Challenge Infection of Healthy Adult Volunteers with RSV A2

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RSV A2 Provided by: Laboratory of Infectious Diseases (LID), NIAID/NIH

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List of Abbreviations

AE	Adverse Event
AGM	African Green Monkey
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHI	Center for Human Immunology, Autoimmunity, and Inflammation
CI	Confidence Interval
CIR	Center for Immunization Research
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case Report Form
CRIS	Clinical Research Information System
CRIMSON	Clinical Research Information Management System of the NIAID
CSO	Clinical Safety Office
CTM	Clinical Trial Material
CXR	Chest X-Ray
DAIDS	Division of AIDS
DCR	Division of Clinical Research
DLM	Department of Laboratory Medicine
DNA	Deoxyribonucleic Acid
cDNA	Complementary Deoxyribonucleic Acid
EBV	Epstein-Barr Virus
EKG	Electrocardiogram
FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in 1 Second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
HEK	Human Embryonic Kidney
HIV	Human Immunodeficiency Virus

HLA	Human Leukocyte Antigen
HRPP	Human Research Protection Program
ICH	International Conference on Harmonisation
Ig	Immunoglobulin
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	Intrauterine Device
JHU	Johns Hopkins University
LID	Laboratory of Infectious Diseases
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	Natural Killer
NP	Nasopharyngeal
NREVSS	National Respiratory and Enteric Virus Surveillance System
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PFT	Pulmonary Function Test
PFU	Plaque Forming Unit
PI	Principal Investigator
PRO	Patient-Reported Outcome
RD	Recommended Dose
RNA	Ribonucleic Acid
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcription–Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SAM	Synthetic Absorptive Matrix
SAR	Serious Adverse Reaction
SCSU	Special Clinical Studies Unit
SERF	Safety Expedited Report Form
SMC	Safety Monitoring Committee
SRCP	Safety Review and Communication Plan
SUSAR	Suspected Unexpected Serious Adverse Reaction
UP	Unanticipated Problem
UPnonAE	Unanticipated Problem that is not an Adverse Event

URI	Upper Respiratory Tract Infection
wt	Wild-type

Protocol Summary

Full Title: Challenge Infection of Healthy Adult Volunteers with RSV A2

Short Title:	RSV Challenge Study II
Clinical Phase:	1
IND Sponsor:	OCRPRO, DCR, NIAID
Conducted by:	LID, NIAID, NIH
PI:	Lesia K. Dropulic, MD
Sample Size:	N = 25
Accrual Ceiling:	Up to 35 subjects may need to be enrolled to compensate for dropouts.
Study Population:	Healthy volunteers 18 to 50 years of age with low pre-challenge respiratory syncytial virus (RSV)–specific neutralizing antibody titers.
Accrual Period:	2 years.
Study Design:	This is a study evaluating safety, viral shedding, and RSV illness after human challenge with high dose wild-type, recombinant respiratory syncytial virus A2 (RSV A2) delivered intranasally using an atomizer. Healthy adult volunteers will receive one dose of RSV A2 intranasally followed by clinical, virologic, and immunologic evaluations performed during an inpatient stay at the NIH Clinical Center. Subjects will be followed prospectively for 56 days.
Study Duration:	Start Date: November 2017 End Date: November 2021 Individual subjects will participate for 56 days from the time of inpatient admission to completion of outpatient follow-up. The inpatient portion of the study for each subject will last approximately 9-14 days.
Study Agent:	Live, recombinant, wild-type RSV A2
Intervention Description:	Intranasal inoculation of healthy volunteers with a single dose of recombinant RSV A2 using a nasal atomizer.

Primary Objective: Determine the safety and infectability of high dose, recombinant RSV A2 administered by nasal atomizer to healthy adult volunteers.

Secondary Objectives:
1. Characterize the onset, duration, and magnitude of viral shedding after RSV A2 challenge infection.
2. Determine the frequency of mild to moderate upper respiratory illness in healthy volunteers challenged with RSV A2.
3. Evaluate the duration and severity of clinical illness after RSV A2 challenge infection.
4. Determine the relationship between the magnitude and duration of viral shedding and the severity and duration of RSV illness.
5. Characterize RSV-specific serum and mucosal antibody responses after RSV A2 challenge infection.

Primary Endpoints: Assess expected and unexpected adverse events (AEs) throughout the trial and detect RSV shedding in nasal wash by FilmArray multiplex polymerase chain reaction (PCR) assay and by reverse transcription (RT)–quantitative PCR and quantitative viral culture after challenge with RSV A2.

Secondary Endpoints:
1. Determine day of onset of shedding, number of days of shedding, peak nasal wash viral titer, and mean sum of daily viral titers after challenge.
2. Calculate the number of subjects with mild to moderate upper respiratory illness after challenge, based on study definition of RSV illness.
3. Determine the number of days RSV illness persists after challenge and degree of illness based on study definition.
4. Correlate the magnitude and duration of viral shedding with the severity and duration of RSV-related symptoms.
5. Determine the frequency, magnitude, and duration of serum and mucosal (nasal wash and/or nasosorption) RSV-specific antibody responses after challenge.

Précis

Respiratory syncytial virus (RSV) is the leading cause of pediatric lower respiratory tract infection. RSV also causes lower respiratory tract disease in the elderly and life-threatening disease in immunocompromised hosts. An RSV monoclonal antibody (palivizumab) is currently available for passive immunoprophylaxis in high-risk infants. Vaccines and antiviral agents are under development for the treatment and prevention of RSV, but none are licensed. The ability to challenge healthy volunteers with RSV could rapidly facilitate efficacy studies of future antivirals and vaccines. In addition, challenge studies would provide critical information on viral pathogenesis, including types of cells infected, mucosal and systemic immune response, and alterations in respiratory microbiota. Clinical trial material for human challenge studies has been prepared from live recombinant (complementary DNA-derived) RSV of subgroup A (RSV A2).

This study will be a phase 1 study in healthy adult male and non-pregnant female subjects 18 years to 50 years of age. The main purpose of the trial is to define the safety profile, determine the frequency of RSV shedding in nasal wash, estimate RSV illness rates, and study immune responses in subjects given 1 dose of 10^7 PFU of RSV A2 challenge virus using a nasal atomizer. If RSV A2 is found to be sufficiently infectious in adults, then it may be used as a challenge virus in future studies evaluating antivirals or the protective efficacy of RSV vaccines, or in studies of the immunopathogenesis of RSV infection.

Subjects will be admitted to the NIH Clinical Center and receive a single intranasal dose of $10^{6.3}$ PFU or 10^7 PFU of RSV A2. Subjects will remain at the Clinical Center for approximately 9-14 days after challenge infection undergoing sequential clinical evaluations. Research specimens, nasal washes and blood, will be collected for various research assays. Subjects will be discharged when their daily nasal wash RSV result is negative for two days in a row, and they do not have any signs or symptoms suggestive of possible RSV-associated lower respiratory tract disease. Subjects will return for follow-up evaluation 28 and 56 days after viral challenge.

