CONSORTIUM NAME

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Clinical Response to Rhinovirus Challenge in Human Asthmatics

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IND Sponsor/Number: Monica Lawrence, M.D., University of Virginia / IND 15162

Study Drug Manufacturer/Provider: Monica Lawrence, M.D., University of Virginia

Principal Investigator-Larry Borish, MD
Professor of Medicine and Microbiology
University of Virginia Health Systems
P.O. Box 801355
Charlottesville, VA 22908-1355
Phone: (434) 243-6570
Fax: (434) 924-5779
E-mail: lb4m@virginia.edu

NIAID MEDICAL OFFICER -PETER GERGEN, MD MPH
Division of Allergy, Immunology and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane 6B58
Rockville, MD 20892
Phone: 240-627-3545
E-mail: pgergen@niaid.nih.gov

BIOSTATISTICIAN- JAMES T. PATRIE, MS
Senior Biostatistician
Department of Public Health Sciences
University of Virginia Health Sciences
Charlottesville, VA 22908
Phone: (434) 924-8576
Fax: (434) 243-5787
E-mail: jp4h@virginia.edu

INDEPENDENT MEDICAL MONITOR – W. GERALD TEAGUE
UNIVERSITY OF VIRGINIA HEALTH SYSTEMS
409 LANE RD, BLDG MR4, ROOM 2112, PC BOX 801349
CHARLOTTESVILLE, VIRGINIA 22908
TELEPHONE: (434) 243-4692
WGT2P@VIRGINIA.EDU

PROJECT MANAGER-SUSAN PERRY, RN
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane 6B55
Bethesda, MD 20892-9828, USA
Phone: 240 669-2865
E-mail: perrys@mail.nih.gov

REGULATORY OFFICER-JULIA GOLDSTEIN, MD
Office of Regulatory Affairs
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane
Bethesda, MD 20892-9828, USA
Phone: 240.669.5369
E-mail: goldsteinj@niaid.nih.gov

INVESTIGATOR SIGNATURE PAGE	
Protocol: U01-UVA-04	**Version/Date:** Version 11.0/ 8/04/2021
Site Principal Investigator: Larry Borish, MD	
Title: Clinical response to rhinovirus challenge in human asthmatics	
Study Sponsor: Monica Lawrence, M.D., University of Virginia	

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 a copy of the signature page sent to the NIAID.

After signature, please return a copy of this form by surface mail to:

DAIT Regulatory Management Center
Pharmaceutical Product Development
3900 Paramount Parkway
Morrisville, NC 27560

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

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

Larry Borish, MD
Site Principal Investigator (Print)

_____
Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Clinical response to rhinovirus challenge in human asthmatics
Short Title	Clinical response to RV challenge in human asthmatics
Clinical Phase	Phase I clinical trial
Number of Sites	1. University of Virginia Health System, Charlottesville, VA
IND Sponsor/Number	Monica Lawrence, M.D., University of Virginia / IND 15162
Study Objectives	We hypothesize that the immune responses generated *in the nose* of asthmatics underlie subsequent *systemic* modulation of the immune system, and that – in susceptible individuals (i.e., those with *pre-existing* asthma) – this modified nasal milieu is responsible for the asthma exacerbation. **Primary Objective(s)** 1. To determine whether RV increases expression of IL-25 transcripts by nasal epithelial cells in the asthmatic and AR but not control cohorts at the peak of infection (days 3 and 4). 2. To determine whether RV increases lower respiratory symptoms in the asthmatic but not AR and control cohorts. 3. To determine whether asthmatics and allergic rhinitics will demonstrate an increased severity of infection in comparison to control subjects. **Secondary Objective(s)** *1.* To determine whether asthmatic and AR cohorts demonstrate increased IL-25 transcript expression over the course of RV infection *2.* To determine whether asthmatic and AR cohorts demonstrate increased expression of mRNA transcripts of a type 2 cytokine-inducing profile (IL-33 and TSLP). *3.* To determine whether increased transcript expression of this type 2 cytokine-inducing profile can be corroborated as increased expression of protein. *4.* To determine whether RV infection in the asthma cohort is associated with increases in biomarkers of inflammation.

	5. To determine whether increased severity of RV infection in the asthma and AR cohorts will be associated with more symptoms. 6. To determine whether increased severity of RV infection in the asthma and AR cohorts is related to decreased innate immunity.
Study Design	Open label single center study in asthmatics as well as allergic rhinitic and healthy controls. All subjects will undergo GMP RV16 inoculation and responses will be compared between the 3 cohorts.
Primary Endpoint(s)	*1.* RV-induced expression of IL-25 mRNA transcripts in the asthma and AR cohorts versus healthy controls as determined in nasal scraping samples obtained on days 3 and 4 (compared to day 0) as determined by qPCR. *2.* RV-induced lower respiratory symptoms in the asthma cohort versus AR and healthy control as determined using modified Jackson scores (1) that will include cough, chest tightness, wheezing, and shortness of breath as well as via the asthma control test (ACT). 3. Severity of infection in the asthma and AR cohorts versus healthy controls as quantified by viral load present in nasal secretion samples as determined by qPCR performed on days 1, 2, 3, 4, 7.
Secondary Endpoint(s)	*1.* IL-25 transcript expression in nasal scraping samples obtained on days 1, 2, and 7 (as compared to day 0). *2.* Expression by nasal epithelial cells of a type 2 cytokine-inducing profile will be determined by also evaluating the time course of IL-33 and TSLP transcript expression by qPCR on nasal scraping samples obtained on days 0, 1, 2, 3, 4 and 7. *3.* Expression of this type 2 cytokine-inducing profile will be corroborated by evaluating IL-25, IL-33, and TSLP protein expression by EIA on nasal secretion samples obtained via immunosorbent sponge. *4.* Airway inflammation will be determined as increases in circulating absolute eosinophil counts, increased FeNO, and as changes in methacholine PD20. *5.* Severity of RV infection will be corroborated as increases in upper respiratory symptoms using modified Jackson scores (1).

	This scoring system includes sneezing, nasal discharge, nasal obstruction, sore throat, headache, malaise, and chills. 6. Severity of RV infection will be correlated with decreased innate immunity as assessed as reduced IFN-α, -ß, -λ, and IL-15 measured as qPCR of nasal scrapings and as enzyme immunoassays (EIAs) of nasal secretions (sponge-derived samples).
Accrual Objective	60 (20 each controls, AR, and asthma)
Study Duration	5 years to enroll, 8- 12 weeks for each participant to complete the study
Treatment Description	Rhinovirus (GMP RV16 HRV-16) inoculation
Inclusion Criteria	All subjects: 1. Subjects must be able to understand and provide written informed consent. 2. Age 18 to ≤40 years of age, any gender, any racial/ethnic origin 3. Female subjects of childbearing potential must have a negative pregnancy test upon study entry (day -7) and before each procedure involving pharmacologic interventions (days 0, 4, and 7). 4. Female (and male) subjects with reproductive potential, must agree to use FDA approved methods of birth control for the duration of the study such as, but not limited to, birth control pills, contraceptive foam, diaphragm, IUD, abstinence, or condoms. 5. Participants must be willing to comply with study procedures and requirements. 6. Negative test for serum neutralizing antibody to RV16 at enrollment visit (<1:8) (Visit 1). 7. Negative CoVID-19 test within 72 hrs of RV inoculation 8. Afebrile and negative CoVID screening questions on day of RV inoculation Allergic Rhinitis Subjects 9. Allergy as determined by ≥1 positive prick skin test (wheal ≥5 mm diameter and 3mm larger than the diluent control) to Virginia inhalant panel within 5 years, and a history of symptoms of sneezing, rhinorrhea, pruritus, nasal congestion,

	and/or allergic conjunctivitis on natural exposure to relevant allergens. 10. Negative methacholine challenge (less than 20% decline in FEV1 at ≤8mg/ml) within 1 year 11. FEV1 ≥70% predicted or FEV1/FVC ≥80%. 12. No history of wheezing with viral infection in the last 6 years, and no use of rescue inhalers or long-term controllers for asthma in the last 6 years. Allergic Asthmatic Subjects 13. Allergy as determined by ≥1 positive prick skin test (wheal ≥5 mm diameter and 3mm larger than the diluent control) to Virginia inhalant panel. Subjects are not required to have allergy symptoms at the time of study. Subjects will report history of symptoms of sneezing, rhinorrhea, pruritus, nasal congestion, and/or allergic conjunctivitis on natural exposure to relevant allergens. 14. Asthma determined by physician diagnosis and by a positive methacholine challenge (at least 20% fall in FEV_1 at a methacholine concentration of ≤16 mg/ml) at screening protocol visit before enrollment (obtained within the past year). 15. Asthma must be controlled as determined by ACT score ≥20 and normal lung function (FEV1≥80% predicted and FEV1/FVC ratio >0.65.
Exclusion Criteria (all subjects)	*1.* Positive test for serum neutralizing antibody to RV16 at enrollment visit (≥1:8) (Visit 1). 2. Upper airway modified Jackson criteria symptom scores ≥7 at time of inoculation. *3.* Chronic heart disease including bradycardia, lung diseases other than asthma, or other chronic illnesses including epilepsy, peptic ulcer disease, thyroid disease, urinary tract infection, vagotonia, autoimmune disease, primary or secondary immunodeficiency or any household contacts who are known to be immune deficient. Any medical conditions that could be adversely affected by the administration of cholinergic agent. *4.* Any use of LT modifiers, omalizumab, nasal corticosteroids within 4 weeks prior to Visit 1.

	5. Current use of ß-blockers or cholinesterase inhibitors (for myasthenia gravis) *6.* ß2-agonist use ≥4 days/week in any week or ≥2 nights during the month before Visit 1. *7.* Recent (within 3-yr) asthma exacerbation requiring urgent care visit (unless the treatment involved only the use of a bronchodilator), hospitalization, or oral CCS *8.* Intubation or management in the ICU for an asthma exacerbation ever. *9.* An upper or lower respiratory tract infection within 2 months prior to enrollment. *10.* Previous nasal or sinus surgery within the last 12 months *11.* >5 pack-year smoking history or any smoking within the past 6 mos. 12. Hemoglobin <11.5 g/dL for non-African American subjects or hemoglobin < 11.0 g/dL for African American subjects detected at Visit 1. 13. Laboratory values (other than hemoglobin and ANC) measured at Visit 1 that are considered to be of clinical relevance by the Investigator. *14.* Absolute neutrophil count (ANC) <1500 cells/mm^3 (or 1.5 K/µL) or absolute lymphocyte count (ALC) <800 cells/mm^3 detected at Visit 1. 15. Use of investigational drugs within 12 weeks of participation *16.* Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
Study Stopping Rules	The enrollment and dosing of RV-16 in the study may be suspended pending review by the NIAID DSMB and the University of Virginia IRB for the following reasons: • Any subject has a severe asthma exacerbation that requires treatment in the emergency room or hospitalization • ≥2 subjects experience severe asthma exacerbations requiring oral corticosteroids for ≥5 days, pending further expedited review.

	• Any subject treated for pneumonia • Any subject dies until expedited review is completed to determine whether the death might have resulted from any of the study procedures. • Complications post biopsy requiring an urgent care visit in ≥2 subjects. • *Any death that occurs in the study, which is possibly or definitely related to study treatment regimen.* • The occurrence of a Grade 3 or higher related SAE in 2 or more of the study participants who have received a study treatment.

Study Contacts: Participating Centers

SITE PRINCIPAL INVESTIGATOR

Larry Borish, MD
Professor of Medicine and
Microbiology
University of Virginia Health System
MR4 Bldg Rm 5041
409 Lane Rd.
Charlottesville, VA 22903
Phone: (434) 243-6570
Fax: (434) 924-5779
Email: lb4m@virginia.edu

SITE SUB- INVESTIGATOR

Peter Heymann, MD
Professor Emeritus of Pediatrics
University of Virginia Health System
MR4 Bldg Rm
Charlottesville, VA 22903
Phone: (434) 924-8523
Fax: (434) 924-5779
Email: pw5ha@virginia.edu

SITE SUB- INVESTIGATOR

Monica Lawrence, MD
Associate Professor of Medicine
University of Virginia Health System
MR4 Bldg Rm 5051
Charlottesville, VA 22903
Phone: (434) 243-6811
Fax: (434) 924-5779
Email: ml4nz@virginia.edu

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Glossary of Abbreviations

ACT	Asthma Control Test
AE	Adverse Event
AEC	Absolute Eosinophil Count
ANC	Absolute Neutrophil Count
AR	Allergic Rhinitis
B	Basophil
CBC	Complete Blood Count
CCS	Corticosteroids
CFR	Code of Federal Regulations
CI	Confidence Interval
CLRTS	Cumulative Lower Respiratory Tract Symptoms
CRC	Clinical Research Center
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CURTS	Cumulative Upper Respiratory Tract Symptoms
CysLT	Cysteinyl Leukotriene
DAIT	Division of Allergy, Immunology, and Transplantation
DSMB	Data Safety Monitoring Board
DPI	Days Post Infection
EC	Epithelial Cell
ECP	Eosinophil Cationic Protein
ED	Emergency Department
EIA	Enzyme immunoassay
Eo	Eosinophil
FDA	Food and Drug Administration
FENO	Fractional Exhaled Nitric Oxide
FEV1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
GM	Geometric Mean
GMP	Good Manufacturing Practice

IB	Investigator's Brochure
ICH	International Conference on Harmonization
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate Lymphoid Cell
IND	Investigational New Drug
IRB	Institutional Review Board
LABA	Long-Acting Beta Agonist
LAMA	Long-Acting Muscarinic Antagonist
LT	Leukotriene
MDI	Metered Dose Inhaler
MOP	Manual of Procedures
NB	Negative Binomial
NIAID	National Institute of Allergy and Infectious Diseases
PBMC	Peripheral Blood Mononuclear Cell
PD	Provocation Dose
Pg	Prostaglandin
PHI	Personal Health Identifiers
PI	Propidium Iodide
PI	University of Virginia Principal Investigator
qPCR	Quantitative Polymerase Chain Reaction
RV	Rhinovirus
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SOP	Standard Operating Procedure
TCID	Tissue Culture Infective Dose
TGF	Transforming Growth Factor
TSLP	Thymic Stromal Lymphopoietin
UVA	University of Virginia
VCAM	Vascular Cell Adhesion Molecule

