Hallmarks of Protective Immunity in Sequential Rhinovirus Infections in Humans

DMID Protocol Number: 16-0055

DMID Funding Mechanism: 1U01AI125056-01

PAR-13-151

IND Sponsor: Ronald B. Turner, MD

Principal Investigators: Judith A. Woodfolk, MBChB, PhD; Ronald B. Turner, MD

DMID Clinical Protocol Manager: Tena Knudsen, RN

DMID Medical Monitor: Venus Shahamatdar, MD

Version Number: 2.0

13 October 2017

UVA IRB-HSR #18936, Version 2.0

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies: 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, 21 CFR Part 11 and 21 CFR Part 312
- Conformity with the International Conference on Harmonization (ICH) E6(R2), and per FDA Guidance for Industry E6: Good Clinical Practice (GCP), Consolidated Summary
- NIH Clinical Terms of Award

Refer to: http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm#46.
http://www.fda.gov/cder/guidance/959fnl.pdf
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html

Compliance with these standards provides public assurance that the rights, safety and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this study) will complete and maintain Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigators:

Signed: _____ Date: _____
Name
Title

Signed: _____ Date: _____
Name
Title

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AIDS	Acquired Immunodeficiency Syndrome
CBC	Complete Blood Count
CD	Cluster of Differentiation
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
CROMS	Clinical Research Operations and Management Support
DCC	Data Coordinating Center
DCF	Data Collection Form
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSR	Health Sciences Research
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IgG	Immunoglobulin G
IND	Investigational New Drug Application
IP	Investigational Product
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
MedDRA®	Medical Dictionary for Regulatory Activities
MHCII	Major Histocompatibility Complex Class II
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine

NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NKT	Natural Killer T Cell
NSAID	Non-Steroidal Anti-inflammatory Drug
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PCR	Polymerase Chain Reaction
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
qPCR	Quantitative Polymerase Chain Reaction
RSV	Respiratory Syncytial Virus
RV	Rhinovirus
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
Tfh	T Follicular Helper
Teff	T Effector
Th	T Helper
US	United States
UVA	University of Virginia
WHO	World Health Organization

PROTOCOL SUMMARY

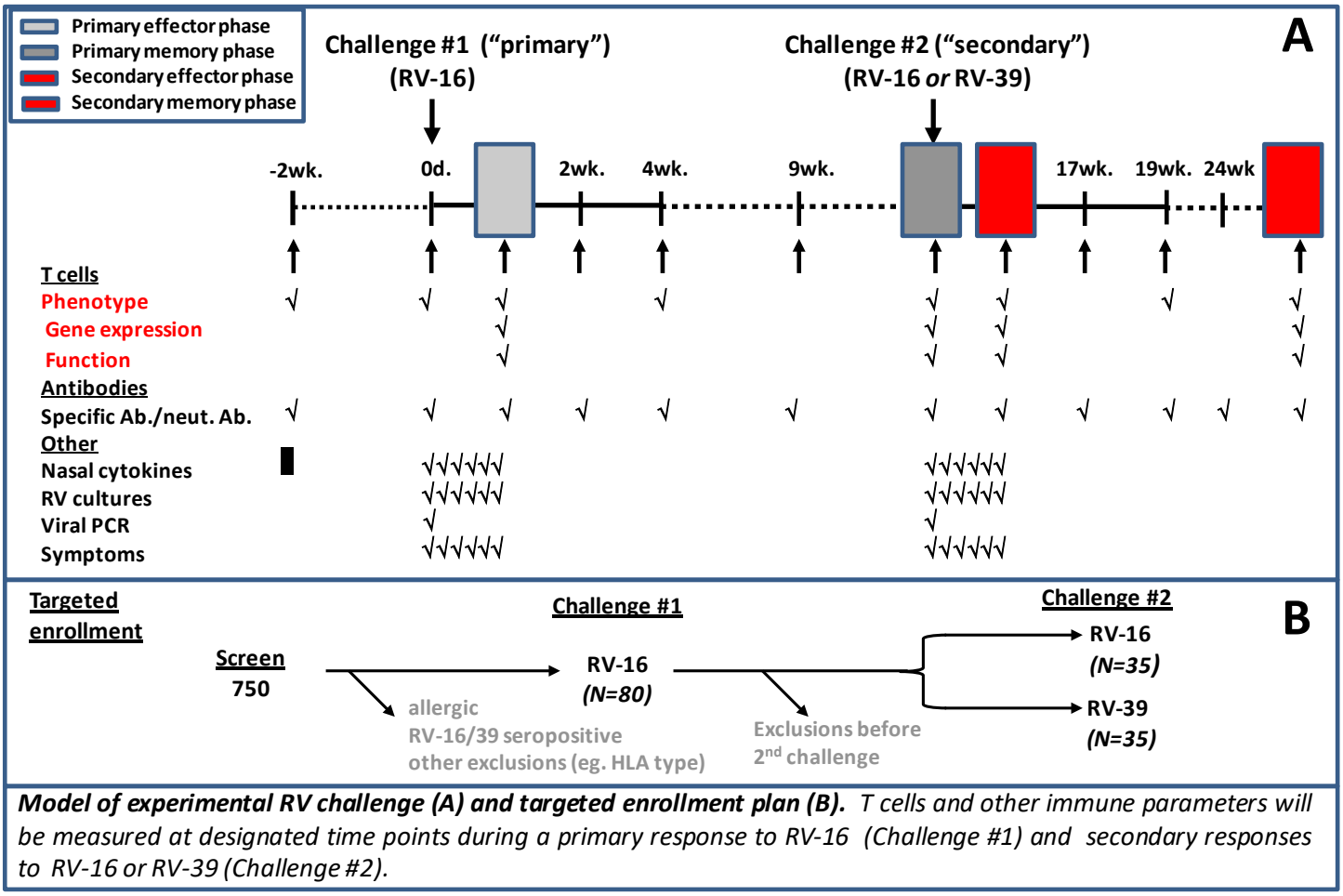
Title:	Hallmarks of Protective Immunity in Sequential Rhinovirus Infections in Humans
Phase:	II
Population:	Healthy young adult volunteers both male and female from the University of Virginia (UVA) community. Approximately 120 healthy, young adult volunteers with an antibody titer ≤1:2 to both rhinovirus (RV) type 39 and RV type 16 will be recruited for participation in the study. Approximately 80 subjects who meet all inclusion and exclusion criteria will be challenged with RV.
Number of Sites:	Single-site
Study Duration:	3 years (for clinical studies, total study duration is 5 years)
Subject Participation Duration:	Each subject will participate in two separate challenge cohorts over a period of 32 weeks.
Description of Agent or Intervention:	Human RV types 39 and 16 by intranasal administration

Objectives:

The primary objective of this study is to assess the relationship between RV-specific T-cell immunity and the human host response to primary RV challenge and subsequent secondary challenge with either homologous or heterologous RV serotypes. The overall hypothesis that will be addressed by the mechanistic studies in this proposal is that T helper (Th) and T follicular helper (Tfh) cells directed against conserved RV epitopes expand upon RV exposure and some of these cells persist as stable cross-reactive memory populations capable of displaying lineage-specific protective functions upon re-infection with related or unrelated strains of RV. The human specimens collected in this study will be analyzed with a variety of state-of-the-art techniques to provide an in depth description of T-cell responses to RV infection, and the correlation of these responses with viral infection, antibody responses, and illness. Beyond this objective, by using a systems biology approach, we aim to gain new insight into the role of diverse cell types involved in adaptive immunity to RV.

Estimated Time to Complete Enrollment: 3 years

Schematic of Study Design:



Model of experimental RV challenge (A) and targeted enrollment plan (B). *T cells and other immune parameters will be measured at designated time points during a primary response to RV-16 (Challenge #1) and secondary responses to RV-16 or RV-39 (Challenge #2).*

1 KEY ROLES

Individuals:

Principal Investigators:
Judith A .Woodfolk, MBChB, PhD
University of Virginia
P.O. Box 801355
Charlottesville, VA 22908
Phone Number: 434-924-1293
Fax Number
Email: jaw4m@eservices.virginia.edu

Ronald B. Turner, MD
University of Virginia
P.O. Box 800386
Charlottesville, VA 22908
Phone Number: 434-243-9864
Fax number: 434-982-4246
Email: rbt2n@virginia.edu

DMID

DMID Scientific Lead:
Teresa M. Hauguel, Ph.D.
Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
5601 Fishers Lane, Room 8E19
Bethesda, MD 20892
Phone: 240-627-3272
Email: hauguelt@niaid.nih.gov

DMID Medical Officer:
Trinka Coster, M.S., M.D. (C)
Medical Officer, Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
Room 8E25, 5601 Fishers Lane
Bethesda, MD 20892
Tel: 301 - 761-7736
Fax: 240 - 627-3139
Email: trinka.coster@nih.gov

DMID Medical Monitor:
Venus Shahamatdar, M.D. [C]
Medical Monitor
Office of Clinical Research Affairs
DMID, NIAID, NIH
Phone: 240-627-3369
Email: venus.shahamatdar@nih.gov

DMID Clinical Project Manager:
Tena Knudsen, B.S.N.
Respiratory Diseases Branch
DMID, NIAID, NIH
5601 Fishers Lane, Room 8E36, MSC 9825
Rockville, MD 20852-9825
Phone: 240-627-3290
Email: knudsent@niaid.nih.gov

DMID Pharmacovigilance Group:
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Institutions:
University of Virginia
P.O. Box 800386
Charlottesville, VA 22908
Contact Person/Local Investigator: Ronald B. Turner, MD
Phone Number: 434-243-9864
Fax number: 434-982-4246
Email: rbt2n@virginia.edu

Institutional Review Board
UVA Institutional Review Board for Health Sciences Research
P.O. Box 800483
Charlottesville, VA 22908
Phone Number: 434-924-2620

School of Medicine Clinical Trials Office
Cobb Hall, Room 1033
135 Hospital Drive
Charlottesville, VA 22908-0786
UVA Messenger Mail: Box 800786
Phone: 434-924-8530

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

RV infections are one of the most common infections of man. These infections are typically associated with upper respiratory symptoms but also cause lower respiratory disease in infants and children and immunocompromised hosts [1]. Approximately 70% of all asthma exacerbations in school-aged children are associated with RV infection and RV infections in young infants may influence the future development of asthma in susceptible hosts [2-4]. RV infections are also associated with exacerbations of chronic obstructive pulmonary disease [5]. Although there has been considerable effort to develop effective antivirals for treatment of RV infections, there are no treatments currently available.

Increased serum concentrations of neutralizing type-specific antibody are associated with protection from infection and illness [6-9]. Although some neutralizing antibodies that cross react with different serotypes have been described [10], efforts to produce antibody-based vaccines have largely been abandoned due to the large number of different serotypes and the limited protection provided by the early vaccines [11, 12].

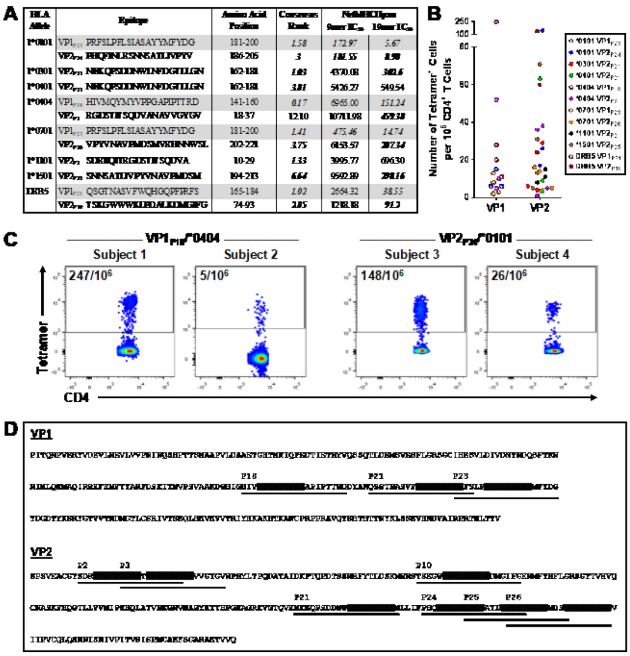


Fig. 1. VP1 and VP2 Epitopes of RV-A16. (A) Epitopes identified by TGEM (VP1 epitopes shaded) and their predicted binding constants. Underline denotes predicted 9mer core epitope. Consensus rank was generated using the IEDB resource. The NetMHCIIpan algorithm was used to compare predictions for 9mer and 19mer epitopes. Bolded values meet defined criteria for MHCII binding (IEDB Consensus Ranks≤ 10; NetMHCIIpan IC_{50}≤ 500). **(B)** Frequencies of RV-specific CD4+ T cells determined by direct *ex vivo* staining with tetramer for each TGEM epitope (29 subjects). **(C)** Representative data showing tetramer+ T cells within the CD4+ T-cell gate stained with $VP1_{P18}$/*0404 and $VP2_{P26}$/*0101 tetramers in 4 subjects with high and low T-cell frequencies. **(D)** Localization of T-cell epitopes within the primary amino acid sequences of VP1 and VP2. Line denotes each 20mer peptide with predicted core epitopes in grey.

Activation of RV-specific cluster of differentiation (CD) 4^+ T cells is a critical antecedent to immunoglobulin G (IgG) responses; however, the nature of the T cells involved remains enigmatic owing to the lack of robust mouse models of RV infection, and challenges in identifying and tracking rare RV-specific T cells in humans. It is now recognized that naïve $CD4^+$ T cells develop into a variety of specialized T-cell subsets under the control of lineage-specifying transcription factors. These subsets include "conventional" T effector (Teff) types (e.g., Th1, Th2 and Th17), as well as Tfh cells. Recent work in animal models indicates that Tfh cells, which provide help to B cells in germinal centers for antibody responses, persist as memory T cells following acute viral infection [13].

1. <u>Identification of Conserved T-Cell Epitopes of RV-16:</u> This study protocol involves sequential RV challenges using the group A viruses, RV-16 and RV-39. Through a

collaboration with Dr. William Kwok, PhD (Benaroya Research Institute), we have comprehensively mapped $CD4^+$ T-cell epitopes of the RV-16 capsid proteins VP1 and VP2 in human leukocyte antigen (HLA)-diverse humans by *in vitro* (tetramer-guided epitope mapping) and *in silico* (computer prediction) methods [14-18]. The capsid proteins VP1 and VP2, encase the RNA genome and contain motifs known to bind neutralizing antibodies, as well as surface receptors for cell entry [19-22]. Thus, they provide candidate T-cell antigens. This work generated 10 unique peptide epitopes of RV-16 that are conserved across RV groups A, B and C (**Fig. 1**). These epitopes bind to HLA class II molecules present in 85% of the general population (listed in **Fig. 1A**). By a multi-step process that involved tetramer guided epitope mapping followed by direct *ex vivo* staining with tetramers [23-25], it was confirmed that circulating memory RV-16-specific $CD4^+$ T cells present at the highest frequencies in healthy uninfected subjects preferentially target these epitopes (**Figs. 1B & C**). Moreover, using a variety of computer prediction algorithms, we have shown that those epitopes identified by tetramer guided epitope mapping are highly conserved across RV-A species, and to a lesser extent across RV-B and C species (**Table I**). Details of these findings are in a manuscript published in the Journal of Immunology [26].