1 Background Information and Scientific Rationale

1.1 Background Information

RSV is an enveloped, single-stranded, negative-sense RNA virus that belongs to the *Pneumovirus* genus of the subfamily *Pneumovirinae* of the family *Paramyxoviridae*.[1] RSV has two subgroups, A and B. Several genotypes exist within each subgroup. RSV subgroups can shift in predominance in 1- or 2-year cycles, which presumably is an advantage for heterologous strains in populations with prior immunity; however, reinfections by the same subgroup may occur frequently. RSV is spread by contact of the nasal or conjunctival mucosa with virus-contaminated large droplets or fomites. The incubation period is about 4 to 5 days (range 2 to 8 days). The virus initially replicates in the nasopharynx and frequently spreads to the lower respiratory tract, especially in primary infection. Viral shedding from the respiratory tract usually occurs from 3 to 14 days, but may last for weeks in infants.[1]

RSV is the most important cause of severe viral respiratory tract disease in the pediatric population worldwide. Approximately 50%-70% of infants are infected with RSV during the first year of life, and nearly all children have been infected by age 2-3 years.[1] In the U.S., the National Respiratory and Enteric Virus Surveillance System (NREVSS) of the Centers for Disease Control and Prevention (CDC) monitors the seasonal occurrence of RSV. From 2012 to 2014, RSV was associated with an estimated 57,527 hospitalizations and 2.1 million outpatient visits among children < 5 years of age.[2] Worldwide, in children < 5 years of age, RSV was estimated to account for at least 3.4 million yearly hospitalizations for acute respiratory tract disease and up to 200,000 deaths each year.[3] Reinfection by RSV occurs throughout life without need for antigenic change. Reinfection is particularly common during the first few years of life.[4]

RSV is an important cause of morbidity and mortality in the elderly. Among adults > 65 years of age, RSV accounts for approximately 10,000 deaths annually in the U.S.[5] RSV also causes wintertime respiratory illnesses among older adults living in long-term care or attending daycare, with infection rates of 5%-10% in nursing home residents per year, complicated by pneumonia and death at rates of 10%-20% and 2%-5%, respectively.[6] In temperate climates, seasonal outbreaks begin in the fall and continue through winter and spring (November to April). There is presently no known viral reservoir.

Disease manifestations vary greatly, ranging from mild upper respiratory tract illness to fever, otitis media, and mild to severe lower respiratory illness with bronchitis, bronchiolitis (inflammation of the small airways), or pneumonia. In children under 1 year of age, RSV is the most common cause of bronchiolitis. When infants and young children are exposed to RSV for the first time, 25%-40% will show signs of a lower respiratory tract illness and 0.5%-2% will require hospitalization.[1] Infants at highest risk of a serious infection are those who are premature or have congenital heart or lung disease or abnormalities of the airways, or neuromuscular disease that compromises management of pulmonary secretions. Reinfection in healthy older children and adults usually involves only a common cold syndrome, although there can be lower respiratory tract involvement. RSV is an important cause of severe respiratory illness in the elderly and in high-risk adults who have chronic lung and/or heart disease or compromised immune systems.

An RSV monoclonal antibody (palivizumab) was licensed in June 1998 by the Food and Drug Administration (FDA) for passive immunoprophylaxis in high-risk infants. Vaccines and antiviral agents are under development for the treatment and prevention of RSV, but none are licensed. There have been a number of obstacles to developing antiviral drugs and vaccines.[1] One obstacle is that RSV grows only to moderate titer in cell culture and is notoriously prone to lose infectivity during handling, which complicates non-clinical and clinical studies. RSV is only semi-permissive in available experimental animals, so evaluation of the properties and efficacy of vaccines and antiviral drugs depends on clinical studies. In this regard, the availability of a challenge model in adults would be an important advance. The development of vaccines for pediatric use has been impeded by reduced immune responses in infancy due to immunologic immaturity and the immunosuppressive effects of maternal antibodies. The development of effective vaccines also is impeded because RSV replicates and causes disease in the superficial cells of the airway epithelium, where immune protection is less effective. In addition, a formalin-inactivated RSV vaccine in trials in the 1960s caused immune-mediated disease enhancement in RSV-naïve recipients, and the same appeared to be true for subunit RSV vaccines in experimental animals. Therefore, inactivated and subunit vaccines have been considered to be contraindicated for pediatric use, where the vaccine need is greatest. However, live-attenuated and vectored vaccines have been shown to be safe for pediatric use and are under development, but identifying a suitably attenuated and satisfactorily immunogenic strain has been challenging.

A better understanding of viral pathogenicity and the host response to RSV infection in humans would aid efforts for improved RSV therapy and immunoprophylaxis. The correlates of immunity to RSV infection are not clearly defined. In adults, protection against reinfection is believed to be due to: (a) cytotoxic T lymphocytes, which likely contribute a short-term protection against reinfection during the same season; (b) local secretory immunoglobulin A (IgA) antibodies, which appear to play the most important role in short-term and long-term protection; and (c) serum antibodies, which confer durable protection that ameliorates severity but incompletely prevents reinfection. [1]

Data from this and future RSV challenge studies will enhance our understanding of the immune response to RSV infection and facilitate efficacy trials for RSV antiviral and vaccine candidates, thereby expediting their potential applications to clinical use.

1.1.1 Description of the Study Agent/Intervention

The RSV sequence on which the study agent, RSV A2, is based was derived from RSV strain A2, initially isolated from a child in Melbourne, Australia, in 1961.[7] RSV strain A2 was isolated using human embryonic kidney cells (HEKs). The study agent of this IND is a recombinant version of RSV A2 that was derived de novo from synthetic complementary DNA (cDNA).[8] Therefore, it is free from any adventitious agents that could have been present at the time of virus isolation and has a short, well-defined passage history. The sequence of the clinical trial material (CTM), RSV A2, corresponds to the sequence of the original isolate, passaged seven times in HEK cells, except for 13 nucleotide differences.[9] Eleven of these nucleotide differences are silent on the amino acid level and considered to be phenotypically inconsequential. Specifically, 4 of the changes involve nucleotide differences which are considered incidental (C6221T; T6386C; T10514G; T13900C). Six nucleotide differences were introduced deliberately to generate marker restriction sites in noncoding regions of the genome (insertion of a C residue [C1099.1], A1138C, G1139C, A5611G, A5615T, A7559C). Another change involves a C4G substitution that was introduced deliberately near the beginning of the genome because it improves recovery from cDNA but appears to have no effect on virus replication or gene expression. In addition to these 11 non-coding changes, there are 2 nucleotide substitutions that result in amino acid changes in the F protein (E66K and P101Q). These changes modestly increase replication in cell culture (and appear to represent an adaptation to cell culture).

1.1.2 Summary of Previous Pre-Clinical Studies

Chimpanzees are the only nonhuman host that approaches humans with respect to susceptibility to RSV infection, replication, and disease. Virus replication of recombinant RSV A2 of identical genomic sequence to the CTM of this study was compared to replication of a biologically derived RSV A2 in RSV-naïve chimpanzees in two historic studies.[10,11] These studies were approved by the NIAID Animal Care and Use committee, and performed more than 10 years before the 2011 recommendations of the Institute of Medicine on restricting the use of chimpanzees in biomedical research. In both studies, replication of recombinant RSV was similar to that of the biologically derived RSV A2, both in the upper and lower respiratory tract. RSV A2 caused moderate rhinorrhea and cough in chimpanzees, similar to biologically derived RSV A2 (Table 1 [10] and Table 2 [9]). RSV A2 induced high serum neutralizing titers to RSV (Table 2 [9]). These results showed that RSV A2 had an in vivo phenotype similar to that of biologically derived RSV A2.

Table 1 The in vivo phenotype of RSV A2 is similar to biologically derived wild-type (wt) RSV in chimpanzees (From: Whitehead et al.)