1. Background and Rationale

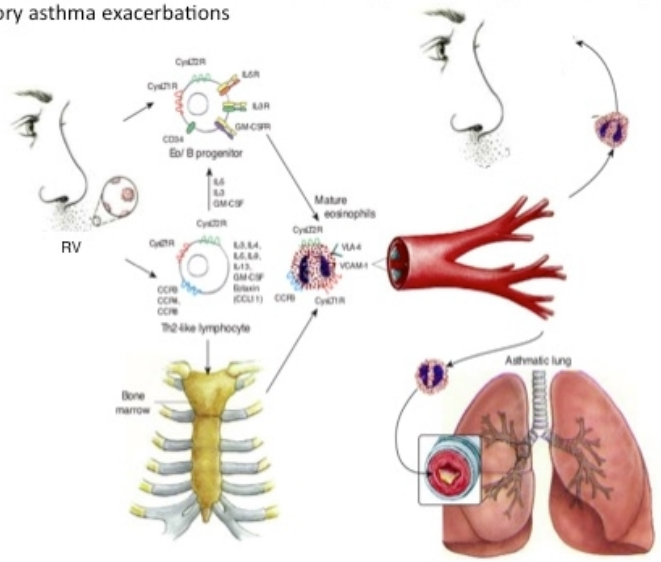
1.1. Background and Scientific Rationale

Determining the mechanisms driving asthma exacerbations caused by RV has remained elusive. It is clear from our studies and others (3-5) that most children and adolescents who experience RV-induced asthma exacerbations are allergic and the presence of high titers of serum IgE antibody to relevant (environmentally present) allergens significantly increases the risk of wheezing with RV. Thus, a surprising feature of RV asthma exacerbations is its tendency in temperate climates to primarily produce asthma exacerbations during particular seasons (September – with a subsidiary peak in May (6). This "September epidemic" has traditionally been ascribed to asthmatics returning to school but epidemiological studies do not support more than a modest seasonal increase in RV incidence in September (7) nor does this explain the surge in exacerbations in May. Our studies support an alternative hypothesis, namely that seasonal exacerbations of asthma reflect sensitization to bystander seasonal allergens. Thus, spring (May) RV-induced exacerbations will occur (and will only occur) in asthmatics sensitive to grass pollen, whereas autumn (September) exacerbations reflect sensitivity presumably to ragweed, dust mites, alternaria and other fall allergens (1). We were able to confirm these concepts in our Costa Rica studies (3). In this environment, where the primary allergen is perennial (dust mites) and there is no seasonality to either dust mite exposure nor – *as in North America* – is there a seasonality to RV infection, there was no seasonal predilection for RV to induce an asthma exacerbation. Even more compelling was the observation that dust mite sensitization was virtually always present in asthmatics who exacerbated to RV and that with sufficient sensitization, an exacerbation was virtually guaranteed (3). Our published data demonstrate evidence of a type 2 cytokine signature associated with RV infections in asthmatics. This is associated with both increased recruitment into nasal secretions of eosinophils and eosinophil-associated inflammatory mediators (ECP and CysLTs) (1). In our current studies, we were also struck by the ability of RV to modestly, *but significantly,* increase *total* IgE in asthmatics, suggestive of a robust Th2 (IL-4/IL-13) response impacting ongoing humoral immune responses. These results are consistent with published observations regarding increased expression of a type 2 cytokine signature in the airway in association with RV infection in asthma (8-10). Increased reactions to bystander allergens can in part reflect the loss of barrier function and their increased access to nasal lymphatics (11). Other ongoing studies by our group will address the influence of RV on adaptive immune responses to both RV and to bystander allergens, so this will not be the focus of the current proposal. However, central to the generation of such a type 2 cytokine response is the generation of an innate immune-driven milieu promoting Th2 immune deviation and expansion. It is increasingly recognized that such a milieu develops in large part from the immune responses of airway epithelial cells (EC) via their capacity to produce IL-25, IL-33, and TSLP (reviewed in (12)). These cytokines have important influences in driving Th2 immune deviation. However, more relevant to the innate immune response and the ***early*** development of an allergic inflammatory milieu (high in eosinophils, ECP, CysLTs, etc.) is the ability of these cytokines to induce secretion of Th2-associated cytokines from mast cells (IL-33, TSLP) and IL-5 and IL-13 from type 2 innate lymphoid (ILC2) cells (IL-25, IL-33)(13-16). Our primary aim will explore the capacity of EC from asthmatics to disproportionately produce Th2-promoting cytokines in response to RV infection. We will then compare levels of these mediators collected in nasal washes and from EC-derived RNA after RV infection of asthmatic subjects and healthy and allergic rhinitis (AR) control individuals. Our preliminary data support the concept that in cultured nasal epithelial cells derived from asthmatics, IL-25, IL-33 and TSLP are produced after RV infection. And, insofar as these assays require multi-generational cell expansion *in vitro*, this predisposition likely reflects prior *in vivo* epigenetic programming, which we plan to confirm experimentally in an exploratory aim.

In addition to promoting a Th2-like inflammatory milieu, a complementary synergistic mechanism is based on the notion that asthmatics have a defective innate immune response to the virus. Studies of *ex vivo* infected ***bronchial*** epithelial

cells (EC) confirmed decreased type I (IFN-ß) and type III (IFN-λ) interferon expression in asthmatics (17-19), as well as deficient IL-15 (20). IL-15 is a type I IFN-induced cytokine that exerts potent anti-viral effects via activation of NK and $CD8^+$ T cells. These investigators posit that these deficiencies lead to increased virus load in the *lower airway* and prolonged symptoms during experimental RV infections. This is also consistent with the realization that with the evolution of allergic inflammation, asthmatics develop enhanced susceptibility to many viral respiratory infections including increased susceptibility to severe RSV and influenza infections. This enhanced susceptibility largely reflects the biological activities of IL-4 and IL-13 in reducing expression of antimicrobial peptides (21-23) along with disruption of epithelial barrier function (11, 24). But also that this increased susceptibility persists even after the disease has been in longstanding remission, demonstrating that these are a combination of genetic (25) and – especially – *epigenetic* changes that can be interpreted as having permanently altered the differentiation state of the epithelium. It should be noted that other studies (26-28) failed to confirm more robust RV replication in asthmatics' *lungs* following RV infection compared to non-asthmatics. Likewise, the extent, or indeed *whether* RV even needs to infect the lower airways to drive an asthma exacerbation remains quite controversial. While perhaps contributing to the severity of the asthma exacerbation, it is our contention that *lower* airway infection is *not* essential for asthma exacerbations to occur. Analogously, it is understood that upper airway inflammation (e.g., a nasal allergen challenge) exacerbates lower airway inflammation (i.e., asthma) in the *absence* of direct inoculation of the lower airway (29, 30). We conceptualize (figure 1) that RV infection in allergic asthmatics exacerbates an allergic inflammatory process with local activation of Th2-like cells, but also numerous innate cells including eosinophil-basophil (Eo/B) progenitors, innate lymphoid type 2 cells (ILC2s), eosinophils and others. Either hormonally or, more likely, via transit of these cells to the marrow, this drives enhanced hematopoiesis of eosinophils, basophils, mast cell precursors, neutrophils and other cells central to this inflammatory process. These cells are selectively recruited back into the sinonasal tissue, reflecting local expression by endothelium of adhesion molecules (e.g., VCAM-1) and numerous relevant chemotaxins (eotaxins, RANTES, CysLTs, etc.). However, in those individuals with pre-existing asthma – and *only* those individuals with *pre-existing* asthma – these same addressins and chemotaxins are expressed in the lower airway and thus a surge in inflammation will concomitantly occur in the lung. Such a model is consistent with published studies showing a spike in G-CSF expression, increased neutrophilia, and a *later* influx of neutrophils into the lung after experimental RV infection, and also with our studies showing similar surges in circulating eosinophilia after RV inoculation (31, 32). We therefore feel strongly that it is the innate response to RV-infected *nasal* epithelium that is the relevant target of inquiry. It is our contention that AR subjects will behave *identically* to asthmatics, however, in their case, the absence of relevant addressins and chemotaxins in their lungs (and the functioning immune suppressive tendencies of the healthy lung) precludes development of a lower airway response. A prediction of this model is that both asthmatic and AR cohorts will display increased circulating mediators of inflammation, but that only in asthmatics will this translate to increased lower airway inflammation. This model will be validated by having an AR control cohort, which should develop the *systemic (humoral)* inflammatory manifestations without any lower airway changes. But even if lower airway infection is, in fact, what is required to drive asthma

Figure 1: Humoral Mechanisms of RV-induced upper respiratory rhinitis driving lower respiratory asthma exacerbations



exacerbations, the designed studies remain valuable by using nasal EC as a surrogate for the programmed response to RV infection of lower airway (bronchial) EC.

1.2. Rationale for Selection of Investigational Product or Intervention

Acute exacerbations are a hallmark of urgent care visits and hospitalizations for asthma, interfere with school and work productivity and as such drive much of the morbidity (and, presumably, all of the mortality) associated with asthma (33). Equally important, the presence of frequent exacerbations defines an asthma phenotype that is most associated with progressive loss of lung function (34, 35). Among children and adolescents, 80 to 85% of asthma exacerbations are associated with upper respiratory viral infections (36, 37) and of these, RV consistently accounts for ~60-70% of the viruses identified (6, 7, 38-43). For example, in our studies, viral infections were identified in 61% of children aged 3-18 years hospitalized with an asthma exacerbation and RV comprised 77% of all positive tests (44). And even into late adulthood, RV remains responsible for 11% of ED visits for acute respiratory illnesses (45). For these reasons, RV will be the agent investigated in the current studies, to safely and prospectively evaluate the mechanisms that lead to asthma exacerbations, and with the potential for identifying future therapeutic interventions. It should be noted, however, that RV infections are common and most do not lead to exacerbations ((46) and unpublished data) and, similarly, asthma exacerbations are not a frequent response to experimental RV challenges, including in our published studies (1, 47). Determining the underlying mechanisms for asthma exacerbations caused by RV has remained elusive and is central to developing interventions that address the associated morbidity. The experimental RV challenge model will be used to test the hypothesis that in asthmatics, nasal RV infection will promote a type 2 inflammatory cytokine milieu. In addition, we hypothesize that in asthmatics these infections will also prove to be more severe. The strain of RV that will be used (RV-16) is a pool provided by Dr. Monica Lawrence's laboratory at the University of Virginia. This pool has been extensively safety tested and has been cleared for experimental challenge studies by the FDA (IND# 15162). 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 and control subjects. Dr. Lawrence, 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. In addition, this pool of RV has undergone extensive clinical trials using the experimental RV challenge model to evaluate subjects at the University of Virginia. Using the Jackson criteria for scoring cold symptoms (48, 49), infection rate and total symptom scores are comparable to our previous experience with other rhinovirus challenge pools (both the RV-16 used in our initial rhinovirus challenges and earlier RV-39 challenges conducted by Dr. Ron Turner) with the gratifying exception of a higher than expected *percentage* of subjects who developed cold symptoms in the infected volunteers. The *severity* of cold symptoms, however, were similar to the severity of symptoms 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 (47, 50, 51). The dose of RV to be used is in this study (approximately 300 $TCID_{50}$) is similar to that used in our previous challenges (1). This inoculum is likely to be higher than the dose of RV associated with a natural infection, although information regarding how low an RV inoculum is sufficient to produce a cold is not known.

Preclinical Experience

N/A

1.3. Clinical Studies

Approximately 2000 volunteers have been challenged in studies conducted by Dr. Borish, Dr. Turner, Dr. Lawrence, Dr. Heymann, and our other colleagues at UVA 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 90-95% 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 (52, 53) 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 (54). Approximately $1/3^{rd}$ 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 still be recovered by culture in 20% of subjects on day 18 after challenge, but no virus shedding is detected by day 20 (55). 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 <1000 cells/mm^3.

In our previous experimental challenge studies in asthmatics, and those of others, lower respiratory tract symptoms (mild wheeze, cough, dyspnea and/or chest tightness) have been reported in subjects with mild asthma (1, 56). Our previous studies have also shown that moderate to severe asthma exacerbations caused by naturally occurring infections with RV occur predominantly in children, but although less common in asthmatic adults (56), even into late adulthood, RV remains responsible for 11% of ED visits for acute respiratory illnesses (45). 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 ≥125 IU/ml) and do our challenges outside of periods of increased relevant allergen exposure (e.g. in the spring or fall months in Virginia) without risks for causing an asthma exacerbation requiring hospital care (1). 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 ED 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.

The total dose of RV-16 used in this study is expected to result in development of lower airway symptoms but not in either of the control cohorts, but 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 approximately 300TCID50 of rhinovirus 16 where we used a modification of the Jackson criteria to score daily upper and lower respiratory tract symptoms (1). Upper and lower respiratory scores were summarized for each individual by computing cumulative scores (CURTS and CLTRS) 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, $P = 0.2$). 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. During the 21 days 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.

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 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 (57). For this reason, the experimental challenge study described below is a much better study design, because subjects will either be seronegative, or have low titers (<1:8) of neutralizing antibody to the strain of RV (i.e. RV-16) used for inoculation.

2. Study Hypotheses/Objectives

2.1. Hypotheses

We hypothesize that the immune responses generated *in the nose* of asthmatics underlie subsequent *systemic* modulation of the immune system, and that – in susceptible individuals (i.e., those with *pre-existing* asthma) – this modified nasal milieu is responsible for the asthma exacerbation.