Table I. Sequence Similarity Between RV-A16 Epitopes and Rhinovirus Strains Belonging to Species A, B, and C.

Epitope	Prevalence of Amino Acid Sequence Identity Among Strains of Each Rhinovirus Species								
	RV A					RV B		RV C	
	100%	99-95%	94-90%	89-85%	Total	Total	Range (% identity)	Total	Range (% identity)
$VP2_{P3}$ RGDSTITSQDVANAVVGYGV	40/77 (51.9%)	17/77 (22.1%)	10/77 (13.0%)	7/77 (9.1%)	74/77 (96.1%)	10/29 (34.5%)	72-67%	1/51 (2.0%)	72%
$VP2_{P2}$ SDRIIQITRGDSTITSQDVA	50/77 (64.9%)	8/77 (10.4%)	13/77 (16.9%)	2/77 (2.6%)	73/77 (94.8%)	23/29 (79.3%)	65%	21/51 (41.2%)	78-72%
$VP1_{P23}$ PRFSLPFLSIASAYYMFYDG	39/77 (50.6%)	14/77 (18.2%)	3/77 (3.9%)	17/77 (22.1%)	73/77 (94.8%)	21/29 (72.4%)	74-68%	7/51 (13.7%)	70-65%
$VP2_{P26}$ VPYVNAVPMDSMVRHNNWSL	8/77 (10.4%)	53/77 (68.8%)	5/77 (6.5%)	6/77 (7.8%)	72/77 (93.5%)	23/29 (79.3%)	82-71%	7/51 (13.7%)	76-70%
$VP2_{P10}$ TSKGWWWKLPDALKDMGIFG	31/77 (40.3%)	33/77 (42.9%)	1/77 (1.3%)	7/77 (9.1%)	72/77 (93.5%)	21/29 (72.4%)	95-84%	4/51 (7.8%)	89-79%
$VP2_{P24}$ PHQFINLRSNNSATLIVPYV	7/77 (9.1%)	43/77 (55.8%)	12/77 (15.6%)	8/77 (10.4%)	70/77 (90.9%)	23/29 (79.3%)	85-70%	7/51 (13.7%)	75-70%
$VP2_{P25}$ SNNSATLIVPYVNAVPMDSM	7/77 (9.1%)	59/77 (76.6%)	4/77 (5.2%)	0/77 (0.0%)	70/77 (90.9%)	22/29 (75.9%)	84-68%	7/51 (13.7%)	74-68%
$VP1_{P21}$ QSGTNASVFWQHGQPFPRFS	2/77 (2.6%)	16/77 (20.8%)	21/77 (27.3%)	29/77 (37.7%)	68/77 (88.3%)	0/29 (0.0%)	—	0/51 (0.0%)	—
$VP2_{P21}$ NEKQPSDDNWLNFDGTLLGN	17/77 (22.1%)	5/77 (6.5%)	20/77 (26.0%)	9/77 (11.7%)	51/77 (66.2%)	6/29 (20.7%)	89%	0/51 (0.0%)	—
$VP1_{P18}$ HIVMQYMYVPPGAPIPTTRD	1/77 (1.3%)	3/77 (3.9%)	8/77 (10.4%)	28/77 (36.4%)	40/77 (51.9%)	3/29 (10.3%)	79-54%	0/51 (0.0%)	—

Data was generated based on sequence alignments obtained by Protein BLAST using the top 5,000 results. Peptides are listed from highest to lowest "identity scores" based on their overall prevalence of identity within RV species A.

Values denote the prevalence of strains within each RV species that showed amino acid sequence identity, with percentages in parentheses. The total number of RV types for each species used as the denominator is based on classifications according to McIntyre et al (63).

For RV species B and C, only the total prevalence with the corresponding range of amino acid sequence identity is shown, owing to lower sequence identities compared with RV species A.

2. <u>Patent:</u> Based on their cross-reactive potential, the UVA, through its affiliated Licensing and Ventures Group, has patented the use of these peptide epitopes as vaccines against RV infection. This patent is declared in the consent form. There is no income from this patent and it therefore does not constitute a financial conflict of interest under Public Health Service guidance. (http://grants.nih.gov/grants/policy/coi/coi_faqs.htm#3181)

3. <u>Tracking RV-specific T Cells During Experimental Infection:</u> The current study will use major histocompatibility complex class II (MHCII) tetramers that display conserved RV-16 peptide epitopes to identify and track circulating RV-specific CD4$^+$ T cells in subjects who receive sequential RV challenges with RV-16 and RV-39 (**Fig. 1** and **Table I**). This will allow us to interrogate adaptive immunity to heterotypic RV strains. Preliminary work performed under a separate pilot study established the feasibility of this approach. In that study, tetramer-guided epitope mapping [16] was used to develop two DRB1*0401 tetramers that displayed conserved RV-39 epitopes (one each for capsid protein VP1 and VP2). The pilot study included 16 healthy DRB1*0401+ subjects (ages 18-60 years old) who participated in a RV-39 experimental challenge study. All subjects had no history of allergies and were seronegative for RV-39 (serum neutralizing antibody titer ≤1:4) (*see enrollment,* **Fig. 2**). Challenge with RV-39 was administered intranasally (2 doses per nostril of a 0.25 mL solution containing 100 $TCID_{50}$/mL). RV-39 antibody titer was re-tested immediately before challenge (day 0) and during convalescence (day 21). Those subjects experiencing ≥4-fold increase in RV-39 serum neutralizing antibody titer, or at least one RV-39 positive nasal culture, were considered infected. Peripheral blood mononuclear cell (PBMCs) were isolated from DRB1*0401+ subjects before RV challenge (days -28 and 0), and during acute (day 5) and convalescent (day 21) phases (*model*, **Fig. 2**). Samples were frozen and then thawed for analysis upon study completion. Tetramer staining and flow cytometry analysis was performed using established methods. A control tetramer containing an irrelevant peptide (GAD555-567) provided a negative control [23-25, 27, 28].

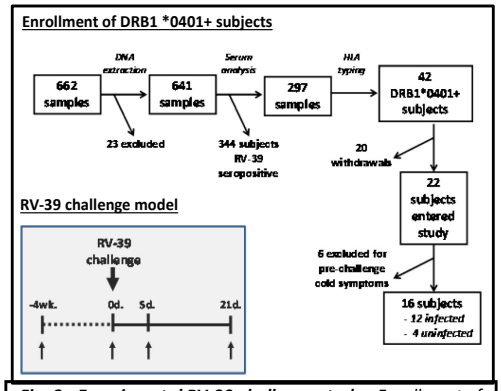


Fig. 2. Experimental RV-39 challenge study. *Enrollment of DRB1*0401+ subjects and RV-39 challenge study design. Arrows in the model denote PBMC sampling time points.*

Of the 16 DRB1*0401+ subjects enrolled, 12 were infected and 4 were uninfected. In *post hoc* tests, 3 subjects had evidence of recent RV exposure based on positive serum neutralizing antibody titers or positive polymerase chain reaction (PCR) for virus on day 0. In addition, two subjects who met criteria for infection based on positive nasal culture failed to seroconvert by day 21. Thus, we could assess modulation of RV-specific CD4$^+$ T cells in the context of different serology and exposure profiles. Circulating CD4$^+$ T cells specific for conserved regions of RV-39 capsid proteins were identified in all subjects immediately before inoculation. Among

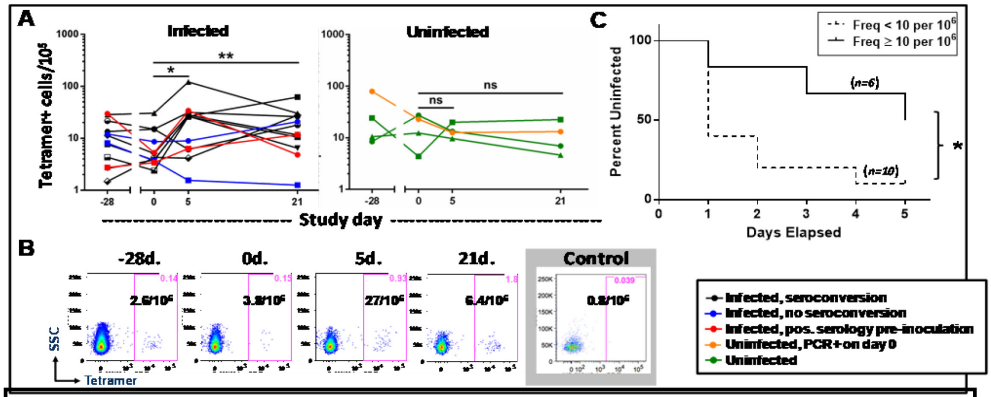


Fig. 3. Tracking RV-39-specific CD4+ T cells during experimental challenge. ***(A)*** *Change in the frequency of tetramer+ CD4+ T cells in infected (n=12) and uninfected (n=4) subjects. *p<0.05, **p<0.01.* ***(B)*** *Representative data from an infected subject with RV-39 seroconversion. Control: GAD555 tetramer.* ***(C)*** *Relationship between the frequency of RV-specific CD4+ T cells at day 0 and time to positive nasal RV culture post-inoculation. *p=0.03.*

infected subjects, expansion of circulating RV-specific $CD4^+$ T cells was observed within 5 days of RV inoculation (**Fig. 3A**). Moreover, T-cell expansion during the acute phase was present in infected individuals who had evidence of recent RV exposure, including one with a high T-cell frequency pre-inoculation (in red, **Fig. 3A**). T-cell frequencies either contracted, or else continued to expand, after resolution of infection (**Figs. 3A & B**). Subjects who failed to seroconvert post-inoculation, showed no expansion of circulating RV-specific $CD4^+$ T cells during the acute phase, regardless of infection status (in blue, **Fig. 3A**). *Notably, higher frequencies of RV-specific T cells pre-inoculation were associated with lower infection rates as judged by time to positive RV culture (**Fig. 3C**) and delayed time to peak symptoms. These observations, coupled with the link between high levels of RV shedding and more severe symptoms [29], imply a protective role for pre-existing T cells directed against conserved epitopes*. A manuscript describing these findings has been submitted for publication (Muehling LM, Turner RB, Brown KB, Wright PW, Lehtinen M, Kwok WW, Woodfolk JA. Single-cell tracking of rhinovirus-specific CD4+ T cells during experimental infection). MHCII tetramers that display conserved epitopes of RV-16 (shown in **Fig. 1 & Table I**) have recently been used by the Woodfolk group to identify, enumerate, and track RV-16-specific $CD4^+$ T cells in healthy and asthmatic subjects following experimental challenge with RV-16 (work in progress). *Together, these findings establish our ability to track circulating RV-specific T cells during experimental infection.*

4. Tracking the Molecular Signature of RV-Specific T Cells: Using both RV-39 and RV-16 challenge models, we can assess the molecular signature of circulating RV-specific T cells as follows:

1. RV-specific T cells that exist prior to infection include central memory Tfh cells.
2. During acute infection, RV-specific T cells expand in numbers and acquire an activated effector memory phenotype consistent with trafficking to inflamed sites.
3. Following infection, RV-specific T cells revert to a central memory phenotype.
4. RV-specific T cells express CCR5, a molecule implicated in T-cell homing to sites of viral infection [30].
5. RV-specific T cells have a Th1 signature, as judged by their capacity to secrete the Th1-associated cytokine IFN-gamma.

Together, these findings confirm the existence of circulating central memory Tfh cells directed against conserved RV epitopes that have the potential to rapidly mobilize upon re-infection with RV, and to survive as long-term memory cells.

5. Measuring RV-Specific Serum Antibodies: Antigen binding radioimmunoassays to measure IgG specific for RV-16 VP1 and VP2 have been developed and IgA assays are in progress. This assay can be used to detect a rise in the levels of capsid protein-specific antibodies following experimental infection with RV (data not shown). *These findings establish the feasibility of tracking serum antibodies specific for viral antigens that are targeted by $CD4^+$ T cells of interest. Such antibodies may be highly relevant to cross-protection.*

6. High-Dimensional Immunophenotyping by Mass Cytometry: T cells will be analyzed in the context of a broad array of cell types in order to construct a comprehensive picture of adaptive immunity to RV. This will be accomplished by a systems biology approach using mass cytometry (CyTOF). This technology, which allows simultaneous analysis of at least 45 immune markers, allows us to capture data on multiple cell types. These include, but are not limited to,

Th1, Th2, Th17 cells, T follicular helper cells, CD8+ T cells, NKT cells, gamma delta T cells, and various B-cell populations. Mass cytometry provides a powerful discovery-based experimental tool that will generate large amounts of data that cannot be predicted based on current knowledge. *The Woodfolk lab has optimized procedures for performing mass cytometry.*

Summary: We have demonstrated the ability to identify and characterize RV-specific $CD4^+$ T cells, to measure RV capsid protein-specific IgG antibodies, and to apply mass cytometry to an experimental RV infection in humans. We are now poised to explore how memory T cells specific for conserved RV epitopes mediate protective responses to RV.

2.2 Rationale

The experimental RV challenge model has been used for many years to study the pathogenesis and treatment of RV infection. In this model, seronegative volunteers are challenged by intranasal inoculation of a safety tested RV. The challenge model is ideal for the purposes of this trial since the $CD4^+$ cells of the volunteers can be carefully characterized at baseline before infection with a known RV serotype, followed over the course of the infection and during convalescence, and then reassessed following re-challenge with either the same virus or a different serotype. This longitudinal study allows us to precisely track the dynamic kinetics of adaptive immunity to RV in a time-controlled fashion. Such a study would not be feasible in a design that used natural RV infections owing to the complex kinetics of anti-viral responses, and the inability to determine at what time point subjects were naturally infected. These barriers would preclude accurate assessment of different phases of the adaptive response to RV.