Virus used to infect animal[a]	Chimpanzee no.[b]	Mean virus titer (log_{10} PFU/ml)				Rhinorrhea score[d]		Days with cough
		Nasal wash sample		Tracheal lavage sample				
		Peak titer	Daily titer[c]	Peak titer	Daily titer[c]	Peak	Mean	
Recombinant RSV A2 (D46)	1	4.9	3.7	3.4	3.2	3	1.1	4
	2	5.1	3.1	4.6	4.5	4	2.0	4
	3	4.4	3.9	4.0	2.5	3	1.4	5
	4	4.6	3.5	4.7	3.0	4	2.6	5
Mean ± SE		4.8 ± 0.16	3.6 ± 0.15	4.2 ± 0.30	3.1 ± 0.33	3.5	1.8	4.0
A2 wt (biologically derived)	5[b]	4.6	3.6	5.1	3.7	3	1.0	2
	6[b]	5.3	3.6	5.9	4.0	3	2.1	0
Mean ± SE		5.0 ± 0.35	3.6 ± 0.30	5.5 ± 0.40	3.8 ± 0.46	3.0	1.6	1.0

a RSV naïve chimpanzees were inoculated by the intranasal and intratracheal routes with 10^4 PFU of the indicated virus in a 1-ml dose per site. Nasal wash samples were collected daily for 10 days, and tracheal lavage samples were collected on days 2, 5, 6, 8, and 10. A2 (D46), recombinant RSV derived from plasmid D46.

b Historic control animal from the work of Crowe et al., 1994, Vaccine 12:783-90.

c Mean daily titers were computed for each chimpanzee by adding together the virus titers from days with detectable levels of virus (≥0.7 PFU/ml) and dividing the sum by the number of days with detectable virus titer.

d The amount of rhinorrhea was estimated daily and assigned a score (0 to 4) that indicated extent and severity. Mean rhinorrhea scores represent the sum of scores during the 8 days of peak virus shedding divided by 8.

Table 2 The in vivo replication, pathogenicity, and immunogenicity of recombinant RSV A2 is similar to that of biologically derived wild-type (wt) RSV in chimpanzees (From: Whitehead et al.)

Virus used to infect chimpanzees[a]	No. of animals	Mean peak virus titer (log_{10} PFU/ml)		Rhinorrhea score[c] (range, 0–4)		Mean serum neutralizing antibody titer (reciprocal log_2) on day:	
		Nasopharyngeal swab specimen	Tracheal lavage fluid	Peak	Mean	0	28
Recombinant RSV A2 (D46)	2	4.9 ± 0.15	5.4 ± 0.05	2.5	1.3	<3.3	10.5
wt RSV A2 (biologically derived)	2[b]	5.0 ± 0.35	5.5 ± 0.40	3.0	1.4	<3.3	11.2

a RSV naïve chimpanzees were inoculated by the intranasal and intratracheal routes with 10^4 PFU of the indicated virus in a 1-ml dose per site. Nasal wash samples were collected daily for 10 days, and tracheal lavage samples were collected on days 2, 5, 6, 8, and 10. A2 (D46), recombinant RSV derived from plasmid D46.

b Historic control animal from the work of Crowe et al., 1994, Vaccine 12:783-90.

c The amount of rhinorrhea was estimated daily and assigned a score (0 to 4) that indicated extent and severity. rhinorrhea scores represent the sum of scores during the 8 days of peak virus shedding divided by 8.

The RSV A2 CTM and an experimental RSV A2 virus derived from the same cDNA as the CTM were also characterized in nonhuman primates studies of RSV vaccine candidates in RSV-seronegative African Green Monkeys (AGMs). AGMs (n = 4) were dosed by intranasal and intratracheal inoculation at a dose of 2×10^6 PFU (equivalent to $10^{6.3}$ PFU). Serum was obtained on days 0, 21, and 28 post-immunization to determine the neutralizing antibody response. Nasopharyngeal (NP) swabs were collected daily on days 0 through 10 and day 12, and tracheal lavage samples were collected every other day from day 2 through day 12 from all animals included in the study. In this study and in several independent studies, RSV A2 replicated over 6 to 11 days in the upper respiratory tract, and up to 13 days in the lower respiratory tract of AGMs. Virus shedding from the upper respiratory tract ranged from 3.9 to 5.4 log_{10} PFU/ml, and shedding from the lower respiratory tract ranged from 3.0 to 5.5 log_{10} PFU/ml. Compared to the attenuated RSV vaccine strains, RSV A2 replicated to higher titers in nonhuman primates. RSV A2 was immunogenic, inducing serum neutralizing antibody titers of 6.7-10 log_2 in naïve AGMs by day 28.

1.1.3 Summary of Relevant Clinical Studies

Eleven studies of safe experimental infection with RSV in healthy human volunteers have been reported in the literature, dating back to 1961.[12-18;20-12;23-25] Experimental infection of healthy adult volunteers (with low pre-challenge RSV titers) using RSV strains A and B in the dose range of 3.7-5.4 $\log_{10}$ PFU have resulted in 70%-92% viral shedding from the upper respiratory tract. When reported, nasal, sinus, and ear symptoms were noted in 38%-70% of people with evidence of viral shedding. The experimental RSV challenge model produces a phenotype of respiratory illness that is similar to that seen in seasonally occurring RSV infection of healthy working adults. In 177 previously healthy working adults with RSV infection, the most frequently reported symptoms involved the nose, ear, throat, and sinus, and 85% of subjects also reported cough.[19]

AEs reported during RSV human challenge studies have included transient mild pulmonary function test (PFT) abnormalities, suppression of neutrophils and platelets, and liver enzyme elevations. Headache was the most commonly reported systemic complaint. In detail, the alanine aminotransferase (ALT) level was abnormal in 2 of 12 subjects receiving a $10^{4.7}$-PFU dose of RSV A2.[13] The ALT level normalized by day 28. AEs also occurred in subjects receiving a 10^{4}-PFU dose of RSV A.[20] The subjects in this challenge study had minor PFT abnormalities (5/53), and mild lab abnormalities with elevated ALT level (5/53), elevated aspartate aminotransferase (AST) level (8/53), reduced absolute neutrophil count (ANC) (3/53), and reduced platelets (1/53). Fever was not reported in any prior challenge subjects (several studies allowed for acetaminophen treatment). Overall, prior challenge studies with RSV have revealed that participants acquire an upper respiratory illness similar to that seen in the community. Importantly, no significant AEs related to challenge infection with RSV have been reported.

We previously conducted a phase 1 challenge study with RSVA2 in healthy volunteers 18-50 years of age (NIH protocol number 15-I-0148; Clinical Trials Identifier NCT02484417). Cumulatively, we challenged 25 volunteers. Three volunteers in cohort 1 and 10 in cohort 2 were challenged with the low dose of RSV A2 (10^{5} PFU) using intranasal droplets. Five volunteers in cohort 3a and 7 in cohort 3b were challenged with a higher dose of RSV A2 ($10^{6.3}$ PFU) using intranasal droplets. Seven of 13 participants in low dose cohorts 1 and 2 had RSV shedding detected by RT-PCR or culture of nasal wash on any day, day 2 or beyond, after challenge infection with RSV A2, resulting in a

shedding frequency of 54%. We observed a total of 3 RSV-associated upper respiratory tract infection (URI) events (1 moderate illness and 2 mild illnesses), as determined by our RSV illness study definition. Hence, the frequency of mild to moderate respiratory RSV illness for the low dose group was 23%. Our third cohort of healthy volunteers that received the high dose of RSV A2 ($10^{6.3}$ PFU) was admitted to the Special Clinical Studies Unit (SCSU) in two separate groups: cohort 3a + cohort 3b. Three of 11 evaluable participants in the entire cohort 3 had RSV shedding detected in nasal wash by culture and/or RT-PCR for a shedding frequency of 27%. Only 2 of the 3 participants had respiratory symptoms associated with RSV shedding and met the study definition of RSV illness, resulting in an illness frequency of 18%. The most common expected AEs related to RSV illness were nasal congestion/rhinorrhea (50%), headache (42%), sore throat (29%), cough (25%), and sinus congestion/pain (17%). The majority of symptoms were of mild-moderate severity (Grade 1 or 2), except for 1 episode of sinus congestion that was Grade 3. Unexpected AEs consisted of other symptoms that may occur with an URI, such as sneezing (32%), nausea (24%), itchy or irritated throat (16% each), watery eyes (20%), and decreased appetite (16%) that were of mild (Grade 1) severity. Five participants had episodes of epistaxis (16%) of Grade 2 or 3 severity that were possibly, probably or unlikely related to the nasal wash, nasal swab, or RSV infection. No serious AEs (SAEs) occurred during the entire study. Per the study design, since the RSV illness criteria were not met for the high dose cohort (>3 subjects/10-12 volunteers), additional subjects were not dosed with the high dose. We concluded that challenge infection with 10^5 PFU and $10^{6.3}$ PFU RSV A2 appears to be safe in healthy volunteers. Disease manifestations were limited in healthy adults because of pre-existing immunity. The participants with RSV shedding in nasal wash had a significantly lower pre-dose mean neutralizing antibody titer compared to participants with no shedding detected (mean neutralizing antibody titer, 6.7 reciprocal $\log_2$ versus 8.3 reciprocal $\log_2$, P=0.0011). The same applied to RSV illness: Participants with RSV illness had a significantly lower pre-dose mean neutralizing antibody titer compared to participants without RSV illness [6.3 reciprocal $\log_2$ ($\log_{(2)}$) versus 8.0 $\log_{(2)}$, P=0.0041]. The pre-dose mean serum F protein IgG was not significantly different in patients with or without RSV shedding or illness.