2.2. Primary Objective(s)

1. To determine whether RV increases expression of IL-25 transcripts by nasal epithelial cells in the asthma and AR but not control cohorts at the peak of infection (days 3 and 4).
2. To determine whether RV increases lower respiratory symptoms in the asthma but not AR and control cohorts.
3. To determine whether asthmatics and allergic rhinitics will demonstrate an increased severity of infection in comparison to control subjects.

2.2. Secondary Objective(s)

1. To determine whether asthmatic and AR cohorts demonstrate increased IL-25 transcript expression over the course of RV infection

2. To determine whether asthmatic and AR cohorts demonstrate increased expression of mRNA transcripts of a type 2 cytokine-inducing profile (IL-33 and TSLP).

3. To determine whether increased transcript expression of this type 2 cytokine-inducing profile can be corroborated as increased expression of protein.
4. To determine whether RV infection in the asthma cohort is associated with increases in biomarkers of inflammation.
5. To determine whether increased severity of RV infection in the asthma and AR cohorts will be associated with more symptoms.
6. To determine whether increased severity of RV infection in the asthma and AR cohorts is related to decreased innate immunity.

3. Study Design

3.1. Description of Study Design

A total of 60 subjects will be enrolled in this open label challenge study over a period of 48-54 months (September 2016 – March 2021). These will include 20 subjects in each of 3 cohorts: healthy controls, allergic rhinitis controls, and asthmatics. At Visit 1 subjects will have baseline assessments of lung function and blood work. Viral inoculation will be performed on day 0 and assessments of cold and allergy symptoms, nasal viral titers, nasal mediators, lung function, and FeNO will be obtained on days 0 and days 1, 2, 3, 4, and 7 post-infection (dpi). In addition, methacholine challenges (days -7 and 4) will be performed. Finally, nasal biopsies will be performed on day 4 to assess extent of viral infection and nature of cell death (apoptosis v. necrosis). Each subject will complete the study at day 14.

Table I: Study Outline***

Visit #:	1	2	3	4	5	6	7	8	9
	Day -7	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14**	Day 21
Symptom Assessment	X	X	X	X	X	X	X	X	X
Negative CoVID19 screen (afebrile/preserved sense of smell)	X	X	X	X	X	X	X	X	X
Pregnancy Test	X	X				X	X		
Viral antibody serology/CBC	X								
Virus inoculation		X							
Viral titers		X	X	X	X	X	X		
FEV1/ FeNO	X	X	X	X	X	X	X	X	
Nasal Scraping		X	X	X	X	X	X		
Nasal Washes / Sponge		X	X	X	X	X	X		
Nasal Biopsy						X			
Blood for Th immune deviation / AEC	X					X			
Methacholine Challenge	X					X			

** If subject is still symptomatic at day 14 they will be contacted at day 21 with the possibility of an unscheduled visit. A Day 21 in-person visit will be scheduled if ANC or ALC falls below 500/µL, and does not return to baseline following the Day 14 blood draw. If disease activity increases after day 7 or other concerns arise either between visits 6 and 7 or after visit 8, participants will be instructed to contact study personnel and may be asked to return to the study site for an "unscheduled" visit.

*****CoVID-19 mitigation plan**. As per UVA requirements for all clinical research, participants will be screened for the presence of fever and symptoms of CoVID-19 at each clinic visit. These guidelines also require all research subjects to come unaccompanied to all clinic visits and wear masks at all times (except when access to nasal samples is required). Research personnel are also required to wear masks, gloves, and eye protection. Additionally, subjects for this study will be required to have a negative CoVID19 test within 72 hrs of the RV inoculation. Subjects are *expected* to develop mild cough and possibly minimal shortness of breath as a consequence of the RV infection. Any subject who develops fever, anosmia, or concerning symptoms of cough or shortness of breath will undergo immediate testing for CoVID-19 and will remain in isolation until the results are available.

For 1 week after inoculation, subjects will be expected to self-quarantine as much as feasible. Subjects will still be permitted to attend classes, places of employment, dining facilities, and other essential activities during this period. However, participants should not engage in any non-essential social activities and will be required to wear masks (except while eating) and to socially distance whenever away from their residence. For volunteers who do not live alone, they will be, as much as possible, to isolate themselves from household contacts for the 7 days after inoculation

RV challenges will only be performed at times of minimal CoVID prevalence in Albemarle County. Specifically, we will require a test positive rate at the University of Virginia to be ≤5% in the week prior to any RV challenge. Additionally, daily cases in Charlottesville/Albemarle County will be <50/100,000 **and** this incidence will be either decelerating or stable (not increasing).

Primary Outcomes

1. RV-induced expression of IL-25 mRNA transcripts in the asthma and AR cohorts versus healthy controls as determined in nasal scraping samples obtained on days 3 and 4 (compared to day 0) as determined by qPCR.
2. RV-induced lower respiratory symptoms in the asthma cohort versus AR and healthy control as determined using modified Jackson scores (1) that will include cough, chest tightness, wheezing, and shortness of breath as well as via the asthma control test (ACT).
3. Severity of infection in the asthma and AR cohorts versus healthy controls as quantified by viral load present in nasal secretion samples as determined by qPCR performed on days 1, 2, 3, 4, 7

3.2. Secondary Outcomes

1. IL-25 transcript expression in nasal scraping samples obtained on days 1, 2, and 7 (as compared to day 0).
2. Expression by nasal epithelial cells of a type 2 cytokine-inducing profile will be determined by also evaluating the time course of IL-33 and TSLP transcript expression by qPCR on nasal scraping samples obtained on days 0, 1, 2, 3, 4 and 7.
3. Expression of this type 2 cytokine-inducing profile will be corroborated by evaluating IL-25, IL-33, and TSLP protein expression by EIA on nasal secretion samples obtained via immunosorbent sponge.
4. Airway inflammation will be determined as increases in circulating absolute eosinophil counts, increased FeNO, and as changes in methacholine PD20.
5. Severity of RV infection will be corroborated as increases in upper respiratory symptoms using modified Jackson scores (1). This scoring system includes sneezing, nasal discharge, nasal obstruction, sore throat, headache, malaise, and chills.
6. Severity of RV infection will be correlated with decreased innate immunity as assessed as reduced IFN-α, -ß, -λ, and IL-15 measured as qPCR of nasal scrapings and as enzyme immunoassays (EIAs) of nasal secretions (sponge-derived samples).

3.3. Exploratory Outcomes

1. Commercial EIAs for IL-33 and TSLP have been problematic and, additionally, IL-33 needs to be activated and, therefore, measuring full length IL-33 (or IL-33 transcripts) will not necessarily reflect biological activity. We therefore propose to complement these assays with functional studies demonstrating the ability of nasal secretions to support Th2 immune deviation in an ongoing immune response. For these studies we will investigate a non-specific pan-activating T cell signal (αCD3/ αCD28 beads) and a universal antigen (tetanus) that consistently expand both Th1 and Th2 lymphocytes. These studies will demonstrate a Th2 immune deviating "milieu" but cannot address the specific cytokine responsible. However, these approaches permit the use of individual or combinations of neutralizing antibodies (and/or in the case of IL-33, blocking anti-ST2 antibody) that will allow us to identify the specific cytokine (or combinations) driving Th2 immune deviation.
2. Epigenetic Modification of the IL-25, IL-33, and TSLP genes will be assessed on nasal epithelial cell at baseline (day -28) using bisulphite modification/methylation-specific PCR and changes in histone acetylation patterns.
3. Nasal biopsies will be performed at day 4 dpi. Most (>90%) virions remain intracellular and, as such, nasal secretions do not accurately reflect the extent of viral infection. More importantly, nasal washes do not accurately sample the primary site of infection (the adenoids). Nor do washes give information regarding the diffuseness or associated cytopathological response of the infection, specifically whether cell death is apoptotic (and, as such, anti-inflammatory with IL-10, TGF-ß, and PgE2 release) or necrotic (and, as such, pro-inflammatory with IL33 release). For these reasons, a key component of the current studies will be to assess these parameters via nasal biopsies. These studies will be used to explore: 1. the extent of viral infection – i.e. whether this occurs diffusely throughout the nares or remains focally within the adenoids; 2. the comprehensiveness of epithelial infection at each site 3. the presence and extent of infection-associated cell death and 4. whether this represents cytopathic (necrotic) or apoptotic cell death.
4. We will also determine whether RV infection in the asthma cohort is associated with increased use of rescue inhaler and decreased lung function. Use of albuterol metered dose inhaler will be assessed by diary and decreased FEV1 monitored by a Microlife hand held monitor

3.4. Stratification, Randomization, and Blinding/Masking

N/A. This is an open label study of a single RV inoculation. Data will be compared between healthy controls, allergic rhinitic controls, and asthmatic subjects.

3.4.1. Procedure for Unblinding/Unmasking

N/A. Study is not blinded.

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

Asthmatics will be studied as the relevant target for RV-induced asthma exacerbations and compared to control and AR subjects to identify the basis for their unique sensitivity. We will study mild asthmatics who are well-controlled with or without long-term controllers and low risk insofar as they have no recent (3-year) history of exacerbations to mitigate the risk of a severe asthma exacerbation during the study. It is our contention that it is the innate response to RV-infected *nasal* epithelium that is the relevant target of inquiry and, as such**, that AR subjects will behave *identically* to asthmatics,** however, in their case, the absence of relevant addressins and chemotaxins in their lungs precludes development of a lower airway response. We will therefore also enroll AR subjects to validate this concept that both asthmatic and AR cohorts will display increased circulating mediators of inflammation, but that only in asthmatics will

this translate to increased lower airway inflammation. Results will be evaluated in comparison to a non-allergic non-asthmatic control cohort and we will consider the potential impact of asthma long-term controllers regarding modulation of measures of airway mediators and markers of inflammation.

4.2. Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants. Participants in this trial will have participated in our separate (IRB protocol #12656 or 19512) screening protocol performed at day -28. Results obtained from the screening protocol will used to initially determine eligibility as described below.

All subjects:

1. Subjects must be able to understand and provide written informed consent.
2. Age 18 to ≤40 years of age, any gender, any racial/ethnic origin
3. Female subjects of childbearing potential must have a negative pregnancy test upon study entry (day -7) and before each procedure involving pharmacologic interventions (days 0, 4, and 7).
4. Female (and male) subjects with reproductive potential, must agree to use FDA approved methods of birth control for the duration of the study such as, but not limited to, birth control pills, contraceptive foam, diaphragm, IUD, abstinence, or condoms.
5. Participants must be willing to comply with study procedures and requirements.
6. Negative test for serum neutralizing antibody to RV16 at enrollment visit (<1:8) (Visit 1).
7. Negative CoVID-19 test within 72 hrs of RV inoculation
8. Afebrile and negative CoVID screening questions on day of RV inoculation

Allergic Rhinitis Subjects:

1. Allergy as determined by ≥1 positive prick skin test (wheal ≥5 mm diameter and 3mm larger than the diluent control) to Virginia inhalant panel within 5 years (done as part of screening protocol), and a history of symptoms of sneezing, rhinorrhea, pruritus, nasal congestion, and/or allergic conjunctivitis on natural exposure to relevant allergens.
2. Negative methacholine challenge (less than 20% decline in FEV1 at ≤8mg/ml) within 1 year
3. FEV1 ≥70% predicted or FEV1/FVC ≥80%.
4. No history of wheezing with viral infection in the last 6 years, and no use of rescue inhalers or long-term controllers for asthma in the last 6 years.

Allergic Asthmatic Subjects:

1. Allergy as determined by ≥1 positive prick skin test (wheal ≥5 mm diameter and 3mm larger than the diluent control) to Virginia inhalant panel with 5 years (done as part of screening protocol). Subjects are not required to have allergy symptoms at the time of study. Subjects will report history of symptoms of sneezing, rhinorrhea, pruritus, nasal congestion, and/or allergic conjunctivitis on natural exposure to relevant allergens.
2. Asthma determined by physician diagnosis and by a positive methacholine challenge (at least 20% fall in FEV_1 at a methacholine concentration of ≤16 mg/ml) at screening protocol visit before enrollment (obtained within the past year).
3. Asthma must be controlled as determined by ACT score ≥20 at Visit 1 and normal lung function (FEV1≥80% predicted and FEV1/FVC ratio >0.65.

4.3. Exclusion Criteria (all subjects)

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

1. Positive test for serum neutralizing antibody to RV16 at enrollment visit (≥1:8)(Visit 1).

2. Upper airway modified Jackson criteria symptom scores ≥7 at time of inoculation.
3. Chronic heart disease including bradycardia, lung diseases other than asthma, or other chronic illnesses including epilepsy, peptic ulcer disease, thyroid disease, urinary tract obstruction, vagotonia, autoimmune disease, primary or secondary immunodeficiency or any household contacts who are known to be immune deficient. Any medical conditions that could be adversely affected by the administration of cholinergic agent.
4. Any use of nasal corticosteroids, LT modifiers, omalizumab, within 4 weeks prior to Visit 1.
5. Current use of ß-blockers or cholinesterase inhibitors (for myasthenia gravis)
6. ß2-agonist use ≥4 days/week in any week or ≥2 nights during the month before Visit 1.
7. Recent (within 3-yr) asthma exacerbation requiring urgent care visit (unless the treatment involved only the use of a bronchodilator), hospitalization, or oral CCS
8. Intubation or management in the ICU for an asthma exacerbation ever.
9. An upper or lower respiratory tract infection within 2 months prior to enrollment.
10. Previous nasal or sinus surgery within the last 12 months
11. >5 pack-year smoking history or any smoking within the past 6 mos.
12. Hemoglobin <11.5 g/dL for non-African American subjects or hemoglobin < 11.0 g/dL for African American subjects detected at Visit 1.
13. Laboratory values (other than hemoglobin and ANC) measured at Visit 1 that are considered to be of clinical relevance by the Investigator.
14. Absolute neutrophil count (ANC) <1500 cells/mm^3 (or 1.5 K/µL) or absolute lymphocyte count (ALC) <800 cells/mm^3 detected at Visit 1.
15. Use of investigational drugs within 12 weeks of participation
16. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

4.4. Selection of Clinical Sites/Labs

N/A

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product or Intervention

Approximately 2000 volunteers have been challenged in studies conducted by Dr. Turner, Dr. Lawrence, Dr. Borish, 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 approximately 300 $TCID_{50}$ of RV produces infection in 90 to 95% 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 (52, 53). 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 (55). 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/mm^3. Should any subject develop an ANC or ALC below 500/µL on Day 4, a repeat CBC will be done on Day 14 to ensure that the value has returned to baseline or into the normal range, whichever is lower. If the value has not recovered at on Day 14, another CBC will be completed on Day 21. If the value remains low following the Day 21 draw, the subject will be referred to his or her regular healthcare provider for follow-up.

