals. No subjects will be excluded because of race, gender, or ethnic background. During screening the atopic status of each subject will be defined with assessments of total IgE in serum and allergen specific IgE sensitization by prick skin testing to eleven aero-allergens which represent common allergen exposures in Virginia and the Mid-Atlantic States. Although many studies of asthmatic subjects define atopy as a positive test for IgE to one or more common aeroallergens, we have observed that the asthmatics with total IgE levels $\geq$ 125 IU/ml enrolled in our RV challenge studies frequently have sensitization to several aeroallergens and the large majority have IgE antibody to dust mite (16). We feel that it will continue to be important to characterize the atopic status of subjects enrolled this study by assessing and reporting their total IgE levels and allergen specific sensitizations.

Based on previous studies, pre-treatment with omalizumab will reduce the levels of serum-free total IgE in subjects to very low levels (i.e. reductions to <5% of baseline values) (1a). By binding to Fc epsilon, omalizumab modifies the expression of allergic symptoms by preventing the binding of IgE to IgE-receptor bearing cells (mast cells and basophils). Omalizumab has also been shown to reduce blood eosinophil counts and expired nitric oxide (eNO) levels during treatment (2). In keeping with this, we predict that treatment with omalizumab in our study will lead to a significant decline in

inflammatory biomarkers in the airway prior to RV inoculation and, thus, reduce the risks for an adverse response to RV during the infection. In clinical trials, omalizumab has already been shown to have steroid-sparing effects in asthmatic children and adults (3, 4). Moreover, treatment with this medication has reduced the frequency of asthma exacerbations leading to hospitalization and ER visits, which previous studies have shown are frequently associated with RV infections (5-10).

In this investigation, we will use experimental RV challenges to provide a time-sequence analysis of the response to RV following viral inoculation in asthmatic subjects. The strain of RV that will be used is a pool of RV (strain 16) that will be provided by Dr. Ronald Turner's laboratory at the University of Virginia (UVA). Dr. Turner, is a co-investigator in this study, and has collaborated with Johnson & Johnson Pharmaceutical Research and Development to produce this pool of RV under GMP conditions. This pool has been safety tested and has been approved for experimental challenge studies by the FDA (BB-IND #15162; IND holder, Dr. Ronald Turner). In addition, this pool of RV has undergone extensive clinical trials during Dr. Turner's evaluation of the new RV-16 pool using the experimental RV challenge model to evaluate subjects at the University of Virginia (see section 1.5 Clinical Experience). Using the Jackson criteria for scoring cold symptoms (11, 12), infection rate and total symptom scores are comparable to our previous experience with other rhinovirus challenge pools (both RV-16 used in our initial rhinovirus challenges and RV-39 challenges conducted by Dr. Turner) with the exception of a higher than expected number of subjects who developed cold symptoms in the infected volunteers. The severity of cold symptoms, however, were similar to the severity of symptom recorded by asthmatics and control subjects in previous studies by Dr. Turner and other investigators at UVA using RV 16 prior to the requirement that the RV pools used for inoculation must be produced under GMP conditions (13-15). In addition, the majority

of published data from experimental rhinovirus challenges in asthmatic individuals, including our own, and including subjects with moderately severe asthma, have used RV-16 which would help us compare our results to these previous studies now that a GMP produced pool of RV-16 is available (e.g., 16, 34, 39).

This study is being done to determine whether anti-IgE antibody will be effective in reducing the adverse effects of viral infections in patients with mild asthma. This study will determine whether anti-IgE can modify the inflammatory effects of viral infections. Moreover, the clinical implications of the results (if they support the hypothesis) are that efforts to reduce allergic inflammation may improve the tolerance of allergic asthmatics to the adverse effects of a viral infection. As in our previous studies of RV, we will make all test results (along with interpretation of their significance) available to patients. Thus, we hope that patients will learn about the effects of the common cold on their asthma and how to minimize symptoms when they have an infection. The experimental RV challenge model will be used to test the hypothesis that treating asthmatics with omalizumab (a humanized monoclonal anti-IgE antibody) for 8 weeks prior to virus inoculation and during the infection will lead to a significant decline in upper and lower respiratory tract symptom scores, inflammatory biomarkers, and changes in lung function (e.g. methacholine sensitivity) compared to the results of these assessments in asthmatics treated with placebo prior to RV inoculation and during the infection.

1.2 Rationale for Selection of Study Population

The experimental RV challenge model will be used to test the hypothesis that treating asthmatics with omalizumab (a humanized monoclonal anti-IgE antibody) for 8 weeks prior to virus inoculation and during the infection will decrease the risk for an asthmatic response to RV during the infection.

In our previous experimental challenge studies, and those of others, lower respiratory tract symptoms (mild wheeze, cough, dyspnea and/or chest tightness) have been reported in subjects with mild asthma (16, 17). Our previous studies have also shown that moderate to severe asthma exacerbations caused by naturally occurring infections with RV occur predominantly in children, and are much less common in asthmatic adults, especially those with mild asthma (17). As a result, we, as well as other investigators, have done our challenges by evaluating allergic adults (mostly college students) with mild asthma. In our studies, we can detect significant differences in lower respiratory tract symptoms, methacholine sensitivity, and markers of airway inflammation if we screen our subjects for atopy (total IgE levels $\geq$ 125 IU/ml) and do our challenges during periods of increased allergen exposure (e.g. in the spring or fall months in Virginia) without risks for causing an asthma exacerbation requiring hospital care (16). In this regard, one of the important exclusion criteria for participation in our challenges is that we will not enroll subjects who have required treatment in the hospital or ER during the previous 3 years. Because RV infections occur frequently, we are confident that subjects who participate in this study will have tolerated natural RV infections over the last 3 years without experiencing a significant asthma exacerbation.

To date, we have inoculated 45 mild asthmatic adults with RV (strains 16 or 39). Only one of these subjects, who was inoculated with RV-39, experienced audible wheezing and a reduction in FEV_1 during peak cold symptoms. Her symptoms responded to a short (5 day) course of oral steroids. She and the other 44 subjects did not experience symptoms requiring a visit to the ER or hospital. We have also completed a surveillance study of 16 adults with mild asthma, using the same inclusion and exclusion criteria proposed for this study to monitor the effects of administering omalizumab Omalizumab on natural RV colds. The treatment (i.e. 8 subjects treated with

omalizumab and 8 treated with placebo) covered a period of 3 months. No subjects withdrew from this study and there were no adverse events. Ten of the 16 subjects (62%) had nasal washes which tested positive for RV by RT-PCR at least once during clinic visits scheduled every other week. No subject experienced significant chest symptoms or reductions in lung function triggered by these natural infections. However, we cannot rule out the possibility that the subjects may have been seropositive to some the strains of RV detected in this surveillance study (18). For this reason, the experimental challenge study described below is a much better study design, because subjects will either be sero-negative, or have low titers (< 1:4) of neutralizing antibody to the strain of RV (i.e. RV-16) used for inoculation.

1.3 Investigational Product(s)/Intervention(s)

A. Omalizumab

Omalizumab (Xolair®) was approved by the FDA on June 20, 2003 for the treatment of adults and adolescents (age 12 and up) with moderate to severe asthma who have a positive skin test or in vitro reactivity to perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids. Omalizumab is a recombinant DNA-derived humanized IgG1k monoclonal antibody that selectively binds to human immunoglobulin E (IgE). Omalizumab is produced by a Chinese hamster ovary cell suspension culture in a nutrient medium containing the antibiotic gentamicin. Note that gentamicin is NOT detectable in the final product. Omalizumab is a sterile, white preservative-free lyophilized powder contained in a single-use vial that is reconstituted with sterile water for injection and is administered subcutaneously.

B. Placebo drug (produced by the same manufacturer: Novartis Pharmaceuticals):

The product described in this module is the placebo to Xolair 150 mg Powder for solution for injection. The placebo is the same mixture of inactive excipients, in quality and quantity, as those used for the drug product, except that the drug substance, omalizumab, is not added.

C. Rhinovirus (strain 16)

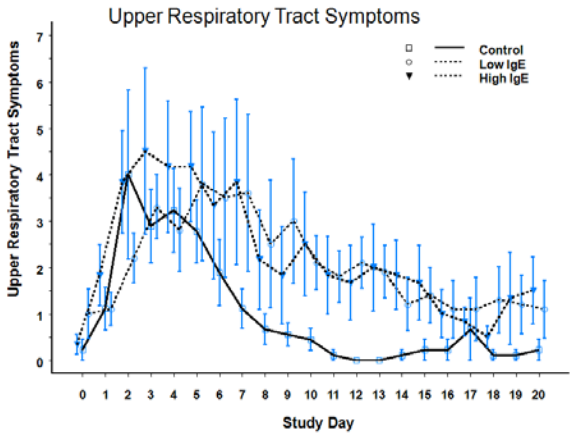
Rhinovirus (strain 16) will be used for inoculation in the RV challenge. The strain of RV that will be used is a pool of RV (strain 16) provided by Dr. Ronald Turner's laboratory at the University of Virginia. This pool has been extensively safety tested and has been approved for experimental challenge studies by the FDA (IND# 15162).

The dose of RV used is in this study (300 - 500 $TCID_{50}$) is similar to that used in our previous challenges (16). This inoculum is likely to be higher than the dose of RV associated with a natural infection, although information regarding how much of an RV inoculum is sufficient to produce a cold is not known. The total dose of RV-16 used in this study is expected to result in a response in the lower airway that is sufficient to differentiate the asthmatic responses to RV-16 between the subjects who are treated with omalizumab prior to and during the infection and those who are treated with placebo, and at the same time will be safe in the study population of mild asthmatics proposed in this study. This is based on our previous investigations of 16 mild asthmatics and 9 controls challenged with a total dose of 100-150 TCID50 of rhinovirus 16 where we used a modification of the Jackson criteria to score daily upper respiratory tract symptoms and lower respiratory tract symptoms (16). Upper and lower respiratory scores were summarized for each individual by computing cumulative scores over study days 0 to 4 and over study days 0 to 21. The study showed that in both asthmatic patient groups (i.e., those with high IgE and those with low IgE), the lower respiratory tract symptoms scores were significantly increased ($P < .001$)

compared with control patients by day 21 (high IgE = 13.5 [6.2 to 29.3]; low IgE = 8.5 [3.2 to 22.3]; control patients = 0.7 [0.3 to 1.5]). Additionally, by day 4, the CLRTS scores for the asthmatic patients with high IgE were greater (mean [CI]: 4.2 [1.4 to 12.5]) than for the asthmatic patients with low IgE (1.0 [0.4 to 2.5, *P* = .05) and control patients (0.4 [0.2 to 1.2] *P* = .0003 (low IgE group versus control patients , *P* = .2) which is the rationale for enrolling asthmatic subjects with total IgE levels $\geq$ 125 IU/ml in our planned study with omalizumab. During the infection, all but 3 of the asthmatic subjects reported mild lower respiratory tract symptoms of cough, wheeze, shortness of breath, or chest discomfort and none of the asthmatic patients had symptoms requiring intervention with anti-inflammatory medications (i.e., inhaled or oral steroids).

Also on the basis of the modified Jackson criteria, the development of peak upper respiratory symptoms in asthmatic patients was in many cases delayed: for example, 8 of 9 control patients (89%) had peak symptoms during the first 4 days compared with 8 of 16 asthmatic patients (50%, *P* = .05) . Also the delayed development of peak symptoms observed for the asthmatic patients was more pronounced in the low IgE group: i.e., 4 of 6 (67%) with high IgE levels had peak symptoms during the first 4 days compared with 4 of 10 (40%) of those with low IgE levels. (See Figure 1 below) During the 21days of monitoring, asthmatic patients had cumulative symptom scores that were increased and prolonged compared with scores in control patients. The difference between scores for the high IgE group and control patients was significant (P < .02); the difference between scores for the low IgE group and control patients was not significant.

Figure 1. Upper respiratory tract symptom scores.*



* Based on the modified Jackson criteria (16) the symptoms recorded daily by asthmatic and controls included sore throat, sneezing, rhinorrhea, and nasal congestion. *Vertical line* represents standard error bars.

A previous study by other investigators has been conducted using doses of 1280-12800 TCID50 of rhinovirus 16 in 8 subjects with allergic asthma (39). In this study, subject-related symptom score was tabulation of 13 cold symptoms with highest possible score of 39 and the highest peak symptom score reported among study subjects was 15. Another study of experimental rhinovirus infection with used doses of 5,000 - 10,000 TCID50 of RV 16 on 2 subsequent days and this resulted in bronchial infection demonstrated by in situ hybridization for RV16 on bronchial biopsy samples. Chest scores were higher in asthmatics than in non-asthmatics, however chest scores did not seem to correlate with a strong in situ hybridization signal (40).

1.4 Rationale for Selection of Investigational Product(s)/Intervention(s) and Regimen

Population surveys have shown a positive correlation between increased levels of total serum IgE and bronchial hyper-reactivity and it is also clear that exacerbations of asthma are frequently triggered by viral respiratory tract infections, especially human RV. Moreover, studies have demonstrated an enhancement of bronchial hyper reactivity during and following colds in atopic individuals. In preliminary studies using the experimental human RV challenge model at UVA, 16 young adults with asthma and 9 non-allergic subjects without asthma were inoculated with human RV (strain 16). The results showed that the upper respiratory tract symptom scores for the asthmatics were significantly increased compared to controls. Six of the 16 asthmatics also had substantially elevated levels of total serum IgE (371-820 IU/mL) and these patients showed greater sensitivity to methacholine and had significantly higher levels of eNO throughout the study. High levels of IgE in the asthmatics were also associated with increased level of eosinophilic cationic protein (ECP) in nasal washes and total blood eosinophil counts, but lower levels of nasal sICAM-1, the epithelial cell receptor for human RV. Because the cell surface expression of ICAM-1 is stimulated by INF-gamma (together with IL-1 and TNF-alpha), the observation of lower levels of sICAM-1 in the high IgE asthmatics raises the hypothesis that increased allergic (Th2) eosinophilic inflammation prior to human RV inoculation may impair the development of INF-gamma production from Th1/Tc1 lymphocytes and hinder viral clearance. These data suggests that patients with asthma and high levels of IgE are more likely to have pre-existing inflammation of the airways, including augmented eosinophil response before virus inoculation and during the infection. The strain of RV that will be used is a pool of RV (strain 16) has been extensively safety tested and has been approved for experimental challenge studies by the FDA (IND# 15162).

Currently, omalizumab would not be a cost-effective medication for individuals with mild asthma. However, there is no reason to believe that mild asthmatics would not derive benefit from this medication, nor is there reason to suspect that they would have a greater risk for adverse events associated with omalizumab treatment. As noted above, we have already treated 16 subjects with mild asthma in a surveillance study for 3 months without any adverse events (IRB-HSR # 10408). This drug has also been shown to improve symptoms in individuals with allergic rhinitis (24-27).

Since June, 2003, omalizumab has been available to treat children and adults (over age 12 years) with moderate to severe asthma. Omalizumab is an expensive medication, but is cost-effective in those with moderate to severe disease. Although we will be using omalizumab in adults with mild asthma, there are several published studies demonstarting efficacy and safety in subjects with allergic rhinitis as noted above (24-27).

In this study, we will use experimental human RV challenges to provide a time-sequence analysis of the response to RV following viral inoculation in mild asthmatic subjects. We predict that treatment with omalizumab will lead to a significant decline in inflammatory biomarkers (section 7.6) prior to inoculation, and thus reduce the risk for an adverse response to RV during the infection. Knowing which biomarkers decline and which do not during the administration of omalizumab should provide new mechanistic insights.

This medication has been shown to decrease the incidence of asthma exacerbations in children and adults, who are atopic along with reductions in the use of oral and inhaled corticosteroids as well as exacerbations leading to hospitalizations and ER visits (3-10). As a result, there is significant cost saving benefits to asthmatics with moderate to

severe disease whose risks for hospital care is increased. In our study Omalizumab will be given to subjects with mild asthma. The FDA has only approved the use of Omalizumab in subjects with moderate to severe asthma. The FDA has previously reviewed this study (IND # 10510) and concluded (as of October 20, 2009) that the clinical trial may be initiated using a GMP produced pool of RV-39 that was available and used for our challenges at the time. More recently, RV-16 was produced under GMP conditions and the FDA has approved the use of RV-16 for experimental inoculations (IND# 15162) which should allow us to use both RV-16 and Omalizumab as proposed for this study after final review by the NIH and the FDA.