The rationale for selecting 10^5 PFU as the starting dose for our first RSV challenge study (NIH protocol number 15-I-0148) was based on existing safety data from prior human and non-human primate challenge studies. Buchman and colleagues challenged 32 healthy volunteers with a 10^6-PFU dose of RSV B without reporting safety issues;

however, their study population was not examined for pre-existing immunity to RSV.[21] The highest dose reported in the literature for RSV challenge in healthy adults is 10^6 PFU. The next highest RSV challenge dose is $10^{5.4}$ PFU of RSV A Memphis strain. The 35 healthy adults receiving this dose had RSV neutralization titers in the lower tertile of the population and the study finished without safety complications. Also in pre-clinical studies, no substantial AEs were seen in non-human primates challenged with RSV A2 at doses of 10^4 PFU (chimpanzees) or $10^{6.3}$ PFU (AGMs) (unpublished data Buchholz).[10,11]

Two prior human adult challenge infection studies specifically using RSV A2 were conducted using doses of 10^3 PFU or $10^{4.7}$ PFU.[13,14] In the study by Noah and colleagues, 3 of 9 subjects (33%) developed symptomatic upper respiratory illness and had evidence of RSV replication in the nasal lavage fluid after challenge with 10^3 PFU.[14] In the study by Lee and colleagues using a higher dose ($10^{4.7}$ PFU), 12 of 13 subjects (92%) with low serum neutralizing antibody activity shed RSV A2 after challenge.[13] Of these subjects, 92% displayed upper respiratory symptoms but none developed lower respiratory disease. This study provides evidence that development of RSV-induced respiratory illness after challenge infection can be done successfully and safely in healthy adults with low pre-inoculation serum anti-RSV antibody titers without complications of lower respiratory tract disease. Please refer to Appendix D for a summary table of completed RSV challenge studies.

We also selected a dose of $10^{6.3}$ PFU for our first RSV challenge study, which is higher than the previously reported 10^6 PFU dose used in human challenge.[21] Safety was acceptable for the $10^{6.3}$ PFU dose of RSV A2 CTM in non-human primate toxicity studies (unpublished data, Buchholz). Since RSV A2 CTM is a recombinant virus passaged through Vero cells, there is theoretical concern that human infectivity may be reduced.[22] Therefore, we chose to evaluate safety of the $10^{6.3}$ PFU dose along with the 10^5 PFU, in case a high titer challenge virus is necessary to overcome a potential loss of infectivity in the setting of pre-existing RSV immunity in healthy volunteers. In addition, prior chimpanzee studies have revealed the RSV A2 CTM to be phenotypically similar in vivo to the biologically derived RSV A2 strain. For this reason, we do not anticipate increased pathogenicity at this higher dose.

In this study, we propose to infect healthy volunteers who have low pre-challenge RSV-specific neutralizing antibody titers with recombinant RSV A2 using a nasal atomizer (MAD Nasal Atomizer, Teleflex). This atomizer has been used safely and successfully

to challenge healthy volunteers with wild-type influenza virus at the NIH Clinical Center.[28] In addition, FluMist is a quadrivalent influenza vaccine that is administered with a nasal atomizer that produces a fine mist primarily deposited in the nose and nasopharynx. The MAD Nasal Atomizer has been developed as a safe and painless way to deliver medications with rapid absorption across mucosal membranes. Delivery via the intranasal route is relatively painless and easy to perform with minimal training. For example, intranasal medication delivery is an effective method for delivering analgesia, anxiolysis, and anticonvulsants to pediatric patients.[26]

The MAD Nasal Atomizer creates a fine mist of particles 30-100 microns in size, which are optimized for deposition across a broad area of mucosa. Administering part of the dose in each nostril doubles the targeted mucosal surface that the challenge virus can infect. A soft conical plug that is part of the atomizer forms a seal with the nostril, preventing expulsion of some of the inoculum via the nostril, which occurred with a few participants in the first challenge study when the virus was administered intranasally using droplets. In addition, the atomization should prevent the dose from dripping down into the oropharynx, which was reported by several participants during the first challenge study. No AEs related to the MAD Nasal Atomizer occurred during the influenza virus challenge studies performed at the NIH Clinical Center (NCT01646138; NCT02594189).

As noted above, our first challenge study resulted in an overall shedding frequency of 42%, and frequency was higher in the cohorts that received the lower dose, 10^5 PFU, of RSV A2 (54%) versus the cohort that received the higher dose, $10^{6.3}$ PFU (27%). The overall frequency of RSV illness for all cohorts was only 21%-23% for the low dose recipients and 18% for the high dose participants. Our goal is to increase the number of participants who shed RSV in nasal wash (RSV shedding is defined as RSV detected in NP wash by PCR and/or viral culture on 2 or more days from day 2 post-challenge or later) or meet the RSV illness study definition (Section 3.1.2) so that our challenge model can be utilized to test antivirals and possibly vaccines in future studies. Our hypothesis is that including an RSV neutralizing antibody titer cut-off in the eligibility criteria for our study participants and administering a higher dose of RSV A2 using a nasal atomizer to provide a larger surface for infection will result in a higher incidence of RSV shedding and a higher frequency of RSV illness after challenge.

1.2 Rationale

This RSV challenge model addresses several unmet scientific and medical needs: 1) to provide a better understanding of the pathogenesis of and immunity to RSV infection in humans, 2) to expedite efficacy trials of new RSV therapeutics and possibly vaccines, and 3) to characterize the nasal microbiota in healthy adults before and after infection with RSV.

The common goals within the RSV research community are to ultimately reduce morbidity and mortality from RSV infection in vulnerable hosts (infants, elderly, immune compromised, and people with chronic cardiopulmonary diseases). *In vitro* models for RSV are well established. However, development of targeted RSV therapies and testing these agents' potential for efficacy and toxicity are better studied *in vivo* in humans. Human, healthy volunteer challenge models offer the ability to generate high quality data, minimize confounding variables, and study viral kinetics and human immune responses to RSV. Prior RSV challenge studies have been conducted without significant AEs. Because healthy volunteers have naturally acquired RSV immunity that puts them at low risk for untoward outcomes, we aim to use data gathered in this population to enhance the development of RSV antivirals and possibly vaccines that will eventually benefit those at greatest risk for severe RSV disease. Data collected from this and future challenge studies will help guide development and testing of RSV antivirals, vaccine candidates, and potentially novel treatment strategies.

This new phase 1 RSV challenge study seeks to optimize and improve the use of RSV A2 as a challenge virus in healthy adult volunteers by: 1) increasing the titer of the virus challenge dose by approximately five-fold; 2) pre-screening potential participants using a RSV neutralization titer cut-off (a more stringent criterion for establishing susceptibility); and 3) intranasal delivery of virus via a nasal atomizer (instead of by liquid droplets).