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 addition, RV challenges have also been performed on mild/moderate persistent asthmatics who are very well controlled on long-term controllers (inhaled corticosteroids with or without long-acting ß-agonists) without untoward reactions.(58) In our 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 (47). 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 (1). 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., (8, 31)).

All subjects in the proposed study will be inoculated with RV-16 and monitored daily for 4 days following inoculation in the clinical research center at UVA hospital and again on day 7 dpi. 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. In addition, monitoring each morning will include a physical examination focused on the respiratory tract, 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. 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. The inclusion of mild-/moderate-persistent asthmatics who are very well controlled on inhaled CCS could lead to more severe exacerbations. However, this has not been the experience of other research groups enrolling this population (58). Furthermore, as noted, the average American has 4 respiratory infections/yr and we would argue that as none of these subjects will have had even a modest asthma exacerbation in the previous 3 years (over which time they will on average have had 12 RV infections) we do not feel this modification adds an untoward risk to the study. And that the current safety monitoring criteria will continue to suffice.

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.

5.2. Risks of Investigational Product or Intervention cited in Medical Literature

Recently, in European studies, challenges with RV-16 were well-tolerated by subjects with moderately severe asthma who were treated with inhaled corticosteroids (9, 59) . However, asthmatic subjects in those studies were using inhaled steroids at the time of inoculation, which would interfere with our goals of judging the effects of RV infection on lower respiratory tract symptoms and bio-markers of inflammation.

5.3. Risks of Other Protocol Specified Medications

Albuterol: Risks of albuterol used to treat RV-induced symptoms or to reverse symptoms induced by methacholine and less likely spirometry/FeNO include palpitations and tremor.

Oral corticosteroids: Short courses of oral CCS (3-5 days) may be indicated to treat more serious asthma exacerbations. Short-term administration of prednisone is associated with appetite stimulation, weight gain, hyperglycemia, transient increases in blood pressure, fluid retention, stomach upset, and agitation/insomnia.

Nasal decongestant/nasal analgesic. Subjects will have a nasal decongestant (oxymetazoline) and analgesic (lidocaine 4%) sprayed into their nose prior to the nasal biopsy. Oxymetazoline will also be given to subjects with directions for use if needed following the procedure to treat mild delayed bleeding. In addition, biopsy sites may be injected with additional lidocaine (1%) and epinephrine (1:100,000). There are no side effects to topical application of oxymetazoline. Lidocaine at very high doses can produce cardiac arrhythmias, however, there is no risk for this occurring at the doses that will be used for this study.

5.4. Risks of Study Procedures

Nasal lavage: may be associated with some discomfort due to <10 second breath hold and the feeling of fluid in the nares. A potential complication is acute bacterial sinusitis (sinus infection) although this has never occurred in thousands of procedures done by us.

Light nasal scraping: An additional nasal specimen will be obtained by using an ASI Rhino-Pro® nasal mucosal curette plastic device to do a light scrapping along the inferior turbinate of each nostril to collect epithelial cells. The nasal scrapings will produce local discomfort. It is possible that nasal scraping may cause temporary epistaxis, which would be treated with local pressure for several minutes.

Nasal lining fluid: An additional nasal specimen will be obtained by applying a small piece (1 X 1 cm) of sterile gauze to inferior turbinate for 4 to 5 minutes in each nostril to collect a sample of epithelial cell lining fluid (about 0.2 ml). Other than local discomfort no complications are known to occur with this procedure.

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 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. This may be associated with mild shortness of breath, cough, chest tightness, wheezing, or chest soreness. Most patients do not have any symptoms. 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. The methacholine challenge will more likely produce mild shortness of breath, cough, chest tightness, and wheezing. These effects will last only a few minutes and will disappear following the inhalation of a bronchodilator.

Nasal biopsy**.** Risks of nasal biopsy include local discomfort and bleeding. Since the biopsies will be performed under direct visualization hemostasis can be readily achieved with application of pressure, topical oxymetazoline or application of silver nitrate cautery. Vasovagal events are expected in a small number of subjects; therefore, subjects will be closely monitored for such symptoms during the biopsy procedure. If signs of a vasovagal episode are present, the chair will put in a reclining position until the subject no longer reports symptoms or demonstrates signs consistent with low blood pressure. There is a theoretical risk of infection although in our experience this has never occurred. To assess the degree of post-procedure signs and symptoms the first 5 number subjects will not undergo the biopsy until the completion of the nasal biopsy in the prior participant.

Silver Nitrate Cautery: Lidocaine will have been applied prior to the cauterization; however, once the anesthetic wears off, subjects may experience tenderness and discomfort for a few days as well as a runny nose for up to a week after the treatment. Silver nitrate cautery also carries a rare risk of infection.

5.5. Potential Benefits

Each subject enrolled will be aware that the results are likely to provide new insights into the mechanism and eventually treatment and ongoing management of asthma triggered by RV. The benefits of participating in these studies are likely to outweigh any risk from the experimental RV challenges, which have been performed safely at our institution for over 20 years without any serious untoward events.

6. Investigational Agent /Device/Intervention

6.1. Investigational Agents/Devices/Interventions

6.1.1. Investigational Agent #1

RV-16 (IND #15162)

6.1.1.1 **Formulation, Packaging, and Labeling**

The strain of RV that will be used is a pool of RV (strain 16) that will be provided by Dr. Monica Lawrence's laboratory at the University of Virginia (UVA). Dr. Lawrence, who is the IND-holder for this this study, has collaborated with Johnson & Johnson, Inc. to produce this pool of RV under GMP conditions.

A single batch has been manufactured. HRV-16 will be supplied in gamma irradiated polypropylene cryovials containing clarified tissue culture lysate (Eagle's Modified Essential Medium containing 10% [8-12% acceptance criteria] gamma irradiated Fetal Bovine Serum (FBS) derived from animals from a Bovine Spongiform Encephalopathy (BSE) free country [Australia] and certified; final pH 6.5 to 8.5) which has been passed through a 0.2 µm filter, filled into gamma-irradiated polypropylene cryovials, snap frozen, and stored at -60°C or below.

Manufacturer: Monica Lawrence, M.D. University of Virginia School of Medicine. P.O. Box 801355. MR4 Bldg Rm 5051, 409a Lane Rd. Charlottesville, VA 22908

The final viral concentration of the cGMP safety tested HRV16 pool is significantly higher than the dose generally used to induce experimental rhinovirus infections.

In advance of a planned inoculation, designated staff at Dr. Lawrence's laboratory will prepare in a laminar flow hood a single-use vial containing approximately 300 $TCID_{50}$ in 1 mL of Lactated Ringer's solution for human use, using aseptic techniques as per the lab's SOP. The actual challenge dose of the virus will be determined by back titration thereby allowing the dilution to be adjusted as necessary to maintain the planned challenge titer should loss of titer occur during storage. The label for the pre-titered vial dispensed for this study label will include at least the following information:

- RV-16 challenge inoculum dose
- Protocol number
- Investigational use statement
- Date and time prepared

Designated study staff will receive the challenge virus dose which will be transported to the Allergy Research Lab on wet ice using an insulated, closed container with a biohazard label. The vial of inoculum is transferred to a -80° freezer and stored until the day of inoculation. It is then thawed as per the SOP. Once thawed the inoculum must be used promptly (within 4 hours) to insure proper dosage potency, although there is evidence that the virus remains viable, and TCID50 stable, for extended periods when stored at 4°C.

6.1.1.2 Dosage, Preparation, and Administration

For virus inoculation, each patient will be inoculated with approximately 300 $TCID_{50}$ after obtaining the pre-virus inoculation nasal wash. Dose selection is described in Section 1.2 (Rationale for Selection of Investigational Product or Intervention)

Approximately 75 $TCID_{50}$/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 for a total of 1 ml.

6.2. Drug Accountability

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

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

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

6.3. Assessment of Participant Compliance with Investigational Agent

N/A (single administration under directly-supervised conditions).

6.4. Toxicity Prevention and Management

Toxicity is prevented by selecting well-controlled, mild asthmatics unlikely to experience a severe exacerbation, by having medications on site and at home for management of symptoms, and by daily study visits to the peak of the viral illness.

6.5. Premature Discontinuation of Investigational Agent

N/A (only a single dosing of the RV will be performed on any subject). No subject will be given more than one dose of the product.

7. Other Medications

7.1. Concomitant Medications

7.1.1. Protocol-mandated

Non investigational drugs that will be available at the clinical site include: Methacholine, oral corticosteroids (prednisone), Albuterol (MDI and nebulizer), and oxymetazoline.

Other permitted concomitant medications

Albuterol MDI 2 puffs q4h prn.

7.2. Prophylactic Medications

N/A

7.3. Prohibited Medications

Topical (nasal or inhaled) corticosteroids (CCS), oral corticosteroids, leukotriene modifiers, antihistamines, omalizumab, theophylline, short- and long-acting anti-muscarinic agents (LAMAs), long-acting ß-agonists (LABAs), cromolyn, and nedocromil.

7.4. Rescue Medications

Albuterol MDI or nebulizer, prednisone

8. Study Procedures

8.1. Enrollment

Potential participants will have had initial screening visits described in the screening study protocol (UVA IRB# 12656 or 19512).

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, and internet as well as referrals from other health care professionals.

8.2. Visit 1 – Screening/Baseline Visit (Day -7)

Participants considered for study will have previously participated in the screening protocol within 1 year before Visit 1. Clinical information obtained from the screening protocol may be applied to eligibility as described in Section 4 above, and will be added to the participants' Visit 1 study record.

For this study, all visits will take place at the Clinical Research Unit on the 1st floor of the Medical Center Barringer Wing or at the University Hospital or the Clinical Research Center (CRC) located on the 3rd floor of the University of Virginia Children's Hospital Outpatient Building (Battle Building) in Charlottesville. The baseline visit will take place ~7 days (5-28 days) before RV inoculation visit. The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Once the informed consent has been signed, the participant is considered enrolled in the (screening) study and will be assigned a unique participant number

The purpose of the baseline visit is to reconfirm eligibility to continue in the study and to perform baseline assessments.

The following procedures, assessments, and laboratory measures will be conducted to confirm participant eligibility and for baseline assessments:

1. Urine pregnancy test for all women of childbearing potential.
2. ACT (asthmatic subjects) – must be ≥20
3. Blood (10 ml) for viral antibody serology and CBC & differential and 50-ml of blood will be obtained for PBMCs (which can be collected at Visit 2 as long as collection is before inoculation).
4. Spirometry
5. Exhaled nitric oxide (FeNO) on asthmatics only
6. Subjects will be instructed on the use of 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.
7. If required, subjects will also be given an albuterol inhaler (MDI) to use as a rescue medicine as needed.
8. Baseline assessment of past medical history. An additional targeted medical history of acute events over the last month will be obtained to use in our assessment of adverse events.
9. A limited physical examination will be performed including chest auscultation.
10. Vital Signs (blood pressure, heart rate, temperature, height, weight)
11. Methacholine challenge (not to be performed if results were obtained within the past year in the separate screening protocol)
12. Between visit 1 and 2 subjects will fill out diary cards weekly.

Visit 1 can completed in multiple visits if they occur within the visit window.

Study Visit 2 - Inoculation (Day 0)

1. Urine pregnancy test for all women of childbearing potential.
2. Vital Signs (blood pressure, heart rate, temperature, height, weight)
3. Prior to inoculation asthma control will be confirmed by the following means -
    a. Diary cards will be reviewed
    b. AEs review
    c. Spirometry – Must demonstrate normal lung function (FEV1>70% predicted or FEV1/FVC ratio > 75% for subjects with FVC values between 80 and 87% predicted whose FEV1 values fall below 70%).
    d. A limited physical examination will be performed including chest auscultation

4. Nasal scraping
5. Nasal wash / sponge

6. Blood draw – 50mls of blood will be obtained for PBMCs ONLY if not collected at Visit 1
7. Exhaled nitric oxide (FeNO)on asthmatics only
8. Inoculation of subjects with RV-16

Continued eligibility must be confirmed during the initial part of this study visit. Sample collection and the inoculation may be delayed if a subject is ill or has evidence of loss of asthma control. The inoculation will not occur if the upper airway modified Jackson criteria symptom scores are ≥7. The participant may be brought back to continue the Day 0 visit. Procedures completed may be repeated to confirm the subject's eligibility. The repeat visit must occur within the stated visit window from Visit 1.