Our group is one of only a handful in the world that is performing RV challenge studies in humans. A key differentiating feature of our study as compared with current models involving experimental inoculation with respiratory viruses in humans, is that we propose a sequential challenge with two different RV strains with known infectivity characteristics in the challenge model (RV-16 and RV-39) in order to establish proof-of concept that conserved RV peptide epitopes are cross-protective. We hypothesize that *Teff and Tfh cells directed against conserved RV epitopes expand upon RV exposure, and a minority of these cells persist as stable cross-reactive memory populations capable of displaying lineage-specific protective functions upon re-infection with related or unrelated strains of RV.* For the first time, we will use novel MHCII tetramers displaying RV epitopes to enumerate, phenotype and isolate RV-specific $CD4^+$ T cells generated during the course of primary and secondary infections. In conjunction, by applying single-cell high dimensional immunophenotyping (mass cytometry), we will capture data on multiple cell types in order to shed light on cellular networks, including T-cell/B-cell interactions, which operate during primary and secondary RV infections. This project capitalizes on novel analytical tools (RV peptide/MHCII tetramers), technologies (mass cytometry and single-cell gene expression platforms) and computational platforms that have been developed and implemented by our group. It leverages synergistic partnerships among experts in T-cell immunology, virology, biochemistry, and single-cell systems biology in order to address fundamental aspects of T cell-mediated RV protection. Additionally, we will gain new insight into cellular and molecular components of the adaptive response to RV that extend beyond T cells. Our findings are expected to impact human health by informing vaccine design through new molecular and functional discoveries related to T cells, B cells and other immune cell types.

RV challenge pools that have been developed under Good Manufacturing Practice conditions and are used under appropriate Investigational New Drug Applications (INDs; IND 12934 for RV39 and 15162 for RV16) will be used to conduct this study of RV immunity. Volunteers will be challenged by intranasal inoculation via nasal drops with 20-100 $TCID_{50}$ of the challenge virus. This dose is selected empirically and is known to produce reproducible infection rates of 80-90% in challenged volunteers. Healthy volunteers are selected for this study to minimize potential adverse events (AEs) associated with experimental infection.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The participants in this study should expect to have a common cold and may experience nasal congestion, runny nose, sneezing, cough, sore throat, malaise, headache and chills but no significant risks to the health of the volunteers are anticipated from participation in this study.

Experience with the study model suggests that adverse outcomes requiring intervention (otitis media, bronchospasm or sinusitis) are rare. The virus pools used for the challenge in this study have been thoroughly safety tested and have been approved for investigational use by the Food and Drug Administration (FDA; IND 12934 for RV39 and IND 15162 for RV16). AEs will be reported to the UVA Human Investigations Committee for review.

RV infection has not been associated with any adverse effects on the fetus or newborn infant, however, women who are pregnant or nursing should not participate in the study. A pregnancy test will be done on all women who decide to participate in the study. All women who participate in the study must have a negative pregnancy test and be using an acceptable method of birth control as determined by the investigator.

RV infections are associated with asthma attacks in people who have asthma. Individuals who have asthma (as evidenced by a history of asthma, a history of wheezing, recurrent severe bronchitis or coughing associated with colds) will be excluded from the study.

The viruses which will be used for the study are RVs which were isolated from volunteers in previous studies. The donor volunteers were tested for human immunodeficiency virus (HIV; acquired immunodeficiency syndrome [AIDS]), hepatitis C and hepatitis B infection, and found to be negative. After isolation, the virus was cultured two times in the laboratory and has been tested for the presence of other pathogens which may cause infections in humans. The production and safety testing of the pools has been reviewed by the FDA and the pools have been granted INDs for experimental RV studies in human volunteers.

2.3.2 Known Potential Benefits

An understanding of the pathogenesis of RV associated illness in humans is of substantial potential benefit to society. There are no benefits to the individual volunteers in this study.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Objectives

3.1.1.1 To assess the relationship between T-cell immunity induced by infection with the rhinovirus serotype RV-16, and the host response to homologous infection with RV-16 or the host response to heterologous infection with RV-39.

3.1.2 Exploratory Objectives

3.1.2.1 The major objective of the study is to perform mechanistic studies that apply state-of-the art experimental technologies to identify, enumerate, and monitor a broad array of known and novel cell types during challenge with RV-16 and re-challenge with RV-16 or RV-39 in order to identify hallmarks of cross-protection. The technologies applied include, but are not limited to, MHCII tetramer staining, mass cytometry, and single-cell gene expression profiling. Exploratory objectives include, , the following: (1) Identify new molecular signatures of RV-specific T cells. (2)Evaluate the quality and quantity of T-cell responses. (3) Identify, enumerate and monitor a broad array of immune cell types (e.g., B-cell populations, CD8+ T-cells, gamma delta T-cells, NKT cells, etc.), and novel cell types. (4) Test multiple relationships between immune cell parameters and infection, serum antibodies, and symptoms (illness), at each sampling time point during RV challenge and re-challenge. Additional objectives may be explored based upon the results of the above mechanistic studies; a protocol amendment will be submitted documenting the additional objectives.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Correlation between the number of pre-existing RV-16-specific T cells at Week 15, and infection after homologous re-challenge with RV-16 or infection after heterologous re-challenge with RV-39 at Week 15.

Numbers of pre-existing RV-specific T cells at Week 15 will be determined by MHCII tetramer staining followed by flow cytometry analysis. Infection will be determined by isolation of virus in culture. Infection will be defined as one positive RV culture obtained in the 5-day time interval after virus re-challenge at Week 15.

3.2.2 Exploratory Outcome Measures

Exploratory outcome measures include the following:

- Tetramer staining and mass cytometry to immunophenotype RV-specific T cells.
- Fluorescent-based flow cytometry and mass cytometry to identify, enumerate, and monitor immune cell types.
- Microarray or single-cell analytical platforms to profile gene expression of immune cells.
- Multiplex cytokine assays to identify cytokines in nasal lavage fluid.
- Antigen binding assays and microtiter neutralization assays will be used to assess serum antibodies. Overall illness will be measured by frequency and severity of symptoms that are solicited and/or expressed by the subjects during follow-up for challenge and rechallenge.

Alternative techniques may be considered, but studies will be limited to addressing the stated exploratory objectives only.

4 STUDY DESIGN

4.1 Description of the Study

This is a mechanistic study to assess the T-cell response to RV infection using the experimental rhinovirus challenge model. All subjects will receive a primary challenge with RV-16 and after 15 weeks, will be re-challenged with either RV-16 or RV-39. The DSMB will review the data and provide recommendations before the re-challenge. The model will be used to assess the correlation between T-cell responses and infection following challenge with homologous or heterologous RV strains. This study is designed to provide a detailed characterization of the T-cell responses to RV infection, to determine whether cross-reacting T-cell responses occur, and to assess how the magnitude of T-cell responses induced by primary challenge with RV-16 relates to infection following re-challenge with RV-16 or RV-39. The timing of the sample collections and the re-challenge is empiric and is designed to correspond with the expected timing of effector and memory adaptive T-cell responses to primary and secondary RV challenge.

The investigational material in this study consists of the virus challenge pools. Although these pools are used under appropriate INDs, these pools are not in a drug/product development pathway and will never be advanced to a New Drug Application (NDA). Under these circumstances, the “phase of the trial” has no relevance or meaning. The size of this trial is comparable to a phase II trial in a drug development process.

The investigational material is being used as a research tool and is not under study in this protocol. We do not plan any changes in dosing or administration.

4.2 Volunteers

Approximately 750 healthy, young adult volunteers will be recruited for participation in the study. These volunteers will be tested for the presence of serum neutralizing antibody to RV type 39 and RV type 16. Volunteer subjects with an antibody titer ≤1:2 to both virus serotypes (~120 volunteers) will be eligible for participation in the challenge trial. Volunteers who are eligible after antibody screening and who agree to participate will complete a brief medical history questionnaire and will have pulse, respiratory rate, body temperature, and blood pressure measured. All female subjects will have a urine pregnancy screen.

Volunteers in this study will be challenged with two different RV serotypes. Volunteers will be free of neutralizing antibody to both serotypes at the start of the study. Two weeks prior to virus challenge, specimens will be collected for phenotyping of T cells and other immune cell types (PBMC specimens); measurement of both neutralizing and capsid-specific antibodies (serum); and assessment of cytokines, quantitative RV culture, and viral PCR (nasal lavage). Two weeks later, all volunteers will have these baseline studies repeated and will then be challenged with RV16 (day 0). After challenge, the volunteers will return to the study site daily for 5 days for collection of nasal lavage specimens for cytokine assays and quantitative RV culture, and for

assessment of common cold symptoms. Specimens will be collected 1, 2, 4 and 9 weeks after challenge for antibody analyses, and 1 and 4 weeks after challenge for T cell analyses. Approximately 15 weeks after the original challenge, all subjects will be assigned to two groups of approximately 35 subjects each. One group will be re-challenged with RV16 and the other group will be challenged with RV-39. The specimen collection after the second challenge will be the same as that after the first challenge. Each volunteer will participate in study activities intermittently over a total period of approximately 32 weeks. The study is designed to be conducted within the school calendar for the UVA which is the primary population for recruitment of volunteers.

The study will be done in three cohorts over 3 years. Approximately 250 volunteers will be screened for each cohort. The feasibility of this enrollment is demonstrated by the fact that our most recent large challenge study in 2013 screened 789 volunteers for three cohorts in 1 year. Based on our experience with our study population, we would expect that screening of 250 volunteers will result in 30-40 volunteers who are seronegative to both virus serotypes, appropriately HLA matched to the available tetramers and meet the other inclusion/exclusion criteria for the study. In the first 2 years, approximately 25 subjects/year will be included in the first challenge with RV16. The sample size included in the final cohort will be adjusted based on attrition in the first two cohorts to ensure that approximately 70 subjects are included in the second challenge with either RV-16 or RV-39. Based on extensive experience with the model, we expect limited attrition (~10%) once volunteers have qualified and been inoculated with virus in the first challenge.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Selection of the Study Population

Participants in the experimental RV challenge studies at the UVA are largely drawn from the University community. These studies are widely known among the students and recruitment is primarily via an established database of individuals who have asked to be notified when a study will occur. Other recruitment activities include mass emails to the student population and "sidewalk chalk" advertising.

Recruitment of eligible volunteers will not be a limitation of this study. Our study site at the UVA has recently completed a separate study in which almost 800 volunteers were screened for participation in a challenge study over a 1-year period. The target of approximately 750 subjects screened over 3 years is well within the capacity of our site.

In this study, subjects will first be screened for neutralizing antibody to the two study viruses under an existing Institutional Review Board (UVA IRB #9948)) approved screening protocol. Volunteers who are eligible to participate by serology (~15%) will then be contacted by email or telephone and offered the opportunity to participate in the challenge studies.

Given the complexity of the study schedule, we will plan to enroll approximately one-third of the study subjects each year for 3 years. The initial challenge will be done during the fall of each year and the second challenge will be done in the winter/spring. This schedule acknowledges the realities of the school calendar and will maximize retention of enrolled subjects. Subjects receive monetary compensation for participation and subject retention is excellent (in a recent study of 150 subjects, no subjects withdrew or were lost to follow-up). The planned enrollment over 3 years will allow variation of the cohort size in years 2 and 3 to ensure that the required sample size is available for analysis.

There are no gender or minority restrictions in this study. An age restriction is included in the study to provide greater homogeneity in the study population. In previous studies of this type at the UVA, approximately 70% of the subjects who participated have been white (72% of the University student body from which volunteer subjects are primarily drawn are white, not Hispanic) and approximately 59% of the participants have been female. The average age of the volunteers in these studies is generally in the early twenties. No gender or racial effects are expected for the analyses in this study. Pregnant women are excluded from the study because of the intentional challenge with RV that is a part of the study. Children are excluded from the study because the intentional challenge with RV cannot be ethically done in children.

5.2 Subject Inclusion Criteria

Subjects must meet all of the inclusion criteria in order to be eligible to participate in the study:
1. Are males and non-pregnant females, 18-40 years of age, inclusive.
2. Provide written informed consent prior to initiation of any study procedures.

3. Are able to understand and comply with planned study procedures and be available for all study visits.
4. Subject must have a serum neutralizing antibody titer of ≤1:2 to RV type 39 and RV type 16.
5. Female subjects* must be using an effective birth control method‡ for at least 30 days prior to study enrollment and through 30 days post-rechallenge.

   *for females who are not surgically sterile or post-menopausal

   ‡§Includes, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill"). Method of contraception will be captured on the appropriate data collection form.

6. Total IgE <200 IU/mL.
7. Hemoglobin ≥ 11 gm/dL for females and ≥ 13 gm/dL for males

5.2.1 Inclusion at Re-challenge

Subject must have participated in the first challenge and meet the following inclusion criteria to be eligible to participate in the re-challenge:

1. Are males and non-pregnant females, 18-41 years of age, inclusive.
2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Female subjects* must be using an effective birth control method‡ for at least 30 days prior to study enrollment and for the remainder of the study.

   *for females who are not surgically sterile or post-menopausal

   ‡§Includes, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill"). Method of contraception will be captured on the appropriate data collection form.

5.3 Subject Exclusion Criteria

5.3.1 Exclusion at enrollment

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation:

1. Any abnormalities of the upper respiratory tract that might interfere with the procedures and assessments in the study
2. Any chronic upper respiratory illness
3. Any chronic lower respiratory disease or any acute lower respiratory illness in the 1 month prior to the challenge
4. Any bleeding tendency by history.
5. History of hypertension or currently on anti-hypertensive medication.
6. History of angina or other cardiac disease.

7. Any medical condition that in the opinion of the Investigator is cause for exclusion from the study.
8. Use of statins
9. Regular use of tobacco in the last 6 months (i.e., more than 2 days out of 7) or inability to refrain from smoking during the study.
10. Participation in any other clinical drug trial in the month prior to the study.
11. Household contacts/room mates who are under protective precautions due to immunosuppression

5.3.2 Exclusion at Challenge

1. Any upper respiratory infection or allergic rhinitis in the 2 weeks prior to challenge.
2. Use of any common cold therapies in the 2 weeks prior to challenge (see list below*)
   * for example: non-steroidal anti-inflammatory medications, benedryl and other antihistamines, Sudafed, pseudoephedrine, phenylephrine, oxymetazoline, inhaled/intranasal steroids, leukotrienes, or any bronchodilators, (such as short acting b-2-agonists, long-acting b2-agonists, anticholinergics).
3. Female subjects* with a positive urine pregnancy screen on day of challenge

5.3.3 *for females who are not surgically sterile or post-menopausalExclusion at Re-challenge

To be considered eligible to participate in the Re-challenge, subjects must meet the eligibility criteria as listed above in both Inclusion and Exclusion Criteria. In addition, they must not have any of the following Exclusion criteria:

1. Subject experienced an infection-related adverse event (e.g. bronchospasm, otitis media, sinusitis, fever (T>100.5º F)) following prior challenge.
2. Female subjects with a positive urine pregnancy screen on day of rechallenge

5.4 Treatment Assignment Procedures

5.4.1 Randomization Procedures

All volunteers will be challenged with RV16 at the first challenge. Randomization of subjects for challenge with either RV16 or RV39 at the second challenge will be done using GraphPad® software with randomization blocks of ten. The challenge pool used will be recorded on source documents and transferred to the data collection form (DCF).