The neutralizing antibody titer cut-off was established based on data from our first RSV challenge study (15-I-0148) that showed a statistical association of low neutralizing antibody titer with RSV shedding and RSV illness, as described above. To determine the optimal cut-off for neutralizing antibody eligibility titer, the proportion of estimated shedders for various antibody titer cut-offs was determined along with the estimated proportion of people who would meet that criteria during screening (Table 3). We initially chose a neutralizing titer cut-off of < 6.8 $\log_{(2)}$ and then, < 7.04 $\log_{(2)}$ to balance the

estimated number of volunteers that will need to be screened to achieve an acceptable RSV shedding rate. However, our screening RSV neutralizing antibody titer data reveals that only approximately 16% of our screened volunteers have an eligible RSV titer. An increase of the eligibility titer cut-off to < 8.00 log $_{(2)}$ will increase the percentage of potentially eligible volunteers to approximately 40%, enabling us to enroll the study and answer the question of whether RSV A2 is a safe and suitable virus for challenge studies.

Table 3 Statistical analysis for determining RSV neutralizing antibody eligibility titer cut-off based on shedding at any time after challenge

Neutralizing antibody titer, [log_2]	Estimated proportion of shedders who meet criteria (CI)	Estimated proportion of screened volunteers who will meet criteria (CI)
≤ 92 (6.53)	1 (1, 1)	0.208 (0.046, 0.371)
≤ 118 (6.88)	0.875 (0.646, 1)	0.333 (0.145, 0.522)
≤ 132 (7.04)	0.788 (0.506, 1)	0.375 (0.181, 0.569)
≤ 151(7.24)	0.667 (0.4, 0.94)	0.5 (0.3, 0.70)

2 Study Objectives

2.1 Primary Objective

Determine the safety and infectability of high dose, recombinant RSV A2 administered by nasal atomizer to healthy adult volunteers.

2.2 Secondary Objectives

1. Characterize the onset, duration, and magnitude of viral shedding after RSV A2 challenge infection.
2. Determine the frequency of mild to moderate upper respiratory illness in healthy volunteers challenged with RSV A2.

3. Evaluate the duration and severity of clinical illness after RSV A2 challenge infection.
4. Determine the relationship between the magnitude and duration of viral shedding and the severity and duration of RSV illness.
5. Characterize RSV-specific serum and mucosal antibody responses after RSV A2 challenge infection.

2.3 Exploratory Objectives

1. Characterize cell-mediated immune responses after RSV A2 challenge infection.
2. Determine cytokine and chemokine levels in serum and NP wash samples after RSV A2 challenge infection.
3. Characterize RNA gene expression patterns after RSV A2 challenge infection.
4. Characterize the phenotype of peripheral blood mononuclear cells after RSV A2 challenge infection.
5. Characterize the nasal microbiome variations in healthy adults before and after RSV A2 challenge infection.

3 Study Design

3.1 Description of the Study Design

This is our second RSV challenge study with a goal of testing a higher dose of live recombinant RSV A2 and administering the dose using a nasal atomizer to healthy 18- to 50-year-old volunteers. The primary co-objectives are the safety of this approach and the frequency of shedding. The secondary objectives include description of the frequency and severity of illness and assessment of various immune responses after challenge.

The first part of the study will be the challenge phase, during which the volunteers will be admitted to the NIH Clinical Center Hospital. The follow-up phase will consist of outpatient visits at Day 28 and Day 56 after challenge.

We plan to administer 10^7 PFU of RSV A2 with a nasal atomizer to 21 healthy adult volunteers with low pre-challenge RSV neutralizing antibody titers. Since this is the first time we will use the nasal atomizer for administration of the 10^7 PFU dose, we will initially challenge 4 study participants with $10^{6.3}$ PFU of RSV A2 (cohort 1), the highest

dose determined to be safe in the first RSV challenge study (15-I-0148). We will assess the participants for AEs and symptoms of RSV illness on a daily basis post-challenge. Nasal swabs, nasal washes, and blood samples will be obtained post-challenge for assessment of RSV shedding and immune responses, as noted in the schedule of procedures (Appendix B). The participants will remain as inpatients for a minimum of 9 days and will be discharged after 2 negative RSV RT-PCR results using the FilmArray assay on consecutive days and if they do not have any symptoms suggestive of lower respiratory tract disease.

After cohort 1 is discharged from the inpatient isolation unit, the safety data will be reviewed by the SMC. If these participants do not experience any significant AEs related to the study agent or its administration, then our next 3 cohorts (cohorts 2-4) will be sequentially enrolled into the inpatient isolation unit. Seven or fewer volunteers per group will be accrued for each inpatient admission until the maximum cohort size (n=7) for each cohort is achieved. Each participant in cohorts 2-4 will receive 10^7 PFU of RSV A2. After discharge of all participants in each cohort from the inpatient unit, the SMC will review the safety data for that particular cohort to determine if it is safe to proceed with the next cohort.

Upon discharge from the inpatient unit, the participants will receive diary cards to enter symptoms or signs that arise in the follow-up period and will attend follow-up outpatient visits on Day 28 and Day 56 after challenge for assessment of safety and follow-up immune studies.

3.1.1 Definitions of RSV Illness

RSV-associated upper respiratory illness for this study is defined as the presence of at least 1 major symptom or 2 minor symptoms that last 2 or more consecutive days. Additionally, there must be evidence of RSV shedding on 2 or more days as detected by culture and/or PCR of NP wash specimens collected on day 2 post-challenge or later.

Major symptoms were selected based on the frequent occurrence (>50%) of these upper respiratory symptoms during RSV infection among previously healthy working adults in a natural history study[19] and include:

- Nasal congestion or rhinorrhea
- Sinus congestion or pain
- Sore throat

- Ear pain

Minor symptoms, based on the less frequent occurrence (<50%) of these symptoms in the natural history study, include:

- Fever, with temperature ranging from 38°C (100.4°F) to 39.4°C (103°F)
- Headache
- Cough
- Hoarseness

Mild upper respiratory illness: RSV-associated upper respiratory illness as defined above and upper respiratory signs or symptoms are no greater than Grade 1 severity. There is no evidence for clinically significant lower respiratory tract illness characteristics of which may include bronchospasm/wheezing, pneumonia, bronchiolitis, and respiratory distress that can manifest as increased work of breathing, increased respiratory rate, and oxyhemoglobin desaturation.

Moderate upper respiratory illness: RSV-associated upper respiratory illness as defined above and upper respiratory signs or symptoms are of Grade 2 or Grade 3 severity. There is no evidence for clinically significant lower respiratory tract illness, as described above.

Severe respiratory illness:

- Any symptom of Grade 4 severity
- Any evidence for clinically significant lower respiratory tract disease as noted above
- Any acute changes in health that are deemed clinically significant by the examining physician.

Sub-clinical illness: Presence of RSV shedding (detected in NP wash by RT- PCR or viral culture for 2 or more days on Day 2 post-challenge or later) and no clinical symptoms.

Symptom Flu-PRO questionnaire: We will use the FLU-PRO (the inFLUenza Patient-Reported Outcome) instrument with additional questions (Appendix F) to evaluate the suitability of this comprehensive patient-reported outcome (PRO) measure to assess RSV illness symptoms in clinical research. The questionnaire will be administered to

study participants on Day -1 to establish a baseline and then on a daily basis to each patient while inpatient, beginning with Day 1. Participants will receive an email generated by the REDCaps project into their personal email accounts (the REDCaps project will be managed by CRIMSON personnel). The email will contain a link to the questionnaire. If a participant is not able to access the questionnaire via their email then a paper questionnaire will be provided to them and the results entered by the research team into the REDCaps database. A recent study revealed that the 37-item FLU-PRO questionnaire is a valid measure of influenza symptoms in adults with a confirmed diagnosis of influenza.[27] We will begin to assess the suitability of FLU-PRO to evaluate symptoms of RSV illness, given the overlap of symptoms between RSV and influenza. Symptoms recorded on the FLU-PRO questionnaire by the study participants will not be utilized to assess and record adverse events. Adverse events will be assessed during the daily interim history and physical exam when the participants will be asked about the presence or absence of the expected adverse events and will be asked open-ended questions to elicit other symptoms.