Study Visit 3, 4, and 5 (Day 1, 2, and 3)

1. Diary cards will be reviewed for symptom control and AEs.
2. A limited physical examination will be performed including chest auscultation and temperature
3. Nasal scraping
4. Nasal wash / sponge
5. Spirometry
6. Exhaled nitric oxide (FeNO) on asthmatics only

Study Visit 6 (Day 4)

1. Urine pregnancy test for all women of childbearing potential.
2. Diary cards will be reviewed for symptom control and AEs.
3. A limited physical examination will be performed including chest auscultation and temperature
4. Nasal scraping
5. Nasal wash / sponge
6. Spirometry
7. Exhaled nitric oxide (FeNO) on asthmatics only
8. Blood sample (50-ml) will be obtained for AEC (part of the CBC & differential) and for collection of PBMCs for later assessment of Th2-immune deviating capacity of nasal secretions.
9. Nasal biopsies will be obtained
10. Methacholine Challenge

Between Visits 6 and 7 (Days 5 and 6)

Subjects will complete the diary card daily

Study Visit 7 (Day 7)

1. Urine pregnancy test for all women of childbearing potential.
2. Diary cards will be reviewed for symptom control and AEs.
3. A limited physical examination will be performed including chest auscultation and temperature
4. Nasal scraping
5. Nasal wash / sponge
6. Spirometry

7. Exhaled nitric oxide (FeNO) on asthmatics only

Study Visit 8 (Day 14) – to occur within a +5 day window (D14 to D19)

Fourteen (14) days after RV inoculation, subjects will return to complete the ACT questionnaire and to report any adverse events. In addition, a limited physical examination will be performed including chest auscultation and a final assessment of FeNO (asthmatics only)/spirometry will be performed. A repeat CBC & differential (3ml purple top) will be drawn if ANC or ALC fell below 500/µL Day 4. If subject is still symptomatic they will be contacted at day 21 with the possibility of an unscheduled visit.

Study Visit 9 (Day 21)

The Day 21 visit will only occur for subjects who remain symptomatic at Day 14 or whose ANC or ALC falls below 500/µL at Day 4 without recovery by Day 14. This visit will occur by phone except in cases where a repeat CBC & differential is needed to check ANC and ALC counts. Participants will be asked if they continue to have symptoms, and whether or not they have had any new or worsening symptoms/conditions since Day 14. If either of these questions is answered affirmatively, the PI will be contacted, and an unscheduled visit will be arranged if the PI feels it is warranted.

8.3. Unscheduled Visits

If disease activity increases after day 7 or other concerns arise either between visits 6 and 7 or after visit 8, participants will be instructed to contact study personnel and may be asked to return to the study site for an "unscheduled" visit. Unscheduled visits will include a physical exam, interim medical history, recording of adverse events and medications, and spirometry testing. Diary cards will also be collected as applicable.

8.4. Visit Windows

Study visits should take place within the time limits specified below. Visit 1 screening/baseline visit will take place between 5 days and 28 days prior to scheduled inoculation. Post-inoculation visits will only take place on the specific day with the exception of visit 7, which will be permitted to have a ±24 hr window, and visit 8, which will have a +5 day window.

9. Mechanistic Assays

Detailed methodology is available in the RO1 application.

1. **Nasal Scrapings.** Will be evaluated for gene expression by quantitative RT-PCR for epithelial-associated genes associated with induction of Th2 profile (IL-25, IL-33, TSLP, and chemokines) and genes involved in anti-viral immunity (IFN-α, IFN-ß, IFN-λ, and IL-15).
2. **Nasal Secretions (and Sponge)**: Will be evaluated for viral load by qPCR and protein expression of cytokine genes as described above for qPCR. In addition, this material will be used to evaluate its Th2 immune deviating propensity.
3. **Blood samples**: Will be obtained for absolute eosinophil count. PBMCs (50-ml) obtained on day -7 will be enriched from the blood sample and used to address presence of immune-deviating propensity of the nasal sponge-derived milieu. Briefly, $CD14^+$ monocytes will be obtained at visit 1 and differentiated into mDC in the presence of GMCSF and IL-4. After 4 days mDC will be pulsed with tetanus and other relevant antigens for 2 additional days. A blood sample (50-ml) will also be obtained on day 4 and enriched for $CD3^+$ T lymphocytes. At

day 6 antigen-pulsed mDC will be allowed to interact with the CFSE-labeled $CD3^+$ T cells from day 4 and co-cultured for 5 days in the presence or absence of the epithelial cell-derived supernatants. Parallel samples of $CD3^+$ T cells will be directly stimulated with anti-CD3/anti-CD28 beads and co-cultured with or without nasal secretion samples. After 5 days intracellular cytokine staining will be performed and % of Th1 (IFN-γ) and Th2 (IL-4) positive T cells determined amongst the $CFSE^{low}$ T cells.

4. **Nasal biopsies***:* Up to three biopsies will be obtained at day 4 dpi. These specimens will be evaluated via immunohistochemistry to determine % of cell infected with RV. In parallel samples, epithelial cells will be identified via staining with APC-conjugated EpCAM and cell death/apoptosis quantified amongst the EpCAM positive population via staining with Annexin V/PI.

10. Biospecimen Storage

No long-term storage of biospecimens is anticipated. Blood samples obtained at screening/baseline visit (for later assessment of Th2 immune deviation) will be stored in liquid N_2 in the PIs laboratory. Similarly, nasal secretion/nasal sponge samples will be stored in a -20°C freezer in the PIs laboratory. The laboratory is always locked and is within a secured building (MR4 Bldg).

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1. Participant Completion

Individuals will have completed their participation when they have completed all of their scheduled study visits.

11.2. Participant Stopping Rules and Withdrawal Criteria

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

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.
5. Individual safety stopping rules:
    a. 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.
    b. A severe asthma exacerbation that requires treatment in the emergency room, hospitalization, or the use of systemic steroids, at any time during the study.
    c. Use of a required but excluded medication such as inhaled corticosteroids or leukotriene modifiers.
    d. Non-adherence to study procedures. Patients who miss visit 3 or 4 may be permitted to remain in the study as long as they comply with all other visits.
    e. Pregnancy
    f. Positive CoVID-19 test

11.3. Participant Replacement

If participants withdraw or are withdrawn, enrollment will continue until the numbers needed in each group complete the study.

11.4. Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant may be asked to complete a final visit and/or final assessments. This will consist of a day 14 visit where participant will be asked about any adverse events and will be paid according to a pro-rated scale. They will be asked to turn in their diary cards and will be evaluated for presence and severity of asthma exacerbation. This will include:

- Administration of ACT
- Limited physical examination (auscultation)
- Spirometry

11.5. Study Stopping Rules

The enrollment and dosing of RV-16 in the study may be suspended pending review by the NIAID DSMB and the University of Virginia IRB for the following reasons:

- Any subject has a severe asthma exacerbation that requires treatment in the emergency room or hospitalization
- ≥2 subjects experience severe asthma exacerbations requiring oral corticosteroids for ≥5 days, pending further expedited review.
- Any subject treated for pneumonia
- Any subject dies until expedited review is completed to determine whether the death might have resulted from any of the study procedures.
- Complications post biopsy requiring an urgent care visit in ≥2 subjects.
- *Any death that occurs in the study, which is possibly or definitely related to study treatment regimen.*
- The occurrence of a Grade 3 or higher related SAE in 2 or more of the study participants who have received a study treatment.

12. Safety Monitoring and Reporting

12.1 Overview

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

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

12.2 Definitions

12.2.1 Adverse Event (AE)

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

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

Study therapy regimen:

- Rhinovirus-16 administration
  Rhinovirus infection is expected to result in cold symptoms among all subjects, and mild asthma symptoms among the asthmatic cohort. In addition, neutropenia and lymphopenia are expected within the first week after rhinovirus inoculation without clinical consequence. Upper and lower respiratory symptom data will be recorded on the RV Dairy Symptom Cards completed by study participants. An adverse event will be recorded in the following circumstances –
  - Treatment with an antibiotic or inhaled or systemic corticosteroid
  - Drop in lung function to ≤70% of predicted or ACT score <19
  - Febrile Neutropenia (ANC <1000/µL with a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4 degrees F) for more than one hour)
  - Lymphopenia (ALC <800/µL)
  - Any cytopenia with appropriate associated symptoms

Study mandated procedures:

- Spirometry / exhaled breath nitric oxide (FeNO) – Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes from the procedure, Coughing requiring treatment with bronchodilators within 30 minutes from the procedure
- Methacholine challenge - Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes from the procedure, Coughing requiring treatment with bronchodilators within 30 minutes from the procedure
- Nasal scrapings – Active epistaxis within 24 hours after the procedure lasting longer than 1 hour
- Nasal wash / sponge – Acute sinusitis diagnosed by a study clinician or based upon assessment of outside health records within 72 hours after the procedure
- Nasal biopsy - Active epistaxis within 24 hours after the procedure lasting longer than 1 hour, or infection needing medical treatment. Vasovagal symptoms and/or vasovagal syncope that do not respond to lowering of the participant's head
- Phlebotomy - Fainting/vasovagal event; Bruising at puncture site larger than 2 cm diameter, Bleeding from puncture site lasting longer than 5 minutes, Swelling at puncture site larger than 2 cm

Events not related to study participation will be recorded on the Adverse Events form if deemed to be Grade 2 or higher in severity.

12.2.2 Suspected Adverse Reaction (SAR)

Any adverse event for which there is a reasonable possibility that the investigational drug [or investigational study therapy regimen] caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event.

A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

12.2.3 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not consistent with the risk information described in the Investigational Brochure.

Expected Risks related to Rhinovirus-16 inoculation and infection:	**Frequency**
• A decline in absolute blood neutrophil counts within first week following inoculation	• Occurs frequently, but not known to be clinically significant.
• Bacterial sinus infection	• Occurs infrequently.
• Lower respiratory tract infection (bronchitis)	• Occurs infrequently. Rhinovirus is not known to cause pneumonia.
• Lymphopenia within first week following inoculation	• Occurs infrequently, and not known to be clinically significant
• 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 • Chest Tightness • Fainting	• Occurs rarely

Expected Risks related to use of Methacholine	**Frequency**
• **Mild asthma symptoms**	• Occurs frequently,

• Coughing • Fainting • Chest tightness • Shortness of breath • Wheezing	• Occurs infrequently
• Bronchoconstriction	• Occurs frequently

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	• Occurs infrequently
• Allergic reaction • Chest pain	• Occurs rarely

Expected Risks related to Nasal Washes / Nasal Sponge / Nasal Scraping	**Frequency**
• Discomfort during the nasal wash procedure • Mild coughing	• 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 nasal biopsy/administration of oxymetazoline, epinephrine (1:100,000), 4% lidocaine, and silver nitrate cautery	**Frequency**
• Pain/tenderness at biopsy site	• Occurs frequently
• Epistaxis	• Occurs infrequently & easily stopped with external pressure applied to nose
• Infection	• Very rarely
• Cardiac arrhythmias / palpitations	• Occurs very rarely or never at the study doses.
• Runny nose	• Occurs frequently
• Vasovagal symptoms and/or vasovagal syncope	• Occurs infrequently

Expected Risks related to Blood Draws	**Frequency**
• Pain at venipuncture site	• Occurs frequently
• Bruising at venipuncture site • Fainting	• Occurs infrequently
• Infection	• Very rarely

12.2.4 Serious Adverse Event (SAE)

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

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

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

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) [version 5.0]. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse

events. The NCI-CTCAE has been reviewed by the Principal Investigator and has been deemed appropriate for the subject population to be studied in this protocol.

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

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

Events grade 2 or higher will be recorded on the appropriate AE case report form [paper CRF] for this study.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not noted above in section 12.2.1 and is also not included in the NCI-CTCAE manual , then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

12.3.2 Attribution Definitions

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

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

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

12.4.2 Collecting Adverse Events

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

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

12.4.3 Recording Adverse Events

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

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

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to IND Sponsor and to DAIT/NIAID

This section describes the responsibilities of the site investigator to report serious adverse events to the IND sponsor and to DAIT/NIAID. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

The Site investigator will report all serious adverse events (see Section 12.2.3, Serious Adverse Event), regardless of relationship or expectedness within 24 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE [paper CRF] will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE [pager CRF] will be updated and submitted.

Reporting to the IND Sponsor:

Monica Lawrence, MD
Department of Medicine
P.O. Box 801355
University of Virginia Health System Charlottesville, VA 22908-1355
(434) 243-6811
ml4nz@virginia.edu

Reporting to DAIT/NIAID:

Peter Gergen, MD, MPH
5601 Fishers Lane 6B58
Rockville, MD 20892
Phone: 240-627-3545
E-mail: pgergen@niaid.nih.gov

12.5.2 Reporting to Health Authority

After an adverse event requiring 24 hour reporting (per Section 12.5.1, *Reporting of Serious Adverse Events to Sponsor*) is submitted by the site investigator and assessed by DAIT/NIAID, there are two options for DAIT/NIAID to report the adverse event to the appropriate health authorities:

12.5.2.1 Annual Reporting

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

- Serious, expected, suspected adverse reactions (see Section 12.2.2, *Suspected Adverse Reaction,* and Section 12.2.3, *Unexpected Adverse Event*).
- Serious and not a suspected adverse reaction (see Section 12.2.2, *Suspected Adverse Reaction*).
- Pregnancies.

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

12.5.2.2 Expedited Safety Reporting

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

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

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

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

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

The sponsor shall report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in

humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

The IND sponsor must notify the FDA within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.3 Reporting of Adverse Events to IRB

All investigators shall report adverse events, including expedited reports, in a timely fashion to their IRB in accordance with applicable regulations and guidelines.

12.6 Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study subject or a partner of a study subject. *A* pregnant subject shall be instructed to stop taking study medication. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report to the DAIT/NIAID all pregnancies within 1 business day of becoming aware of the event using the Pregnancy paper CRF. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy paper CRF shall be updated and submitted to the DAIT/NIAID when details about the outcome are available. When possible, similar information shall be obtained for a pregnancy occurring in a partner of a study subject.

Information requested about the delivery shall include:

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

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion - an SAE shall be submitted to the IND Sponsor and to DAIT/NIAID using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

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

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT/NIAID Medical Officer shall receive periodic reports from the protocol investigator compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site(s) on appropriate paper CRFs.

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

Additionally, the Independent Medical Monitor who is a physician who is independent from the study team and will, at minimum, review all SAEs to assess for possible changes to the overall risk of the study. This person will be expected to communicate with the Protocol Chair/Principal Investigator and the NIAID Medical Officer regarding any safety issues and may be requested to review study safety documentation.

12.8.2 DSMB Review

12.8.2.1 Planned DSMB Reviews

The Data and Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

The DSMB will be informed of an Expedited Safety Report in a timely manner.