5.4.2 Masking Procedures

This study does not involve any blinding or masking procedures. Volunteers will not be told which virus they are receiving for the second challenge. The identity of the virus inoculated for the second challenge is of no clinical significance and this information will not be shared with the subjects.

5.4.3 Reasons for Withdrawal

Withdrawal Criteria

Subjects may be withdrawn from the study (i.e., from any further study procedure but not from analyses) for the following reasons:

- At their own request or at the request of their legally authorized representative.
- If the volunteer fails to comply with study procedures.
- If they are lost to follow-up.

In all cases, the reason for and date of withdrawal must be recorded in the DCF. The subject must be followed up to establish whether the reason was an AE, and, if so, this must be reported.

The investigator or appointed representative must make every effort to contact subject lost to follow-up. Attempts (3 attempts) to contact such subject, and the method of contact, must be documented in the subject's records (e.g., dates and times of attempted contact).

Any subject may be withdrawn from the study at the discretion of the investigator. The subject is also free to terminate participation at any time.

Subjects who meet exclusion criteria for the re-challenge will not be followed beyond the week 15 study visit unless they have an adverse event that must be followed by protocol.

5.4.4 Handling of Withdrawals

Withdrawn Subject Data Collection

The principal investigator (PI) and/or other investigator involved in the study will document the reason for the subject withdrawal on the termination page of the DCF as follows:

- Lost to follow-up: subjects who leave the study without notification, or do not attend the study visit or cannot be contacted by phone. Intensive efforts should be made to locate and recall them if possible and to determine their health status at a minimum.
- AE: an AE event form must be completed.
- Adverse laboratory event: this reason should be stated when a laboratory value is interpreted as a clinically significant abnormal value not explained by a laboratory error, or being not a known or abnormal value commonly observed in this type of population.
- Deviation from protocol.
- Consent withdrawn.
- Other: if the above mentioned reasons are not applicable, then the reason will be specified.

Subjects who withdraw from the study during the symptomatic portion of the virus challenge will be re-contacted approximately 14 days after the challenge to document resolution of the viral illness. Subjects who withdraw at other times in the study will not require any study follow-up. Attempts to contact subjects who are lost to follow-up will be as described in section 5.4.3.

5.4.5 Termination of Study

Although the study Sponsor has every intention of completing the study, it reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to Data and Safety Monitoring Board (DSMB) review and recommendation, and at the discretion of DMID.

6 INVESTIGATIONAL PRODUCT

6.1 Investigational Product Description

6.1.1 Acquisition

The challenge viruses used for this study are in the possession of the sponsor-investigator (Dr. Turner).

6.1.2 Formulation, Packaging, and Labeling

The virus pools consist of clarified cell culture supernatants of Eagle's minimal essential media supplemented with 10% fetal bovine serum. The pools are packaged in cryovials labeled to identify the pool and lot number, and are stored at -60 °C.

6.1.3 Product Storage and Stability

Vials of the RV pool are stored at -60 °C until thawed for use for the challenge. The vials are diluted in lactated ringer's solution for human use to produce the desired dilution for the human challenge. The diluted virus is prepared on the day of challenge and is kept cool on wet ice until the inoculation of volunteers is competed. The stability of the virus is documented by back titration of the diluted inoculum pool to demonstrate that the expected virus titer was delivered.

The virus pools are stored at -60 °C or below. The challenge pools are stable under these conditions for an indefinite period of time measured in decades.

6.2 Dosage, Preparation and Administration of Investigational Product

6.2.1 Challenge and Re-challenge with RV

The challenge viruses to be used in this study are human RVs A16 and A39. Both of these challenge pools are used under FDA regulation (IND 12934 for RV39 and IND 15162 for RV16). Both pools have a concentration of 3.9×10^4 $TCID_{50}$/mL. The virus stock is diluted into Lactated Ringer's (LR) solution for human parenteral administration to provide a final concentration of 20-100 $TCID_{50}$/mL for the inoculation. Following a nasal wash, each subject will be given the challenge virus by intranasal drops. The virus is administered in a volume of 0.25 mL/nostril with a calibrated pipette. The 0.25 mL inoculum is administered twice in each nostril 1-3 minutes apart to provide the total virus inoculum. The lot number of the LR solution will be recorded and the virus challenge material will be back-titrated to document the continued potency of the challenge pool.

6.2.2 Modification of Investigational Product for a Participant

There will be no modification of dose in this study. Only one dose of virus is delivered for each challenge.

6.3 Accountability Procedures for the Investigational Product(s)

This study is being conducted by the sponsor-investigator. The investigational product will be under the control of the sponsor-investigator at all times. A dispensing log will be maintained to document administration of the challenge virus to the individual subjects. The investigator will administer the challenge virus. There will be no shipping or distribution of the investigational product.

6.4 Assessment of Subject Compliance with Investigational Product

Compliance is assured since the product is administered to the volunteer by the investigator.

6.5 Concomitant Medications/Treatments

Volunteers who use any common cold therapies in the 2 weeks prior to the study (see list below*) will be excluded from study participation.

* non-steroidal anti-inflammatory medications, benedryl and other antihistamines, Sudafed, pseudoephedrine, phenylephrine, oxymetazoline, inhaled/intranasal steroids, leukotrienes, any bronchodilators (such as short acting b-2-agonists, long-acting b2-agonists, anticholinergics), or dietary supplements.

In the 5 days after each RV challenge the volunteers will be asked to refrain from the use of common cold therapies (see list below*) to avoid interference with assessment of the endpoints of the study. * non-steroidal anti-inflammatory medications, benedryl and other antihistamines, Sudafed, pseudoephedrine, phenylephrine, oxymetazoline, inhaled/intranasal steroids, leukotrienes, or any bronchodilators, (such as short acting b-2-agonists, long-acting b2-agonists, anticholinergics).

All medications used by the volunteers during the study (Day -14 through week 15 post second RV challenge) will be recorded to assist with assessment of compliance and to permit assessment of the symptoms and symptom scoring (See Section 8.2.2).

7 STUDY SCHEDULE

7.1 Study Schedule

Each challenge cohort will be conducted on an identical schedule of events. Volunteer interactions in each cohort are illustrated in the Study Schedule Table in Appendix A

7.2 Screening

7.2.1 Screening and Enrollment Visit (Study day -14 to -7, study visit 1)

- Review study and obtain informed consent (volunteers who consented are provided with a copy of the signed consent)
- Assessment of inclusion/exclusion criteria
- Recording of demographic data to include date of birth, gender, race and ethnic origin
- Complete and review medical history questionnaire
- Vital signs to include pulse, respiratory rate, blood pressure, temperature
- Blood specimen for HLA typing, hemoglobin, total IgE, and RV serology (neutralizing antibody and capsid protein-specific IgG and IgA) (30 ml)
- Blood specimen for baseline PBMC collection (90 ml)
- Recording of concomitant medications.

7.3 First Virus Challenge

7.3.1 Study day 0 (study visit 2)

- Baseline symptom assessment
- Review of interval medical history
- Review of AEs
- Review of concomitant medications
- Blood draw for acute serology and PBMC collection (105 ml)
- Nasal lavage for respiratory virus PCR, and cytokines
- Urine pregnancy screen for females who are not surgically sterile or post-menopausal (results must be known prior to the RV Challenge) Challenge with RV

7.3.2 Study days 1-5 (study visits 3-7)

- Daily symptom assessment
- Assessment of subjective feeling of a cold (day 5 only)
- Daily assessment of AEs
- Daily assessment of concomitant medications
- Daily nasal lavage for RV qPCR, quantitative RV culture, and cytokines

7.3.3 Study day 7 (study visit 8)

- Review of AEs
- Review of concomitant medications
- Blood draw for PBMC collection and RV serology (105 ml)

7.3.4 Study day 14 (study visit 9)

- Review of AEs
- Review of concomitant medications
- Blood collection for RV serology (15 ml)

7.3.5 Study day 28 ± 2 (study visit 10)

- Review of AEs
- Review of concomitant medications
- Blood draw for PBMC collection and RV serology (105 ml)

7.3.6 Study day 63 ± 2, Week 9 (study visit 11)

- Review of AEs
- Review of concomitant medications
- Blood collection for RV serology (15 ml)

7.3.7 15 Weeks after Virus Challenge (study visit 12)

(Note that this visit coincides with Study day 0 for the second virus challenge.)

- Review of AEs
- Review of concomitant medications
- Blood draw for RV serology and PBMC collection (105 ml)

7.4 Second Virus Challenge

7.4.1 Study day RC0 (study visit RC 1)

- Baseline symptom assessment
- Review of interval medical history
- Urine pregnancy screen for females who are not surgically sterile or post-menopausal
- Review of AEs
- Review of concomitant medications
- Nasal lavage for respiratory virus PCR, and cytokines
- Challenge with RV

7.4.2 Study days RC1-RC5 (study visits RC 2-6)

- Daily symptom assessment
- Assessment of subjective feeling of a cold (day 5 only)
- Daily assessment of AEs
- Daily reassessment of concomitant medications
- Daily nasal lavage for RV qPCR, quantitative RV culture and cytokines

7.4.3 Study day RC7 (study visit RC 7)

- Review of AEs
- Review of concomitant medications
- Blood draw for PBMC collection and RV serology (105 ml)

7.4.4 Study day RC14 (study visit RC 8)

- Review of AEs
- Review of concomitant medications
- Blood collection for RV serology (15 ml)

7.4.5 Study day RC28 ± 2 (study visit RC 9)

- Review of AEs
- Review of concomitant medications
- Blood draw for PBMC collection and RV serology (105 ml)

7.4.6 Study day RC63 ± 2, RC Week 9 (study visit RC 10)

- Review of AEs
- Review of concomitant medications
- Blood draw for RV serology (15 ml)

7.4.7 15 Weeks after Second Virus Challenge (study visit RC 11)

- Review of AEs
- Review of concomitant medications
- Blood draw for PBMC collection and RV serology (105 ml)

7.5 Follow-up

Volunteers will be followed for 15 weeks after each virus challenge as described in sections 7.2 and 7.3.

7.6 Final Study Visit

The final study visit will occur at week 15 after the second virus challenge. The procedures done at this visit are described in section 7.3.7. We will not collect AEs beyond this visit.

7.7 Early Termination Visit

If a volunteer withdraws or is removed from the study due to an AE as described in section 9.1, we will continue to follow the subject until the appropriate resolution of the AE. Early termination due to withdrawal of consent will require no further follow-up or procedures. However, in the event of early termination due to an AE or any other cause, the subject may be asked whether they consent to continue the remaining study procedures.

7.8 Unscheduled Visit

If an unscheduled visit occurs, it will be documented in the notes section of the DCF.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

8.1.1 Medical history

A medical history will be obtained at the time of the enrollment visit. The history is obtained via questionnaire in an interactive interview with a member of the study staff who is a health care professional. The information is recorded on a structured source document to be reviewed by the PI for eligibility prior to enrollment. The medical history will include the use of prohibited medications in the 2 weeks prior to enrollment. Medical records will not be reviewed.

8.1.2 Vital Signs

Vital signs will be done at the enrollment visit.

8.1.3 Concomitant medications

Volunteers will be asked to report all medication use during an interactive interview with the study staff. This information will be collected at each study visit and will be used to assist with assessment of compliance and to permit assessment of the symptoms, symptom scoring, and study analyses. Use of cold medications could impact eligibility (see Section 5.3.1 and Section 6.5), and will impact symptom scoring (see Section 8.2.2).

8.2 Study Procedures

8.2.1 Nasal Lavage

Nasal lavage will be collected for quantitative viral cultures, viral PCR and qPCR, and for determination of cytokine concentrations. These specimens are collected by instillation of 5 mL of sterile 0.9% saline into each nostril. This wash is then immediately expelled into a waxed paper cup and kept chilled until processed. Lavage for viral culture is diluted 3:1 in 4X concentrated viral collection broth and then frozen at -80 °C until cultured for detection of RV. Lavage for cytokine assays is stored undiluted at -80 °C.

8.2.2 Symptom Scoring

Symptom assessment will not be the primary outcome variable for any of the experiments in this proposal. Symptom assessment will be done as part of the secondary analysis of the correlation between different T-cell subsets and symptomatic illness following homologous or heterologous re-challenge.

Volunteers will be asked to refrain from the use of common cold therapies during the 5 days after each RV challenge. Symptom scoring is done using a standardized method [31, 32]. Each

subject is asked in an interactive interview to assess the severity of the symptoms of rhinorrhea, nasal obstruction, cough, sore throat, sneezing, headache, malaise and chilliness as follows:

- **Absent** (0 points)
- **Mild** (1 point) Barely noticeable, does not interfere with daily activity.
- **Moderate** (2 points) Noticeable, but does not prevent daily activity.
- **Severe** (3 points) Interferes or prevents daily activity; or the volunteer used the cold medications listed below* to treat cold symptoms,
  * non-steroidal anti-inflammatory medications, benedryl and other antihistamines, Sudafed, pseudoephedrine, phenylephrine, oxymetazoline.
  Or any use of bronchodilators, leukotrienes, or inhaled/intranasal steroids.

The symptom scores will be recorded prior to the nasal wash procedure on Study Days 0-5.

Symptom scores will be collected on Days 0-5, however only the scores on Days 1-5 will be used to calculate the Total Symptom Score (TSS).

8.2.3 Assessment of infection and illness

Volunteers who have challenge virus detected in nasal lavage by culture or PCR on any of the Study Days 1-5 after virus challenge and/or have a four-fold rise in neutralizing antibody titer to the study virus are considered infected. Volunteers who have a positive culture for the challenge virus on any of the Study Days 1-5 after virus challenge are defined as “virus shedders” and the time from Day 0 to the first positive virus culture is defined as the “time to virus shedding”.

The total symptom score will be the sum of the symptom scores for all symptoms on Study Days 1-5. Infected volunteers who have a total symptom score of at least 6 and either at least 3 days of rhinorrhea or the subjective impression that they have had a cold will be defined as having a symptomatic infection.

8.3 Laboratory Evaluations

8.3.1 Virology

Detection of virus in the Study day 0 nasal lavage is done by a multiplex PCR (Luminex xTAG® Respiratory Viral Panel [Austin, TX]) in the Clinical Laboratory of the UVA Medical Center.