1.5 Clinical Experience

The safety and efficacy of omalizumab has been evaluated and the results reported in three randomized, double-blind, placebo-controlled, multicenter trials. (3-7).
The human RV type 16 was produced in human fetal lung fibroblasts -MRC-5 cells- under GMP conditions in collaboration with Johnson & Johnson, Inc. and has been used for experimental challenges and evaluated for safety and efficacy in clinical studies involving human volunteers during the fall (2011). Fifty-one non-asthmatic volunteers (mean age 25 years) were enrolled and inoculated with the new pool of RV-16 (29 with a 1000 $TCID_{50}$ dose and 22 with a smaller 100 $TCID_{50}$ dose to learn whether the larger dose would increase the infection rate and severity of symptoms. The primary purpose of this clinical trial was to assure that the new challenge pool was comparable to previous pools with regard to infection rate and symptom production. Forty-eight (94%) of the 51 evaluable subjects were infected, and 85% of the 48 infected volunteers developed a symptomatic illness. The results revealed no difference between the two doses used in infection rate or illness. The infection rate and total symptom scores were comparable to our previous experience with other rhinovirus challenge pools with the exception of a higher than expected rate of illness (i.e. development of cold symptoms)

of the volunteers developed in the infected volunteers using the new RV-16 pool. One subject developed a respiratory illness 17 days after RV-inoculation.. After her acute post-challenge cold symptoms had resolved, she developed a respiratory illness 17 days after RV-16 inoculation that was diagnosed as *bronchitis* by her primary care physician who treated her with azithromycin starting 24 days after RV inoculation. She was seen 29 days after inoculation by Dr. Turner. At that time her symptoms were resolving. No other significant virus related respiratory adverse events were reported among the subjects in this trial. Taken together, this new pool has been approved for experimental challenge studies by the FDA (IND# 15162; IND holder Dr. Turner) and has advantages for use in future challenge studies to assess pathogenesis or treatment and is the same strain used in our original published study of experimental rhinovirus challenges in asthmatic subjects (16), and has been the strain used by the majority of other investigators who have published results using the experimental RV challenge model to evaluate the response to rhinovirus in asthmatic subjects.

1.6 Risks

Omalizumab:
A recent review of the literature and the manufacturer's prescribing/product information report (PLI) focused on post-marketing reports leading to an analysis of the safety and the risk of immune-system effects, hypersensitivity reactions, malignant neoplasia, parasitic infections, serum sickness and thrombocytopenia (28,29). The outcome of this analysis, based on an estimated exposure of 57,300 patients, who received treatment between June, 2003 and December, 2006, confirmed a small risk for anaphylactic symptoms (0.2 %), but no deaths occurred (or have ever been reported) and there were no increased risks for other symptoms or reactions in this analysis. More recently (October 30, 2015) the manufacturers of omalizumab (Xolair®) reported that asthmatics with a history of anaphylaxis to foods, medications, or other causes had a

higher risk of anaphylaxis with omalizumab injections compared to those without a history of anaphylaxis. However, the overall risk is still 0.2% and lower (0.1%) in clinical studies. This information will be reviewed in the consent process and all subjects enrolled will be given an Epi-Pen, along with demonstrations how and when to use it. In keeping with AAAAI recommendations and standard practice in the University of Virginia allergy clinics, subjects will also be observed for 2 hours after the first 3 injections of omalizumab or placebo and for 30 minutes after receiving subsequent injections. Each subject will be receiving 3 or 6 injections of study drug during the investigation depending on body weight and baseline serum total IgE levels. A more recent, 5-year safety study found that a slightly higher rate of heart and brain blood vessel problems occurred in 5007 patients treated with Xolair® compared to 2829 patients not treated with Xolair® . These heart and brain blood vessel problems include chest pain, heart attack, blood clots in the lungs or legs, temporary symptoms of weakness on one side of the body, slurred speech, and change in vision. There were no increases in the rates of stroke or death from heart disease in the patients treated with Xolair® compared to patients not treated with Xolair®. It is not known whether the slightly higher rate of heart and brain blood vessel problems is caused by Xolair®. This study included adults with an average age of 45. There were more patients who had severe asthma in the group treated with Xolair® than there were in patients who were not treated with Xolair®. It is possible that other medications used to treat severe asthma raised stroke and heart disease risks and not the Xolair®, but there is no way to know that for sure.

Placebo drug (produced by the same manufacturer: Novartis Pharmaceuticals):
The product described in this module is the placebo to Xolair 150 mg Powder for solution for injection. The placebo is the same mixture of inactive excipients, in quality and quantity, as those used for the drug product, except that the drug substance, omalizumab, is not added.

Rhinovirus (strain 16)

Approximately 2000 volunteers have been challenged in studies conducted by Dr. Turner, Dr. Heymann, and their colleagues over the last 30 years and no serious complications attributable to the viral infection have been detected. The clinical syndrome associated with experimental infection is well-described. Challenge of volunteers with 100 $TCID_{50}$ produces infection in 90 to95% of susceptible volunteers. Symptoms first appear within 24 hours after inoculation and peak at 48-72 hours after challenge. The clinical syndrome is comparable to that reported in natural colds (35, 36) although in one study with identical definitions for duration the median duration of illness was 3.5 days in volunteers with experimental colds and 5.5 days in volunteers with natural colds (37). Approximately one-third of RV infections, whether natural or experimental, are asymptomatic. Virus shedding in infected volunteers follows a pattern similar to that of the symptoms. Virus concentrations in nasal lavage fluid, based on titers in culture, generally peak 2-3 days after challenge and then rapidly decrease. Virus can be recovered by culture in 20% of subjects on day 18 after challenge, but no virus shedding is detected by day 20 (38). To the best of our knowledge, human RV-16 has not been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness. In our studies, we have observed neutropenia in most of the 29 asthmatic subjects and also 16 controls following inoculation with RV-16 in our experimental challenges, which we believe is in keeping with the recruitment of neutrophils into the airways stimulated by RV. However, except for the possibility of bronchitis, which occurs infrequently in experimental infections, no subject has developed clinically significant bacterial infections in our studies, including bacterial sinusitis. Additionally, no subject in our studies with RV-16 has developed ANC's less than 1000 cells/mm3.

The results from experimental challenges in asthmatics with RV-16 and RV-39 at the University of Virginia indicate that RV challenges can be done safely in mild asthmatics who are not using anti-inflammatory medications. This has also been the experience of others using the RV challenge model elsewhere. In the first study of asthmatics enrolled in challenge studies at the University of Virginia using RV-39, one of 19 subjects needed intervention with inhaled steroids during peak cold symptoms (14). One college student enrolled in our studies needed a short course of oral steroids when challenged with RV-39 produced under GMP conditions. This student was one out of 23 asthmatic subjects who were inoculated with this pool in our experimental challenges. Additionally, 3 of the last 12 asthmatic subjects challenged with this pool of RV-39 more recently used albuterol more frequently for chest symptoms during peak symptoms, but they experienced no significant declines in their FEV1 values monitored at home or observed in our clinical research center. No subject enrolled in any of our studies who have been challenged with either RV-16 or 39, or in studies published by others using RV-16 that we are aware of, has experienced an exacerbation requiring hospitalization or treatment in the ER. In our previous published study with RV-16 prior to the virus being manufactured under GMP conditions, 13 of the 16 subjects with mild asthma reported mild symptoms of cough, wheeze, or chest discomfort during the infection, but none required intervention with anti-inflammatory medications such as inhaled or oral corticosteroids (16). We are interested to use this new pool because the majority of published data from experimental challenges with RV have been done using RV-16 which is helpful in comparing our results (e.g., 34, 39).

Recently, in a European study, challenges with RV-16 were well-tolerated by subjects with moderately severe asthma who were treated with inhaled corticosteroids (Adura P, Reed E, MacIntyre J, et al. Experimental rhinovirus-16 challenge in moderate asthma. 2010 ATS Conference abstract presentation (#5516))(41). However, all asthmatic subjects in that study were using inhaled steroids at the time of inoculation which would interfere with our goals of judging the effects of omalizumab on lower respiratory tract symptoms and bio-markers of inflammation.

All subjects in the proposed study will be inoculated with RV-16 and monitored daily for 4 days following inoculation in a hotel. Daily monitoring will include upper and lower respiratory

symptoms scores, and FEV1 measurements (using a hand held FEV1 monitor given to each subject) which will be recorded twice daily (morning and evening) on diary cards. Subjects will also be recording the number of puffs of albuterol used daily from metered dose inhalers with puff counters (which will also be checked daily by the study nurse coordinator). In addition, monitoring each morning will include a physical examination focused on the respiratory tract (performed and recorded by a physician), along with spirometry and measurements of expired nitric oxide (FeNO). This monitoring will be done to enhance our ability to detect changes during the acute infection and to ensure safety. Both the DoubleTree and Holiday Inn hotels in Charlottesville have had an on-going relationship with our research team. The hotels are located within 5 miles of the main medical campus. Each subject will have their own room and a member of our research team will stay in an adjacent room for 24 hours daily for 4 days during the entire hotel stay. The research team member will be a licensed physician or nurse and will be readily available should any problems occur. Thus far, no significant problems or serious adverse events have occurred for any of the asthmatics inoculated with either RV-16 or RV-39.

To date, there is no evidence from extensive clinical trials with omalizumab, or since its use beginning in 2003 after FDA approval, that there have been any serious complications that might suggest that omalizumab might potentiate the effects of RV. Although there have been no studies to specifically examine our stated hypothesis, we are confident that many of the patients who have received omalizumab for their asthma have experienced naturally-occurring RV infections without serious consequences. As for any adverse long-term safety effects of an experimental RV-16 infection, the cold symptoms observed have been generally similar to, but a bit shorter in duration than a natural RV infection (see above). Because most adults will experience at least one or two viral induced colds a year, it would be very difficult to differentiate any adverse effects of an RV-16 experimental infection from the effects of a subsequent natural respiratory tract infection in an effort to evaluate any safety problems long-term.

1.6.1 Risk of Study Procedures

- Inoculation: it is expected that challenge with GMP RV-16 will cause a common cold comparable to that reported in natural colds although of a shorter duration than natural colds

Nasal lavage: it may be associated with some discomfort due to < 10 second breath hold and the feeling of fluid in the nares. A rare potential complication is acute bacterial sinusitis (sinus infection)Light nasal scraping: After the nasal wash an additional nasal specimen will be obtained by using a ASI Rhino-Pro® nasal mucosal curette plastic device to do a light scrapping along the inferior turbinate of each nostril to collect epithelial cells. This will be done using a headlight and a disposable nasal speculum to spread the nares (Bionix) to enhance visibility of the turbinates. The nasal scrapings may be associated with discomfort during the procedure and sometimes sneezing. Minor problems with nose bleeding are infrequent because we are just scraping surface cells called epithelial cells. The nasal scrapings are being done to investigate how genes expressed by the lining cells in the nose (called epithelial cells) respond to changes in the environment, specifically in this study in response to exposure to the common cold virus (rhinovirus) or to environmental allergens. Importantly, the results from these scrapings will not be used to identify a participants genetic background or risk factors, and can't be used for genetic profiling.

- Nasal lining fluid: An additional nasal specimen will be obtained by applying a small piece (1 X 1 cm) of sterile gauze (Surgicel®), routinely used by ENT surgeons at UVA during surgical procedures to provide hemostasis, to inferior turbinate for 4

to 5 minutes in each nostril to collect a sample of epithelial cell lining fluid (about 0.2 ml).

- Monitoring nasal mucous weights and secretions during the 1st four days after virus inoculation: There are no risks involved.
- Injection: the use of sterile technique will make infection at the site where Omalizumab injections are given extremely unlikely, but infection at injection site is a rare potential AE.
- Blood draw: drawing blood causes transient discomfort and may cause fainting. Ecchymosis (bruising) at the blood draw site may occur but can be prevented or lessened by applying pressure to the draw site for several minutes. The use of sterile technique will make infection at the site where blood will be drawn extremely unlikely.
- Lung function tests, exhaled nitric oxide testing and methacholine challenge: The risk of lung function tests and eNO testing is the discomfort of exhaling forcefully. The inhalation of aerosols may be associated with mild shortness of breath, cough, chest tightness, wheezing, chest soreness or headache. Many patients do not have any symptoms at all. Symptoms (if they occur) are mild, last only a few minutes, and disappear following the inhalation of a bronchodilator medication, which will be administered as needed.
    - Impulse oscillometry: Mild light-headedness and coughing, shortness of breath, and/or chest tightness, occurs infrequently and is easily reversed with inhaled albuterol.
- Skin testing: it is expected to result primarily in discomfort, wheal and flare responses and pruritus. Approximately 2-3 in 10,000 skin prick tests results in allergic symptoms away from the site of the skin testing, such as sneezing, rhinorrhea, or rash. Very rarely, an individual who experiences this type of reaction may develop life-threatening symptoms such as anaphylaxis.

2. OBJECTIVES

2.1 Primary Objective(s)

To test the hypothesis that the reduction of total free IgE in asthmatics treated with omalizumab for 8 weeks prior to and during an experimental RV challenge will lead to a significant decline in lower respiratory tract (chest) symptoms [i.e., cumulative lower respiratory symptoms (CLRTS)] recorded by subjects during the first four days of infection following RV challenge compared to lower respiratory tract symptoms recorded during the same time period by asthmatics treated with placebo. The CLRTS scores will be recorded by subjects on diary cards twice daily throughout the study (see example of diary card in Appendix 1: Lower Respiratory Tract Symptoms). For the primary outcome, the evaluation of lower respiratory tract symptoms, which will be derived from the diary cards, will include shortness of breath, chest discomfort, and wheezing.

2.2 Secondary Objective(s)

Secondary endpoints will include a comparison of CLTRS scores in the asthmatic and non-asthmatic subjects over the first 4 days of acute infection, and will include symptoms of shortness of breath, chest discomfort, wheezing, and cough. Secondary endpoints will also include a comparison of changes from baseline in:

- methacholine sensitivity (PC20),
- expired nitric oxide (eNO),
- impulse oscillometry,
- the frequency of circulating RV and allergen specific regulatory and effector T-cells,
- concentrations of nasal wash mediators and cytokines,

- cumulative upper respiratory tract symptom (CURTS) scores. See example of diary card for subjects to record their CURTS scores twice daily throughout the study in Appendix 2).

The changes from baseline for these endpoints will be compared to the results for these assessments measured during peak cold symptoms over the first 4 day s of the infection among subjects enrolled in the omalizumab and placebo treated groups.
Diary cards will be scored daily for runny nose, sneezing, nasal congestion, sore throat, headache, chills/fever, fatigue, itchy eyes, watery eyes and red eyes using a modification of the Jackson criteria as previously described (16).

Using the same statistical methods described for the primary endpoint, we will also compare changes in the CURTS and CLTRS scores between the treatment groups from baseline over the first 7 and 21 days of the infection to detect differences stimulated by RV, and we will compare other the parameters monitored during the infection as listed above over these same time points. Additionally, using our measurements focused on daily symptoms and the same parameters monitored during clinic visits as listed above, we will examine differences between the omalizumab and placebo groups during the 8 weeks of drug administration before RV inoculation.

Currently, there is no validated scoring system for monitoring asthma symptoms on a daily basis as planned for our study. As a result, in parallel with our daily assessments of lower respiratory tract symptoms, we will also be administering the validated Asthma Control Questionnaire (ACT) [included Appendix #3] to enhance our evaluation of lower respiratory tract symptoms before and after the infection. These questionnaires only take minutes to fill out and will be given directly to the study nurse. By itself, the ACT questionnaire, which monitors symptoms over a one month

period (or other questionnaires which evaluate symptoms over a one week period), would not satisfy the goals of monitoring symptoms daily for this investigation and would not be sensitive to our evaluation over the first 4 days of the infection. The ACT questionnaire, as noted below in Section 7.2, will be completed by subjects in clinic:

- When subjects are enrolled 8 weeks before the RV challenge,
- At the clinic visit 4 weeks before the challenge, On the day of RV inoculation in the hotel
- One week after they complete the study (i.e. 28 days after RV inoculation at the final visit) when subjects will come to clinic to receive their payment for participation.

Another secondary endpoint will be rate of subjects whose FEV1 drops by more than 20% from their baseline value at the time of enrollment.

2.3 Exploratory Objective(s)

This study will also evaluate whether establishing an IgE blockade with omalizumab will have an effect on symptoms (i.e., CURTS and CLRTS scores), lung function, biomarkers of inflammation, and allergen and rhinovirus specific lymphocytes in the circulation, in order to improve our understanding for the mechanisms of rhinovirus induced flares of asthma. .

Thus, by establishing an IgE blockade, the administration of omalizumab may be a novel method for deciphering the mechanisms of RV induced changes in the lower airway and may help determine which Th2 associated pathways will be directly affected by the reduction in serum IgE. We also expect to see significant differences in the frequencies of CD4+ and CD8+ regulatory T-cell subsets present in the circulation of subjects from the omalizumab and placebo treated groups during the infection. Of interest is whether we will be able to detect differences in cytokines and chemokines

generated in direct response to RV (e.g. IL-8, IP-10, IL1beta, IL-12, INF-γ, and IFN-α2) between subjects enrolled in these two groups. Based on preliminary data generated in collaboration with Drs. Woodfolk, Borish, and Braciale, we expect to see significant differences in the frequencies of CD4+ and CD8+effector and regulatory T-cell subsets present in the circulation of subjects from the omalizumab and placebo treated groups during the infection.