3.1.2 Study Phases

Inpatient phase:

A volunteer participant is considered enrolled if eligibility criteria continue to be met at Day 0. Subjects will receive one intranasal inoculation of RSV A2 using a nasal atomizer on Day 0 and will be evaluated daily for microbiologic evidence of RSV infection and for clinical signs and symptoms. Daily safety assessments will also be performed. In addition, the RSV RT-PCR results (FilmArray) obtained from NP washes will be double-blinded from Day 0 through the duration of the inpatient stay to minimize bias during assessment of symptoms post-challenge.

If a participant is withdrawn for any reason within the 3 business days prior to admission, then efforts will be made to maintain the intended cohort size by identifying another volunteer from a reserve pool of qualified participants. An optional “stand-by” participant may be enrolled in the inpatient cohort. The “stand-by” participant will be an eligible volunteer who is able to come on Day -1 for an abbreviated, outpatient, clinical evaluation, during which a nasal swab and wash will be performed under the LID screening protocol (#12-I-0121, Screening of Volunteers for Clinical Trials of Investigational and Licensed Vaccines, Antiviral Products, or Live Virus Challenge Studies) for which the participant has already been consented. If the nasal wash is

negative for respiratory pathogens as determined by the FilmArray assay and a scheduled volunteer withdraws from the cohort on Day -1 for any reason, after signing informed consent for the RSV study, the “stand-by” participant may be offered a position in the cohort on Day -1.

Outpatient phase:

All subjects will return for outpatient follow-up at 28 days and 56 days after viral challenge.

3.2 Rationale for Study Design

In our first challenge study, we tested two different doses of RSV A2 in a dose-escalation manner. Neither of these doses caused sufficient infectivity or illness in pre-immune healthy adults aged 18-50 years to establish a reliable challenge model. Therefore, we have decided to try a higher dose of RSV A2 and administer this dose using a nasal atomizer to attempt to increase the frequency of viral shedding and mild-moderate illness amongst our healthy volunteers. We plan to challenge 25 healthy volunteers who have lower RSV neutralizing antibody titers, which correlated with viral shedding and RSV illness in our first challenge study. We hope that, with this sample, we will detect shedding in at least 55% of our study volunteers and have a higher probability of detecting SAEs in our study population (see Section 13.1). Our goal is to test that the higher dose administered using an atomizer is safe and to establish a sufficient frequency of RSV shedding in our study population to indicate that this approach may be useful to evaluate potential antivirals and vaccines in future studies.

3.3 Study Endpoints

3.3.1 Primary Endpoint

Assess expected and unexpected adverse events (AEs) throughout the trial and detect RSV shedding in nasal wash by FilmArray multiplex polymerase chain reaction (PCR) assay and by reverse transcription (RT)–quantitative PCR and quantitative viral culture after challenge with RSV A2

3.3.2 Secondary Endpoints

1. Determine day of onset of shedding, number of days of shedding, peak nasal wash viral titer, and mean sum of daily virus titers after challenge.

2. Calculate the number of subjects with mild to moderate upper respiratory illness after challenge, based on study definition of RSV illness.
3. Determine the number of days RSV illness persists after challenge and degree of illness based on study definition.
4. Correlate the magnitude and duration of viral shedding with the severity and duration of RSV-related symptoms.
5. Determine the frequency, magnitude, and duration of serum and mucosal (nasal wash and/or nasosorption) RSV-specific antibody responses after challenge.

3.3.3 Exploratory Endpoints

1. Perform transcriptome profiling of whole blood after RSV A2 challenge infection.
2. Perform fluorescence-activated cell sorting (FACS) analysis of peripheral blood mononuclear cells after RSV A2 challenge infection.
3. Perform next-generation sequencing using NP wash specimens before and after RSV A2 challenge to identify communities of microorganisms.
4. Perform functional RSV-specific T-cell assays to evaluate T-cell responses after challenge.
5. Measure cytokine and chemokine concentrations in serum and NP wash samples and correlate with RSV shedding, illness, and clearance of RSV.

4 Study Population

4.1 Rationale for Subject Selection

Because we will be infecting subjects with a virus that has the potential to cause substantial illness in people with underlying pulmonary or chronic debilitating conditions, we will enroll only healthy volunteers. We have selected the age group from 18 to 50 years because during this stage, most people have mature and robust immune responses. All adults have experienced prior natural RSV infections and are, as a result, seropositive to RSV.

4.2 Recruitment Plan

Healthy subjects will be recruited from within a 350-mile radius of the NIH Clinical Center. Potential participants who live outside this radius may be eligible to participate, per the PI's discretion, if they provide evidence of an ability to comply with follow up visits. For example, financial means are available, or family or friend support in the area. Subjects will respond to posted flyers, newspaper advertisements, Craigslist, Research

Match, Clinical Connection, the NIAID study web page, NIH employee list serve emails, and by telephone call or email to the clinical trial site. The clinical research staff will conduct an initial phone pre-screening, provide background information about the trial, and review basic inclusion and exclusion criteria before inviting potential subjects to undergo screening.

4.3 Subject Inclusion Criteria

1. Age 18-50 years inclusive.
2. General good health, without significant medical illness, physical exam findings, or significant laboratory abnormalities as determined by the investigator.
3. Willingness to stay confined to the inpatient unit for required study duration.
4. Willingness to have samples stored for future research.
5. Female subjects must be of non-childbearing potential (e.g., surgically sterilized [bilateral oophorectomy, bilateral tubal ligation, hysterectomy] or menopausal defined as 50 years old with amenorrhea for at least 1 year) or, if of child-bearing potential, they must agree to remain abstinent or if sexually active with a partner who can get them pregnant, they must have in place an effective method of contraception for at least 30 days prior to administration of the challenge virus and until 30 days after challenge virus administration:
    - intrauterine device (IUD) or equivalent
    - hormonal contraceptives (e.g., consistent, continuous use of contraceptive pill, patch, ring, implant, or injection)
        - if participant uses contraceptive pill, patch, or ring, they must also use a barrier method at the time of potentially reproductive sexual activity (e.g., [male/female condom, cap, or diaphragm] plus spermicide)
    - be in a monogamous relationship with a partner who has undergone a vasectomy at least 180 days prior to first dose of study agent
6. A plaque reduction RSV neutralization titer < 8.00 $\log_{(2)}$.

4.4 Subject Exclusion Criteria

The presence of any one of the following criteria is sufficient to exclude a prospective subject from enrolling in this study:

1. Subject who was previously challenged with RSV A2.

2. Female subject who is pregnant or lactating OR planning to become pregnant from 30 days prior to inoculation through 30 days after inoculation.
3. Presence of self-reported or medically documented significant medical condition(s) including but not limited to:
    a. Respiratory disease (e.g., chronic obstructive pulmonary disease, emphysema, rhinitis, sinusitis) in adulthood, and additionally:
        i. A history of asthma within the past 5 years, or a current diagnosis of asthma or reactive airway disease associated with exercise, seasonal hay fever or allergic rhinitis
        ii. Presence of any febrile illness or symptoms suggestive of a respiratory infection within 2 weeks prior to inoculation.

    b. Any significant abnormality of the nose or nasopharynx, including recurrent epistaxis within 90 days prior to viral inoculation or nasal or sinus surgery within 180 days prior to viral inoculation.
    c. Chronic cardiovascular disease (e.g., congestive heart failure, cardiomyopathy, ischemic heart disease).
    d. Chronic neurological or neurodevelopmental conditions (e.g., cerebral palsy, epilepsy, stroke, seizures).
    e. Ongoing malignancy.
    f. Chronic medical condition requiring close medical follow-up or hospitalization during the past 5 years (e.g., diabetes mellitus, renal dysfunction, hemoglobinopathy, autoimmune disease).
    g. An immunodeficiency.