RV cultures will be done by routine methods [33]. Nasal lavage collected on Study days 1-5 are cultured for RV by inoculation into two tubes of human embryonic lung fibroblast cells (either MRC-5 or WI-38) and incubation on roller drums at 33 °C for 14 days. RV is identified by the development of typical cytopathic effect. One isolate from each subject who sheds virus is confirmed as the challenge RV serotype by a neutralization assay with HRV type 39 or type 16 antiserum. Subjects who have at least one positive culture for the challenge virus or who have at least a four-fold rise in antibody titer to RV type 39 are considered infected. Previous studies have established that the challenge inoculum is not re-isolated from uninfected volunteers, thus a single positive culture is evidence of virus replication in the upper respiratory tract [34]. Serial 10-fold dilutions of the nasal lavage will be cultured in microtiter plates for semi-quantitative determination of viral shedding. Titers are calculated using the method of Karber.

We will use a qPCR assay that has been previously described by other members of our research group [35]. Briefly, RNA is extracted using QIAamp RNA isolation kits (Qiagen, Crawley, UK) and cDNA is generated. The cDNA is amplified using primers and probes specific for conserved regions of RV and detected by qPCR (RV forward 5′-GGCCCTGAATGTGGCTAA-3′; RV reverse 5′-ATCCCCGCAATTGCTCGTTAC-3′; probe 5′-FAM/ CTTGCAGCCAATGCA-BHQ-3′) (Integrated DNA Technologies, Coralville, IA). Virus quantity is determined by comparison to a standard curve.

8.3.2 Serology

Sera are tested for neutralizing antibody to RV16 and RV39 by a standard microtiter assay [33]. In addition to neutralizing antibodies, specific IgG and IgA antibodies directed against RV-16 and RV-39 capsid proteins, VP1 and VP2, will be measured in serum by antigen binding radioimmunoassay.

8.3.3 Other Laboratory Evaluations

HLA-DRB1 typing will be performed on whole blood DNA specimens obtained on subjects who meet criteria for RV challenge in order to select the relevant MHCII/RV peptide tetramer for T-cell phenotyping studies.

8.3.4 Special Assays or Procedures

At specified time points, PBMC specimens will be collected in order to perform high-dimensional immunophenotyping of circulating T cells and other immune cell types by mass cytometry. In addition, gene expression profiling of discrete T-cell subtypes will be performed using standard microarrays or single-cell analytical platforms (BioMark, Fluidigm).

Screening lab studies (Hgb and IgE) and the respiratory virus PCR will be done in the Clinical Laboratory of the UVA Medical Center. Specimens will be shipped to the laboratory using standard hospital procedures.

For details of laboratory evaluations, please refer to the Manual of Procedures (MOP), which contains the following standard operating procedures (SOPs):

1. Collection of whole blood for serum
2. Microtiter screening neutralization assay
3. RV isolation in cell culture
4. Quantitative RV cultures
5. RV typing neutralization assay
6. qPCR for RV
7. Serum IgG and IgA binding assays
8. DNA Isolation and HLA typing from whole blood.
9. PBMC isolation and cryopreservation
10. PBMC specimen thawing
11. Staining PBMCs with tetramers and antibodies
12. Analysis of samples by multi-color flow cytometry
13. Staining PBMCs for mass cytometry analysis (protocol 1)
14. Staining PBMCs for mass cytometry analysis (protocol 2)
15. Analysis of samples by mass cytometry
16. Single-cell gene expression profiling
17. Single-cell gene expression profiling (Fluidigm)
18. Gene expression profiling using standard microarrays
19. Multiplex cytokine assay for cytokines in nasal lavage fluid

8.3.5 Specimen Preparation, Handling, and Shipping

8.3.5.1 Instructions for Specimen Preparation, Handling, and Storage

All specimen collection and processing will be done in the research laboratories of the PIs using established procedures documented in the laboratory manuals of the respective laboratories (see MOP for details). Specimens will be stored at appropriate temperatures in monitored freezers. Consent for future use of the specimens will be obtained. All specimens will be identified by study number, date, and specimen type, and linked to the DCF and study database.

8.3.6 Specimen Shipment

DNA specimens may be shipped for HLA typing services to external sites (listed in the MOP). Additionally, assay plates containing cDNA specimens may be shipped for single-cell gene expression profiling (see MOP for details).

9 ASSESSMENT OF SAFETY

9.1 Methods and Timing for Assessing, Recording, and Analyzing Adverse Events and Serious Adverse Events

9.1.1 Adverse Event Definition

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject who was administered a pharmaceutical product, regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs. Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, worsening of pre-existing medical condition will be recorded as an AE.

For the purposes of this study using experimental RV infection, physical signs and symptoms that are expected to be associated with the common cold and occur in the seven days after virus challenge should not be reported as AEs. The following physical signs and symptoms are expected to be associated with the common cold: nasal obstruction (nasal stuffiness), coryza (runny nose), sore throat, cough, sneezing, headache, chills, and malaise. If these symptoms are worsening beyond seven days after virus challenge they will be recorded as AEs. All other untoward medical occurrences during the study will be recorded as adverse events (such as fever, bronchospasm, asthma, otitis media, bronchitis and/or sinusitis).

9.1.2 Serious Adverse Event Definition

A Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (SADR) is any Adverse Event (AE) that meets at least one of the following criteria:

1. Death.
2. Life-threatening adverse event;*
3. Inpatient hospitalization or prolongation of an existing hospitalization (see below).
4. Persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life function (see below).
5. Is a congenital anomaly or birth defect.
6. Important medical events **

*Life-threatening refers to immediate risk of death as the event occurred per the reporter. A life-threatening experience does not include an experience that had it occurred in a more severe form might have caused death, but as it actually occurred, did not create an immediate risk of

death. For example, hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though hepatitis of a more severe nature can be fatal. Similarly, an allergic reaction resulting in angioedema of the face would not be life-threatening, even though angioedema of the larynx, allergic bronchospasm, or anaphylaxis can be fatal.

**Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Hospitalization is official admission to a hospital. Hospitalization or prolongation of a hospitalization constitutes criteria for an AE to be serious; however, it is not in itself considered a SAE. In absence of an AE, a hospitalization or prolongation of a hospitalization should not be reported as a SAE. This is the case in the following situations:

- The hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol.
- The hospitalization or prolongation of hospitalization is part of a routine procedure followed by the center (e.g., stent removal after surgery). This should be recorded in the study file.

In addition, hospitalization, for a pre-existing condition that has not worsened, does not constitute an SAE.

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

If there is any doubt about whether the information constitutes a SAE, the information is treated as a SAE.

9.1.3 Assessment and Reporting of Adverse Events:

9.1.3.1 Recording of Adverse Events

All AEs should be captured on the appropriate DCF. All AEs occurring while on study must be documented appropriately regardless of relationship, severity or seriousness criteria. All AEs will be followed to adequate resolution or stabilization. All AEs must be graded for severity and relationship to study product.

All volunteers will be specifically asked about any unexpected events at each study visit. All volunteers will be instructed to contact the study site if they experience any unexpected symptoms during the time they are in the study (from day 0 - day 63 for each challenge cohort). At the final study visit (day 63 for each challenge cohort), volunteers will be asked if they have

had any unusual symptoms or problems during the study. All positive responses will be recorded. Medically significant responses will be followed until the symptom/problem is resolved or judged unrelated to the study and the subject has been referred for appropriate care.

9.1.3.2 Severity

The severity of AEs will be graded on a three–point scale as described below as assessed by the volunteer and reported in detail as indicated on the DCF.

- **Mild** - Barely noticeable, does not interfere with daily activity,
- **Moderate** - Noticeable, but does not prevent daily activity,
- **Severe** - Interferes or prevents daily activity

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

9.1.3.3 Relationship to Study Intervention

All AEs must be graded for severity and relationship to study intervention. The investigator's assessment of an AE's relationship to study is part of the documentation process, but it is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- **Related:** There is a reasonable possibility that the study caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event. All causality relationship of probably not related, possibly related, probably related, and definitely related will be considered related regardless of form of documentation.

- **Not Related:** The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.

9.1.3.4 Type and Duration of Follow-up of Subjects after Adverse Events

Adverse events requiring medical intervention will be followed until the symptom/problem is resolved or the subject is considered stable. Subjects who experience AEs judged unrelated to the study will be referred for appropriate care as needed.

9.1.4 Assessment and Reporting of Serious Adverse Events

Any SAE occurring in a subject after Day 0 in each challenge cohort until Day 28 of the cohort must be reported. Any AE should be recorded on the appropriate DCF.

Unexpected SAEs that are fatal or life-threatening must be filed as soon as possible with the Ethics Committee as soon as possible but not later than 7 calendar days after first knowledge, followed by as complete a report as possible within 8 additional calendar days.

Unexpected SAEs that are not fatal or life-threatening must be filed as soon as possible but no later than 15 calendar days after first knowledge.

AEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

All SAEs will be:

- recorded on the appropriate SAE DCF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID Medical Monitor and Clinical Protocol Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

9.1.5 Reporting Plan for Serious Adverse Events, Unanticipated Problems, Protocol Deviations and Data Breaches

AEs will be reported to the UVA Human Investigations Committee for review as follows.

Type of Event	To whom will it be reported:	Time Frame for Reporting	How reported?
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study participation	IRB-HSR Medical Monitor	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, Unexpected adverse event	IRB-HSR Medical Monitor	Within 7 calendar days from the time the study team received knowledge of the event.	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations This would include a Data Breach.	IRB-HSR Medical Monitor	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. *http://www.virginia.edu/vprgs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc)*
Protocol Violations/Noncompliance *The IRB-HSR only requires that MAJOR violations be reported.*	IRB-HSR Medical Monitor DMID	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation, Noncompliance and Enrollment Exception Reporting Form *http://www.virginia.edu/vprgs/irb/hsr_forms.html*
Data Breach	The UVA Corporate Compliance and Privacy Office Medical Monitor ITC: if breach involves electronic data UVA Police if breach includes such things as stolen computers.	As soon as possible and no later than 24 hours from the time the incident is identified. As soon as possible and no later than 24 hours from the time the incident is identified. IMMEDIATELY	UVA Corporate Compliance and Privacy Office Phone: 924-9741 **ITC:** Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html Phone: (434) 924-7166

UVA PI HELD IND			
Life-threatening and/or fatal unexpected events related or possibly related to the use of the investigational agent.	FDA	Within 7 calendar days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
Serious, unexpected and related or possibly related adverse events	FDA	Within 15 calendar days after the study team receives knowledge of the event	Form FDA 3500A (MedWatch) or narrative
All adverse events	FDA	Annually	IND annual report

9.1.6 Reporting of Pregnancy

Any pregnancy occurring between the screening and final visits for each cohort will be recorded and reported to the IRB, DMID and the Data Safety Monitoring Board (DSMB). No further study inoculations will be administered to pregnant subjects, but all study-mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Pregnancies will be followed to pregnancy outcome pending the subject's permission.

9.2 Methods and Timing for Assessment of the Performance of the Human Rhinovirus Challenge Model

9.2.1 Historical performance of the challenge model

The experimental RV challenge model is a well-established model that has been used for the study of the pathogenesis and treatment of rhinovirus infections since the 1960s. There have been a number of variations on the model but the proposed procedures and evaluations in this protocol (described in section 8.2 above) and have been used by the PI for 35 years. Under these conditions, the expected infection rate for volunteers in the study is 85-90%. Mean symptom scores for the RV39 used in the study have typically been in the range of 14-18 with standard deviations of ± 13. The mean total symptom scores for the RV16 was 20 ± 10 in the initial evaluation of the challenge pool.

The investigators are not aware of any previous rhinovirus challenge study that was discontinued because of unexpected risk to the volunteers in the study. In light of this experience, it is unlikely that events that would require stopping the study would occur.

9.2.2 Evaluation of viral infection

Infection performance will be monitored and assess in two ways:

9.2.2.1 Back titration of the challenge pool

After each challenge a portion of the challenge inoculum is back titrated in cell culture to assure that viable virus is present at the approximate titer anticipated. The accuracy of the quantitative culture method is generally accepted to be ± 0.5 $\log_{10}$ TCID50/ml. Failure to detect virus in the back titration or detection of greater than 1 $\log_{10}$ TCID50/ml over the intended challenge inoculum will be reported to the Medical Monitor and DSMB.

9.2.2.2 Infection rate

Evaluation of viral infection to assess the performance of the model will be limited to the initial RV16 challenge in each cohort. The purpose of the study is to evaluate the effect of RV16 induced immunity on subsequent homotypic and heterotypic challenges so any change in the expected characteristics of the model will be more likely an effect of the experimental design than a change in the performance of the model. If the infection rate in the first challenge of any cohort is less than 75% this will be reported to the Medical Monitor and DSMB. Note that a decreased infection rate would potentially impact the data analysis and benefit of the study but would not pose a direct increased risk to the volunteers.

9.2.3 Evaluation of symptoms

Evaluation of symptoms to assess the performance of the model will be limited to the initial RV16 challenge in each cohort. The purpose of the study is to evaluate the effect of RV16 induced immunity on subsequent homotypic and heterotypic challenges so any change in the expected characteristics of the model in the re-challenged cohorts will be more likely an effect of the experimental design than a change in the performance of the model. Should the initial challenge result in an average total symptom score of <5 this will be reported to the Medical Monitor and the DSMB. Note that a decreased symptom burden would potentially impact parts of the data analysis and the benefit of the study but would not pose a direct increased risk to the volunteers.

Given the historical mean and standard deviation of the total symptom scores in the model, it would be an unexpected event if more than 10% of the volunteers in any cohort (initial challenge or re-challenge) had a total symptom score >40. Should this occur we will report the event to the Medical Monitor and stop the study pending DSMB review.

9.2.4 Halting Rules

Each study cohort will be challenged with virus over one to two days so application of halting rules cannot be done during the cohort. The following halting rules will be applied and require DSMB review before proceeding to subsequent challenges.

9.2.4.1 More than 10% of volunteers in any cohort with a total symptom score >40
9.2.4.2 Any subject experiences type 1 hypersensitivity immediately after receiving the study product
9.2.4.3 Any SAE judged by the investigator to be related to the challenge virus infection.

9.3 Safety Oversight (ISM plus DSMB)

9.3.1 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs and other AEs as needed and provide an independent assessment to DMID. UVA will have an ISM with experience in infectious diseases or internal medicine.

9.3.2 Data and Safety Monitoring Board (DSMB)

This clinical trial will utilize a DSMB, which will be comprised of an independent group of experts with experience with early phase clinical research studies. The primary responsibility of the DSMB is to monitor subject safety. The DSMB is external to DMID and composed of at least three voting members. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial. Committee activities will be delineated in a DSMB charter that will delineate membership, responsibilities, and the scope and frequency of data reviews. The DSMB will operate on a conflict-free basis independently of the study team. DMID, the DSMB, the ISM or the PI may convene ad hoc meetings of the DSMB according to protocol criteria or if there are concerns that arise during the study. The DSMB will have access to unblinded data during its closed session, if applicable. After its assessment, the DSMB will recommend continuation, modification, or termination of the clinical trial.