12.8.2.2 *Ad hoc* DSMB Reviews

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

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

12.8.2.2.1 Temporary Suspension of enrollment for *ad hoc* DSMB Safety Review

- Any participant develops a severe asthma exacerbation that requires treatment in the emergency room, hospitalization, or the use of systemic steroids, at any time during the study.

A temporary halt in enrollment will be implemented if an *ad hoc* DSMB safety review is required.

13. Statistical Considerations and Analytical Plan

13.1 Overview

Objectives: Our primary objective will be to address the hypothesis that epigenetic changes selectively develop in nasal epithelial cells (EC) during the evolution of allergic airway disease as a result of which nasal EC are programmed to produce cytokines central to orchestrating an allergic inflammatory immune response in the upper – and lower – airways. We will determine whether nasal epithelium from allergic asthmatics and rhinitics when infected with RV, is programmed to secrete cytokines that promote a Th2 cytokine “signature” (IL-25, IL-33, and TSLP) as well as mediators directly driving engagement of an allergic inflammatory process (including CCL5, CCL11, CXCL1, and GMCSF). A total of 60 subjects will be enrolled including 20 each that are normal controls, allergic rhinitis controls, and asthmatics. Cytokine/chemokine will be collected at day 0, 1, 2, 3, 4, and 7 post-infection and evaluated by qPCR and EIA.

We will correlate the expression of these cytokines with engagement of an allergic inflammatory response, including eosinophil co-recruitment at days 1-4. In addition, we predict that in asthma and AR systemic increases in eosinophilia will be observed, but that only in asthmatics will increased asthma symptoms, bronchial hyperreactivity, and eNO be observed.

Analytical approach: The RV challenge model is an example of a serial study design, in which subjects will receive a nasal presentation of RV-16 on challenge day 0 and then they will be serially monitored in the CRU for 7 days. *Analytical method selection*: Due to the fact that most of our data collection will be serial in nature, i.e. data will be collected on challenge days 0, 1, 2, 3, 4, and 7, the analytical methods that we select to use to analyze our primary and secondary outcome data will take into account the *repeated measure* aspect of the RV challenge model. *Missing data*: We will make a concretive effort to limit the occurrence of missing data, and if deemed necessary, multiple-imputation methods will be utilized to control biases associated with incomplete data. *Levels of detection*: If specimen levels are below the level of assay detection, we will use the mid-point between 0 and the minimum detection level of the assay as the unit of analysis.

13.2 Outcomes

- IL-25, IL-33, TSLP qPCR in nasal scraping-derived specimens (days 1-7)
- IL-25, IL-33, TSLP protein in nasal sponge-derived specimens (days 1-7)
- Th2 immune deviating activity in nasal sponge-derived specimens (days 1-7)
- Upper and lower respiratory symptoms via ACT and questionnaire (days 1-7)
- FEV_1 (days 1-7)
- FeNO (days 1-7)
- Circulating AEC (day 4)
- Bronchial hyperreactivity (methacholine PD20) (day 4)
- RV load in nasal secretions (qPCR) (days 1-7)
- IFN-α, ß-, λ and IL-15 by qPCR (days 1-7)
- Nasal biopsy samples (day 4) will be evaluate for % of infected cells and amongst the cells whether they display evidence of apoptosis (annexin V positive) or necrosis (PI positive).

13.3 Measures to Minimize Bias

N/A

Analysis Plan

13.4.1 Analysis Populations.

Normal control subjects, allergic rhinitis controls, and asthmatics

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

Analysis of RV-induced IL-25 expression: The endpoint data will be the challenge study day 0, 1, 2, 3, 4, and 7 nasal scraping derived measurements of IL-25 qPCR expression. *Endpoint transformations*: Since we expect that the IL-25 expression measurements on any particular challenge study day (i.e. day 0, 1, 2, 3, 4, and 7) will be distributed approximately Log-Normal (positive skewed distribution), we will transform the IL-25 expression data to the natural logarithmic scale.

Units of analysis: The day 0 $\log_e$(IL-25 qPCR expression) measurements will subtracted from the post-challenge day 1, 2, 3, 4, and 7 $\log_e$(IL-25 qPCR expression) measurements and these *subject-specific* pre- versus post-challenge changes in $\log_e$(IL-25 qPCR expression) will be the units of analysis. *Analytical approach*: The changes in $\log_e$(IL-25 qPCR expression) on post-challenge days 1, 2, 3, 4, and 7 will be analyzed as repeated measures by way of a linear mixed model (LMM). *LMM fixed effects*: Two sources of variability in the pre- versus post-challenge changes in $\log_e$(IL-25 qPCR expression) will be of primary interest. One source of variability will be the study-group to study-group variability (i.e. asthmatics, allergic rhinitis controls, and healthy-controls) in the changes in $\log_e$(IL-25 qPCR expression), while the other source of variability will be the post-challenge day to day variability in the changes in $\log_e$(IL-25 qPCR expression). *Concomitant variable variability*: Variability in the $\log_e$(IL-25 qPCR expression) changes due to inherent differences in day 0 $\log_e$(IL-25 qPCR expression) will be extracted from the residual error to increase statistical power. *Random effects*: *Subject-specific* and *time-specific* random effects will be incorporated into the LMM to allow *intra-subject* measurement correlation to be accounted for in variance estimation and hypothesis testing. *Hypothesis testing*: *Between-study-group comparisons*: With regard to hypothesis testing, the pivotal comparisons will be the *between* study-group comparisons of the mean changes in $\log_e$(IL-25 qPCR expression) on post-challenge study days 3 and 4 when cold symptoms are expected to peak. More specifically, we will compare if the study-day 3 and study-day 4 mean changes in $\log_e$(IL-25 qPCR expression) differ between the 3 different underlying study-populations after taking into account the inherent variability in challenge study-day 0 $\log_e$(IL-25 qPCR expression). These comparisons will require conducting a total of 6 hypothesis tests (i.e. 3 per study day), and in order to maintain an *intraday* experimental-type I error rate of 0.05, the null hypothesis rejection rule for the "*intraday*" *between-group* comparisons will be based on a Bonferroni corrected two-sided $p \leq 0.0167$ (i.e. 0.05/3) rejection rule. *Within-study-group comparisons*: On a per *study-group* basis, we will conduct statistical tests to determine if the means of the $\log_e$(IL-25 qPCR expression) distributions on post-challenge days 1, 2, 3, 4, and 7, differ from the mean of the $\log_e$(cytokine expression) distribution on challenge study day 0. The *within-study-group* comparisons will be considered *secondary* in nature, and this set of comparisons will be primarily focused on the challenge study-day 3 and challenge study-day 4 changes in mean $\log_e$(IL-25 qPCR expression) when symptoms are expected to peak. More specifically, we will test if the mean of the $\log_e$(IL-25 qPCR expression) distribution is the same on challenge study-day 0 and challenge study-day 3 and if the mean of the $\log_e$(IL-25 qPCR expression) distribution is the same on challenge study-day 0 and challenge study-day 4. A Bonferroni corrected two-sided $p \leq 0.025$ (i.e. 0.05/2) decision rule will be utilized as the null hypothesis rejection criterion for this set of tests. *Confidence intervals*: To aid in data interpretation, a 95% confidence interval will be constructed for each *between-group* comparison and for each *within-group* comparison. *Scale of interpretation*: The point-estimate and the lower and the upper 95% confidence interval limits will be anti-log transformed so that point-estimate and the lower and the upper limits of the 95% confidence interval represent ratios of geometric mean cytokine expression. Alternative analytical approach: If the linear mixed model assumptions are deemed to be violated for this endpoint, we will use a linear mixed model based permutation test strategy to derive empirical based *distribution free* tests for testing the aforementioned *between-group* and *within-group* hypotheses.

Analysis of RV-induced lower-respiratory symptoms: The lower respiratory symptom scores from challenge study-days 0, 1, 2, 3, 4, and 7, will be analyzed in two different ways. In the first approach; due to the expect infrequency of lower respiratory symptoms in the allergic rhinitis and healthy control cohorts, cumulative lower respiratory total symptom (CLRTS) will be computed for challenge period; day 0 to day 4, and for challenge period; day 0 to day 7. In the second approach the daily total lower respiratory symptom scores will be analyzed.

Analytical approach #1: We will use the total lower respiratory symptom scores of challenge days 0, 1, 2, 3, and 4 to compute to a cumulative lower respiratory symptom score (CLRTS) for challenge period day 0 to day 4, and we will use the total lower respiratory symptom scores of challenge days 0, 1, 2, 3, 4, and 7 to compute a CLRTS for challenge period day 0 to day 7. *Analytical approach*: The CLRTS will be analyzed as integer scaled random variables by way of a Negative Binomial (NB) generalized estimating equation (GEE) model. *GEE model specification:* The GEE model specification for the CLRTS analysis will include two predictor variables. One predictor variable will identify the study-group (i.e. asthmatics, allergic rhinitis controls, and healthy-controls) while the other predictor variable will identify the challenge period (i.e. day 0 to day 4, or day 0 to day 7). *Variance estimation*: An "unstructured" variance-covariance matrix form will be utilized to account for the inherent correlation between the first set of CLRTS scores (challenge day 0 to challenge day 4) and the second set of CLRTS scores (challenge day 0 to challenge day 7). *Hypothesis testing*: Wald tests will be conducted to determine if the means of CLRTS distributions for each challenge period differ from study-population to study populations. A Bonferroni two-sided $p \leq 0.0167$ (0.05/3) criterion will be utilized as the null hypothesis rejection rule for the intra-challenge-period, between-group comparisons.

Analytical approach # 2: We will analyze the lower respiratory total symptom scores for challenge days 0, 1, 2, 3, 4, and 7 as repeated measures in this approach. *Analytical approach*: The lower respiratory total symptom score data will be analyzed by way of a negative-binomial generalized estimating equation (GEE) model. *Model specification:* The GEE model specification will include two predictor variables. One predictor variable will identify the study-group (i.e. asthmatics, allergic rhinitis controls, and healthy-controls), while the other predictor variable will identify the challenge study-day (i.e. day 0, 1, 2, 3, 4, or 7). *Variance estimation*: A "working independence" variance covariance matrix form will be utilized to account for the inherent *intra-subject* measurement correlation induced by the repeated measures aspect of the RV challenge study design. *Hypothesis testing:* Wald chi-square tests will be utilized to test if the means of total lower symptom score distributions on a particular challenge day differed between the 3 different study-groups. The primary focus will be to determine if the means of total lower symptom score distributions differed between the 3 different study-groups on study-days 3 and 4, when symptoms are expected to peak.

Analysis of the severity of RV-induced infection: The post-challenge (i.e. challenge days 1 to 7) RV loads in the nasal secretions of challenged subjects will be derived via qPCR. Quantification is based upon serial dilutions of a standardized pool of the virus with a known $TCID_{50}$/ml. *Data transformation*: Since the RV load data will be quantified in terms of "virion frequency per unit volume of nasal section", it is expected that based on previous RV challenge studies that the RV load data will have to be analyzed on the $\log_{10}$ scale. *Analytical approach:* The nasal secretion RV load data will be analyzed via a LMM. *Model specification*: Two sources of nasal secretion RV load variability will be examined. One source of variability will be the study-group to study group variability (i.e. asthmatics, allergic rhinitis controls, healthy-controls) and the other source of variability will be the post-challenge day to day variability (i.e. challenge day 1, 2, 3, 4, or 7). *Hypothesis testing:* The pivotal *between-group* comparisons will be focused on the post-challenge day 3 and day 4 nasal secretion RV loads when symptoms are expected to peak. Post-challenge day 3 and day 4 mean $\log_{10}$(nasal secretion RV load) will be compared in a pairwise manner between the 3 different study-populations. In order to maintain an *intraday* experimental type I error rate 0.05, a Bonferroni corrected two-sided $p \leq 0.05/3$ decision rule will be utilized as the null hypothesis rejection rule.

Post-challenge day 1, 2, and 7 *between-group* comparisons of mean $\log_{10}$(nasal secretion RV load) will be consider as secondary comparisons and the same hypothesis testing procedures will be employed to conduct these *between-group* comparisons. *Within-group* related hypotheses will also be considered as secondary hypotheses. *Scale of interpretation*: The point estimates and 95% confidence interval limits for the *between-group* and the within-group comparisons of mean $\log_{10}$(RV load) will be anti-log transformed so that the *between-group* comparisons and the *within-group* comparisons of nasal secretion RV load can be interpreted as ratios of "nasal secretion RV load" geometric means.

13.4.3 Supportive Analyses of the Primary Outcome(s)

Supportive analyses for the primary outcomes: As supportive analyses we will examine the IL-25 cytokine expression in the nasal washes collected on study days 1, 2, 3, 4, and 7 of the RV 16 challenge. These data will be analyzed in exactly the same way as the nasal scraping derived IL-25 expression data (see above).

13.4.4 Analyses of Secondary and Other Outcome(s)

As indicated in section 3.1 and 13.2 there will be several secondary outcome variables and the analytical plans related to these outcome variables will be briefly described in this subsection.

Analysis of expression of nasal epithelial cells of a type 2 cytokine-inducting profile: We will evaluate the time course of IL-33 and TSLP transcription expression by qPCR on nasal sample obtained on challenge days 0, 1, 2, 3, 4, and 7. *Endpoint transformations*: Since we expect that the IL-33 and TSLP expression measurements on any particular challenge day (i.e. day 0, 1, 2, 3, 4, and 7) will be distributed approximately Log-Normal (positive skewed distribution), we will transform the IL-33 and TSLP expression data to the natural logarithmic scale. *Units of analysis:* The day 0 $\log_e$(cytokine qPCR expression) measurements will subtracted from the post-challenge day 1, 2, 3, 4, and 7 $\log_e$(cytokine qPCR expression) measurements and these *subject-specific* pre- versus post-challenge changes in $\log_e$(cytokine qPCR expression) will be the units of analysis. *Analytical approach*: The changes in $\log_e$(IL-33 qPCR expression) on post-challenge days 1, 2, 3, 4, and 7, and the changes in $\log_e$(TSLP qPCR expression) on post-challenge days 1, 2, 3, 4, and 7, will be analyzed as repeated measures by way of linear mixed models (LMM). *Model specification*: Per outcome variable, the LMM specification will the same as the LMM specification for the analysis of the post-challenge days 1, 2, 3, 4, and 7 changes in $\log_e$(IL-25 qPCR expression) (see section 13.4.2 above). *Hypothesis testing*: The between-group hypothesis, and the within-group hypotheses, will be identical to those described in "Hypothesis testing" section of the IL-25 qPCR analytical approach (see section 13.4.2 above).