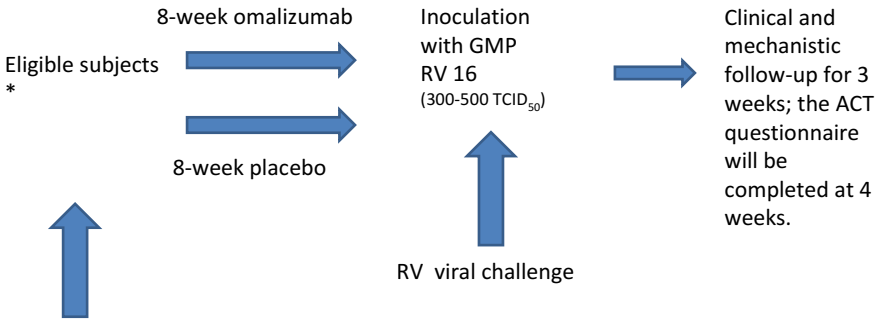
The nasal scrapings are being done to investigate how genes expressed by the lining cells in the nose (called epithelial cells) respond to changes in the environment, specifically in this study in response to exposure to the common cold virus (rhinovirus) or environmental allergens. The changes in gene expression will be monitored by RNA sequencing and epigenetic testing.

3. STUDY DESIGN

A total of 42 subjects will be enrolled in this randomized, double-blind, placebo-controlled study over a period of 33 months (February, 2013 through November, 2015). The 42 subjects will be randomized in a 1:1 ratio within each season to receive either Omalizumab or placebo for a total of 8 weeks. At the end of 8 weeks, participants will receive four inoculations over a 15 minute period of RV strain 16 in the evening when they enter the hotel and are then required to stay in a hotel over the next four days for close monitoring and observation. A final dose of the study drug (omalizumab or placebo) will be administered 2 weeks after inoculation. Subjects will be monitored daily for 21 days post RV 16 inoculation and for an additional week to complete the ACT questionnaire on day 28. Thus, each subject will also be seen for their final follow-up visit at 28 days for completion of the ACT questionnaire, a validated questionnaire which reviews their recall for symptoms over the previous month (i.e., since RV inoculation) and when they will also receive their payment for their participation.

Study Design

Investigator initiated, double-blind placebo controlled, 1:1 randomization omalizumab : placebo N=42, (21 subjects treated with omalizumab and 21 with placebo) enrolled over 33 months starting in the Spring of 2013



* Enrollment will begin either in the Fall (end of August), or the Spring (mid to end of February). Randomization of subjects will occur at enrollment.

Rhinovirus challenges will be conducted annually in the spring or the fall. To reduce the chance of seasonal imbalance (i.e., spring vs fall seasons) in the treatment assignments, we will generate two randomization lists. One list will be utilized for the spring rhinovirus challenges, while the remaining list will be utilized for the fall rhinovirus challenges. Each randomization list will be generated using a block randomization scheme in which the block sizes will all be an even number (e.g., 2,4,6,8), but variable in size and each block will have an equal number of assignments to the group treated with Omalizumab (Group 1) and group treated with placebo (Group 2). James Patrie, M.S. a Senior Biostatistician in the Department of Public Health Sciences at UVA will be providing the randomization. The block sizes used in generating the randomization will not be divulged to any members of the research team involved in the enrollment, monitoring, and evaluation of subjects. Amy Adams, Pharm.D., a coordinator of investigational drug studies at the University of Virginia, will be receiving the drug and placebo from Novartis, Inc. and will be supplying the drug to us in a blinded fashion according to the randomization plan.

3.1Study Endpoints

3.1.1 Primary Endpoint(s)

The primary endpoint will be based on the comparison of cumulative lower respiratory tract symptoms scores (CLRTS) recorded daily by the asthmatic subjects treated with omalizumab compared to the CLRTS scores among subjects treated with placebo over the first 4 days following RV inoculation. Lower respiratory tract symptoms to be included in this analysis are shortness of breath, chest discomfort and wheezing. These symptoms will be recorded twice daily by the asthmatic subjects as previously described (16). Each symptom will be scored on a severity scale of 0 to 3 (i.e. 0 = symptoms not present; 1 = mild, but clearly present; 2 = moderate and uncomfortable; and 3 = severe, interfering with sleep or activity as shown on diary cards included in Appendix 3. Thus, the maximum total score recorded by a subject on any one day for these three parameters is 18 (16).

3.1.2 Secondary Endpoint(s)

Secondary endpoints will include a comparison of CLTRS scores in the asthmatic and non-asthmatic subjects over the first 4 days of acute infection, and will include symptoms of shortness of breath, chest discomfort, wheezing, as well as cough. Secondary endpoints will also include a comparison of changes from baseline in:

- methacholine sensitivity (PC20),
- expired nitric oxide (eNO),
- impulse oscillometry,
- the frequency of circulating RV and allergen specific regulatory and effector T-cells,
- concentrations of nasal wash mediators and cytokines,

- cumulative upper and lower respiratory tract symptoms scores (CURTS and (CLRTS) detected during peak cold symptoms over the first 4 day s of the infection among subjects enrolled in the omalizumab and placebo treated groups.

The upper respiratory tract symptoms monitored will include the following symptoms recorded twice daily by each subject on their diary cards: : runny nose, sneezing, nasal congestion, sore throat, headache, chills/fever, fatigue, itchy eyes, watery eyes and red eyes using a modification of the Jackson criteria as previously described (16).

Additionally, using the same statistical methods described for the primary endpoint, we will compare changes in the symptom scores between the treatment groups from baseline over the first 7 and the 21 days of monitoring during the infection to detect differences stimulated by RV. We will also compare changes in biomarkers of inflammation and bronchial hyper-reactivity between the omalizumab and placebo treatment groups during the 8 weeks of drug administration before RV challenge to evaluate the influence of the study drug on these mechanistic parameters and symptoms in both treatment groups. To do this, we will be evaluating the levels of well accepted inflammatory biomarkers in subjects with asthma (i.e. expired nitric oxide [FeNO] and blood eosinophil counts), which have been shown to decrease during the administration of omalizumab (2,6). These markers of inflammation will be measured together with assessments of methacholine sensitivity (to judge bronchial hyper-reactivity) and impulse oscillometry (to judge airway resistance), so that we can evaluate the effects of study drug (omalizumab vs placebo) on the levels of inflammation and sensitive measures of lung function prior to and during the infection. For each subject, we will be measuring FeNO levels (ppb) and absolute blood eosinophil counts (cells/mm3) at each clinic visit and daily during the hotel stay as well as PC20 (mg/ml) for methacholine, impulse oscillometry, and lymphocyte analyses at 4 time points, beginning at enrollment (8 weeks before the RV challenge), on the day of

inoculation (day 0), during peak cold symptoms (day 4), and at (day 21). The statistical plan, focused on comparing changes in these parameters from baseline between the treatment groups at each time point is described in Section 9.

The Asthma Control Questionnaire (ACT) [included Appendix #3] will be administered and evaluated to enhance our assessment of lower respiratory tract symptoms before and after the infection. The ACT questionnaire, as noted below in Section 7.2, will be completed by subjects in clinic:

- When subjects are enrolled 8 weeks before the RV challenge,
- At the clinic visit 4 weeks before the challenge,
- On the day of RV inoculation in the hotel
- One week after they complete the study (i.e. 28 days after RV inoculation at visit the final visit) when subjects will come to clinic to receive their payment for participation.

In addition, another secondary endpoint will be rate of subjects whose FEV1 drops by more than 20% from their baseline value at the time of enrollment.

3.2 Study Completion

This study will be considered "completed" when all participants have completed the study and data required to meet the chosen objectives are analyzed.

After the study is completed, the Principal Investigator or Data Center will compile a final study report as per ICH E6 and 21CFR312. The study report will be submitted to the local IRB, NIAID, SMC and the FDA.

4 SELECTION AND WITHDRAWAL OF PARTICIPANTS

4.1 Inclusion Criteria

Subjects who meet all of the following criteria at screening will be eligible for enrollment as a study participant. Subjects must also be able to understand and provide written informed consent

- Age 18 to ≤ 40 years of age, any gender, any racial/ethnic origin
- Physician-diagnosed asthma
- ACT score > 19 at enrollment before the administration of study drug
- Short-acting beta-agonist use < daily in last 4 weeks
- FEV1 > 70%, or FEV1/FVC ratio > 75% for subjects with FVC values between 80 and 87% predicted whose FEV1 values fall below 70%.
- Positive Methacholine challenge test (i.e. at least 20% fall in FEV_1 at a Methacholine concentration of ≤ 16 mg/ml) at screening protocol before enrollment (20).
- Total serum IgE level ≥ 125 IU/ml documented during screening protocol.
- Positive test for allergen specific IgE antibody by prick skin testing documented during screening protocol to allergens associated with current allergen exposure at the time of the RV challenge (e.g., dust mite, *Alternaria*, and/or ragweed for subjects challenged with RV in the fall, or positive tests to tree and/or grass allergens for those challenged with RV in the spring). In keeping with the study design goals of inoculating subjects during periods of allergen exposure, sensitization to other allergens (e.g., cat or dog) will also qualify for enrollment if subjects are currently exposed to these allergens at home.
- Participant must be willing to comply with study procedures and requirements

- Participant must be considered eligible for participation based on results of screening procedures conducted by protocol number 20100686 at VCU and IRB# 12656 at UVA). Refer to Appendix 4.

4.2 Exclusion Criteria

Subjects who meet any of these criteria are not eligible for enrollment:

- Inability or unwillingness of a participant *or subject's legal representative* to give written informed consent *and HIPPA authorization*
- Positive test for serum neutralizing antibody to RV-16. Subjects with a neutralizing antibody titer $\geq$ 1:4 will be excluded at screening within 6 weeks of enrollment.
- To avoid RV-16 inoculations in subjects with more restrictive lung volumes, those whose FVC is < 80% predictive will also be excluded.
- Total IgE levels measured at screening protocol that are too elevated based on a subjects weight, to meet the recommendations proposed by Novartis for treatment with Omalizumab (21).
- Chronic heart disease, lung diseases other than asthma, or other chronic illnesses including primary and/or secondary immunodeficiency.
- Hospitalization or treatment in the ER (unless the treatment involved the use of a bronchodilator only) for asthma during the last three years.
- Subjects who have had one or more night time awakenings caused by asthma symptoms and/or who have needed their SABA (albuterol) inhaler for asthma symptoms $\geq$ 4 days during the week before enrollment, or during the week before the inoculation with RV-16.
- Intubation or management in the intensive care unit for an asthma exacerbation

- An upper or lower respiratory tract infection within six weeks prior to enrollment
- Previous nasal or sinus surgery within the last 12 months.
- Who have a 5 pack/year history of smoking, or any smoking within the last 6 months.
- Female subjects who are or who plan to become pregnant during the study, or who are nursing a baby. Additionally, to be included in this study, a woman of child-bearing potential must have a negative urine pregnancy test at screening, during the run, and prior to viral inoculation and agree to use an effective method of birth control such as, but not limited to, birth control pills, contraceptive foam, diaphragm, IUD, abstinence, or condoms during the entire duration of the study (through Day 28 after RV 16 inoculation).
- Subjects who have used omalizumab within 6 months prior to enrollment,or have been using inhaled corticosteroids, inhaled cromolyn or nedocromil, a long acting beta agonist, inhaled ipratropium bromide, or systemic leukotriene modifiers for their asthma on a daily basis within 4 weeks prior to enrollment, or subjects using nasal corticosteroids on a daily basis within 4 weeks prior to enrollment.
- Subjects who are currently receiving beta-adrenergic blocking agents.
- Subjects who are currently receiving allergen immunotherapy (IT), or who have received allergen IT within the last 3 years..
- Hemoglobin <11.5 g/dL for non-African American subjects or hemoglobin < 11.0 g/dL for African American subjects detected during screening within 6 weeks of enrollment.
- Absolute neutrophil count (ANC) < 1500 cells/mm3 (or 1.5 K/uL) detected during screening within 6 weeks of enrollment or Day -2.

4.3 Participant Withdrawal Criteria

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

a. The participant elects to withdraw consent from all future study activities, including follow-up.

b. 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).

c. The participant dies.

d. The participant develops a medical condition or is started on new medication(s) 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.

e. The participant meets any of the individual stopping rules as delineated in section 8.

f. A severe asthma exacerbation that requires treatment in the emergency room, hospitalization, or the use of systemic steroids, at any time during the study.

g. Severe anaphylaxis secondary to Omalizumab dosing

h. Poor control or persistent activation of secondary atopic disease.

i. Non-adherence to dosing protocol and or study procedures.

j. Pregnancy

k. If a subject misses an Omalizumab dose, the dosing schedule will be resumed as soon as possible. If a dose is missed > _1__ time the subject will be discontinued from dosing due to significant non-adherence. At each visit when Omalizumab is administered, subjects will be reminded about the importance of receiving all injections of this medication.

5 INVESTIGATIONAL PRODUCT(S)/INTERVENTION MATERIAL(S), OTHER STUDY PRODUCTS (CONTROLS/PLACEBOS)

5.1 Investigational Product(s)/Intervention(s)

Omalizumab (Xolair®)

The study drug omalizumab and placebo will be provided at no cost by Novartis Pharmaceuticals.

Placebo drug (produced by the same manufacturer: Novartis Pharmaceuticals):

The product described in this module is the placebo to Xolair 150 mg Powder for solution for injection. The placebo is the same mixture of inactive excipients, in quality and quantity, as those used for the drug product, except that the drug substance, omalizumab, is not added.

Rhinovirus (strain 16)

The strain of RV that will be used is a pool of RV (strain 16) that will be provided by Dr. Ronald Turner's laboratory at the University of Virginia (UVA). Dr. Turner, who is a co-investigator in this study, has collaborated with Johnson & Johnson, Inc. to produce this pool of RV under GMP conditions. This new pool was deemed by the FDA to be safe to use for the research experimental challenges to be conducted in this study.
Please refer to sections 1.3 and 5.2 for more complete information on RV 16.

5.2 Preparation, Administration, and Dosage

Before the experimental challenge with RV (strain 16), each subject will be treated for 8 weeks with subcutaneous injections of either omalizumab (**Group 1**) or placebo (**Group 2**) prior to the experimental RV challenge. The 42 asthmatic subjects enrolled in this

study will be randomized 1:1. All subjects and study team members will be blinded as to which treatment group the subjects are randomized to.

Omalizumab (Xolair®)

Injections of either omalizumab -150 to 375 mg- or placebo will be given monthly, or bi-monthly, depending on the subject's weight and total IgE level using the prescribing guidelines provided by the manufacturer (27).

The dose (mg) and dosing frequency will be determined by serum total IgE level (IU/mL), measured before the start of treatment, and body weight (kg). See the dose determination charts below (Table 1 and Table 2) for appropriate dose assignment. Doses less than or equal to 300 mg will be given every 4 weeks.

Table 1

Administration Every 4 Weeks

Xolair Doses (milligrams) Administered by Subcutaneous Injection Every 4 Weeks for Adults and Adolescents 12 Years of Age and Older

Pre-treatment Serum IgE (IU/mL)	Body Weight (kg)			
	30–60	> 60–70	> 70–90	> 90–150
≥ 30–100	150	150	150	300
> 100–200	300	300	300	
> 200–300	300			
> 300–400	**SEE TABLE 2**			
> 400–500				
> 500–600				

Table 2

Administration Every 2 Weeks

Xolair Doses (milligrams) Administered by Subcutaneous Injection Every 2 Weeks for Adults and Adolescents 12 Years of Age and Older

<table>
<tr><th rowspan="2">Pre-treatment Serum IgE (IU/mL)</th><th colspan="4">Body Weight (kg)</th></tr>
<tr><th>30–60</th><th>> 60–70</th><th>> 70–90</th><th>> 90–150</th></tr>
<tr><td>≥ 30–100</td><td colspan="4" rowspan="2">SEE TABLE 1</td></tr>
<tr><td>> 100–200</td><td>225</td></tr>
<tr><td>> 200–300</td><td></td><td>225</td><td>225</td><td>300</td></tr>
<tr><td>> 300–400</td><td>225</td><td>225</td><td>300</td><td rowspan="4"></td></tr>
<tr><td>> 400–500</td><td>300</td><td>300</td><td>375</td></tr>
<tr><td>> 500–600</td><td>300</td><td>375</td><td colspan="2">DO NOT DOSE</td></tr>
<tr><td>> 600–700</td><td>375</td><td colspan="3"></td></tr>
</table>

Doses will be adjusted for significant changes in body weight during the study (See Table 1 and Table 2).

Preparation and administration of Omalizumab will be conducted according to manufacturer. Each vial of omalizumab from the manufacturer contains 150 mg of drug and will be reconstituted with 1.4 ml of sterile water for injection. The injection may take 5-10 seconds to administer because the solution is slightly viscous. Each vial delivers 1.2 mL (150 mg) of Omalizumab. We will not administer more than 150 mg per injection site. We will divide doses of more than150 mg among two or more injection sites. (Table 3).

Table 3
Number of Injections and Total Injection Volumes

Xolair Dose (mg)	Number of Injections	Total Volume Injected (mL)
150	1	1.2
225	2	1.8
300	2	2.4
375	3	3.0

Inoculation with RV-16 Procedures:

A nasal wash will be performed prior to inoculation in order to obtain baseline assessments including tests for RV, cell counts, and inflammatory biomarkers.For virus inoculation, each patient will be inoculated with 0.25 ml of RV type 16 in each nostril after obtaining the pre-virus inoculation nasal wash. The RV-16 virus solution used for inoculation will be diluted in HANK's balanced salt solution and will be pre-tittered to contain 300-500 $TCID_{50}$/ml. (tissue culture infectious dose 50/ml.). Thus, 75 – 125 $TCID_{50}$/0.25 ml will be instilled into each nostril during the initial inoculation. Five to 10 minutes after the initial inoculation, another inoculum (using the same amount of RV) will be administered.