The DSMB will conduct a review of all available safety data after each cohort has completed the day 8 study visit (7 days following the first study inoculation). The review will also include all available safety data on second inoculations accrued through the time of data freeze for the DSMB meeting and the DSMB will recommend continued enrollment of the remaining subjects, modification, or discontinuation of enrollment.

If any of the halting rules are met following the first or second study challenge, the study will not proceed with the remaining enrollment or study challenge without a review by and recommendation from the DSMB to proceed.

Upon completion of this review and receipt of the advice of the DSMB, DMID will determine if study entry or study dosing should be interrupted, or if study entry and study dosing may continue according to the protocol.
A final review of Safety data will be conducted after the study is completed. This review may be performed electronically if the DSMB Chair agrees.

9.3.3 Safety Monitoring

This study is not blinded and involves an intervention that is well-characterized and has an extensive safety record. The investigational material for this study is the challenge pools that will be used for the RV infections. The previous studies that have been done with these pools, as well as the extensive experience with the RV challenge model, in general, mitigate concern about the safety of the model.

Safety will be monitored during the challenge studies by the PI. Results of the challenge studies and collection of AEs will be reviewed by the DSMB as described above.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Under the U01 mechanism, site monitoring is the responsibility of the investigators. To ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, CGP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs, and protocol specific MOP, the study will be monitored by an external, independent entity, the UVA School of Medicine Clinical Trials Office (https://research.med.virginia.edu/clinicalresearch/research-resources/offices-supporting-clinical-research/clinical-trials-office/available-services/).

In brief, site visits will be made at standard intervals as defined by the Site Monitoring Plan. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, case report forms, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions. See detailed protocol monitoring plan for details. The details of the site monitoring process for this study are given in a separate document (Protocol Monitoring Plan). This Monitoring process is separate and independent from the internal Quality Management Process to be conducted by the PI as described in Section 12 of this protocol.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

The overall hypothesis that will be addressed by this proposal is that Th and Tfh cells directed against conserved RV epitopes expand upon RV exposure and some of these cells persist as stable cross-reactive memory populations capable of displaying lineage-specific protective functions upon re-infection with related or unrelated strains of RV. The IP is not the object of this study and there are no hypotheses related to the study product.

11.2 Study Outcome Measures

This study is a mechanistic study, and thus, the conventional framework of primary and secondary outcome measures used in standard clinical trials does not apply.

11.2.1 Primary Objectives

To assess the relationship between T-cell immunity induced by infection with the rhinovirus serotype RV-16, and the host response to homologous infection with RV-16 or the host response to heterologous infection with RV-39.

11.2.2 Exploratory Objectives

The major objective of the study is to perform mechanistic studies that apply state-of-the art experimental technologies to identify, enumerate, and monitor a broad array of known and novel cell types during challenge with RV-16 and re-challenge with RV-16 or RV-39 in order to identify hallmarks of cross-protection. The technologies applied include, but are not limited to, MHCII tetramer staining, mass cytometry, and single-cell gene expression profiling. Owing to the large amounts of data that will be generated and the exploratory nature of the study, outcomes cannot be predicted. Exploratory objectives include, but are not limited to, the following: (1) Identify new molecular signatures of RV-specific T cells. (2) Evaluate the quality and quantity of T-cell responses. (3) Identify, enumerate and monitor a broad array of immune cell types (e.g., B-cell populations, CD8+ T-cells, gamma delta T-cells, NKT cells, etc.), and novel cell types. (4) Test multiple relationships between immune cell parameters and infection, serum antibodies, and symptoms (illness), at each sampling time point during RV challenge and re-challenge.

11.2.3 Primary Outcome Measure

Correlation between the number of pre-existing RV-16-specific T cells at Week 15, and infection after homologous re-challenge with RV-16 or infection after heterologous re-challenge with RV-39 at Week 15. With regard to measurement transformation, in our past characterization of T-cell responses to RV-16 and RV-39, we have found that the measurement distribution of many of the aforementioned outcome measures (e.g., T-cell response, viral load) exhibit prominent right-tailed skewness, which depending upon whether a parametric or a non-parametric analysis approach is utilized to analyze the data, has required either rescaling the outcome data to a logarithmic scale (e.g., log10 scale), or rescaling the data to a rank scale, respectively.

11.3 Sample Size Considerations

11.3.1 Sample Size Calculation Summary

The number of subjects enrolled for initial challenge with RV16 (80) is determined based on power analyses related to the primary outcome measure in Aim 2. In Aim 2, our sample size is designed to address the hypothesis that ***high frequencies of pre-existing RV-16-specific memory T cells directed against conserved epitopes protect against RV-39 infection***. **Our primary objective is to test the relationship between the frequency of RV-16-specific memory T cells at 15 weeks and viral infection in response to re-challenge with RV-16 or RV-39.** In order to do this, we must first establish that there is a scientifically meaningful reduction in the RV infection rate for secondary versus primary RV challenge. A sample size of 35 volunteers per re-challenge will provide a two-sample test of equal proportions with 85% statistical power (with a one-sided α=0.025 type I error rate) to detect a reduction of as small as 28% between the infection rate following primary challenge and the infection rate following secondary challenges.

If we have 35 volunteers per re-challenge, and if the underlying bivariate correlation between the frequency of RV-16-specific memory T cells at 15 weeks and viral infection in response to secondary challenge with RV-16 or RV-39 is at least 0.46 units in absolute magnitude - which if true, would be considered a scientifically meaningful association - we expect to have at least 0.80 statistical power to reject the null hypothesis that there is no correlation between the frequency of RV-16-specific memory T cells at 15 weeks and viral infection in response to secondary challenge with RV-16 or RV-39.

11.3.2 Power Calculation Details

The “TWOSAMPLEFREQ” statement of the SAS version 9.4 (SAS Institute Inc., Cary, NC) “POWER Procedure” was used to conduct the first portion of the power analysis. As user input, the infection rates for the primary and secondary RV challenges were specified as 0.90 and 0.65, respectively. A zero difference in the infection rates of the primary and secondary RV challenges was specified as the null difference and the alterative difference was specified as a 0.25 unit reduction (i.e., 0.90 - 0.65) in the secondary RV challenge infection rate compared to the primary challenge infection rate. In accordance with the alternative hypothesis “less than” direction, an α=0.025 one-sided type I error rate was specified, and the group sample sizes were specified as n=80 for the primary RV challenge and n=35 for the secondary RV challenge. Based on this set of input parameters, a sample size of 35 volunteers per re-challenge should provide a statistical test with at least 85% power (with a one-sided α=0.025 type I error rate) to detect a reduction of as small as 28% (i.e., [(0.90-0.65)]/0.90 x 100%) between the infection rate following primary challenge and the infection rate following secondary challenge.

The “ONECORR” statement of the SAS version 9.4 “POWER PROCEDURE” was used to conduct the second portion of the power analysis. As user input, the null correlation was specified to be zero and the alternative correlation was specified to be 0.46. The test statistic was specified as the “Fishers z statistic”, and the two-sided type I error rate (α) was specified as α=0.05. Based on this set of input variables, the power to detect a correlation of 0.46 in absolute magnitude is 0.81.

The rationale for the number of subjects screened is as follows:

- 750 volunteers will be screened for antibody to provide approximately 120 volunteers who are seronegative to both RV-16 and RV-39.
- Of the 120 resulting seronegative volunteers, approximately 96 will have a matched RV tetramer available for the T-cell studies.
- 80 eligible subjects (after review of other inclusion/exclusion criteria) will be challenged with RV-16, approximately 72 (90%) will be infected.
- 70 subjects (35 per virus) will be rechallenged with either RV-16 or RV-39.

Note that volunteers who have a positive respiratory virus panel (PCR for respiratory syncytial virus [RSV], influenza, parainfluenza, metapneumovirus, rhino/enterovirus, and adenovirus) from nasal lavage on the day of RV challenge, or who shed a RV other than the challenge virus in nasal lavage over the course of the study, will be excluded from the analysis.

Owing to the powerful, yet exploratory, nature of mass cytometry experiments, we anticipate that a much smaller sample size will be necessary to discover novel cell types induced by RV exposure that are biologically relevant. Our proposed work is at the forefront of science in this regard. As an example, recent work describing longitudinal monitoring of Tfh cells during HIV infection in a primate model using mass cytometry was published in *Science Translational Medicine* with a sample size of 8 [36].

11.4 Planned Interim Analyses

There are no planned interim analyses in this study.

11.4.1 Safety Review

There are no specific safety outcome measures in this study. Reported AEs will be reviewed after each challenge cohort. Unexpected or serious events judged to be related to the virus challenge will be reviewed with the ISM, the DSMB and DMID before the next cohort is challenged.

11.4.2 Immunogenicity or Efficacy Review

Immunogenicity or efficacy are not relevant to this study.

11.5 Analysis Plan

11.5.1 General Statistical Plan

General statistical support related to the primary outcome measure, and many of the parameters assessed under exploratory objectives will be provided by Mr. James Patrie, MS (Pstat® Accredited Senior Biostatistician, Department of Public Health Sciences). Analyses performed by Mr. Patrie will include, but are not limited to: (a) comparing infection rates resulting from primary challenge with RV-16 and re-challenge with RV-16 or RV-39, (b)

comparing RV-specific T-cell numbers before and after primary RV-16 challenge, (c) examining correlations between specific T-cell numbers and quantitative shedding as assessed by culture and qPCR as well as the time to virus shedding, (d) comparing clinical outcomes (e.g., cytokine response, and symptom scores) between primary RV challenge and RV re-challenge, and (e) examining correlations between RV specific T-cell numbers and clinical outcomes.

11.5.2 Primary Outcome Analyses (Correlation between the number of pre-existing RV-16 specific T-cells (15 wks.) and infection following re-challenge with RV-39 as assessed by virus shedding in nasal lavage)

Infection rates at primary challenge and the infection rates at secondary challenge will be analyzed by way of a binomial generalized estimating equation (GEE) model [37]. The GEE model response variable will be a binary indicator variable that will be assigned the value 1 if the volunteer is deemed infected on RV challenge and the value 0 will be assigned otherwise. The GEE model predictor variable will be 3 level classification variable. One level will differentiate the primary RV-16 challenge outcome data from the secondary RV challenge outcome data, a second level will identify the secondary RV-16 challenge outcome data, and the third level will identify the secondary RV-39 challenge outcome data. Since we expect that approximately 90% of the volunteers who undergo the primary RV-16 challenge will be eligible to undergo either RV-16 or RV-39 re-challenge, we cannot legitimately assume that the primary challenge infection status and re-challenge infection status of the volunteers who undergo re-challenge are statistically independent. To account for possible *intra-subject* measurement correlation, the components of the GEE model variance and covariance matrix will be estimated by the Huber and White sandwich estimator [38, 39].

With regard to hypothesis testing, the GEE version of the Wald test will be used to test whether the log-odds for infection is the same for the primary RV-16 challenge and the RV-16/RV-39 re-challenge. The Wald test will also be utilized to test whether the log-odds of infection is the same for the RV-16 and RV-39 re-challenges. For the primary challenge versus re-challenge comparisons, a 0.025 one-side decision rule will be utilized as the null hypothesis rejection criterion, while for the RV-39 re-challenge versus RV-16 re-challenge comparison, a two-sided 0.05 decision rule will be utilized as the null hypothesis rejection criterion. With regard to odds ratio confidence interval construction, the GEE version of Wald confidence interval will be utilized to estimate the lower and upper limits of the 95% confidence interval. The relationship between the number of pre-existing RV-16 specific T-cells (15 wks.) and infection following re-challenge with RV-39, as assessed by virus shedding in nasal lavage, will be quantitatively expressed in terms of the Pearson Product-Moment correlation coefficient if the bivariate normal assumption is deemed creditable based on the observed data, or quantitatively expressed in terms of the Spearman Rank correlation coefficient if the bivariate normal assumption is not supported by the observed data. In either case, the pivotal quantity for the null hypothesis test that the true correlation coefficient is equal to zero will be the Fishers z-statistic, and a two-side $p \leq 0.05$ decision rule will be utilized as the null hypothesis rejection criterion. Confidence interval construction for estimating the underlying correlation coefficient will be based on the Fishers z-transformation method [40].

11.5.3 Potential analyses for exploratory endpoints

Infection rates at primary challenge and the infection rates at secondary challenge will be analyzed by way of a binomial generalized estimating equation (GEE) model [37]. The GEE model response variable will be a binary indicator variable that will be assigned the value 1 if the volunteer is deemed infected on RV challenge and the value 0 will be assigned otherwise. The GEE model predictor variable will be 3 level classification variable. One level will differentiate the primary RV-16 challenge outcome data from the secondary RV challenge outcome data, a second level will identify the secondary RV-16 challenge outcome data, and the third level will identify the secondary RV-39 challenge outcome data. Since we expect that approximately 90% of the volunteers who undergo the primary RV-16 challenge will be eligible to undergo either RV-16 or RV-39 re-challenge, we cannot legitimately assume that the primary challenge infection status and re-challenge infection status of the volunteers who undergo re-challenge are statistically independent. To account for possible *intra-subject* measurement correlation, the components of the GEE model variance and covariance matrix will be estimated by the Huber and White sandwich estimator [38, 39].

With regard to hypothesis testing, the GEE version of the Wald test will be used to test whether the log-odds for infection is the same for the primary RV-16 challenge and the RV-16/RV-39 re-challenge. The Wald test will also be utilized to test whether the log-odds of infection is the same for the RV-16 and RV-39 re-challenges. For the primary challenge versus re-challenge comparisons, a 0.025 one-side decision rule will be utilized as the null hypothesis rejection criterion, while for the RV-39 re-challenge versus RV-16 re-challenge comparison, a two-sided 0.05 decision rule will be utilized as the null hypothesis rejection criterion. With regard to odds ratio confidence interval construction, the GEE version of Wald confidence interval will be utilized to estimate the lower and upper limits of the 95% confidence interval. We will examine potential relationships between RV-16 specific Tfh/Teff cells and post-RV-16/RV-39 challenge infection as assessed by viral load. Bivariate relationships will be quantified either by the Pearson Product Moment correlation coefficient or by the Spearman Rank correlation coefficient. In either case, the pivotal quantity for the null hypothesis test that the correlation coefficient is equal to zero will be the Fishers z-statistic, and a Bonferroni corrected two-sided $p \leq 0.05/c$ decision rule will be used as the null hypothesis rejection criterion, where the “c” represents the total number of null hypothesis tests. Correlation coefficient confidence interval construction will be based on the Fishers z-transformation method. As part of the correlation analysis, we will also compare the correlation coefficients for RV-16 specific Tfh/Teff cells and post-RV-16 challenge viral load relationships to the correlation coefficients for the RV-16 specific Tfh/Teff cells and post-RV-39 challenge viral load relationships by way of *distribution-free* random permutation tests [41], where virus re-challenge type (i.e., RV-16 or RV-39) will be the variable utilized to generate the pseudo null permutation distribution. A Bonferroni corrected two-sided $p \leq 0.05/c$ decision rule will be used as the null hypothesis rejection criterion, where the “c” represents the total number of null hypothesis tests. Cox Proportional Hazards regression will be use examine multivariate relationships between the pre-challenge RV specific T-cell frequencies and time to RV-16 virus shedding.