Analysis of IL-25, IL-33 and TSLP protein expression by EIA. We will evaluate the time course of IL-25, IL-33 and TSLP protein expression by EIA on nasal sample obtained via immunosorbent sponge on challenge days 0, 1, 2, 3, 4, and 7. ***Analytical approach:*** IL-25, IL-33 and TSLP protein expression data will be analyzed as repeated measures via linear mixed models in exactly the same way as the IL-25, IL-33 and TSLP qPCR expression data (see sections 13.4.2 and immediately above).

Analysis of albuterol metered dose inhaler utilization: Albuterol metered dose inhaler utilization will be evaluated on challenge days 0, 1, 2, 3, 4, and 7 within the asthma cohort to determine if the frequency of albuterol inhaler utilization is related to severity of symptoms.

Analytical approach: Based on diary records, the total number of times that the subjects used their albuterol metered dose inhaler will be determined for study days 0, 1, 2, 3, 4, and 7, and these frequencies will be analyzed via a Negative Binomial generalized estimating equation (GEE) model. *Hypothesis testing*: Our primary interest, will be to compare albuterol metered dose inhaler utilization on post-challenge days 3 and 4 when lower and upper respiratory symptoms are expected to peak to challenge days 0, 1, 2, and 7. The Wald test will be the pivotal quantity for the *inter-day* comparisons and the multiple comparison experimental type 1 error rate for the inter-day comparisons will 0.05.

Secondary analyses: As secondary analyses, Negative Binomial GEE regression models will be utilized to determine if RV loads and total upper and lower respiratory symptoms scores on challenge days 0, 1, 2, 3, 4, and 7 predict albuterol metered dose inhaler utilization on challenge days 0, 1, 2, 3, 4, and 7.

Analysis of FEV1: Measurements of FEV1 will be acquired on challenge study days: 0, 1, 2, 3, 4, and 7. *Data transformation*: If the FEV_1 measurement distributions for challenge study days 0, 1, 2, 3, 4, and 7 are positively skewed, the FEV_1 will be transformed to the natural logarithmic scale, otherwise the FEV_1 data will be analyzed on the mL scale. *Units of analysis:* The challenge study day 0 FEV_1 measurements will subtracted from the post-challenge FEV_1 measurements of challenge study days 1, 2, 3, 4, and 7 and these *subject-specific* pre- versus post-challenge changes in FEV_1 will be the units of analysis. *Analytical approach*: The post-challenge changes in FEV_1 will be analyzed via a LMM. *LMM specification*: Two source of variability in the post-challenge changes in the FEV_1 measurements will be examined. One source of variability will be the study-group to study group variability (i.e. asthmatics, allergic rhinitis controls, and healthy-controls), while the other source of variation will be the post-challenge day to day variability (i.e. challenge study day 1, 2, 3, 4, or 7). Variability in the FEV_1 changes due to inherent differences in the challenge study day 0 Fev_1 (baseline) will be extracted from the residual error to increase statistical power. *Random effects*: *Subject-specific* and *time-specific* random effects will be incorporated into the LMM to allow *intra-subject* measurement correlation to be accounted for in variance estimation and hypothesis testing. *Hypothesis testing:* The pivotal *between-group* comparisons will be focused on the post-challenge day 3 and day 4 changes in FEV_1 when symptoms are expected to peak. Baseline adjusted post-challenge day 3 and day 4 mean changes in FEV_1 will be compared in a pairwise manner between the 3 different study populations. In order to maintain an *intraday specific* experimental type I error rate 0.05, a Bonferroni corrected two-sided $p \leq 0.05/3$ decision rule will be utilized as the null hypothesis rejection rule. Post-challenge day 1, 2 and 7 *between-group* comparisons of the changes in FEV_1 will be consider as secondary comparisons, and the same hypothesis testing procedures will be employed to conduct these *between-group* comparisons. *Within-group* related hypotheses will also be considered as secondary hypotheses.

***Analysis of FeNO*:** FeNO will be derived from lung-function tests that will be conducted on challenge study days 0, 1, 2, 3, 4, and 7. *Data transformation*: If the FeNO measurement distributions for challenge study days 0, 1, 2, 3, 4, and 7 are positively skewed, the FeNO data will be transformed to the natural logarithmic scale, otherwise the FeNO data will be analyzed on the ppb scale. *Analytical approach*: The FeNO data will be analyzed via a LMM. *LMM specification*: The LMM specification will an exactly the same as the LMM model specification for the FEV_1 analysis (see above). *Hypothesis testing:* The pivotal *between-group* comparisons will be focused on the post-challenge day 3 and day 4 changes in FeNO when symptoms are expected to peak. Baseline adjusted post-challenge day 3 and day 4 mean changes in FeNO will be compared in a pairwise manner between the 3 different study-populations.

In order to maintain an *intraday specific* experimental type I error rate 0.05, a Bonferroni corrected two-sided p≤0.05/3 decision rule will be utilized as the null hypothesis rejection rule for this set of tests. Post-challenge day 1, 2, and 7 *between-group* comparisons of the changes in FeNO will be consider as secondary comparisons, and the same hypothesis testing procedures will be employed to conduct these *between-group* comparisons. *Within-group* related hypotheses will also be considered as secondary hypotheses.

***Analysis of bronchial hyperreactivity (methacholine PD20)*:** The changes in bronchial hyperactivity from before challenge to after challenge will be quantified in terms of the changes in methacholine challenge PD20 from pre-challenge day -7 to post challenge day 4 (Δ PD20). *Analytical approach*: The Δ PD20 data will be analyzed by way of analysis of variance (ANOVA). *Model specification*: The primary source of Δ PD20 variation that will be examined will be the variation due to study-group to study-group variation. *Hypothesis testing*: We will test if the 3 underlying study-population Δ PD20 distributions share the same mean. If rejected a Bonferroni corrected two-sided p≤0.05/3 decision rule will be utilized as the null hypothesis rejection rule for between-group comparisons. *Alternative analytical approach*: If the ANOVA model assumptions are violated, the Δ PD20 data will be analyzed by *distribution-free* permutation methods.

Analysis of circulating eosinophil count: Changes in the absolute eosinophil count (AEC) from day -7 to post-challenge day 4 will be compared between the 3 different groups via ANOVA. *Model specification*: The primary source of Δ AEC variation that will be examined will be the variation due to study-group to study-group variation. *Hypothesis testing*: We will test if the 3 underlying study-population Δ AEC distributions share the same mean. A p≤0.05 decision rule will be utilized to null hypothesis rejection rule for this test. If rejected a Bonferroni corrected two-sided p≤0.05/3 decision rule will be utilized as the null hypothesis rejection rule for between-group comparisons. *Alternative analytical approach*: If the ANOVA model assumptions are violated, the Δ %EC data will be analyzed by *distribution-free* permutation methods.

Analysis of eosinophil percent of total cells: Changes in the eosinophil percent of total cells (Δ %EC) from day -7 to post-challenge day 4 will be compared between the 3 different groups via ANOVA. *Model specification*: The primary source of Δ %EC variation that will be examined will be the variation due to study-group to study-group variation. *Hypothesis testing*: We will test if the 3 underlying study-population Δ %EC distributions share the same mean. A p≤0.05 decision rule will be utilized to null hypothesis rejection rule for this test. If rejected a Bonferroni corrected two-sided p≤0.05/3 decision rule will be utilized as the null hypothesis rejection rule for between-group comparisons. . *Alternative analytical approach*: If the ANOVA model assumptions are violated, the Δ %EC data will be analyzed by *distribution-free* permutation methods.

Analysis of upper respiratory symptom scores: The upper respiratory symptom scores that will be reported daily from challenge study day 0 to challenge study day 7, will be analyzed in two ways. In the first approach, cumulative upper respiratory total symptom (CURTS) will be computed for challenge days 0 to 4, and for challenge days 0 to 7. In the second approach the daily total upper respiratory symptom scores will be analyzed. *Please see the analytical plan for the analysis lower respiratory symptom scores for further details (section 13.4.2)*.

Analysis of innate immune deviating activity: IFN-α, ß-, λ and IL-15 data will be derived via qPCR from the RV challenge study day 0, 1, 2, 3, 4 and 7 nasal secretion samples. *Data transformation*: We expect that anti-viral

immunity expression data will be positively skewed, and therefore the expression data will have to be analyzed on the logarithmic scale. *Units of analysis:* Per endpoint (e.g. IFN-α), the challenge study day 0 $\log_e$(expression) levels of the endpoint will subtracted from the day 1, 2, 3, 4, and 7 post-challenge $\log_e$(expression) levels of the endpoint and these *subject-specific* pre- versus post-challenge changes in $\log_e$(expression) will be the units of analysis. *Analytical approach*: The logarithmic transformed expression data of each anti-viral immunity endpoint (e.g. IFN-α) will be analyzed via a LMM. *LMM specification*: Two source of variability in the post-challenge changes in $\log_e$(expression) will be examined. One source of variability will be the study-group (i.e. asthmatics, allergic rhinitis controls, and healthy-controls), while the other source will be the post-challenge day (i.e. challenge study days 1, 2, 3, 4, or 7). Variability in the post-challenge changes in $\log_e$(expression)of the endpoint due to inherent differences in the $\log_e$(expression) of the endpoint on challenge study day 0 (baseline) will be extracted from the residual error to increase statistical power. *Random effects*: *Subject-specific* and *time-specific* random effects will be incorporated into the LMM to allow *intra-subject* measurement correlation to be accounted for in variance estimation and hypothesis testing. *Hypothesis testing:* The pivotal *between-group* comparisons will be focused on the post-challenge day 3 and day 4 changes in $\log_e$(expression) when peak symptoms are expected to occur. Baseline (i.e. challenge study day 0) adjusted day 3 and day 4 mean changes in $\log_e$(expression) will be compared in a pairwise manner between the 3 different study-groups. In order to maintain an *intraday* experimental type I error rate 0.05, a Bonferroni corrected two-sided $p \leq 0.05/3$ decision rule will be utilized as the null hypothesis rejection rule. Post-challenge day 1, 2, and 7 *between-group* comparisons of the changes in $\log_e$(expression) will be consider as secondary comparisons, and the same hypothesis testing procedures will be employed to conduct these *between-group* comparisons. *Within-group* related hypotheses will also be considered as secondary hypotheses. *Scale of interpretation*: The point estimates and 95% confidence interval limits for the *between-group* and the *within-group* comparisons of mean $\log_e$(expression) will be exponentiated so that the *between-group* comparisons and the *within-group* comparisons of the anti-viral immunity expression can be interpreted as a ratio of geometric means.

Analysis of nasal biopsy samples: Post-challenge day 4 nasal biopsy samples will be evaluated for % of infected cells and amongst the cells whether they display evidence of apoptosis (annexin V positive) or necrosis (PI positive). *Analytic approach*: Since the underlying distributions of these cell characteristics are complicated, we will use *distribution-free* permutation tests to conduct the *between-group* comparisons. *Hypothesis testing*: Based on the pseudo permutation test null distribution, a Bonferroni corrected two-sided $p \leq 0.05/3$ decision rule will be utilized as the null hypothesis rejection rule for the *between-group* comparisons.

***Bivariate correlation analyses*:** As secondary analyses, we intend to examine bivariate relationships between several of the outcome variables. As one example, we intend to examine bivariate relationships between post-challenge day 0 to day 4 cumulative RV load and post-challenge day 3 and post-challenge day 4 expression of Th2 immune deviating activity (IL-25, IL-33 and TSLP). As a second example, we intend to examine bivariate relationships between post-challenge day 0 to day 4 post challenge cumulative RV load and post-challenge day 1, 2, 3, and day 4 innate expression of anti-immunity (IFN-α, ß-, λ and IL-15). Bivariate associations will be quantitatively expressed in terms of the non-parametric Spearman Rank correlation coefficient. Since multiple hypotheses will be test, Bonferroni type I error rate corrections will be implemented in establishing the null hypothesis rejection rules.

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

Overview: As listed in section 3.4, there will be several exploratory endpoints. The majority of these endpoints will be analyzed descriptively by providing descriptive statistics. For continuous scaled variables the descriptive

statistics will include the mean, standard deviation, median, interquartile range, and the range; i.e. minimum and the maximum, of the empirical distribution, while for categorical variables, the descriptive statistics will include category frequencies and percentages.

13.4.6 Descriptive Analyses

Not planned

13.5 Interim Analyses

Not planned for this study

13.6 Statistical Hypotheses

All of the hypotheses related to the primary and secondary outcomes will be two-sided. Three types of hypothesis tests will be conducted. The underlying motivation of one set of hypotheses (*between-group comparisons*) will be to examine if the distributions of the outcome random variable in the underlying study populations of the 3 study groups share the same location parameter value (e.g. mean, geometric mean). The underlying motivation of a second set of hypotheses (*within-group comparisons*) will be to examine if the underlying distributions of the outcome random variable pre- and post- RV challenge share the same location parameter value, and the underlying motivation of a third set of hypotheses (*bivariate-correlation*) will be to examine if the bivariate correlation between two random outcome variables is zero.

Between-group comparisons: With regard to the *between-group* comparisons, for each primary and secondary outcome variable the central *null hypothesis* is that the location parameter values (i.e. means or geometric means) of underlying distributions of the 3 study populations (i.e. asthmatics, allergic rhinitis controls, healthy-controls) are the same, whereas the *alternative hypothesis* is that at the underlying distributions of the 3 study populations have location parameter values that are not the same.