Non investigational drugs that will be available at the clinical site include: EpiPen, Methacholine and Albuterol. EpiPen, oral steroids, and albuterol will also be available with the physicians and study nurses in the hotel over the entire 4 day period after RV-16 inoculation.

5.3 Accountability of Investigational Product(s)/Intervention(s)

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

Records for receipt, storage, use, and disposition will be maintained by the study site. A dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of investigational product(s)/intervention material(s) dispensed.

All records regarding the disposition of the investigational product(s)/intervention material(s) will be available for inspection by the site monitor, and NIAID. At the end of the study, drug accountability will be done and all unused drug will be returned to Novartis.

5.4 Modification or Discontinuation of Investigational Product(s)/Intervention Material(s)

5.4.1 Modification of Investigational Product(s)/Intervention(s)

N/A

5.4.2 Premature Discontinuation of Investigational Product(s)/Intervention(s)

Study stopping rules and individual stopping rules are described in sections 8.1.1 and 8.1.2, respectively. Participant withdrawal criteria are described in section 4.3
If a subject withdraws from the study at anytime during the 8 week pre-treatment period, they will come to clinic within one week of their withdrawal and turn in their diary cards. They will also be asked about any adverse events and will be paid according to a pro-rated scale which is described in the consent form. If the subject withdraws following virus inoculation, the same plan for follow-up will apply and subjects will be asked to return on day 28 to receive their payment, complete the ACT questionnaire, and a blood sample will be obtained (5ml) to check for serum neutralizing antibody (i.e. sero-conversion) to RV-16.

6 OTHER MEDICATIONS

6.1 Concomitant Medications

N/A

6.2 Prophylactic Medications

N/A

6.3 Rescue Medications

Following the injection, subjects will stay in the clinic for 2 hours after receiving the first 3 injections and for 30 minutes after subsequent injections An EpiPen unit will be given to each subject and they will be instructed how to treat any allergic symptoms observed after the Omalizumab or the placebo is given. Other rescue medications available at the study site and the hotel (for use after administration of omalizumab and rhinovirus challenge, respectively) are described in section 5.2.

6.4 Prohibited Medications

Include inhaled corticosteroids, leukotriene modifiers, inhaled cromolyn sodium, inhaled nedocromil sodium, long acting beta agonists, and ipratropium bromide. Nasal corticosteroids are also part of the exclusion criteria because using these medications can confound the interpretation of the effects of omalizumab on asthmatic symptoms in atopic subjects. Use of antihistamines is discouraged at all times during the study and prohibited on the day of and for the 4 days following inoculation with RV-16.Beta-adrenergic blocker agents are also prohibited medications.

7 STUDY VISITS AND PROCEDURES

7.1 Enrollment and Randomization

This research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any screening study procedures. Participants who are deemed eligible for the study will be enrolled and assigned a unique participant number.

Subjects will be contacted by telephone or e-mail if they have met the inclusion and exclusion criteria for this study determined after they have successfully completed the screening visits described in the screening study protocol (UVA IRB# 12656) and briefly described below.

It is anticipated that study participants will be enrolled from UVA, Virginia Commonwealth University, James Madison University, Liberty University, University of Richmond, Mary Baldwin College, Washington and Lee, and other universities located within 90 minutes by car from the UVA campus. Participants will be recruited through the use of posters/flyers, newspaper ads, internet and referrals from other health care professionals. No participants will be selected from the laboratories of Dr Heymann,Dr Turner, or other key investigators.

7.2 Screening and study Visit(s)

All visits will take place at the UVA Asthma and Allergic Disease Center in Charlottesville. The hotel stay post RV-16 challenge will take place at either the Holiday Inn (1901 Emmet Street North) or the DoubleTree Hotel (900 Hilton Heights Road) in Charlottesville, VA 22901. Procedure done during the visits will take up to 90 minutes. Additionally, during the visits when the injections of omalizumab or placebo

are given, subjects will be observed for 2 hours after the first 3 injections and for 30 minutes after subsequent injections to provide treatment in the event of an episode (uncommon) of anaphylaxis.

Subjects meeting entry criteria include those selected from a previous screening study summarized in Appendix 4. Unless otherwise stated, physiological values obtained from the screening protocol are valid for eight months.
Subjects who are eligible and who decide to participate will undergo the following Study Visits (See Appendix 5: Schedule of Events for an outline of the study schedule):

Study Visit 1 (- 8 Weeks); Note: To establish baseline values at enrollment, participants will be seen in the morning (between 7 AM and noon) at Elson Student Health Center, or at the Clincal Research Unit (CRU) located on the 1st floor of the Medical Center Barringer Wing at UVA, or at the Clinical Research Center (CRC) located on the 3rd floor of the new UVA Children's Hospital Outpatient Building (Battle Building). All locations are approximately 10 minutes walking distance from our laboratory and the Asthma and Allergic Diseases Center[Note: All 3 locations will be used based on availability and convenience for our subjects and our staff who will be doing all of the assessments.] The following assessments will be completed at the enrollment visit (Visit 1):

Potential subjects will sign the consent form at this visit prior to any additional study procedures. Subjects will then be randomized. The following procedures will take place at this visit:

- Urine Pregnancy Test for all women of childbearing potential.
- Nasal Wash
- Nasal scraping using the ASI Rhino-Pro®

- Spirometry
- Exhaled Nitric Oxide (eNO)
- Methacholine challenge
- Impulse oscillometry
- 8 mls of blood will be collected for differential cell counts (CBC with differential), assessments of total and allergy specific IgE antibody, and for serum antibody titers (serology) to RV- 16.
- 155 mls of blood (~ 5 ounces) will be collected (during the same venipuncture done to collect the 8 ml for blood counts) to interrogate the status of RV and allergen specific T cells in response to the infection in both groups and for basophil and dendritic cell experiments.
- Subjects will be instructed on the use of the diary cards which will be used to record upper and lower respiratory tract symptom scores twice daily, as well as how to use the ATS approved Microlife Electronic PEF and FEV1 monitor and the number of puffs of albuterol required for resolution of symptoms.
- Subjects will be instructed on the use of the EpiPen and given an EpiPen to use in case of emergency. Subjects will also be given an albuterol inhaler (MDI) with a puff counter to use as a rescue medicine as needed. Subjects will use this inhaler instead of their own albuterol MDI.
- Baseline assessment of past medical history over the last month to use in our assessment of adverse events.
- A limited physical examination will be performed including weight (Kg)
- Subjects will be randomized
- Subjects will receive their first dose of study drug (omalizumab vs. placebo). Please refer to section 5.2 (Preparation, Administration and Dosage)

- Subjects will fill out the ACT questionnaire.

Omalizumab and placebo will be provided for this research by Novartis Pharmaceuticals. After enrollment, 4 follow-up visits every other week (i.e., 6, 4, and 2 weeks and just before the RV challenge) will be scheduled to monitor the effects of the study drug on allergic inflammation lung function.

Between Visit 1 and 2:

- Subjects will complete the diary card information on a twice daily basis.

Study Visit 2 (- 6 Weeks)

- At - 6 weeks, subjects receiving study drug injections every 2 weeks will come into the clinic for an injection of study drug (omalizumab vs. placebo) and a urine Pregnancy Test for all women of childbearing potential.
- Assessment for Adverse Events and review of diary cards if subject is scheduled for study drug injection.

Study Visit 3 (- 4 weeks)

The following study procedures will be performed:

- Urine Pregnancy Test for all women of childbearing potential
- Nasal Wash
- Spirometry
- Exhaled Nitric Oxide (eNO)
- 8 mls of blood will be collected for differential cell counts (CBC with differential).
- Limited physical examination
- Subjects will receive a dose of study drug (omalizumab vs. placebo).

- Review of the daily diary cards and assessment of Adverse Events
- Subjects will fill out the ACT questionnaire.

Between Visit 3 and 4:

- Subjects will complete the diary card information on a twice daily basis.

Study Visit 4 (- 2 weeks)

The following study procedures will be performed:

- Urine Pregnancy Test for all women of childbearing potential
- Nasal Wash
- Nasal scraping using the ASI Rhino-Pro®
- Spirometry
- Exhaled Nitric Oxide (eNO)
- 8 mls of blood will be collected for differential cell counts (CBC with differential).
- Limited physical exam
- Subjects who will be receiving their study drug injections every 2 weeks will receive a dose of study drug (omalizumab vs. placebo)
- Review of the daily diary cards and assessment for adverse events

Between Visit 4 and Day of Admission to the hotel for evaluation and RV-16 inoculation (Day 0)

- Subjects will complete the diary card information on a twice daily basis.

Study Visit 5 (within 2 days before virus inoculation; i.e., either 2 days or 1 day before, or on the day of admission to the hotel and virus inoculation):

To establish baseline values just prior to inoculation with RV-16, subjects will be seen in the morning (between 7 AM and noon) at the Elson Student Health Respiratory

Research Unit, the Battle Building CRC, or the CRU at UVA for the following assessments:

- Urine LTE_4 for all subjects. [Note: LTE4 is a cysteinyl leukotriene which can be measured in urine. This biomarker of inflammation will be checked at this visit and during peak cold symptoms (day #4 after virus inoculation).]
- Nasal Wash
- Nasal lining fluid
- Spirometry
- Exhaled Nitric Oxide (eNO)
- Urine pregnancy test
- Methacholine challenge
- Impulse oscillometry
- 8 mls of blood will be collected for differential cell counts (CBC with differential) and for serum neutralizing antibody titers to RV- 16.
- 155 mls of blood will be collected for T-cell, basophil, and dendritic cell studies.
- Limited physical exam
- Subjects will receive a dose of study drug (omalizumab vs. placebo).
- Review of the daily diary cards and assessment for Adverse Events.

Admission to the hotel: (Day 0) Inoculation Day

On Day 0 (after 8 weeks of study drug treatment) subjects will check into a local hotel (Holiday Inn Hotel in Charlottesville). They will stay in this hotel for the next 4 days and will not be allowed to leave their rooms. Each subject will have their own room that they cannot leave until the day of discharge (Day 4). A member of the study team

(physician or nurse) will be staying in an adjacent room (day and night) throughout the hotel stay. Each subject will be admitted to the hotel in the evening (between 5 and 7 PM) of Day 0 and the following procedures will be done:

- Urine Pregnancy Test for all women of childbearing potential
- Collect and review completed diary card and issue new diary cards
- Limited physical exam and temperature (PO)
- Complete the Asthma Control Test
- Expired nitric oxide
- Nasal wash
- Spirometry
- Inoculation of subjects with RV-16
- Each subject will be given boxes of tissues and ZIP lock freezer bags to put all used tissues into after nose blowing to monitor mucous/secretion weights during the hotel stay.

Hotel Stay (Days 1-4 in the morning before noon)

On hotel stay days 1 – 4 the following procedures will be performed:

- Nasal Wash
- Nasal scraping using the ASI Rhino-Pro® on day 2 only.
- Nasal lining fluid on day 4 only.
- Spirometry [Note: The spirometry scheduled on Day 4 may be done in the hotel, or later in the morning in the Battle Building CRC before the methacholine test.]
- Exhaled Nitric Oxide (eNO)
- 8 mls of blood will be collected for complete blood count and differential cell counts (CBC with differential) on days 2 and 4 only in the morning.
- Evaluation by research staff every morning with limited physical exam and temperature (PO)
- Review of the daily diary cards and assessment for adverse events

- Prior to discharge from the hotel in the morning and additional 110 ml of blood will be obtained (during the same venipuncture done to collect the 8 ml for blood counts) for the assessments of circulating T-cells, basophils, and dendritic cells. Another urine sample will be checked in the morning on day 4 from all subjects for LTE4 measurements.

- Shortly after leaving the hotel (within 24 hours), subjects will return to the Elson Student Health Respiratory Research Unit, the Battle Building CRC, or CRUat UVA for a urine pregnancy test, methacholine test and impulse oscillometry to detect changes during peak cold symptoms.

Between discharge from the hotel and Visit 6 (Day +7, i.e., one week after inoculation):

- Subjects will continue to complete the diary card on a twice daily basis.

Study Visit 6 (Follow-Up Visit on Post-Inoculation Day 7)

On post-inoculation days 7 the following procedures will be performed:

- Nasal Wash
- Nasal scraping using the ASI Rhino-Pro®
- Spirometry
- Exhaled Nitric Oxide (eNO)
- 8 mls of blood will be collected for differential cell counts (CBC with differential) and an additional 60 ml of blood will be obtained for T-cell and dentritic cell studies.
- Impulse oscillometry
- Evaluation by research staff with limited physical exam.
- Review of the daily diary cards and assessment for adverse events

Between visit 6 and 7:

- Subjects will complete the diary card information on a twice daily basis.

Study Visit 7 (Follow Up Visit on Post-Inoculation Day 14)

- Nasal Wash
- Nasal lining fluid obtained before the nasal wash
- Spirometry
- Exhaled Nitric Oxide (eNO)
- 8 mls of blood will be collected for differential cell counts (CBC with differential)
- Evaluation by research staff with limited physical exam
- Subjects will receive a dose of study drug (omalizumab vs. placebo) if receiving study drug every 2 weeks
- Review of the daily diary cards and assessment for adverse events

Between Visit 7and 8:

- Subjects will complete the diary card information on a twice daily basis.

Study Visit 8: Evaluation Visit (Post-Inoculation Day 21)

- Nasal Wash
- Nasal scraping using the ASI Rhino-Pro® done after the nasal wash
- Spirometry
- Exhaled Nitric Oxide (eNO)
- Methacholine challenge
- Impulse oscillometry
- Evaluation by research staff with limited physical exam
- Urine for pregnancy screen for women of childbearing potential

- 8 mls of blood will be collected for differential cell counts (CBC with differential) and serum neutralizing antibody (sero-conversion) to RV-16. During the same venipuncture, an additional 110 ml of blood will be obtained for T-cell, basophil, and dendritic cell studies.
- Review of the daily diary cards and assessment for adverse events

Study Visit 9: Twenty-eight days after RV inoculation, subjects will return to receive their payment (check) for completing the study and to complete the ACT questionnaire and to report any adverse events.

Note: The total blood volume obtained during the study (i.e., 342 ml during the 8 weeks before RV inoculation and 336 ml during the infection over 3 weeks) is 678 ml over a period of 11 weeks and falls within the limits approved by our IRB.

7.3 Visit Windows

Study visits should take place within the time limits below:
Visits 1 (8 weeks before the virus challenge), 6 (7 days after the virus challenge), 8 (21 days after the virus challenge), and 9 (28 days after the virus challenge) will occur within a 24 hours window prior to or 48 hours following the planned visit scheduled according to the protocol. As noted above, Visit 5 can be scheduled 2 days before, 1 day before, or on the day of the virus inoculation. To accommodate subjects with difficult school or work schedules, Visits 2 (6 weeks before the challenge), 3 (4 weeks before the virus challenge), 4 (2 weeks before the virus challenge), and 7 (14 days after the virus challenge) will also occur within a 24 hour window prior to or 48 hours after the planned visit scheduled according to the protocol.

7.4 Study Procedures

Specifications and Guidelines

1. **Pregnancy Tests:** Fifteen to 30 mls of urine will be collected from all women of childbearing potential at -8, weeks, -4 weeks, -2weeks, Day 0 and 21. As in our previous studies (e.g. IRB-HSR #'s 12673) we will be doing these tests at study visits using the Answer, Early Result Pregnancy Test; Scantibodies Laboratory, Inc. Santee, CA).
2. **Hotel Stay:** Post inoculation with RV-16 all subjects will stay in a local hotel. (Holiday Inn Hotel on Rt. 29 North in Charlottesville Virginia) where several studies of RV challenges involving asthmatic and non-asthmatic subjects have already been done by UVA investigators (Drs. Heymann and Turner). Subjects will not be allowed to leave their rooms and are instructed to call for room service. Each subject will be evaluated and samples will be collected on the day of virus inoculation and daily over the first four days during peak cold symptoms. Physicians and nurses from the University of Virginia Asthma and Allergic Diseases Center will be available in the hotel for this study 24 hours a day.
3. **Inoculation with RV-16 Procedures:** Virus inoculation will be done after performing a nasal wash as described in procedure #10 as described below. Each patient will be initially inoculated with 0.25 ml of RV type 16 in each nostril after obtaining the pre-virus inoculation nose wash. The RV-16 virus solution used for inoculation will be diluted in HANK's balanced salt solution and will be pre-tittered to contain 300-500 $TCID_{50}$/ml. (tissue culture infectious dose 50/ml.).
    - Five to 10 minutes after the initial inoculation, another inoculum of 0.25 ml. of RV type 16 in each nostril will be administered.
4. **Diary Cards:** Each subject will be responsible for the completion of a daily diary card. Most subjects now have computers and our diary cards have been

established on-line to enhance capturing data that is being submitted and checked for compliance in real-time by our laboratory. However, subjects will also have the option to use hard copies of the diary cards which have been used successfully in the past and which are reviewed in detail for completeness by our study coordinator at each study visit. At this review, the subject will turn in their diary card with their data entries since their last study visit and a new diary will be issued. Subjects will be instructed to complete these diary cards twice daily in the morning and evening . Diary cards will be used by each subject to record their upper and lower respiratory tract symptom scores beginning at Visit 1 and ending at Visit 8. PEF and FEV_1 values and the puffs of albuterol required for chest symptoms. Upper respiratory tract symptoms recorded will include sneezing, nasal discharge, nasal obstruction, sore throat, headache, malaise, or chills, using a modification of the Jackson criteria as previously described (16). Each symptom will be scored on a severity scale of 0 to 3 (i.e. 0 = symptoms not present; 1 = mild, but clearly present; 2 = moderate and uncomfortable; and 3 = severe, interfering with sleep or activity (see Appendix 2). The lower respiratory symptoms monitored will include cough, chest tightness, wheeze, and shortness of breath. Again each symptom will be recorded using the same severity scale (see Appendix 1 at the end of this protocol). Each subject will be given an albuterol metered dose inhaler containing a puff counter (Ventolin, GlaxoSmithKline) which will be used when needed during the study. The puffs of albuterol used by each subject during the study will also be recorded twice daily on the diary cards. The use of albuterol used to reverse the response to methacholine during the study, or used prophylactically prior to exercise will not be included in the analysis of albuterol puffs used in response to asthma symptoms encountered by the subject during the study. The tests for PEF and FEV_1 will be done using a Microlife hand held monitor (www.microlifeusa.com).