We will examine potential relationships between the levels of RV-16- and RV-39-specific antibodies and illness (i.e., total symptom score on day of peak symptom) following heterotypic re-challenge with RV-39, as well as potential relationships between the levels of RV-16- and RV-39-specific antibodies and illness following homotypic re-challenge with RV-16. Bivariate

relationships will be quantified either by the Pearson Product-Moment correlation coefficient or by the Spearman Rank correlation coefficient. In either case, the pivotal quantity for the null hypothesis test that the true correlation coefficient is equal to zero will be the Fishers z-statistic, and a Bonferroni corrected two-sided $p \leq 0.05/c$ decision rule will be implemented as the null hypothesis rejection rule, where the c represents the total number of null hypothesis tested. Correlation coefficient confidence interval construction will be based on the Fishers z-transformation method. As part of analysis, we will also compare the correlation coefficients for the relationships between RV-16- and RV-39-specific antibodies and illness following heterotypic re-challenge with RV-39 to the correlation coefficients for the relationships between RV-16- and RV-39-specific antibodies and illness following homotypic re-challenge with RV-16 by way of distribution free random permutation tests [40], where virus re-challenge (i.e., heterotypic or homotypic) will be the variable utilized to generate the pseudo null permutation distribution. A Bonferroni corrected two-sided $p \leq 0.05/c$ decision rule will be used as the null hypothesis rejection criterion, where the "c" represents the total number of null hypothesis tests.

Pre and post-RV-16-specific Tfh/Teff cells frequencies and pre- and post-challenge RV-16- and RV-39-specific antibody frequencies will be analyzed via linear mixed effects models. For each outcome variable, the linear mixed model will be specified to include a single fixed-effect, which will identify the outcome assessment time (i.e., pre- or post-RV-16 challenge), and a single random effect that will identify the subject. Based on our prior work, we anticipate that the majority of these outcomes variables will be have to be analyzed on the $\log_{10}$ scale to reduce the impact of right tailed distribution skewness, and hence, the pre- to post-change in the response will be quantified as a ratio (post:pre) of geometric means. The pivotal quantity for hypothesis testing will be the "linear-contrast of means" t-statistic, and a two-sided $p \leq 0.05$ decision rule will be utilized as the rejection criterion for testing the null hypothesis that there is no underlying pre- to post-challenge change in the mean/geometric mean of outcome distribution.

The longitudinal clinical outcome data such as viral load, cytokine concentration, and cold symptom scores, that will be collected on the day of RV inoculation (i.e., day 0) and on post-challenge days 1, 2, 3, 4, and 5 will be analyzed via linear mixed models (e.g., cytokine response) and via generalized estimating equation models (e.g., total daily symptom score). The linear mixed models and generalized estimating equation models will be specified so that the longitudinal response profiles of the secondary challenges can be compared with the longitudinal response profiles of the primary challenge. With regard to hypothesis testing, all statistical tests will be two-sided and a Bonferroni corrected $p \leq 0.05/c$ decision rule will be implemented as the null hypothesis rejection criterion, where c denotes the number of between-challenge comparisons.

11.5.4 Mass Cytometry Data Analysis

Mass cytometry data will be analyzed using Cytobank tools [http://www.cytobank.org] [42] including new packages (SPADE, viSNE, Wanderlust) [43-47], as well as "classic" tools (biaxial plots, principal component analysis, heat maps). Statistical and bioinformatics support for high-dimensional immunophenotyping data will be provided by Dr. Irish's lab under a subcontract agreement, in conjunction with the UVA Flow Cytometry Core. New and emerging computational tools will be shared between the Irish and Woodfolk labs on an ongoing basis.

The UVA Bioinformatics Core will provide bioinformatics support for gene expression data sets and for integrative analytics aimed at combining proteomic and transcriptomic data sets.

High content single cell data analysis and associated bioinformatics. Team members at UVA and Vanderbilt University will analyze mass cytometry experiment data using appropriate computational tools, including recently development tools for cytometry (viz. SPADE, viSNE, Wanderlust) and classic tools (viz. biaxial plots, principal component analysis, heat maps). Dr. Irish's lab has developed several tools to automatically identify enriched features of cell populations in mass cytometry data. Dr. Irish's lab is an expert in the bioinformatics and statistics of connecting surface marker and signaling features of cells to other immune parameters (e.g., antibodies) and clinical features of patient populations [48-51]. Dr. Irish also co-created the Cytobank bioinformatics cloud computing platform for flow cytometry data in order to analyze the data for these studies and has led the integration of key informatics tools (SPADE, viSNE, heat maps) into the web interface of Cytobank.

Mass cytometry experiment design and instrumental tracking and optimization. In order to ensure uniformity within the mass cytometry data set, the Woodfolk lab will perform all mass cytometry experiments. Dr. Irish's lab will work together with UVA team members and the UVA Flow Cytometry Core to monitor the quality, signal to noise, and signal stability of the mass cytometry (CyTOF) data generated. Personnel in the Woodfolk and Irish labs, as well as in the UVA Core, have all received advanced training in the operation of the instrument and are experienced in connecting mass cytometry experiment analysis, instrument troubleshooting, and experiment design. These collaborations have been in place for over 18 months. A variety of quality control (QC) measures are in place to minimize variability among samples and to limit "batch" effects that may arise from running multiple samples on different days. These include running all specimens harvested from the same subject on the same day, and using a barcoding system to maximize sample throughput on the CyTOF and minimize variability in staining among specimens. QC measures encompass mass cytometry panel design, antibody conjugation and titration, and instrument tracking controls. These controls, which are conceptually the same as those used for tracking fluorescent cytometers, are evidenced by production of data at the same or higher level of quality by the UVA CyTOF instrument as compared with the Vanderbilt CyTOF.

11.5.5 Gene Expression Data Analysis

To ensure the reliability of the data generated in single-cell analyses, validated primers and SOPs have been provided by the Roederer lab (NIH/NIAID) [52]. Microarray data will be analyzed by the UVA Bioinformatics Core using a combination of custom-built and open-source application-specific software in the R statistical computing environment (www.r-project.org), including limma packages from Bioconductor. Data generated using the Biomark platform will be analyzed using application-specific tools implemented in the SINGuLAR package developed by Fluidigm in the R statistical computing environment [53, 54]. Hierarchical cluster analysis of the full set of gene expression results will be performed and cross-platform concordance of transcriptomic versus proteomic data will be analyzed as a QC check through collaborative efforts between the Woodfolk lab and UVA Bioinformatics and Flow Cytometry Cores. As data becomes available, the UVA Bioinformatics Core will also work in conjunction with other

components of the scientific team to develop integrative analytical pipelines that link gene expression profiles with immune parameters, infection, and symptoms.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The study site will maintain appropriate research records for this trial, in compliance with Section 4.9 of ICH E6 GCP, and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of DMID and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance (QA) reviews, audits and evaluation of the study safety and progress. All data collected directly from the volunteers will be collected on paper DCFs. If a subject is withdrawn from the study the principal investigator (PI) and/or other investigator involved in the study will document the reason for the subject withdrawal on the termination page of the DCF. Data that result from the laboratory procedures will be collected as laboratory reports/source documents.

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original. Entries into the DCF will be attributed to the staff that completed them with a signature and date.

Data reported in the DCF should be consistent with the source documents or any discrepancies should be explained.

13 QUALITY CONTROL AND QUALITY ASSURANCE

This is a single-site study and all human subject activity will be conducted under the supervision of Ronald Turner, MD, one of the protocol PIs. All clinical protocol related data will be recorded on appropriate source documents or directly into the paper DCF. Entries that are transcribed from source documents will be audited by the study coordinator or her designee. The process and tools for QC and QA are detailed in the associated Clinical Quality Management Plan. A final audit of the DCFs will be conducted by the study monitor. After the study monitor has reviewed the original DCFs, data will be entered into the database for subsequent analysis.

An independent internal audit is provided by the Post-Approval Monitoring Program that is conducted by the IRB at the UVA. This program is a monitoring program that addresses regulatory compliance review rather than data integrity.

The investigational site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The Principal Investigator will provide direct access to all source data/documents, and reports for the purpose of monitoring and auditing, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site. The process and tools for QC and QA are detailed in the associated Clinical Quality Management Plan.

Clinical site monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. An independent regulatory compliance audit is conducted by the IRB at the UVA.

Quality control procedures will be implemented by the database which will generate reports to ensure data quality, accuracy and completeness and audit of data entered. Any missing data or data anomalies will be communicated to the investigative site for timely clarification/resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Good Clinical Practice

The investigators will assure that this study is conducted in full conformity with GCP. All study personnel who are involved with the clinical study will have documentation of up-to-date training in HSP and/or GCP through the UVA IRB (Collaborative Institutional Training Initiative training).

14.3 Institutional Review Board

This study will be conducted under the authority of the UVA IRB for Health Sciences Research.

Federalwide Assurance (FWA) #: 00006183
Exp. Date: June 13, 2018

IRB for HSR (IRB-HSR) Registered with Office for Human Research Protections (OHRP) and FDA
IRB#: 00000447
Expires August 6, 2015

Physical Address:
UVA IRB-HSR
One Morton Drive
Suite 400, Box 5
Charlottesville, VA 22903

The IRB will provide review of the informed consent documents, recruitment materials, protocol, amendments to the consent documents or protocol, safety reports, deviation report and continuing reviews. Before they are placed into use, the study protocol and informed consent documents will be reviewed and approved by the UVA IRB. Any amendments to the protocol or consent materials will first be reviewed and accepted by DMID, then reviewed and approved by the UVA IRB before such changes are placed into use.

Collaborating institutions will not receive identifiable private information about specific volunteers and therefore are not conducting human subjects research as defined under the common rule, and will not be required to obtain IRB approval for the study.

14.4 Informed Consent Process

The site principal investigator will choose subjects in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, subjects must sign an informed consent form and subjects must provide consent as appropriate for age that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to the individuals agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, including pre-screening of subjects for eligibility, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products, including the nature and risks of this trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum samples. Subjects will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them, and have the opportunity to discuss the trial with their family, friends or legally authorized representative or think about it prior to agreeing to participate.

Informed consent forms describing in detail the study interventions/products, study procedures, risks and possible benefits are given to the subjects. The informed consent form must not include any exculpatory statements. Informed consent forms will be IRB-approved and the subject will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain the research study to the subject and answer any questions that may arise. Subjects must sign the informed consent form, and written documentation of the informed consent process is required prior to starting any study procedures/interventions being done specifically for the trial, including administering study product.

DMID will provide the site principal investigator, in writing, any new information that significantly impacts the subjects' risk of receiving the investigational product. This new information will be communicated by the site principal investigator to subjects who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented, if necessary.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the subjects consent; however, before any study procedures are performed to determine protocol eligibility an informed consent form must be signed. Subjects will be given a copy of all informed consent forms that they sign.

By signing the informed consent form, the subject agrees to complete all evaluations required by the trial, unless the subject withdraws voluntarily, or is withdrawn or terminated from the trial for any reason.

Volunteers for the screening blood draw will be recruited from the UVA community by posted, newspaper, or email advertisements or by contacting volunteers who have added their contact information to an IRB approved recruitment database (IRB-HSR #13653). All advertising material will be reviewed and approved by the IRB. Volunteers who express interest will be asked to come to the study site where they will be given a consent form (IRB #9948) to read and all questions will be answered by study staff. Volunteers will be provided with a brief description of the various studies under recruitment that will be potentially available for participation. Volunteers who wish to participate will have their blood drawn and tested for serum neutralizing antibodies to the study virus.

Volunteers who are seronegative will be contacted and offered the opportunity to participate in this clinical trial. Those who express interest will be emailed a copy of the consent form for this study and a time for the enrollment visit will be scheduled. At the enrollment visit, the consent form will be reviewed with the volunteer and all questions answered. Volunteers who wish to participate will provide written affirmation of their consent prior to any study procedures. All volunteers who consent to participation will be given a copy of the signed consent form.

14.5 Exclusion of Women, Minorities, and Children (Special Populations)

14.5.1 Participation of Women

There are no gender restrictions for participation in these studies. Experience from previous studies using the human experimental RV infection model at the UVA suggests that about 59% of subjects who participate will be female. This gender distribution reflects the current gender distribution of undergraduates at UVA (56% female). Pregnant women are excluded from the study due to the intentional challenge with RV that is a part of the study.

14.5.2 Participation of Minorities

There are no racial or ethnic restrictions in this study. In previous studies using this experimental model at UVA, approximately 83% of volunteers have identified as white, 5% Black or African-American, and 10% Asian. The UVA undergraduate population is approximately 72% White, 6% African-American, and 12% Asian. Approximately 6% of the undergraduate students identify themselves as Hispanic or Latino.

14.5.3 Participation of Children

Children are excluded from the study because the intentional challenge with RV cannot be ethically done in children.

14.6 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

Health Insurance Portability and Accountability Act (HIPAA) identifiers will be collected in the source documentation and recorded for purposes of payment of volunteers. Identifying information will be retained in a secure location by the clinical study site. Study records will be maintained behind two locked doors, and computer records will be password protected. All volunteers will be assigned a study number and laboratory specimens will be identified only by study number, type of specimen, study visit number and date of collection. The electronic database will be coded by study number and will contain no HIPAA identifiers.

14.7 Study Discontinuation

This study does not involve a therapeutic intervention. If the study is discontinued during the symptomatic portion of the virus challenge, challenged subjects will be re-contacted approximately 14 days after the challenge to document resolution of the viral illness. If the study is discontinued at other times in the study subjects will not require any study follow-up. Attempts to contact subjects who are lost to follow-up will be as described in section 5.4.3.

14.8 Future Use of Stored Specimens

This is a mechanistic study and there is an expectation that there may be unexpected results that will lead to further evaluation that cannot be predicted *a priori*. We will seek consent from the volunteers to use stored specimens for unanticipated studies that are related to the overall objectives of the protocol. No genetic testing will be performed on specimens stored for future use.

Subjects will be asked for permission to keep any remaining specimens for possible use in future research studies. All samples are de-identified at the clinical site prior to transfer to the laboratory. Samples will be stored in the investigator's laboratory in -70 C freezers located in laboratory areas that are accessible only to laboratory personnel. These specimens may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. Each sample will be labeled with a study number, date, and specimen type. Within two years of study completion the study specimens will be de-linked from subject identifiers.