4. Use of systemic corticosteroids exceeding 10 mg/day of prednisone equivalent and nasal steroid preparations or immunosuppressive drugs within 30 days before inoculation and within 60 days after. Low dose topical steroid preparations used for a discrete period of time are permitted.
5. Inhaled bronchodilator or inhaled steroid use within the last 360 days or use after upper respiratory tract infections.
6. Behavioral or cognitive impairment or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and cooperate with the study protocol.
7. Complete blood count (CBC), AST, ALT, creatinine values or other screening labs or tests (e.g. electrocardiogram [EKG], chest x-ray [CXR]) are outside of the

NIH Department of Laboratory Medicine normal reference range and deemed clinically significant by the PI.

8. Positive FDA-approved HIV test obtained during screening procedures.
9. Positive serology for hepatitis C virus obtained during screening period.
10. Presence of hepatitis B surface antigen obtained during screening period.
11. A smoker of tobacco products or a routine marijuana smoker currently or in the past year.
12. Current alcohol abuse or addiction.
13. Current illicit drug abuse or addiction.
14. Receipt of a licensed vaccine within 30 days prior to RSV A2 inoculation and planned vaccination within 60 days after inoculation.
15. Receipt of blood or blood-derived products (including immunoglobulin) within 180 days prior to viral inoculation. Receipt of packed red blood cells given for an emergent indication in an otherwise healthy person, and not required as ongoing treatment, is not exclusionary.
16. Receipt of an investigational agent or vaccine within 90 days prior to scheduled RSV A2 inoculation and planned receipt within 60 days after inoculation.
17. A body mass index (BMI) ≤ 18.5 or ≥ 37.0.
18. A medical, occupational, or family problem that would preclude the participant from complying with all study requirements.
19. Shares household, works closely with, or has routine contact with a child (children) < 5 years of age or with immunocompromised individual(s), adult(s) with significant cardiopulmonary disease or asthma, institutionalized persons or persons with functional disability, or any other individual that, in the judgment of the PI, might be at increased risk for complications if exposed to RSV.
20. Deprived of freedom by an administrative or court order or in an emergency setting.
21. Any condition that in the opinion of the PI would jeopardize the safety or rights of a person participating in the trial or would render the person unable to comply with the protocol.

Co-enrollment Guidelines: For the first month of the study, co-enrollment in other trials is restricted. Co-enrollment in other trials examining investigational agents or devices within 90 days prior to or 60 days following viral inoculation is restricted. After a subject completes the first month, they may co-enroll in trials that do not involve investigational agents and in which subject participation is not expected to alter normal responses of

the immune system or upper and lower respiratory tract function. Study staff should be notified of subject's desire to participate in another study as it may require approval by the PI.

4.5 Justification for Exclusion of Special Populations

Exclusion of Women:

1. **Pregnancy:** Pregnant women are excluded from this study because the effects of RSV A2 on the developing human fetus are unknown with the potential for teratogenic or abortifacient effects. Although there is no evidence of an association between RSV infection and problems during pregnancy or harm to the fetus, for the sake of caution pregnant women will be excluded.
2. **Breastfeeding:** Because there is an unknown but potential risk for AEs in nursing infants secondary to challenge infection of the mother with RSV A2, breastfeeding women are excluded from this study.

Exclusion of Children: Because there are insufficient data regarding dosing or AEs available in adults to judge the potential risk in children, children are excluded from this study.

4.6 Recruitment of NIH Employees

NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH information sheet on Employee Research Participation."

- NIH staff may be a vulnerable class of participants.
- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation.
- The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees.
- The employee subject's privacy and confidentiality will be preserved in accordance with NIH Clinical Center and NIAID policies, which define the scope and limitations of the protections.

- For NIH employee subjects, consent will be obtained by an individual independent of the employee's team. Those in a supervisory position to any employee and co-workers of the employee will not obtain consent.
- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

5 Study Agent/Interventions

5.1 Disposition and Dispensation

The RSV A2 challenge virus was manufactured by Meridian Life Sciences. RSV A2 is stored at Fisher BioServices (a NIAID-designated commercial repository). About 2 weeks prior to admitting a cohort of healthy volunteers, the NIH Investigational Drug Management and Research Section pharmacist or PI will submit a Challenge Virus Request form to OCRPRO, who will request a shipment from Fisher BioServices. Upon receipt of the shipment by the NIH Pharmacy, the study agent will be stored in an access-controlled freezer at –80°C ± 15°C in the NIH Pharmacy. The RSV A2 will be diluted to dose with Lactated Ringer's Solution for Injection (USP), obtained from the NIH Central Supply room and will be dispensed by the pharmacist on Day 0 as a one-time dose (0.5 ml per nostril) using a 1-ml MAD Nasal Intranasal Atomizer (Teleflex, MAD300 atomizer). The study agent will be released to the study team after it is ordered in the Clinical Research Information System (CRIS) by the PI or by a designated study investigator and approved by a pharmacist. The challenge virus doses will be transported on wet ice to the inpatient unit and administered by the PI or by a designated study investigator.

5.1.1 Formulation, Packaging, and Labeling

Each 1-ml atomization device will be individually labeled with the subject ID number, expiration date, and time. The device carrier bag will be labeled with subject's study ID number, the date the dose was dispensed, dosing instructions, recommended storage conditions, the expiration date and time, the name and address of the manufacturer, and the Investigational Use Statement ("Caution: New Drug – Limited by Federal [USA] Law to Investigational Use").

5.2 Study Agent Storage and Stability

The challenge virus will be stored in an access-controlled –80°C freezer in the NIH Pharmacy at –80°C ± 15°C. The diluent (Lactated Ringer's Solution for Injection, USP) will be stored at room temperature, as recommended by the supplier. Once thawed, RSV A2 must be diluted and used within 4 hours of thaw.

5.3 Preparation, Administration, and Dosage of Study Agent

Preparation: The dose of study agent will be prepared by the NIH research pharmacy as described in the Drug Fact Sheet developed by the research pharmacy. The concentration of stock RSV A2 is $10^{7.3}$ PFU/ml. Lactated Ringer's Solution for Injection, USP, will be used as diluent. Pharmacy personnel will prepare the dose of challenge virus for each study participant using aseptic technique under Biosafety Level 2 conditions. The diluted challenge virus dose, $10^{6.3}$ PFU for cohort 1 or 10^{7} PFU for cohorts 2-4, will be drawn up in the atomization device 1-ml syringe.

Dosing and Administration: The research pharmacist will prepare and dispense one dose of either $10^{6.3}$ PFU or 10^{7} PFU for each enrolled participant in cohort 1 or cohorts 2-4, respectively. In detail, the virus will be diluted to the appropriate concentration in Lactated Ringer's Solution for Injection, USP. Doses will be delivered using a 1-ml syringe atomization device. While supine, subjects will be dosed intranasally with 0.5 ml per nostril, administering a total RSV A2 dose of $10^{6.3}$ PFU in one ml or 10^{7} PFU in one ml. Doses will be administered by study staff trained to administer the study agent. Participants will be observed for 30 minutes after inoculation.

Duration of Therapy: Single dose given on Day 0.

Use of Ancillary Medications/Over-the-Counter Products/Foods: Study staff will administer any concomitant medications, vitamins, or supplements as appropriate.

Disposal of Used, Partially Used, and Unused Virus: Each vial of stock virus will be used to prepare doses only once and any unused agent will be discarded after use. Unused vials that have been thawed will also be discarded. Vials will be discarded by incineration following standard operating procedures of the NIH Pharmacy.

5.4 Assessment of Subject Compliance with Study Agent

A one-time challenge virus dose will be directly administered to each subject intranasally using a nasal atomizer by the PI or other trained clinician on the study team. Doses of RSV A2 will be ordered in CRIS. The Clinical Research Information Management System of the NIAID (CRIMSON) will be the primary source for dose administration, where it will be documented in case report forms (CRFs). The dose administration will also be documented in CRIS.