The Microlife monitor is ATS approved for evaluating peak expiratory flows (PEF) and FEV1 assessments at home. Daily recordings (before breakfast and dinner) will be established at baseline, during treatment with omalizumab or placebo, and during the experimental infection with RV. The lung function data will be downloaded at every clinic visit (i.e. whenever omalizumab or placebo is administered and at follow-up visits during the infection) and will be reviewed by the study nurses who will check that proper monitoring and record keeping is being done.

During the hotel stay, subjects will continue to monitor and record their FEV1 values along with their symptom scores. If any subject experiences a decline in FEV1 below 70% predicted, or more than 20% from their baseline value at the time of enrollment, they will notify a member of the study team (licensed physician or nurse who will be staying in an adjacent hotel room day and night during the hotel stay) immediately. Epinephrine, albuterol, and oral steroids will also be readily available to the member of the study team (in their hotel room) to use for treatment.

5. **Spirometry**:

   Spirometry (i.e. basic lung function tests done routinely in clinic to judge lung volumes during a maximal expiratory effort for 12 seconds) will be done at each visit and in the morning of each day of the hotel stay using methods and equipment (e.g. Koko Spirometer , Inspire Health Care, Louisville, CO) approved and standardized by the American Thoracic Society (ATS). Subjects will wear a nose clip, and be asked to take a deep breath, and then blow it out for as long as possible into the Spirometer.

6. **Methacholine challenge:**

   Methacholine challenges will be done according to ATS guidelines (20). During this test, subjects will receive an FDA-approved drug called Provocholine. This

drug will be inhaled and cause mild symptoms of asthma to occur. Subjects will be asked to breathe in different mists of methacholine that are each slightly stronger. Spirometry testing will follow each dosing strength. The test will stop when lung function decreases by 20%, or after the 10th mist is given. The test will also be stopped if the subject has difficulty breathing. After the test is stopped, the subject will be given a treatment of albuterol to open up the airways. Each subject will already have done a methacholine challenge as part of the screening process in order to be eligible for this study. The methacholine challenges in this study will be done at Visit 1 (-8 weeks), at Visit 5 (within 2 days prior to RV-16 inoculation), on Day 4 of the hotel stay, and at Visit 8 (Day 21).

7. Impulse oscillometry:

Impulse oscillometry is a means of assessing respiratory system impedance in a spontaneously breathing patient. In practice, the subject breathes through a mouth seal in line with a loudspeaker that generates a pulse wave lasting 0.1 second that is transmitted down the airways. By generating sound waves of varying frequencies throughout the respiratory cycle, the instrument can evaluate airway patency and resistance at many different levels within the respiratory tree during normal breathing. While spirometry is relatively insensitive with regards to the small airways, oscillometry may provide us with the precision required to evaluate these airways that are more likely to be involved in asthma, particularly during a RV infection. Repeated measurements do not alter pulmonary function, it is suitable for bedside measurements, and it is not effort dependent.

8. Limited Physical Examination:

At each study visit a physical examination focused on the respiratory tract will be done. This exam will include an examination of the nose, throat, ears, lungs, and cervical adenopathy which may manifest changes during the RV cold.

9. Exhaled Nitric Oxide (eNO)

Exhaled Nitric Oxide (eNO) measurement will be performed using a portable NioxMino® Nitric Oxide analyzer (Aerocrine AB, Sweden). These measurements are easily done by having subjects exhale normally for 12 seconds into a hand held eNO monitor.

10. Nasal Washes:

Nasal washes will be obtained at study visits planned at enrollment (8 weeks prior to virus inoculation) and at 6 and 2 weeks before virus inoculation. Washes will also be collected on the day of inoculation (day 0), daily during the hotel stay post inoculation days 1-4, and at follow-up visits (days 7, 14, and 21 after virus inoculation as note in Appendix 1. The nasal washes will be obtained as described in our previous studies, but with minor modifications to improve subject comfort and the quality of the sample obtained (8, 16). A small amount (2 ml) of phosphate buffered saline (PBS), pH 7.4 will be instilled, using a Mucosal Atomization Device (Wolfe Tory Medical, Inc; www.wolfetory.com) into each nostril (i.e. each nostril treated separately) with the subject sitting upright and holding their breath. Wash fluid and secretions will then be aspirated with gentle suction through a BBG Nasal Aspirator (Codan, US Corp., Santa Ana, CA) inserted only into the anterior nares. The combined washes from both nares result in volumes of 3 to 4 ml of fluid and secretions. After the wash; a plastic transfer pipette will be used to vigorously mix secretions with wash fluid. One ml of this mixture will be transferred to a vial containing veal infusion broth (Bartels Viral Transport Medium, Trinity Biotech, U.S.A.), which will be transported to Dr. Turner's laboratory for culture. (IBC # 129-01) The remaining

wash solution and secretions will be frozen and stored at -80°C for PCR-based viral analyses and for immunoassays. The replication, and clearance, of RV in the upper airway during the 21 days post RV inoculation will be evaluated by titers of virus in culture in Dr. Turner's laboratory and by qPCR in our laboratory. The Bio-Rad Cytometric Bead Assay (Bio-Plex System, Hercules, CA) will be used to measure cytokines and chemokines selected to evaluate (1a) the response to RV (IL-8, IL-12, IFN-γ, IL-1β, IP-10, and IFN-α2 and IFN-beta, the latter type I interferons), and (2) Th_2 associated inflammation (IL-5, IL-13, and eotaxin). Cys LT's will also be measured (Cayman Chemical Co.). To help differentiate the local production of these biomarkers from vascular leakage, the same assessments will be done in serum and ratios of each metabolite/biomarker detected in the nasal fluid and serum will be compared. In addition, albumin will also be measured in nasal wash supernatants and sera to judge vascular leakage as described in our previous investigation (16).

11. Light nasal scraping: After the nasal wash an additional nasal specimen will be obtained by using a ASI Rhino-Pro® nasal mucosal curette plastic device to do a light scrapping along the inferior turbinate of each nostril to collect epithelial cells. This will be done using a headlight and a disposable nasal speculum to spread the nares (Bionix) to enhance visibility of the turbinates.

12. Nasal lining fluid: An additional nasal specimen will be obtained by applying a small piece (1 X 1 cm) of sterile gauze (Surgicel®), routinely used by ENT surgeons at UVA during surgical procedures to provide hemostasis, to inferior turbinate for 4 to 5 minutes in each nostril to collect a sample of epithelial cell lining fluid (about 0.2 ml).

13. Each subject will be given boxes of tissues and ZIP lock freezer bags to put all used tissues into after nose blowing to monitor mucous/secretion weights during the hotel stay. A detailed protocol for how the tissues and bags will be

replaced and the used tissues in bags weighed (3 times daily) is included in the Regulatory Binder for this investigation.

14. Blood tests: Blood samples will be obtained by venipuncture for isolation and analysis of basophils, dendritic cells, and RV and allergen specific T-cells as indicated in the protocol (Section 7.2). The evaluation of these cells will be done in collaboration with Drs. Judith Woodfolk and Larry Borish in the UVA Asthma and Allergic Diseases Center. (IBC #s: 134-02 and 115-00). Blood samples (8ml) for eosinophil counts (8 ml) and to test for sero-conversion to RV-16 will also be obtained as described in the protocol (Section 7.2) and noted in Appendix 5.

14. Administration of Study Drug (Omalizumab vs. Placebo)

After subjects are randomized, injections will be given monthly, or bi-monthly throughout the study, depending on the subject's weight and total IgE level using the prescribing guidelines provided by the manufacturer (21). All injections will be given in clinic as indicated at UVA. Following the injection, subjects must stay in the clinic for 2 hours after receiving the first three injections and for 30 minutes after subsequent injections. . An EpiPen unit will be given to each subject and they will be instructed how to treat any allergic symptoms observed after the Omalizumab or the placebo is given, although reactions following injections of Omalizumab are uncommon as described below.

- Throughout the study, each subject will receive $75/week for a total of 7 weeks and $125/week for the last 4 weeks of daily monitoring of their symptom (CURTS and CLRTS) scores and lung function tests at home. This is included as part of the payment of $1870 given to subjects who complete the study and is noted in a pro-rated scale given to subjects in their informed consent form. Each subject will also receive an additional $45 for each of the 3 clinic visits for sample collection scheduled at 8 weeks, 4 weeks, and 2 weeks before virus inoculation.

Additionally, each subject will receive $575 for the RV challenge, and $45 for each of the 3 clinic visits (on days 7, 14, and 21 after virus inoculation) during the resolution of the infection. Total payment/subject will be $1870.

Participants from the Richmond area (VCU and J. Sargent Reynolds), Staunton/Harrisonburg area (JMU and Mary Baldwin), Lexington area (Washington and Lee), or the Lynchburg area (Liberty University), will also be reimbursed $30.00 per trip for their travel expenses to UVA for visits number 1 through 8. No travel or reimbursement is needed for Visit # 9, because the ACT questionnaire for this visit will be completed on-line.

Note: Subjects will receive study drug or placebo and an EpiPen at no cost to them. The cost of all study procedures and the hotel stay are paid for by the study.

7.5 Study Arm Assignment Procedures7.5.1 Blinding and Randomization

Individual assignment to the Omalizumab or placebo during randomization for this blinded study will be displayed on occluded labels [or sealed disclosure envelopes] provided to the site pharmacist. The occluded label must be removed at the time drug is dispensed and must be then attached to a medication label form.

7.5.2 Securing Blinding and Randomization Information

Randomization lists will be maintained in a secured area by the individual(s) responsible for maintaining the blind. During site visits, the site monitor will check the occluded labels (or disclosure envelopes) to ensure that they are intact and in a secure, yet accessible, location for study personnel. If any of the occluded labels or disclosure envelopes is opened, the site monitor will verify that the Principal Investigator, the

SMC of the study and the NIAID Medical Officer have been notified and that a written account has been completed and forwarded to the above individuals.
Jim Patrie from the Department of Public Health Sciences will carry out the statistical plans for this study and will keep the blind which will only be accessed if the stopping rules described above (8.1.1 and 8.1.2 below) apply.

7.5.3 Requirements for Unblinding

Prior to the end of the study, a subject may be unblinded only for safety reasons.
If a clinically significant event occurs and knowledge of treatment assignment is required, the blind may be broken.. The Principal Investigator will immediately notify NIAID Medical Officer that an unblinding might or has occurred and provide the reasons for the unblinding.

7.5.4 Documenting an Unblinding

Any unblinding (opening of a label or disclosure envelope) will require a full written account of the event(s) that necessitated the unblinding of the treatment assignment for an individual participant (s). This account will be made in the participant (s) study file and in the final study report and will include the reason(s) for the unblinding, the name of NIAID Medical Officer who was notified, the names of the unblinded individual staff members, and the date and time the unblinding occurred. Documentation of an unblinding will be promptly provided to the SMC and NIAID Medical Officer and must be incorporated in the final study report.

7.6 Mechanistic studies

This investigation will also evaluate whether establishing an IgE blockade with omalizumab will have an effect on Th2 inflammatory mediators (e.g. eosinophil cationic

protein and cysteinyl leukotriene measured in nasal washes) which may be of importance to measure in order to understand the mechanisms of rhinovirus induced flares of asthma. In addition, Th2 and Th1 associated biomarkers, along with several innate immune biomarkers and airway pH will be monitored in this study. Thus, by establishing an IgE blockade, the administration of omalizumab may be a novel method for deciphering the mechanisms of RV induced changes in the lower airway and may help determine which Th2 associated pathways will be directly affected by the reduction in serum IgE. We also expect to see significant differences in the frequencies of CD4+ and CD8+ regulatory T-cell subsets present in the circulation of subjects from the omalizumab and placebo treated groups during the infection. Using nasal wash supernatants (i.e. from the end organ where it is known that RV replicates vigorously), it will be of interest to determine whether we will be able to detect differences in cytokines and chemokines generated in direct response to RV (e.g. IL-8, IP-10, IL1beta, IL-12, INF-γ, and IFN-α2) between subjects enrolled in these two groups.

8. SAFETY PROCEDURES

8.1 Stopping Rules

8.1.1 Study Stopping Rules

Study enrollment and investigational product(s)/intervention(s) and study procedures will be suspended pending expedited (within 48 hours) review of all pertinent data by the Institutional Review Board, the SMC, and the NIAID, if any one of the following occurs:

- Consistent with the study design, we plan to enroll 8 subjects in during the spring months of 2014, 2015, and 2016 and 7 subjects in the fall of 2014, and 2015.

  If, after inoculation with the RV-16 pool, two subjects experience severe asthma exacerbations requiring oral steroids for $\geq$ 5 days annually (during the spring and fall enrollments), or if 1 subject requires treatment in the ER, or hospitalization, the stopping rule will apply until further expedited review by the SMC, the UVA IRB, and the NIAID. Among the last 23 asthmatic subjects challenged with RV, only one subject required intervention with a 5 day course of oral steroids, but did not require treatment in the ER or hospitalization. Because the exclusion criteria (see below) obviates the enrollment of subjects who have required treatment in the ER, or hospitalization during the last 3 years (or any intubation ever requiring management in an intensive care unit), we suspect that it is not likely that the above stopping rules will be encountered.
- Lower respiratory tract infections have not yet occurred during experimental (or even natural) infections with rhinovirus in an immune competent host, because this pathogen is not known to be a cause of pneumonia. It is conceivable that one or two subjects could acquire another respiratory tract

pathogen that could cause a lower respiratory tract infection. If 2 subjects developed a significant lower tract infection (not including bronchitis) following RV-16 inoculation and this will lead to stopping the study for further review.

- Death: One death will lead to stopping the study until an expedited review is completed to determine whether the death might have resulted from any of the study procedures.
- An anaphylactic reaction leading to death or two reactions leading to hospitalization as a result of Omalizumab administration, will lead to stopping the study until an expedited review is complete.
- Periodic safety reviews identify specific events (serious and non-serious) occurring more commonly in the drug treatment group than in the placebo group (such as known consequences of asthma or other events that commonly occur in the study population independently of drug therapy) the enrollment will pause pending a safety data review by the SMC, and the NIAID.

8.1.2 Individual Stopping Rules

- At subjects request – Subject withdrawal
- At PIs request and discretion (for medical reasons or if subject was noncompliant with appointments, or completion of diary cards, etc.)
- Pregnancy
- A severe asthma exacerbation that requires management in the emergency room (ER) including treatment with systemic steroids, hospitalization, or the

use of systemic (oral) steroids at any time during the study. Thus far, no subjects infected with rhinovirus experimentally have required treatment in the ER or hospitalization for asthma symptoms.

- The use of inhaled cortico-steroids during the study will also exclude the subject from further participation, because this medication will confound the anti-inflammatory effects of omalizumab. However, the need for ICS by subjects during our RV-16 challenges to date has not occurred.
- Anaphylactic reaction to Omalizumab
- A decrease in platelet count of grade 1 severity or higher after the first injection of Omalizumab.
- A blood hemoglobin level < 10.0 g/dL, in keeping with a NCI-CTCAE grade one adverse event.
- An absolute neutrophil count of < 1000/ mcl
- Signs and symptoms similar to serum sickness (fever, arthralgia and rash) as noted in the manufacturer's prescription/product information report (PLI).

8.1.3 Early Discontinuation of Investigational Product(s)/Intervention(s) with continued study participation/follow-up

Participants terminated from the study due to safety reasons linked to product (s)/intervention (s) will be monitored as described in Section 8.1.4.

8.1.4 Follow-up after early study termination

Participants who are prematurely terminated from the study will be followed to monitor safety for a minimum of 30 days or until resolution or stabilization of the

disqualifying event whichever is longer or until the SMC, the NIAID Medical Officer and the Principal Investigator determine that the follow-up is complete.