There are no benefits to subjects in the collection, storage, and subsequent research use of specimens. Reports about future research done with a subject's samples will not be kept in

their health records. Subjects can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A subject's decision can be changed at any time by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood has already been used for research purposes, the information from that research may still be used. Participant consent for stored specimens will be part of the study Informed Consent Form (ICF) which will be asked for during the enrollment visit.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the DCF will be provided for use as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the DCF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

DMID and/or it designee will provide guidance to investigators on making corrections to the source documents and DCF.

15.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs must be graded, assessed for severity and causality, and reviewed by the site PI.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

Source documentation and DCF completion will be the responsibility of the study coordinator and designated study staff. The PI will have overall responsibility for the study data.

15.2 Data Capture Methods

Primary data will be collected on DCFs.

The symptom score data for this study will be entered into an existing challenge model database created in FoxPro. These data will be merged with laboratory assay data as required for the mechanistic analyses proposed.

15.3 Types of Data

The data collected for this study by the clinical site will include demographic, safety, virology, serology, and symptom score data. The mechanistic data from the assays supported by the clinical trial will be collected by the individual laboratories and merged with the clinical data for analysis.

15.4 Timing/Reports

The AEs will be reviewed on an ongoing basis by the study investigator and a report will be made to the ISM/DSMB at the end of each challenge cohort. Reports for the DSMB will be prepared for the DSMB according to a schedule determined at the first convened DSMB meeting. All data will be stored and manipulated as coded data. The data analysis for the outcomes defined in this study will occur after all clinical interventions are complete. There is no planned interim analysis. This is a mechanistic study and the study endpoints will be evaluated using experimental laboratory procedures. It is anticipated that the performance of laboratory analyses will be an iterative process with the results of initial analyses informing the performance of subsequent analyses. A final study report will be produced when all analyses proposed in the study are complete.

15.5 Study Records Retention

Study records and reports including, but not limited to, eDCFs, source documents, informed consent forms, and study drug disposition records shall be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the drug, until 2 years after the investigation is discontinued and the FDA has been notified. The participating site must contact DMID for authorization prior to the destruction of any study records. Informed consent forms for future use will be maintained as long as the sample exists.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:
4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity.

Protocol deviations will be recorded in the source documents and via the UVA IRB-HSR protocol violation form placed in the regulatory binder. Protocol deviations will be reviewed by the PI who is also the sponsor of the IND. Any protocol deviation meeting the criteria to be reported to the UVA IRB-HSR must also be reported to DMID in the same time-frame.

16 PUBLICATION POLICY

It is anticipated that the results of this study will be published in scientific journals. This study has been registered with clinicaltrials.gov. Any publications resulting from this proposal will be made publicly available as required by NIH policy.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH OER Grants and Funding, http://grants.nih.gov/grants/oer.htm

Following completion of this trial, the lead principal investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editor (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry, such as ClinicalTrials.gov* (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy.

It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the trial for publication.

For trials in which DMID is not the IND/IDE sponsor, or there is no IND/IDE, and DMID does not provide data management services, it is the responsibility of the investigator to

register the trial and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases

*Journal Citation: De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004; 351:1250-1.

17 LITERATURE REFERENCES

1. Brownlee JW, Turner RB. New developments in the epidemiology and clinical spectrum of rhinovirus infections. Current Opinion in Pediatrics **2008**; 20(1): 67-71.
2. Johnston SL. Natural and experimental rhinovirus infections of the lower respiratory tract Review. Am J Respir Crit Care Med **1995**; 152: S46–S52.
3. Lemanske RF, Jr., Jackson DJ, Gangnon RE, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. Journal of Allergy & Clinical Immunology **2005**; 116(3): 571-7.
4. Rakes GP, Arruda E, Ingram JM, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. Am J Respir Crit Care Med **1999**; 159(3): 785-90.
5. McManus TE, Marley AM, Baxter N, et al. Respiratory viral infection in exacerbations of COPD. Respir Med **2008**; 102(11): 1575-80.
6. Alper CM, Doyle WJ, Skoner DP, Buchman CA, Cohen S, Gwaltney JM. Prechallenge antibodies moderate disease expression in adults experimentally exposed to rhinovirus strain hanks. Clin Infect Dis **1998**; 27(1): 119-28.
7. Alper CM, Doyle WJ, Skoner DP, et al. Prechallenge antibodies: moderators of infection rate, signs, and symptoms in adults experimentally challenged with rhinovirus type 39. Laryngoscope **1996**; 106(10): 1298-305.
8. Cate TR, Couch RB, Johnson KM. Studies with rhinovirus in volunteers: Production of illness, effect of naturally acquired antibody, and demonstration of a protective effect not associated with serum antibody. J Clin Invest **1964**; 43: 56-67.
9. Cate TR, Rossen RD, Douglas R, Jr., Butler WT, Couch RB. The role of nasal secretion and serum antibody in the rhinovirus common cold. Am J Epidemiol **1966**; 84(2): 352-63.
10. Cooney MK, Fox JP, Kenny GE. Antigenic groupings of 90 rhinovirus serotypes. Infect Immun **1982**; 37(2): 642-7.
11. Douglas RG, Jr., Couch RB. Parenteral inactivated rhinovirus vaccine: Minimal protective effect. Proc Soc Exp Biol Med **1972**; 139: 899–902.
12. Perkins JC, Tucker DN, Knope HL, et al. Evidence for protective effect of an inactivated rhinovirus vaccine administered by the nasal route. Am J Epidemiol **1969**; 90(4): 319-26.
13. Hale JS, Youngblood B, Latner DR, et al. Distinct memory CD4+ T cells with commitment to T follicular helper- and T helper 1-cell lineages are generated after acute viral infection. Immunity **2013**; 38(4): 805-17.
14. Nielsen M, Lund O. NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. BMC Bioinformatics **2009**; 10: 296.
15. Nielsen M, Lundegaard C, Lund O. Prediction of MHC class II binding affinity using SMM-align, a novel stabilization matrix alignment method. BMC Bioinformatics **2007**; 8: 238.
16. Novak EJ, Liu AW, Gebe JA, et al. Tetramer-guided epitope mapping: rapid identification and characterization of immunodominant CD4+ T cell epitopes from complex antigens. J Immunol **2001**; 166(11): 6665-70.

17. Wang P, Sidney J, Dow C, Mothe B, Sette A, Peters B. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoS Comput Biol **2008**; 4(4): e1000048.
18. Wang P, Sidney J, Kim Y, et al. Peptide binding predictions for HLA DR, DP and DQ molecules. BMC Bioinformatics **2010**; 11: 568.
19. Che Z, Olson NH, Leippe D, et al. Antibody-mediated neutralization of human rhinovirus 14 explored by means of cryoelectron microscopy and x-ray crystallography of virus-Fab complexes. J Virol **1998**; 72: 4610–22.
20. Neumann E, Moser R, Snyers L, Blaas D, Hewat EA. A cellular receptor of human rhinovirus type 2, the very-low-density lipoprotein receptor, binds to two neighboring proteins of the viral capsid. J Virol **2003**; 77(15): 8504-11.
21. Olson NH, Kolatkar PR, Oliveira MA, et al. Structure of a human rhinovirus complexed with its receptor molecule. Proc Natl Acad Sci USA **1993**; 90(2): 507-11.
22. Xiao C, Tuthill TJ, Bator Kelly CM, et al. Discrimination among rhinovirus serotypes for a variant ICAM-1 receptor molecule. J Virol **2004**; 78(18): 10034-44.
23. James EA, LaFond R, Durinovic-Bello I, Kwok W. Visualizing antigen specific CD4+ T cells using MHC class II tetramers. J Vis Exp **2009**; (25).
24. Kwok WW, Ptacek NA, Liu AW, Buckner JH. Use of class II tetramers for identification of CD4+ T cells. J Immunol Methods **2002**; 268(1): 71-81.
25. Reijonen H, Kwok WW. Use of HLA class II tetramers in tracking antigen-specific T cells and mapping T-cell epitopes. Methods **2003**; 29(3): 282-8.
26. Muehling LM, Mai DT, Kwok WW, Heymann PW, Pomes A, Woodfolk JA. Circulating memory CD4+ T cells target conserved epitopes of rhinovirus capsid proteins and respond rapidly to expermental infection in humans. J Immunol.
27. Day CL, Seth NP, Lucas M, et al. Ex vivo analysis of human memory CD4 T cells specific for hepatitis C virus using MHC class II tetramers. J Clin Invest **2003**; 112(6): 831-42.
28. Novak EJ, Liu AW, Nepom GT, Kwok WW. MHC class II tetramers identify peptide-specific human CD4(+) T cells proliferating in response to influenza A antigen. J Clin Invest **1999**; 104(12): R63-7.
29. Douglas RG, Jr., Cate TR, Gerone JP, et al. Quantitative rhinovirus shedding patterns in volunteers. Am Rev Respir Dis **1966**; 94: 159–67.
30. Kohlmeier JE, Miller SC, Smith J, et al. The chemokine receptor CCR5 plays a key role in the early memory CD8+ T cell response to respiratory virus infections. Immunity **2008**; 29(1): 101-13.
31. Hendley JO, Edmonson WP, Jr., Gwaltney JM, Jr. Relation between naturally acquired immunity and infectivity of two rhinoviruses in volunteers. J Infect Dis **1972**; 125: 243-8.
32. Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. Arch Intern Med **1958**; 101: 267-78.
33. Gwaltney JM, Jr., Colonno RJ, Hamparian VV, Turner RB. Rhinoviruses. In: Schmidt NJ, Emmons RW. Diagnostic Procedures for Viral Rickettsial and Chlamydial Infections. 6th ed. Washington, DC: Am Public Health Assoc, **1989**:579-614.

34. Harris JM, II, Gwaltney JM, Jr. Incubation periods of experimental rhinovirus infection and illness. Clin Infect Dis **1996**; 23: 1287-90.
35. Kennedy JL, Shaker M, McMeen V, et al. Comparison of viral load in individuals with and without asthma during infections with rhinovirus. Am J Respir Crit Care Med **2014**; 189(5): 532-9.
36. Yamamoto T, Lynch RM, Gautam R, et al. Quality and quantity of TFH cells are critical for broad antibody development in SHIVAD8 infection. Sci Transl Med **2015**; 7(298): 298ra120.
37. Hardin J, Hilbe J. Generalized estimating equations. London: Chapman and Hall/CRC, **2003**.
38. Huber P. The behavior of the maximum likelihood estimates under nonstandard conditions. Berkeley, CA: University of California Press, **1967**.
39. White H. Maximum likelihood estimation of misspecied models. Econometrica **1982**; 50(1): 126.
40. Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. Applied Linear Statistical Models: Fourth Edition. London: IRWIN, **1996**.
41. Good P. Permutation, parametric and bootstrap tests of hypotheses. 3rd ed. New York: Springer, **2005**.
42. Kotecha N, Krutzik PO, Irish JM. Web-based analysis and publication of flow cytometry experiments. Curr Protoc Cytom **2010**; Chapter 10: Unit10 7.
43. Amir el AD, Davis KL, Tadmor MD, et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nat Biotechnol **2013**; 31(6): 545-52.
44. Irish JM. Beyond the age of cellular discovery. Nat Immunol **2014**; 15(12): 1095-7.
45. Newell EW, Sigal N, Bendall SC, Nolan GP, Davis MM. Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8+ T cell phenotypes. Immunity **2012**; 36(1): 142-52.
46. Qiu P, Simonds EF, Bendall SC, et al. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. Nat Biotechnol **2011**; 29(10): 886-91.
47. Sen N, Mukherjee G, Sen A, et al. Single-cell mass cytometry analysis of human tonsil T cell remodeling by varicella zoster virus. Cell Rep **2014**; 8(2): 633-45.
48. Diggins KE, Ferrell PB, Jr., Irish JM. Methods for discovery and characterization of cell subsets in high dimensional mass cytometry data. Methods **2015**; 82: 55-63.
49. Irish JM, Hovland R, Krutzik PO, et al. Single cell profiling of potentiated phospho-protein networks in cancer cells. Cell **2004**; 118(2): 217-28.
50. Irish JM, Myklebust JH, Alizadeh AA, et al. B-cell signaling networks reveal a negative prognostic human lymphoma cell subset that emerges during tumor progression. Proc Natl Acad Sci U S A **2010**; 107(29): 12747-54.
51. Myklebust JH, Irish JM, Brody J, et al. High PD-1 expression and suppressed cytokine signaling distinguish T cells infiltrating follicular lymphoma tumors from peripheral T cells. Blood **2013**; 121(8): 1367-76.
52. Dominguez MH, Chattopadhyay PK, Ma S, et al. Highly multiplexed quantitation of gene expression on single cells. J Immunol Methods **2013**; 391(1-2): 133-45.

53. Buganim Y, Faddah DA, Cheng AW, et al. Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. Cell **2012**; 150(6): 1209-22.
54. Flatz L, Roychoudhuri R, Honda M, et al. Single-cell gene-expression profiling reveals qualitatively distinct CD8 T cells elicited by different gene-based vaccines. Proc Natl Acad Sci U S A **2011**; 108(14): 5724-9.

APPENDIX A: SCHEDULE OF EVENTS

First Challenge	Day -14	Day 0[1]	Days 1-5	Day 7	Day 14	Day 28	Week 9	Week 15[1]
Re-Challenge= RC		RC Day 0[1]	RC Days 1-5	RC Day 7	RC Day 14	RC Day 28	RC Week 9	RC Week 15'
Informed consent[2]	x							
Medical history questionnaire	x							
Urine pregnancy screen [3]		x						
HLA typing	x							
Hemoglobin	x							
Serum IgE	x							
Serum RV-specific antibody	x	x		x	x	x	x	x
Nasal lavage for multiplex PCR		x						
RV challenge		x						
Nasal lavage for cytokines		x	x					
Nasal lavage for quantitative RV culture			x					
Nasal lavage for RV qPCR			x					
Symptom Score		x	x					
Blood collection for PBMC isolation (90cc)	x	x		x		x		x
Adverse event evaluation		x	x	x	x	x	x	x
Review of concomitant medication use	x	x	x	x	x	x	x	x

[1]Note that the Week 15 visit is the final specimen collection for the primary challenge and Day 0 for the re-challenge. The first challenge will be with RV16 and the second challenge will be with either RV16 or RV39. The complete study involves two virus challenges with sample collection over a total of 32 weeks.
[2]Volunteers will be screened for neutralizing antibody to the challenge virus strains under a separate antibody screening protocol (UVA HSR-IRB #9948) 2-4 weeks prior to enrollment in this protocol. Only seronegative volunteers will be asked to participate in this protocol.
[3] A urine pregnancy screen is required before each challenge (Study Day 0 and Study Day RC 0)