Within-group comparisons: With regard to the *within-group* comparisons, for each primary and secondary outcome variable the *null hypothesis* is that the underlying value of distribution location parameter (i.e. means or geometric means) is the same before and after RV-challenge, whereas the *alternative hypothesis* is that the underlying value of the distribution location parameter is not the same before and after RV-challenge.

Bivariate-correlation: With regard to bivariate correlation, the *null hypothesis* is that the underlying correlation between the two outcome random variables is zero, whereas the *alternative hypothesis* is that the underlying correlation between the two outcome random variables is non-zero.

13.7 Sample Size Considerations

Overview (Aim 1): The primary null hypotheses of Aim 1 will test if the pre- to post-challenge day-3 and day-4 changes in ex vivo IL-25 are the same for allergic asthmatics, allergic-rhinitis-controls, and health controls. *Hypothesis testing*: To combine the post-challenge day-3 and day-4 comparisons we will first construct a 4 degree of freedom linear contrast of means to test the "global" null hypothesis that irrespective of the study group, the mean pre- to post-challenge day-3 change in $\log_e$(IL-25) (i.e. $\Delta \log_e(\text{IL-25})_{[\text{Day 3}]} = \log_e(\text{IL-25}_{\text{Post [Day 3]}}) - \log_e(\text{IL-25}_{\text{Pre [Day 0]}})$) is the same, and irrespective of the study group, the mean pre to post-challenge day-4 change in $\log_e$(IL-25) (i.e. $\Delta \log_e(\text{IL-25})_{[\text{Day 4}]} = \log_e(\text{IL-25}_{\text{Post [Day 4]}}) - \log_e(\text{IL-25}_{\text{Pre [Day 0]}})$) is the same. *Scenario #1*: If this "global" null hypothesis fails to be rejected at the $p \leq 0.05$ significance level, then no post-challenge day-3 or post-challenge day-4 between-group comparisons will be conducted. *Scenario #2:* If this "global" null

hypothesis is rejected at the p≤0.05 significance level, then 3 linear contrasts of the post-challenge day-3 mean $\Delta \log_e(\text{IL-25})_{[\text{Day 3}]}$ will be constructed to compare the mean $\Delta \log_e(\text{IL-25})_{[\text{Day 3}]}$ between the 3 study-populations, and 3 linear contrasts of the post-challenge day-4 mean $\Delta \log_e(\text{IL-25})_{[\text{Day 4}]}$ will be constructed to compare the mean $\Delta \log_e(\text{IL-25})_{[\text{Day 4}]}$ between the 3 study-populations. Since under scenario # 2, three post-challenge day-3 between-group comparisons of mean $\Delta \log_e(\text{IL-25})_{[\text{Day 3}}$will be conducted, a two-sided Bonferroni p≤0.05/3 decision rule will be utilized as the null hypothesis rejection criterion for this set of between-group comparisons. Similarly, since under scenario #2 three post-challenge day-4 between-group comparisons will be conducted, a two-sided Bonferroni p≤0.05/3 decision rule will be utilized as the null hypothesis rejection criterion for this set of between-group comparisons.

***Power analysis* (AIM 1):** If 20 subjects per study-group have challenge day-0, day-3 and day-4 *ex vivo* IL-25 expression measurements we expect to have at least 0.80 statistical power to detect between study-population differences in the post-challenge day 3 and post-challenge day 4 changes in *ex vivo* IL-25 expression if the underlying pre- to post-challenge IL-25 expression geometric mean (GM) ratios of any two study populations differ in magnitude by at least a factor of 1.14 (column 3 of Table II).

Effect- size terminology: *Since the primary endpoint is $\log_e(\text{IL-25}_{\text{Post-challenge}}) - \log_e(\text{IL-25}_{\text{Pre-challenge}})$, this variable can be re-expressed as $\log_e(\text{IL-25}_{\text{Post-challenge}}/\text{IL-25}_{\text{Pre-challlenge}})$. If we let $\mu_{\text{log-Ratio}}$ =mean $\log_e(\text{IL-25}_{\text{Post}}/\text{IL-25}_{\text{Pre}})$, then $\exp(\mu_{\text{log-Ratio}})$ is the geometric mean of $\text{IL-25}_{\text{Post}}/\text{IL-25}_{\text{Pre}}$ (i.e. a geometric mean (GM) ratio). The factor by which the GM ratios of two study-populations must differ in magnitude in order to have 0.80 statistical power to reject the null hypothesis that the geometric mean ratio is same for the two study populations is what is reported in column 3 of Table II.*

Power analysis details: In computing the minimum detectable factor by which the pre to post-challenge *ex vivo* IL-25 expression geometric mean ratios must differ between any two study-populations in order to have at least 0.80 statistical power to detect this difference we assumed: 1) that the correlation between the pre- and post-challenge *ex vivo* $\log_e$(IL-25 qPCR expression) will be no less than 0.30, 2) that the random variable for the pre-challenge to post-challenge day-3 change in $\log_e$(IL-25 qPCR expression) (i.e. $\Delta \log_e(\text{IL-25})_{[\text{Day 3}]}$) is normally distributed in all 3 study populations and that the random variable for the pre-challenge to post-challenge day-4 change in $\log_e$(IL-25 qPCR expression) (i.e. $\Delta \log_e(\text{IL-25})_{[\text{Day 4}]}$) is normally distributed in all 3 study populations, 3) that the *between-subject* standard deviations for the post-challenge day 3 and post-challenge day 4 changes in $\log_e$(IL-25 qPCR expression) (i.e. $\Delta \log_e(\text{IL-25})_{[\text{Day 3}]}$, and $\Delta \log_e(\text{IL-25})_{[\text{Day 4}]}$, respectively) is less than or equal to 0.12, and 4) that per post-challenge day (e.g. post-challenge day-3) the *experimental* type I error rate of the hypothesis testing procedure will be less than or equal to 0.05 after Bonferroni correction for 3 between-group comparisons.

Between-subject variance estimation: The between-subject variability of 0.12 standard deviation units for the post-challenge day-3 and the post-challenge day-4 changes in $\log_e$(IL-25 qPCR expression) (i.e. $\Delta \log_e(\text{IL-25})_{[\text{Day 3}]}$, and $\Delta \log_e(\text{IL-25})_{[\text{Day 4}]}$, respectively) was derived from our own pilot data. Five asthmatics and 7 healthy controls contributed ex vivo measurements of IL-25 at 48 hours post-challenge. Based on natural logarithm values of these measurements, the empirical standard deviation of $\log_e$(IL-25) was 0.10 units. The variance for the pre- to post-challenge change in $\log_e$(IL-25) (i.e. $\Delta \log_e$(IL-25)) was then computed using the formula: $\text{var}(\Delta \log_e \text{IL} - 25) = \text{var}(\log_e \text{IL} - 25_{\text{Post}}) + \text{var}(\log_e \text{IL} - 25_{\text{Pre}}) - 2\rho * \text{var}(\log_e \text{IL} - 25_{\text{Pre}})^{\frac{1}{2}} * \text{var}(\log_e \text{IL} - 25_{\text{Post}})^{\frac{1}{2}}$, where $\text{var}(\log_e \text{IL-25}_{\text{Pre}})$ was assumed equal to $\text{var}(\log_e \text{IL-25}_{\text{Post}}) = 0.010$ and the within-

subject correlation (ρ) between the $\log_e$ IL-25_{Pre} and the $\log_e$ IL-25_{Post} measurements was conservatively estimated as 0.30. Based on this set of assumptions the between-subject variability for $\Delta \log_e$(IL-25) was 0.0144 and standard deviation for $\Delta \log_e$(IL-25) was $(0.0144)^{1/2}$= 0.12.

Table II. Minimum detectable factor by which the geometric mean (GM) ratios for the pre- to post-challenge change in ex vivo IL-25 expression must differ in magnitude between two study populations in order to have at least 0.80 statistical power to reject the null hypothesis that the geometric mean ratios are same for the two underlying study populations.

Endpoint	*Standard Deviation $\Delta \log_e$(IL-25) Expression*	*Factor by which the GM Ratios of the two study-populations must differ in magnitude.*
Ex-vivo IL-25 Expression	0.12‡	1.14†

†Effect size with Bonferroni type I error rate correction for 3 between-group comparisons. ‡ denotes the *between-subject* estimate of variability $\Delta \log_e$(IL25) in standard deviation units that was used in the 2-sample t-test sample-size formula.

Overview (Aim 2): The primary null hypothesis of Aim 2 will test if the post-challenge day-4 geometric mean "cumulative RV load"[†] is the same for allergic asthmatics, allergic-rhinitis-controls, and the health controls. Note that this is equivalent to testing if the post-challenge day-4 mean $\log_{10}$(cumulative RV load) is the same for allergic asthmatics, allergic-rhinitis-controls, and the health controls. <u>*Hypothesis testing*</u>: To test the primary null hypothesis we will first conduct a 2 degree of freedom linear contrast of the post-challenge day-4 means for $\log_{10}$ (cumulative RV load). *Scenario #1*: If this "global" null hypothesis fails to be rejected at the p≤0.05 significance level, then no pairwise between-group comparisons will be conducted. Scenario #2: if this "global" null hypothesis is rejected at the p≤0.05 significance level, then 3 linear contrasts of the $\log_{10}$ (cumulative RV load) means will be constructed to compare the mean $\log_e$(cumulative RV load) between the 3 study-populations. Since under scenario #2 three hypothesis tests will be conducted, a two-sided Bonferroni p≤0.05/3 decision rule will be utilized as the null hypothesis rejection criterion to maintain an experimental type I error rate of 0.05.

†Definition: Post-challenge day-4 "cumulative RV load" represents the sum of the post-challenge day-0, day-1, day-2, day-3, and day-4 RV loads.

Power analysis (AIM 2): If 20 subjects per study-group have RV load measurements for post-challenge days: 0, 1, 2, 3, and 4, we expect to have at least 0.80 statistical power to detect between study-population differences in the post-challenge day-4 geometric mean "cumulative RV load" if the underlying post-challenge day-4 "cumulative RV load" geometric means of two study populations differ in magnitude by at least the factor of 25 (column 3 of Table III).

Effect-size terminology: Since the primary endpoint is log_{10}(cumulative RV load), a between-group comparison of mean log_e(cumulative RV load) is simply $\Delta\mu$= mean log_{10}(cumulative RV load)$_{Group\ A}$ - mean log_{10}(cumulative RV load)$_{Group\ B.}$ Therefore, $10^{\Delta\mu}$ = geometric mean (cumulative RV load)$_{Group\ A}$/ geometric mean (cumulative RV load)$_{Group\ B}$, which is a ratio of geometric means. The factor 25 is the ratio of geometric means for post challenge day-4 cumulative RV load that is required in order to have at 0.80 statistical power to reject the null hypothesis that the geometric mean day-4 "cumulative RV load" is the same for the two study- populations.

Details: In computing the minimum detectable factor by which the geometric means for post-challenge day 4 cumulative RV load must differ in magnitude in order to have at least 0.80 statistical power to reject the null hypothesis that the two geometric means are equal we assumed: 1) that the random variable for post-challenge day-4 log_{10}(cumulative RV load) is normally distributed in all 3 study populations, 2) that the *between-subject* variability in post-challenge day-4 log_{10}(cumulative RV load) in standard deviation units is less than or equal to 1.32 in each study population, and 3) that the *experimental* type I error rate of the test will be less than or equal to 0.05 after Bonferroni correction for 3 between-group comparisons.

Between-subject variance estimation: The estimate of 1.32 units for the *between-subject* variability in post-challenge day-4 log_{10} (cumulative RV load [virions]) in standard deviation units was derived from the day-4 post HRV16 challenge log_{10} (cumulative RV load [virions]) measurements of 8 controls and 16 asthmatics who participated in a HRV16 challenge study that was conducted at the University of Virginia by our group.

Table III. Minimum detectable factor by which the geometric means (GM) for post-challenge day-4 cumulative RV load must differ between two study-populations in order to have at least 0.80 statistical power to reject the null hypothesis that the geometric mean post-challenge day-4 cumulative RV load is the same for the two study populations.

Endpoint	*Standard Deviation Post-challenge Day-4 log_{10}(Cumulative RV Load [virions])*	*Factor by which the Geometric Means of the two study-populations must differ in magnitude.*
Cumulative RV load	1.32	25.0†

† Effect size with Bonferroni type I error rate correction for 3 between-group comparisons. ‡ denotes the estimate for the between-subject variability in post-challenge day-4 log_{10} (cumulative RV load) in standard deviation units that was used in the 2-sample t-test sample-size formula.

14. **Identification and Access to Source Data**

14.1. Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial. Data derived from source documents will be transferred to protocol-specific CRFs. Source documents will include print-outs from spirometer, qPCR machine, EIA, flow cytometer, clinical laboratory

14.2. Access to Source Data

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

15. Protocol Deviations

15.1. Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

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

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

15.2. Reporting and Managing Protocol Deviations

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

Upon determination that a protocol deviation has occurred, the study staff will a) notify the site Principal Investigator, b) if warranted, notify the NIAID Project Manager, and c) will complete a Protocol Deviation form. The DAIT/NIAID Medical Officer will make the decision as to whether the Deviation is major or not and what the impact of the Deviation on the study participant or the entire study may be. Subjects may at their discretion choose to participate in all or only parts of this study. If a subject chooses not to participate in all aspects of this study (e.g., nasal biopsies) that will not be considered a protocol deviation nor will it impair their ability to enroll and complete the study.

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. Statement of Compliance

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

16.2. Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the FDA 1572 form will present the consent 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. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

16.3. Privacy and Confidentiality

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

16.4. Quality Assurance and Control

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all CRFs are completed for every participant entered in the trial.

The IND sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

17. Publication Policy

No specific policy on the publication of study results will apply to this trial.

18. References

(see pages 60-63)

Appendix 1: 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. Borish 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* (60).

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. Borish, together with back-up from the allergy fellow on call will be contacted (paged) for evaluation.\

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