8.1.5 Participant Replacement

The protocol accounts for a 20% drop out rate to achieve the power estimated to test the hypothesis of this investigation. Although it is not anticipated that more than 20% of subjects enrolled will be unable to complete the study, we are prepared to enroll additional subjects to meet the target sample enrollment of 42 subjects. Subjects who do not get inoculated will be replaced.

8.2 Adverse Events

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with 21 CFR 312; ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting; and ICH E6: Guideline for Good Clinical Practice and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events version (Version 4.0, May 2009)). These criteria have been reviewed by the study investigators and the IND sponsor and have been determined appropriate for this study population.

8.2.1 Definitions

8.2.1.1 Adverse Events and Suspected Adverse Reactions

Adverse events (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation participant administered a Study Agent(s)/Intervention(s) and that does not necessarily have a causal relationship with this 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) Study Agent(s)/Intervention(s), whether or not related to the medicinal (investigational) Study Agent(s)/Intervention(s). Any medical condition that is present at the time that the subject is screened will be considered as baseline and not recorded as an AE. However, if the condition deteriorates or changes in severity at any time during the study it will be recorded and reported as an AE.

Suspected Adverse Reactions (SAR)

Any adverse event for which there is a reasonable possibility that the drug caused the event." For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

8.2.1.2 Adverse Events Associated with Study Procedures

The following clinical situations, when associated with study procedures are defined as **expected** adverse events and will be recorded on the AE CRF. These situations do not limit the principal investigator from reporting any other events, associated or not with these procedures, from being recorded and reported as AEs.

What risks are **expected** due to the intervention in this protocol?

Expected Risks related to Omalizumab vs. placebo:	**Frequency**
• Injection Site Reaction	☒Occurs frequently,

• Body Pain • Fatigue • Arthralgia • Hair loss • Fracture • Leg pain • Arm pain • Dizziness • Pruritus • Dermatitis • Earache	☒Occurs infrequently (however, none of these symptoms have been shown to occur more frequently than in subjects treated with placebo in controlled trials).
• Anaphylaxis	☒Occurs rarely

Expected Risks related to Rhinovirus-16 inoculation and infection:	**Frequency**
• . Cold symptoms such as mild fever, wheezing, cough, and chest tightness. • A decline in absolute blood neutrophil counts. • Bacterial sinus infection • Lower respiratory tract infection (bronchitis)	☒Occurs frequently, Occurs frequently, but not known to be clinically significant. Occurs infrequently. Occurs infrequently. Rhinovirus is not known to cause pneumonia.
• Cold symptoms could cause severe wheezing and shortness of breath. requiring additional; treatment	☒Occurs infrequently

Expected Risks related to Spirometry Testing (excluding use of Methacholine)	**Frequency**
• Mild light-headedness and coughing	☒Occurs frequently,
• Chest soreness • Mild respiratory fatigue • Mild shortness of breath	X Occurs rarely

• Chest Tightness • Fainting	

Expected Risks related to use of Methacholine	**Frequency**
• **Mild asthma symptoms**	☒Occurs frequently,
• Coughing • Fainting • Chest tightness • Shortness of breath • Wheezing	X Occurs infrequently
• Bronchoconstriction	X Occurs rarely

Expected Risks related to impulse oscillometry	**Frequency**
• Mild light-headedness and coughing, shortness of breath, and/or chest tightness	X Occurs infrequently and is easily reversed with inhaled albuterol

Expected Risks related to Albuterol (if needed following Methacholine testing)	**Frequency**
• Headache • Dizziness • Insomnia • Tremor • Sweating • Nausea • Vomiting • Dry Mouth • Increased pulse rate • Mild throat irritation • Cough	X Occurs infrequently
• Allergic reaction • Chest pain	X Occurs rarely

Expected Risks related to Nasal Washes	**Frequency**
• Discomfort during the nasal wash procedure • Mild coughing	X Occurs infrequently
Expected Risks related to Nasal Scrapings	
• Mild to moderate discomfort for 30 seconds during the procedure • Sneezing • Epistaxis	• Occurs frequently • Occurs frequently • Occurs infrequently & easily stopped with standard treatment (external pressure applied to the nose).
Expected Risks related to obtaining nasal lining fluid	
• **Mild discomfort for 3 to 5 minutes during the procedure** • **Sneezing**	• **Occurs frequently** • **Occurs frequently**
Expected Risks related to monitoring nasal mucous and secretion weights	**None.**
Expected Risks related Blood Draws	**Frequency**
• Pain at venipuncture site	X Occurs frequently,
• Bruising at venipuncture site • Fainting	X Occurs infrequently
• Infection	X Occurs rarely

Expected Risks related to hotel stay (confinement)	**Frequency**
• Boredom • Sense of isolation	X Occurs occasionally, although subjects are predominantly college students who study for their classes during the hotel stay.

Expected Risks related to use of EpiPens (supplied for this study)	Frequency
• Tachycardia • Nausea and Vomiting • Pale Skin • Dizziness • Tremors • Headache • Anxiety	X Occurs infrequently

Reproductive Risks	Frequency
• Potential risk to fetus if become pregnant during study	X Occurs rarely

Privacy Risks	Frequency
• Privacy risk	X Occurs rarely

A summary of safety tests/procedures/observations to be performed is described in Section 7 and Appendix 5.

- Note:

  1. - Only subjects at low risk for significant asthma symptoms will be enrolled (as described above in the exclusion criteria).

  2. -Clinical monitoring will be conducted during RV challenge. Additionally, after subjects receive injections of omalizumab they will be monitored for signs and symptoms of anaphylaxis as described in Section 7.2. All subjects will be provided with EpiPen and instructions about how and when to use it. However, needing to use EpiPen, based on the reports of the frequency of anaphylaxis, is unlikely.

8.2.1.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the outcomes described below:

1. *Death.* A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up.
2. *A life-threatening event*. An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death..
3. An inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Congenital anomaly or birth defect.

Additionally, SAEs will be reported as described in Section 8.2.4.

8.2.1.4 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or it is not listed at the specificity or severity that has been previously observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the IND application.

The Principal Investigator will ensure the timely reporting of unexpected adverse events as described in table in Section 8.2.2.2.

8.2.1.5 Safety Monitoring Committee

The Safety Monitoring Committee (SMC) will provide independent safety monitoring in a timely fashion and to provide recommendations regarding the safe continuation of the study. The SMC will evaluate adverse events, including SAE, against the known safety profile of the study product to assess for possible changes to the overall risk of the study. The SMC , along with the PI and the NIAID Medical Officer, will conduct periodic reviews of cumulative safety data including, but not limited, to SAEs. The SMC will be available to communicate with the NIAID Medical Officer on safety events as needed to discuss individual specific SAEs or clusters of SAEs developing during the study and results of periodic safety reports.

8.2.2 Collecting, Recording and Managing Adverse Events

8.2.2.1 Identifying Adverse Events

Any adverse event that occurs from the moment the subject has signed the consent form will be recorded and is reportable. Collection of AEs/SAEs will be completed after the completion of the 8 week period of treatment with omalizumab (before virus inoculation) and again through the final visit after the infection (Day 28).

Adverse events may be discovered through any of these methods:

1. Observing the participant.
2. Questioning the participant, with standardized questions/procedures.
3. Receiving an unsolicited complaint from the participant.
4. An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event.

8.2.2.2 Recording AEs

Throughout the study all identified adverse events (serious and non-serious) will be recorded for all randomized participants on all appropriate source document and adverse event case report forms regardless of their severity or relation to the study.

A complete description of all adverse events will include event description, time of onset, investigator assessment of severity, relationship to study agent(s) or procedures/intervention(s), time of resolution/stabilization of the event, expectedness, determination of whether the AE qualifies as a SAE, and action taken. A change in the severity of the AE will also be documented. The PI will document assessment of severity and relationship on the source documents or the on the CRF.

Will all adverse events, as defined in this protocol, be collected/recorded?

☒ Yes

How will adverse event data be collected/recorded?

☒ Paper AE forms/source documents (please attach a copy of this form if possible)

How will AEs be classified/graded?

Please refer to Section 8.2.3.1

What scale will the PI use when evaluating the relatedness of adverse events to the study participation?

Please refer to Section 8.2.3.2

When will recording/reporting of adverse events/unanticipated problems begin?

☒ After subject signs consent

When will the recording/reporting of adverse events/unanticipated problems end?

☒ At the end of Visit 9 (28 days post inoculation)

Please complete the following table to describe details of Adverse Event, Unanticipated Problem, and Protocol Violation reporting to the IRB.

Type of Event	To whom will it be reported*:	Time Frame for Reporting	How reported?
Any event resulting in death that is deemed DEFINITELY related to (caused by) study participation (Note: An internal event is one that occurs in a subject enrolled in an UVa protocol.)	IRB-HSR	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, Unexpected adverse event And Unexpected Non-Serious Adverse Event	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. *Timeline includes submission of signed hardcopy of AE form.*	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. *http://www.virginia.edu/vprqs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc)*

Protocol Violations (Note the IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by your sponsor, if applicable.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation and Enrollment Exception Reporting Form http://www.virginia.edu/vprgs/irb/hsr_forms.html *Go to 3rd bullet from the bottom.*

How will the endpoint data be collected/recorded?

☒ Protocol specific case report forms (attach if in-house)

☒ Source documents

Data and Safety Oversight Responsibility

Who is responsible for overseeing safety data for this study (*i.e., Who is looking at data* in aggregate form to identify trends?)(Check all that apply)

☐ No additional oversight body other than PI (skip question 5.2)

☒ NIAID Medical Officer

☒ NIAID SMC

☒ PI

Please check aggregate reviews that will occur by the PI? *(Answer this question* ONLY if the PI is the ONLY person overseeing the safety data for this study. (*Note: in this case you will have checked the first box for question* #5.1 "*No additional oversight body other than* PI.")

N/A

How often will aggregate review occur? *Answer this question ONLY if the PI is the ONLY person responsible for overseeing the safety if the study*

N/A

How will a report of the information discussed in questions above be submitted to the IRB?

☒ Part of IRB-HSR Continuation Status Form

☐ Separate report from sponsor/ SMC

☐ Other: *(specify)*

8.2.2.3 Recording SAEs

Serious adverse events will be recorded on the serious adverse event case report form and will include a narrative of the event signed and dated by the Principal Investigator.

8.2.2.4 Managing Adverse Events

The site investigator must apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from further treatment under the protocol. The investigator must institute any necessary medical therapy to protect a participant from any immediate dangers. In this regard, if any subject experiences asthma symptoms which are not controlled with their albuterol metered dose inhaler, or if they experience a decline in their FEV1 by 20% below their baseline FEV1 at baseline, they will be treated acutely by the study physicians as described in Appendix 6 and followed carefully until the symptoms flare has resolved.

An adverse event will be followed until any of the following takes place: a) it is resolved, b) participant is stable, c) a minimum of 30 days after participant is terminated

from the study and the NIAID Medical Officer and the Principal Investigator determine that follow-up is complete.

If an abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) is determined to be an AE (refer to National Cancer Institute's Common Terminology Criteria for Adverse Events Version , Version 4.0, May 2009) , then the evaluation that produced the value or result can be repeated until the value or result returns to normal, or the result can be explained, or the usual standard of care does not require further follow-up, and the participant's safety is not at risk.

8.2.3 Grading and Attribution

8.2.3.1 Grading criteria

In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version (Version 4.0, May 2009) This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates 'or' within the description of the grade.):

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using he toilet, taking medications, and not bedridden).
Grade 4 = Life-threatening consequences; or urgent intervention indicated.
Grade 5 = Death related to AE.

Anaphylaxis is a disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death.
Severity grading of anaphylaxis as per the NCI-CTCAE manual is as follows:
Grade 1=
Grade 2=
Grade 3= Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension
Grade 4= Life-threatening consequences; urgent intervention indicated
Grade 5= Death

8.2.3.2 Definition of attribution:

When evaluating the relatedness of adverse events to the study participation the PI will determine the relationship of adverse events to the study
Unrelated: The event is temporally independent of the study product; and/or the event appears to be explained by another etiology. Alternative etiology must be described in subject's records.

Related: There is an association between the event and administration of the study product, a plausible mechanism for the event to be related to study product and causes other than study product have been ruled out **and /or** the event re-appeared on re-exposure to study product.

8.2.4. SAE Reporting and Procedures

The Principal Investigator will be notified by the study staff as soon as a staff member becomes aware ANY SAE. In the absence of the Principal Investigator, a physician sub-investigator will be notified.

8.2.4.1. Notifying the NIAID Medical Officer

The NIAID Medical Officer will be notified by the Principal Investigator by email no later than 24 hours after the investigative site becomes aware of the SAE, regardless of the presumed relationship to the study product.

Within another 24 hours, the NIAID Medical Officer must receive the corresponding SAE case report form or MedWatch form generated and signed by the Principal Investigator with the description of the SAE and the Principal Investigator's assessment of causality and expectedness . The SAE case report form or MedWatch form will be re-submitted by the PI to the NIAID Medical Officer with updated relevant medical information in the narrative section of the form as needed until the case is closed.

Submission timelines of MedWatch forms to the FDA is captured in section 8.2.4.3. below.

Contact information for the NIAID Medical Officer is listed below:

Lisa M Wheatley, MD, MPH
Division of Allergy, Immunology and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane Rm 6B56
Rockville, MD 20892, USA
Phone: 240-627-3573
Email: lisa.wheatley@nih.gov

8.2.4.2 Unexpected, Non-Serious Adverse Events

An unexpected, non-serious adverse event that is of Grade 3 severity or higher and study related will be recorded and reported to the NIAID Medical Officer under the serious adverse event reporting procedure outlined in the SAE Reporting and Criteria Section (Section 8.2.4.1) of the protocol (i.e. within 24 hours).

8.2.4.3. Notifying the FDA

The IND Sponsor is responsible for FDA safety submissions as follows:

The following process for reporting a serious adverse event ensures compliance with the ICH guidelines, 21 CFR 46 and 21CFR §312.32.

- **Expedited reporting** to the FDA applies if the adverse event is considered to be:
    - Serious (ref. Section 8.2.1.3), **and**
    - A Suspected Adverse Reaction (ref Section 8.2.1.1), **and**
    - Unexpected (ref. Section 8.2.1.4)

      These events are called SUSARs (a serious, unexpected suspected adverse reaction)

  SAEs that do not strictly fit the above criteria may be reported to the FDA in an expedited manner if the IND Sponsor or the NIAID Medical Officer chooses to do so. Expedited events will be reported by the IND Sponsor within 15 calendar days after the IND sponsor becomes aware of the SAE; fatal or life-threatening events will be reported within 7 calendar days. Each 7-day report must be followed up by a 15-day report.

- **Standard reporting (non-expedited)** to the FDA (report in the IND Annual Report) applies to all SAEs not meeting the criteria for expedited reporting. The Principal Investigator will be responsible for compiling the IND Annual Report.

Adverse Events that are study endpoints do not need to be captured in an expedited or standard IND safety report, **unless** there is evidence suggesting a causal relationship.

8.2.4.4. Notifying the Safety Monitoring Committee

The NIAID Medical Officer will submit all expedited SAEs on an ongoing basis to the SMC. Individual or clusters of SAEs may be reported expeditiously to the SMC either when specified by the SMC, or upon determination of the NIAID Medical Officer.

8.2.4.5. Notifying the Institutional Review Board

The Principal Investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB in accordance with IRB regulations and guidelines as described in table in Section 8.2.2.2.

8.2.4.6 Reporting Pregnancy

The investigator will be informed immediately of any pregnancy and will report all pregnancies within 24 hours to the NIAID Medical Officer (as described in Section 8.2.4.1) utilizing the SAE report form. This report is for tracking purposes only. All pregnancies that are identified during the study will be followed to conclusion and the outcome of each will be reported. The investigator will discuss with the participant and/or the treating physician the known possible risks of the investigational product(s) on the fetus. Monitoring of the participant will continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy

will be submitted to the NIAID Medical Officer and Project Manager. Subjects will be terminated from the study due in the event of pregnancy.

8.2.5 Non Serious Adverse Events Reporting

8.2.5.1 Notifying the Safety Monitoring Committee

The study biostatistician or Principal Investigator will provide the NIAID Medical Officer with periodic cumulative safety reports (tables and data listings of all AEs, including SAEs) during the 3 years of the study.
Reports are due as described below and must capture cumulative safety information for the entire cohort of subjects participating in the study in the spring or the fall of each year of the study.

- 1 week prior to RV inoculation
- After each subject enrolled have completed their last study visit.

The NIAID Medical Officer will review tables and data listings and send the safety reports to the SMC for further review. Individual or clusters of AE's will be reported expeditiously to the SMC either when specified by the SMC, or upon determination of the NIAID Medical Officer.
The PI, the NIAID Medical Monitor will review safety data on an ongoing basis including in addition to the periodic reviews listed above.

8.2.5.2 Notifying the Institutional Review Board

The Principal Investigator will ensure the timely dissemination of AE information to the IRB in accordance with applicable regulations and guidelines described in table in Section 8.2.2.2

8.2.5.3 Notifying the FDA

The IND Sponsor will file all adverse events per 21 CFR 312.32, whether expedited or part of the IND Annual Report. The Principal Investigator will be responsible for compiling the IND Annual Report. The SMC and the NIAID Medical Officer will conduct the oversight of safety data filed with the FDA by the Principal Investigator.

8.3 Protocol Deviations (Protocol Violation)

The practices below are consistent with Good Clinical Practice (GCP ICH E6) Sections:

- Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, section 5.1.1
- Noncompliance sections 5.20.1, and 5.20.2.

8.3.1 Protocol Deviation Definition

A protocol violation is defined as any change, deviation, or departure from the study design or procedures of research project that is NOT approved by the IRB-HSR prior to its initiation or implementation, OR deviation from standard operating procedures, Good Clinical Practices (GCPs), federal, state or local regulations. Protocol violations

may or may not be under the control of the study team or UVA staff. These protocol violations may be major or minor violations.

Protocol Deviations:

The Principal Investigator is responsible for reporting protocol deviations to the IRB using the standard reporting form as described in table in Section 8.2.2.2 As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

Major Protocol Deviation - is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.

If the deviation meets any of the following criteria, it is considered a major protocol deviation (example list is not exhaustive):

1. The deviation has harmed or posed a significant or substantive risk of harm to the research subject.

   Examples:

   - A research subject received the wrong treatment or incorrect dose.
   - A research subject met withdrawal criteria during the study but was not withdrawn.
   - A research subject received an excluded concomitant medication.

2. The deviation compromises the scientific integrity of the data collected for the study.

   Examples:

   - A research subject was enrolled but does not meet the protocol's eligibility criteria.
   - Failure to treat research subjects per protocol procedures that specifically relate to primary efficacy outcomes.(if it involves patient safety it meets the

first category above)

- Changing the protocol without prior IRB approval.
- Inadvertent loss of samples or data.

3. The deviation is a willful or knowing breach of human subject protection regulations, policies, or procedures on the part of the investigator(s).
   Examples:
   - Failure to obtain informed consent prior to initiation of study-related
   - Procedures
   - Use of outdated or incorrect consent forms
   - Falsifying research or medical records.
   - Performing tests or procedures beyond the individual's professional scope or privilege status (credentialing)
4. The deviation involves a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.
   Examples:
   - Working under an expired professional license or certification
   - Failure to follow federal and/or local regulations, and intramural research
   - Repeated minor deviations.
5. The deviation is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles.
   Examples:
   - A breach of confidentiality.
   - Inadequate or improper informed consent procedure.

Minor Protocol Deviation - A minor protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that has not been approved by the IRB and which DOES NOT have a major impact on the subject's rights,

safety or well-being, or the completeness, accuracy and reliability of the study data.

8.3.2 Reporting Protocol Deviations

Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator, b) notify the NIAID Project Manager (refer to investigator's signature page for contact information) , and the NIAID medical Monitor and c) will complete the Protocol Deviation form. All major protocol violations must be reported to the IRB-HSR immediately upon discovering them, and no later than seven (7) calendar days from the time the study team receives knowledge of the event.

NIAID may request discussion with the Principal Investigator to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study and corrective actions. The Principal Investigator will complete and sign the Protocol Deviation form and submit it to the NIAID Medical Officer and Project Manager and to the site IRB, per IRB regulations (refer to table in Section 8.2.2.2). Major protocol deviations will be reported to the SMC by the NIAID Medical Officer. The IND sponsor will be responsible for notifying the FDA.

9 SAMPLE SIZE CALCULATIONS AND STATISTICAL PLAN

9.1 Sample Size, and Power Calculations

9.1.1 General considerations

Data will be provided in *listings sorted by patient #ID, cohort or treatment group as applicable.* Tabular summaries will be presented by *cohort and treatment group as applicable.* Categorical data will be summarized by *the number and percent of subjects in each category.* Continuous variables will be summarized by descriptive statistics using *mean, standard deviation, median, minimum and maximum. Describe here the formal statistical tests planned.*

All of the data analyses associated with this Aim will be conducted following the guidelines of the intention to treat principle. Missing data will not be imputed.

9.1.2 Patient populations

Sample Size Justification: To evaluate the asthmatic response to RV, the primary outcome variable for this study will focus on changes in the cumulative lower respiratory tract symptom (CLRTS) scores computed as the sum of the lower respiratory tract symptom scores from the day of the RV inoculation through the first four days after virus inoculation in the hotel. A total of 42 subjects will be enrolled. Twenty one subjects will be randomized to Omalizumab and 21 subjects will be randomized to placebo. **Power Analysis**: To account for a possible 20% drop out rate, our power calculations assume that 17 subjects per study-group will complete the study. Participants who are determined to have developed a naturally acquired RV-16 infection prior to experimental inoculation (4-fold rise in RV antibody titers or a positive RV RCR in nasal washings) will be dropped from the analysis. **Details**: A Monte Carlo simulation was carried out to estimate the statistical power that we would have with 17 subjects per group to detect a clinically meaningful difference between the day 4 mean CLRTS of the placebo group and the day 4 mean CLRTS of the Omalizumab group. In the simulation we

assumed that the underlying distributions of the day 4 CLRTS in the placebo and Omalizumab study populations are negative-binomial, with means 4.2 and 1.8, respectively. Note that in our previous challenge study of six asthmatics with high total IgE, ten asthmatics with low levels of total IgE, and the nine healthy controls (16), 4.2 was the observed day 4 mean CLRTS in the high IgE group, while the mean day 4 CLRTS was 1.0 in the low IgE group. The power of the statistical test was determined based on 10,000 simulation repetitions, in which at each repetition we generated 17 observations from each of the two aforementioned negative-binomial distributions. We then used a negative-binomial generalize linear model (log-link) with one predictor variable, which identified the distribution from which the response (CLRTS) was generated, to test the null hypothesis that the means of the two negative-binomial distributions are equal. The power to detect a 2.4 unit absolute difference between the day 4 mean CLRTS (i.e. $|4.2 - 1.8| = 2.4$) was calculated by determining the proportion of times that the null hypothesis was rejected when the rejection rule was based on a Wald test with two sized type I error rate 0.05. The power of the test was determined to be 0.83.

Statistical Analyses (Primary Endpoint): The lower respiratory tract symptom scores from the day of RV inoculation (study day 0) through study day 4 of monitoring in the hotel will be tallied in consecutive sequence to produce a CLRTS score for each subject. The CLRTS score will be integers and they will be analyzed by way of a negative-binomial generalized linear model (GLM). The GLM model specification will include two variables. One variable will be a classification variable that will identify the group (placebo, or omalizumab) to which the subject was randomly assigned, while the second variable will denote the subject's total lower respiratory symptom score on the day prior to RV-inoculation. A linear contrast of means will be used to test our primary null hypothesis that the mean of the CLRTS score measurement distribution over the 4 days after RV inoculation is the same for the 2 study-populations (i.e. placebo and omalizumab). The null hypothesis will be rejected if the p-value of the Likelihood Ratio test is less than or equal to 0.05. A 95% confidence interval will be constructed to

estimate a plausible range of values for the CLRTS score means determined over the first 4 days after RV inoculation (i.e. omalizumab CLRTS score mean: placebo CLRTS score mean) after adjusting for the lower respiratory tract symptom scores recorded on the day prior to RV inoculation.

As a secondary analysis, we will add the subjects' baseline IgE values to the negative binomial regression model as covariate information to insure that the primary pivotal comparison findings hold when the CLRTS mean scores of the placebo and omalizumab groups are estimated at a common reference baseline IgE value.

Alternative Approach: If we observe a systematic pattern in the residual plot or the qq-plot of the standardized Pearson residuals of the negative-binomial GLM (indicative of lack of goodness of model fit) we will analyze the CLRTS scores via a randomization (permutation) test, and use a bootstrapping procedures to obtain a 95% confidence interval for the mean difference between the CLRTS scores of the two study-arms.
Statistical Analyses (Secondary Endpoints): In addition to comparing the CLTRS scores between subjects enrolled in the omalizumab and placebo groups over the first 4 days after RV inoculation (primary endpoint), we are also prepared to compare the CLTRS scores between the 2 study groups over the first 7 days and during the entire 21 days of monitoring after RV inoculation. These comparisons will be carried out as described above. We are prepared to do this recognizing that we won't be able to administer omalizumab to subjects whose total IgE levels are too high to receive the study drug as noted in the exclusion criteria for this study (section 4.2). As a result, the total IgE levels for subjects enrolled in this proposed study may, on average, be lower than for the subjects enrolled in our previously published study (16). Thus, it is possible that the CLRTS scores for the subjects enrolled in this proposed study may peak later

than we anticipate, as was observed for asthmatics who had lower levels of total IgE in our previous study (16).

We will use one-way analysis of variance (ANOVA) to compare the mean post-inoculation day 4 change (from baseline prior to RV Inoculation) in methacholine sensitivity (MS) [PC20/FEV1 (mg/ml)] between the subjects who were randomized to the placebo and omalizumab groups. A linear contrast of means will be conducted to test our primary null hypothesis that the mean of the underlying distribution of measurements of change in MS on post-inoculation day 4 is the same for the 2 study-populations. The null hypothesis will be rejected if the p-value of the t-test is less than or equal to 0.05. A 95% confidence interval will be constructed to estimate a plausible range of values for the mean difference in the change in MS on day 4 between the 2 populations. In addition, we will use a binomial exact test to compare between the 2 treatment groups the number of subjects whose FEV1 drops below 70% predicted, or by more than 20% from their baseline value (measured prior to RV inoculation), during the post RV inoculation phase.

The FeNO data that we collect on study days 0, 1, 2, 3, and 4 in the hotel will be analyzed by way of a linear-mixed effect ANCOVA. The response data will represent a set of delta values that will quantify the change in the FeNO concentrations on post-inoculation study days 0 through 4, from the baseline FeNO values measured just prior to the RV inoculation. The ANCOVA model specification will include two factors. One factor will identify the group to which the subject was randomly assigned, while the second factor will identify the day on which the FeNO was assessed. The FeNO value measured just prior to RV inoculation will function as the ANCOVA adjustment factor. Linear contrasts will be used to test our primary null hypotheses that on post-inoculation day 4, the mean change in FeNO was the same in the 2 study groups after

adjusting for their baseline FeNO. A two-sided ≤0.05 decision rule will be utilized as the rejection criterion for this test. Linear contrasts will also be used to test our secondary null hypotheses that over post-inoculation days 0,1,2,3 and 4 the mean change in FeNO will be the same in the 2 study-groups after adjusting for baseline FeNO. The effects of omalizumab on FeNO during the months of treatment before the infection will be analyzed in a similar manner as the FeNO data collected after RV inoculation.

Data for additional secondary outcome variables will be collected at baseline before inoculation, and on post-challenge days 0,1, 2, 3, and 4 in the hotel, and on follow-up days 7, 14, and 21 after the subject leaves the hotel. Many of these additional secondary outcomes will be analyzed in exactly the same manner as in our previous experimental RV challenge study (16). Additionally, the frequency of the $CD4^+$ and $CD8^+$ T-cells expressing Foxp3 on post-inoculation day 3 will be compared to the frequency of these cells prior to RV inoculation by way of the non-parametric Wilcoxon sign rank test. The between group comparison of the change in these cells on post-inoculation day 4 will be conducted via the non-parametric Wilcoxon rank sum test. We will also examine the relationship between pairs of the secondary outcome variables via linear mixed effects regression models.

We will also compare changes in biomarkers of inflammation and bronchial hyperreactivity between the omalizumab and placebo treatment groups during the 8 weeks of drug administration before RV challenge and after RV inoculation to evaluate the influence of the infection on these parameters. For each subject, we will be measuring FeNO levels (ppb), absolute blood eosinophil counts (cells/mm3), impulse oscillometry and PC20 (mg/ml) for methacholine at 4 time points, beginning at enrollment (8 weeks before the RV challenge), on the day of inoculation (day 0), during peak cold symptoms (days 4 and 7), and at study day 21). The statistical analyses will be

focused on comparing changes in theses parameters from baseline between the treatment groups at each time point and the analyses will be conducted by way of linear-mixed effects models. For each analysis, the set of repeated measurements will identify the changes in the biomarker measurements at days: 0, 4, 7 and 21 from the measurements at the baseline (i.e. beginning of enrollment). Three potential sources of variation in the biomarker change data will be consider in the analysis. One source will be the treatment group (omalizumab vs. placebo), the second source will be the assessment times (days 0, 4, 7 and 21), and the third source will be treatment group by assessment time interaction. The subjects" biomarker measurements at enrollment will be treated as covariate information in the analysis. Between-group comparisons of the mean biomarker change at days 0, 4, 7 and 21 will be based on a set of linear contrasts of the least-squared means. A Bonferroni multiple comparison hypothesis rejection rule will be utilized in hypothesis testing to maintain an overall false positive rejection rate of 0.05. The ACT questionnaire will be evaluated using statistical methods similar to evaluating the changes in the CURTS and CLRTS scores. The results will be compared at 4 time points when the ACT questionnaire will be completed (i.e., at enrollment, at Visit 3 and 5, and at 28 days after viral inoculation when subjects return to receive their payment for completing the study).

Missing data: The longitudinal data analysis methods that we propose to use to analyze the primary and secondary outcome data in this study are data efficient (i.e. they utilize all of the available data from each individual in the estimation of the statistical model parameters). If data imputation is deemed necessary, we will use the SAS PROC MI procedure (SAS version 9.2.2 EAS, Cary, NC) to carry out the data imputation. The subsequent data analysis will be conducted in accordance with the analytical guidelines proposed by Schafer (30).

9.1.3 Study Participant Baseline Characteristics and Demographics

Summary of descriptive statistics for baseline and demographic characteristics (age, race, sex, body weight, and height) will be provided for all enrolled participants.

9.1.4 Study Endpoints

The primary endpoint is defined in section 3.1.1
The secondary endpoints are defined in section 3.1.2.

9.1.5 Study Completion

The percent of participants who complete the study, losses to follow-up, time to lost to follow-up, and reasons for discontinuation (e.g., adverse events) will be presented.

9.2 Interim Analyses

Interim analyses are not planned for this study.

9.3 Deviations from Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principal features will require a protocol amendment and will be described in the final report. These changes will be subject to review by the IRB, SMC, NIAID, and the FDA

10 IDENTIFICATION AND ACCESS TO SOURCE DATA

10.1 Identifying Source Data

The investigator will keep accurate records to ensure that the conduct of the study is fully documented. Data derived from source documents will be transferred to protocol-specific CRFs. The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical records or hospital database and the data will be transferred to clinical CRFs, as applicable.

10.2 Updating Source Documentation

Documents describing the safety profile of an investigational product(s)/intervention material(s), such as the investigator's brochure and the package insert, will be amended as needed by the investigational product(s)/intervention material(s) manufacturer to ensure that the description of safety information adequately reflects any new clinical findings.

For purchased investigational product(s)/intervention material(s), the Principal Investigator will promptly notify , the NIAID Medical Officer, and the IRB about changes to the package insert.

10.3 Permitting Access to Source Data

The investigational site participating in this study will maintain the highest degree of confidentiality for the clinical and research information obtained from the participants in this clinical trial. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site will permit authorized representatives of the IND sponsor(s), NIAID and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information will be removed). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals.

11 QUALITY CONTROL AND QUALITY ASSURANCE

The Principal Investigator will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all CRFs and participant study files are legible and complete for every participant.

The Principal Investigator, through the use of an independent site monitor will be responsible for the regular review of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data and accuracy of source documentation verification. The reports of the site monitor will be submitted to the Principal Investigator and the NIAID Project Manager. NIAID will independently review these reports.

When the CRFs are complete, they will be reviewed and signed by the Principal Investigator. All discrepancies identified by the site monitor or NIAID will be reviewed, and any resulting queries will be resolved with the Principal Investigator and the CRFs will be amended as needed.

12 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 Statement of Compliance

This study was designed to ensure the protection of subjects according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human subjects. This clinical study will be conducted using current good clinical practice (cGCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance [1], and according to the criteria specified in this study protocol. Before study initiation, the protocol the informed consent documents and the CRFs will be reviewed and approved by NIAID, SMC , IRB, as well as any other appropriate health authorities. Any amendments to the protocol or to the consent materials will also be approved by the appropriate bodies listed above prior to implementation.

12.2 Informed Consent and Assent

The informed consent form will provide information about the study to a prospective participant or subject's legal representative to allow for an informed decision about participation in the study. Prospective participant or subject's legal representative must be given ample opportunity to review the informed consent and inquire about the results of the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form prior to study participation. Consent materials for participants who do not speak or read English will be translated into the participants' appropriate language.

The informed consent form will be revised and receive IRB approval whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent form will be given to a prospective participant for review. The Principal Investigator or an approved designee will discuss the consent with the prospective participant and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

12.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used during collection, storage, and reporting of participant information.

Tissue banking

What samples will be banked? Nasal wash specimens and blood.

Information Accompanying Specimens

What information will be on the label of the specimen to be banked? Study code (i.e. X for Omalizumab), type of sample (i.e. N = nasal wash, B = blood), subject number, and date sample collected. For example, XN 01 (01/01/09) = nasal wash from subject number one collected on January 1st 2009).

1. **What information will be "linked to" or will accompany the specimen?** Subject number will be on the specimen collected. This subject number will also be on the enrollment log that will be linked to the subject's name.
2. **If the samples are identifiable please explain why they cannot be coded or de-identified**. N/A

Collection and/or Storage of Specimens

3. **How much material (e.g. blood, tissue) will be collected and how will it be collected?** Blood samples and nasal washes will be collected in the amounts and at the frequency and using methods described previously in this protocol.

4. **Who will be responsible for storing the specimens?** Peter Heymann MD

5. **Where will the specimens be stored?** Freezer in MR 4-Peter Heymann's lab

6. **Could the loss of confidentiality of the subject's health information potentially have a negative impact decisions of health coverage, employment, insurability or any other benefit, or cause social stigmatization?** No

7. **Will another research institution or entity outside of UVa ever have control over the specimens?**

Nasal scraping samples for analyzing epithelial cell gene expression via RNA seq will be sent to a research collaborator at the University of Cincinnati. These samples will be de-identified. The only information recorded on the samples is decribed above on page 109 (see "Information Accompanying Specimens"). No access to HIPAA identifiers will be available to our collaborators.

8. **Can participants withdraw their specimens or request that they be destroyed?** Yes

Privacy Plan for Studies With Consent

1. **Describe your plan to protect the identifiable data from improper use and disclosure.**

___X _ Option # 2 - Health information and/or specimens will be stored with HIPAA identifiers.

- Will any of the data be stored in electronic format?` Yes`
  If yes, where will it be stored?
  __X___ a Health Systems Computing Services (HS/CS) managed server that is configured to store data regulated by HIPAA,
- Will any of the data be stored in hard copy format (*e.g.- on paper*)? YES
  If yes, where will it be stored?
  ___**X**__ case report forms, including diary cards from each subject, will be stored in a secure area with limited access.

_____questionnaires/ surveys will be stored in a secure area with limited access.

- Only investigators for this study and clinicians caring for the patient will have access to the data. They will each use a unique log-in ID and password that will keep confidential.
- If specimens stored: The following security precautions will be implemented for specimens stored at UVa:
  - Specimens will be stored in a locked freezer/ or locked room
  - Access to the freezer/room will be limited to study personnel
  - A log book will maintained documenting specimens added to or taken from the freezer/room.
- Each investigator accessing electronic data will sign the University's Electronic Access Agreement available at http://www.itc.virginia.edu/policy/form/eaa.pdf and forward the signed agreement to the appropriate department as instructed on the form.
  If you currently have access to clinical data it is likely that you have already signed this form. You are not required to sign it again.
- Accessing an electronic database holding the HIPAA identifiers and health information from an external site will be done only via the use of a VPN#.
- No identifiable data (data with health information and HIPAA identifiers) will be transferred to any other location such as a desktop, laptop, memory stick, CD etc. The researcher will follow all health system security policies found at https://www.healthsystem.virginia.edu/Intranet/security/Security_Policies/home.cfm
- The study team may temporarily move data out of the electronic database for analysis under the following conditions:
  - HIPAA identifiers, such as name, SS#, (see Table A for full list HIPAA identifiers) WILL NEVER be transported at the same time as the health information.
  - HIPAA identifiers will be kept in a secure location with limited access.
  - If analysis of data is done on another computer-:
    - That computer will be secured according to, at a minimum, security guidelines described at http://itc.virginia.edu/security/checklistforPCs.html.
    - the HIPAA identifiers and health information WILL NEVER be stored on the additional computer at the same time,

        - The data will be deleted from the hard drive of the additional computer as soon as possible.
        - the data will be securely removed from the server, additional computer(s), and electronic media according to the University's Electronic Data Removal Policy described at https://etg07.itc.virginia.edu/policy/policydisplay?id=IRB-004
    - The data may not be analyzed for any other study without additional IRB approval

2. Describe your/central registry's plan to destroy the HIPAA identifiers at the earliest opportunity consistent with the conduct of the research.
Check one option below:

__**X**___ NA- the identifiers will not be destroyed. The identifier will be needed to be able to continue to add data in the future.

3. Do you confirm that you will not reuse the identifiable data (HIPAA identifiers or health information) or disclose any of this information to any other person or entity except as outlined in this protocol, except as required by law, for authorized oversight of the research study, or use it for other research unless approved by the IRB-HSR?

This means that after the study is closed at UVA:

- You cannot contact the subject by any method (you cannot call them, send a letter, talk to them in person about the study, etc) without additional IRB approval
- You cannot use the data for any research that is not already described in your IRB protocol without additional IRB approval (if you change your hypothesis you must modify your protocol)
- You cannot share your research data with another researcher outside of your study team without additional IRB approval
- Any health information with HIPAA identifiers will be shredded or discarded by using recycling bins for confidential material found in clinic settings. *For large item disposal of confidential material contact Environmental Services at 2-4976 or University Recycling at 2-5050.*

YES

TABLE A: HIPAA Identifiers

1. Name
2. All geographic subdivisions smaller than a state, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of the zip code if, according to the current publicly available data from the Bureau of the Census: (1) The geographic unit formed by combining all zip codes with the same 3 initial digits contains more than 20,000 people and (2) The initial 3 digits of a zip code for all such geographic units containing 20,000 is changed to 000.
3. All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older. *[This means you may record the year but not record the month or day of any date related to the subject if the subject is under the age of 89. In addition if the subject is over the age of 89 you may not record their age and you may not record the month, day or year of any date related to the subject]*
4. Telephone numbers
5. Fax numbers
6. Electronic mail addresses
7. Social Security number
8. Medical Record number
9. Health plan beneficiary numbers
10. Account numbers
11. Certificate/license numbers
12. Vehicle identifiers and serial numbers, including license plate numbers
13. Device identifiers and serial numbers
14. Web Universal Resource Locators (URLs)
15. Internet Protocol (IP) address numbers
16. Biometric identifiers, including finger and voice prints
17. Full face photographic images and any comparable images
18. Any other unique identifying number, characteristic, code that is derived from or related to information about the individual (e.g. initials, last 4 digits of Social Security #, mother's maiden name, first 3 letters of last name.)

13 PUBLICATIONS

Publication of any data from this study must be carried out in accordance with the clinical or mechanistic study agreement.

14 REFERENCES

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Diary Cards

Appendix 1: Lower Respiratory Tract Symptoms – RV-16 Challenges in Volunteers With Asthma

Patient I.D. Number: _____
Patient Name: _____

Never leave a box empty.
If a symptom is not present enter a 0.
Only record whole numbers (i.e. 0, 1, 2, etc. not 0.5, 1.5, etc.)

Date																
Study Day	**D**	**N**	**D**	**N**	**D**	**N**	**D**	**N**	**D**	**N**	**D**	**N**	**D**	**N**	**D**	
Symptoms																
Cough																
Shortness of Breath																
Chest Discomfort																
Wheezing																
Night Awakenings																
Asthma Monitor																
Peak Flow																
FEV 1																
Medication																
1. Albuterol # of puffs																
Other Medications																
1.																
2.																

Symptom Score Guide
0=Not Present
1=Mild, clearly present
2=Moderately severe, uncomfortable
3=Severe, interfering with sleep or activity

FEV1 and Peak Flow Values
Record FEV_1 and peak flow results in boxes provided for the day time (D) & night time (N) measurements.

Medications:
List current medications at the beginning of each new diary card. Each day, record your usage of that particular medicine. If new medicines are started, list them as well. Please call your contact person before you start any new medicine.

Diary Card

Appendix 2: Upper Respiratory Tract Symptoms – RV-16 Challenges

Patient I.D. Number: _____
Patient Name: _____

Never leave a box empty.
If a symptom is not present enter a 0.
Only record whole numbers (i.e. 0, 1, 2, etc. not 0.5, 1.5, etc.)

Days -7 to 0

Date																
Study Day	**-7/D**	**-7/N**	**-6/D**	**-6/N**	**-5/D**	**-5/N**	**-4/D**	**-4/N**	**-3/D**	**-3/N**	**-2/D**	**-2/N**	**-1/D**	**-1/N**	**0/D**	
Symptoms																
Runny Nose																
Sneezing																
Nasal Congestion																
Sore Throat																
Headache																
Chills/Fever																
Itchy/Watery/and or Red Eyes																
Total Score:																

Symptom Score Guide
0=Not Present
1=Mild, clearly present
2=Moderately severe, uncomfortable
3=Severe, interfering with sleep or activity

* **Day -7 = day run-in starts**
D = day time symptom score (~ 8 am – 10 pm)
N = night time symptom score (~10 pm – 8 am)

Appendix 3: Asthma Control Test

Asthma Control Test™

This survey was designed to help you describe your asthma and how your asthma affects how you feel and what you are able to do. To complete it, please mark an ☒ in the one box that best describes your answer.

1. **In the past 4 weeks, how much of the time did your asthma keep you from getting as much done at work, school or at home?**

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. **During the past 4 weeks, how often have you had shortness of breath?**

More than once a day	Once a day	3 to 6 times a week	Once or twice a week	Not at all
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. **During the past 4 weeks, how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?**

4 or more nights a week	2 to 3 nights a week	Once a week	Once or Twice	Not at all
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. **During the past 4 weeks, how often have you used your rescue inhaler or nebulizer medication (such as Albuterol, Ventolin®, Proventil®, Maxair® or Primatene Mist®)?**

3 or more times per day	1 or 2 times per day	2 or 3 times per week	Once a week or less	Not at all
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. **How would you rate your asthma control during the past 4 weeks?**

Not Controlled at all	Poorly Controlled	Somewhat Controlled	Well Controlled	Completely Controlled
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

To score the ACT

Each response to the 5 ACT questions has a point value from a 1 to 5 as shown on the form. To score the ACT, add up the point values for each response to all five questions.

If your total point value is 19 or below, your asthma may not be well-controlled. Be sure to talk to your healthcare professional about your asthma score.

Take this survey to your healthcare professional and talk about your asthma treatment plan.

Appendix 4: Outline for Screening Visits to Determine Eligibility for RV Challenges for Subjects Receiving Omalizumab or Placebo Prior to and During the Experimental Infection (all under the screening protocol IRB-HSR# 12656)

Screening subjects for this study was started under IRB HSR #12656 during the fall of 2012 and will be on-going until study completion. Screening to participate will require 2 visits and those who meet all of the criteria described below will be eligible for enrollment as study participants. The following screening procedures will be done:

Screening Visit 1: (Done at UVA for those subjects attending UVA or living in the Charlottesville area, or at VCU for those attending VCU or living in the Richmond area.)

- Signing the screening consent form
- Questionnaire ("Rhinovirus Infection and Asthma" screening questionnaire).
- Blood for total IgE and RV-16 Serology.
- Subject's weight (kg)

Screening Visit 2: (This visit will be competed in Charlottesville at the Elson Student Health Respiratory Research Unit, or the CRU at UVA)

- Skin testing
- Methacholine challenge
- FeNO
- Blood for HLA typing (needed for developing tetramers for subjects who qualify for participation), and CBC and differential cell count to document neutrophil counts and hemoglobin values to meet the enrollment criteria.

Note: The test results for neutralizing antibody to RV-16 and the CBC and differential cell count need to be documented within 6 weeks prior to the enrollment and will be repeated (in 5 to 10 ml of blood) for subjects who complete their screening more than 6 weeks before enrollment.

Appendix 5. Schedule of Events (*only for those on biweekly dosing of omalizumab/ placebo

	Visit 1	Visit 2* ONLY if receiving bi-monthly injections	Visit 3	Visit 4	Visit 5	Hotel Admission Rhinovirus Inocu-lation	Hotel Stay	Visit 6	Visit 7	Visit 8	Visit 9
	8 weeks before hotel stay	6 weeks before hotel stay	4 weeks before hotel stay	2 weeks before hotel stay	Within 2 days before Day 0	Day 0 Cold virus Inocu-lation	Post Inocu-lation Days 1 - 4	Post Inocu-lation Day 7	Post-Inocu-lation Day 14	Post-Inocu-lation Day 21	Post-Inocu-lation Day 28
Sign consent	X										
Randomization	X										
Urine Pregnancy Testing	X	X	X	X	X	X x	Day 4 only			X	
Urine LTE4					X		Day 4 only				
Injection of study drug	X	X*	X	X*	X				X*		
Nasal Wash	X		X	X	X	X	X	X	X	X	
Nasal lining fluid						X	Day 4 only		X		
Nasal scraping	X				X		Day 2 only	X		X	
Nasal mucous weights						X	X				
Spirometry	X		X	X	X	X	X	X	X	X	
Exhaled Nitric Oxide	X		X	X	X	X	X	X	X	X	
Blood for blood counts	X		X	X	X		X	X	X	X	
Blood for immune cells	X				X		Day 4 only			X	
Blood for antibody titers	X				X					X	
Completion of Diary Cards **	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	
ACT questionnaire	X		X			X					X
Impulse oscillometry	X				X		Day 4 only	X		X	
Methacholine Challenge	X				X		Day 4 only			X	

Rhinovirus -16 Inoculation* **						X					
Limited Physical Exam	X		X		X	X	X	X	X	X	

Appendix 5 Legend:

* As described in the protocol (Section 7.2), the dose and frequency (monthly, or bi-monthly) of omalizumab administered will be based on each subject's weight and total IgE level as recommended by the manufacturer. Because the inclusion criteria for this study require each subject to have a total IgE level ≥ 125 IU/ml, it is expected that most subjects will be receiving the study drug bi-monthly.

** Refer to Appendix 1 and Appendix 2

* * * Subjects will not be challenged with RV 16 if their FEV1 drops below 70 % predicted [in keeping with the exclusion criteria for this study (Section 4.2)], or if their FEV1 drops by more than 20% from their baseline value at the time of enrollment.

Note:

- AE's will be evaluated by the MO and SMC for all enrolled subjects 1) during the week prior to RV inoculation and 2) at the end of their participation in the study. All SAEs will evaluated by the NIAID MO as soon as the SAE CRF form or MedWatch form is received from the PI.

-All visits, except for the visits on the day of virus inoculation (day 0) and days 1 – 4 (during the hotel stay), and on day 7 will occur within a 24 hours window prior to or following the planned visit scheduled according to the protocol

- At each study visit a physical examination focused on the respiratory tract will be done. This exam will include an examination of the nose, throat, ears, lungs, and cervical adenopathy which may manifest changes during the RV cold.

- Tests to be conducted in nasal washes will include eosinophil cationic protein, cysteinyl leukotriene, Th2 and Th1 associated biomarkers (e.g. IFN-γ), innate Immune biomarkers (e.g. IL-8, IP-10, IL-1beta, IFN-α2)

- Blood will be also tested for eosinophil cationic protein along with CBC and differential count at each at all visits. Immune cell analyses will include CD4+ and CD8+ regulatory and effector T-cell subsets present in the circulation along with dendritic cell and basophil analyses.

- Diary cards, lung function (FEV1) monitors, albuterol inhalers with puff counters, and EpiPens (with instructions provided by research nurses) will be provided to each participant at enrollment.

- Injection of study drug is shown for those who will receive their injections every other week. Even though subjects will need to have total IgE levels ≥ 125 IU/ml to participate

in this study, it is possible that a small number of subjects will have a low enough weight to receive their injections monthly. These subjects will receive their injections on Visits number 1, 3, 5, and 8.
- Twenty-eight (28) days after inoculation with RV-16 (i.e., Visit 9), subjects will return to clinic to complete the ACT questionnaire (see Appendix 3), report any adverse events, and receive their payment for participation.

Appendix 6: Treatment Guidelines for Asthma Symptom Exacerbations

Treatment guidelines should symptom exacerbations occur during the study:

If a study participant experiences an increase in lower respiratory tract symptoms, or a decline in lung function, the following plan describes how subjects will be treated.

- For FEV1 value dropping between 20-29%20% from the baseline value not responding to albuterol, start on inhaled corticosteroids (Flovent 110 mcg./puff, 2 puffs bid) and discontinue when symptoms return to baseline.
- For FEV1 value dropping between 30- 49% from the baseline value not responding to albuterol, check oximetry and start on inhaled corticosteroid as above and 3 to 5 days of oral steroids (Prednisone 50 mg/day).
- For FEV1value dropping 50% or more from the baseline value. Transportation to the emergency room will be recommended and arranged. Individual stopping rules will apply as described in the Complete Clinical Protocol (Section 8.1.2). Further follow-up and management with the subject's primary care doctor; Dr. Heymann and his study team will continue to be involved and available for follow-up and evaluation 30 days afterward for any subject who requires oral steroids, a visit to the ER, or hospital admission.

These treatment plans are in keeping with recommendations from the *Guidelines for the Diagnosis and Management of Asthma* (16).

Note: Baseline FEV1 values apply to the FEV1 measurements established at the scheduled visit with 2 days of RV-16 inoculation. Also note that young adults rarely become febrile during a natural or experimental infection with rhinovirus. However, if subjects develop a temperature greater than or equal to 101 degrees F, Dr. Heymann, together with back-up from the allergy fellow on call will be contacted (paged) for evaluation.