5.5 Concomitant Medication and Procedures

All concomitant prescription medications taken during study participation will be entered into CRIMSON. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be documented in CRIMSON are concomitant prescription medications, over-the-counter medications, and non-prescription medications taken at the time of AEs (all grades).

5.6 Prohibited Medications and Procedures

Prohibited medications during the inpatient hospitalization include decongestants, nasal sprays (including nasal saline), antihistamines, expectorants, cough suppressants, anti-inflammatory medications, throat lozenges, herbal remedies, or naturopathic remedies. Any participant requesting symptomatic relief for persistent symptoms associated with RSV illness that significantly interfere with usual activities will be evaluated as outlined in Section 6.2.1.

The interventions listed below have the potential to interfere with virus-induced immune responses and will be reviewed with the subjects during each outpatient visit. If a subject uses or receives any of these interventions during the study, then it will be noted in the subject's record.

1. Use of any investigational drug or investigational vaccine other than the study article within 60 days after inoculation with RSV A2.
2. Receipt of a licensed vaccine within 60 days after inoculation with RSV A2.
3. Receipt of immunoglobulins and/or blood derived products, with the exception that packed red blood cells are not prohibited if/when given with emergent indications and not needed for ongoing therapy.
4. Administration of systemic corticosteroids exceeding 10 mg/day of prednisone equivalent within 60 days after inoculation with RSV A2.

5. Nasal corticosteroids are prohibited after inoculation with RSV A2, but may be used after the Day 28 study visit.
6. Receipt of any immune suppressive or immune modulatory medications within 60 days after inoculation with RSV A2.

6 Study Schedule and Clinical Procedures

The study schedule is provided in tabulated form in Appendix B. Total volumes of blood to be collected for research purposes are provided in Appendix C.

6.1 Screening

Potential volunteers for the RSV challenge protocol will undergo screening after enrollment into the LID screening protocol #12-I-0121 (Screening of Volunteers for Clinical Trials of Investigational and Licensed Vaccines, Antiviral Products, or Live Virus Challenge Studies).

To summarize, the following screening procedures will occur while the volunteer is enrolled in the screening protocol:

Screen 1 Visit. (Day -180 to Day -14)

- Review health history questionnaire
- Vital signs (blood pressure, temperature, heart rate, respiratory rate, and pulse oximetry), height and weight
- CBC with differential, acute care panel, mineral panel, and hepatic panel
- Urine drug screen
- Urine pregnancy test (females)
- RSV-specific neutralizing antibody titer
- HIV, hepatitis B, and hepatitis C testing
- EKG
- CXR

If any of the above tests were performed at NIH within 30 days of the Screen 1 Visit, and the investigators deem the results to be within limits of eligibility for the study, then duplicate screening labs do not need be repeated at the Screen 1 Visit. Volunteers deemed to be healthy and who meet eligibility criteria for the challenge study will continue to the Screen 2 Visit.

Screen 2 Visit. This visit will occur between Days −56 to −2. The following procedures will be performed on this visit:

- Complete medical history and physical examination
- Vital signs (blood pressure, temperature, heart rate, respiratory rate, and pulse oximetry)
- Review and signing of reproductive counseling form.
- PFT using standard equipment to measure forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1)
- FLU-PRO symptom questionnaire (baseline)
- NP wash for baseline cytokine assessment (see Section 7)
- NP swab sampling may be performed for microbiome analysis (see Section 7)
- Labs from the screen 1 visit may be repeated to follow-up on results outside of the laboratory reference range.

Screen 1 visit must occur prior to Screen 2 visit for all participants.

Volunteers will be contacted 2 weeks prior to Day −1 via email to provide instructions for arrival on Day −1. In addition, baseline health will be re-assessed by inquiring via email or phone whether there have been any changes in a volunteer's health (e.g. new medical diagnoses/surgeries, unexpected visits to the doctor's office, urgent care or ER) and if any new medications have been started.

A volunteer who reports symptoms of an URI during the 2 weeks prior to inpatient enrollment may be offered an additional screening visit for NP wash and multiplex FilmArray PCR evaluation. If the volunteer has a positive result for a respiratory pathogen, then the volunteer may be offered a position in a future cohort, and the open spot in the upcoming cohort will be offered to another volunteer from a reserve pool of qualified participants.

If the volunteer meets eligibility criteria for this study, then signed informed consent will be obtained at the screen 2 visit or afterwards, but prior to protocol-specific testing and procedures.

6.2 Inpatient Study Phase (Day −1 until discharge)

Subjects will be admitted to the Special Clinical Studies Unit in Clinical Center on study Day −1, where they will be familiarized with the study procedures and monitored for

signs or symptoms of acute illness that would preclude their further participation in the study. Visitors are not allowed in the Special Clinical Studies Unit where the study participants will remain hospitalized under isolation for the duration of the inpatient phase of the study. Subjects manifesting any signs or symptoms of illness or who are now uncomfortable with or unwilling to adhere to study procedures will be discharged before administration of challenge virus. If a volunteer has a respiratory or febrile illness or a significant deviation of baseline health at the time of admission, as assessed by the PI, they will be discharged prior to virus administration.

Admission to Clinical Center (Day −1)

1. Reconfirm or obtain signed informed consent, if not already obtained.
2. Record vital signs (blood pressure, temperature, heart rate, respiratory rate, and pulse oximetry), height, and weight.
3. Provide baseline FLU-PRO questionnaire to be filled out by subject.
4. Perform interim history and history-directed physical examination.
5. Obtain CBC with differential, acute care panel, and hepatic panel (these labs do not need to be repeated if they were obtained <56 days prior to Day −1)
6. Obtain urine drug screen.
7. Serum pregnancy test and pregnancy risk assessment (i.e., last menstrual period and any recent history of potentially reproductive sex that is too early to be detected) for subjects who are capable of becoming pregnant. A positive serum pregnancy test will exclude the subject from the trial. Suspicion of a possible very early pregnancy based upon recent unprotected, potentially reproductive sex may result in exclusion or deferral (i.e., until absence of pregnancy is assured) at the discretion of the PI.
8. Review and signing of reproductive counseling form with female participants.
9. Obtain HIV rapid antibody screen.
10. Obtain hepatitis B and C serology testing, EKG, and CXR if obtained >120 days prior to Day -1; obtain PFT if performed > 56 days prior to Day −1.
11. Placement of synthetic absorptive matrices (SAM) in on naris for assessment of antibody and/or chemokine and cytokine levels in mucosal lining fluid.
12. Obtain NP wash for FilmArray assay, qualitative and quantitative viral culture, lab-based assay for RT-PCR, antibody assays, including secretory IgA, and cytokine/chemokine assays.
13. Obtain nasal swabs from each nares for microbiome analysis.
14. Obtain blood for HLA testing.

15. Obtain blood for Epstein-Barr virus (EBV) serology (required for control for T-cell assays).
16. Obtain blood for quantitative immunoglobulins and research serum for total IgA and IgG.
17. Obtain blood for lymphocyte phenotyping panel T, B, and NK cell subsets.
18. Obtain research blood for RSV antibody and neutralization assays, T- and B-cell assays, transcriptome profiling, serum cytokine analyses, and for more extensive molecular immune cell phenotyping.

Day of Inoculation (Day 0)

1. Perform interim history and directed physical examination, focusing on any acute complaints.
2. Obtain research blood for T- and B-cell assays, immune cell phenotyping, transcriptome profiling, and cytokine analysis.
3. Record vital signs pre-inoculation and 30 ± 10 minutes post-inoculation.
4. RSV challenge virus inoculation: subjects in cohort 1 will receive $10^{6.3}$ PFU of RSV A2 and subjects in cohorts 2-4 will be receive 10^{7} PFU. Subjects will be supine and 0.50 ml of inoculum will be administered to each nostril. Subjects will be asked to remain supine for approximately 10 minutes.
5. Observe subjects for at least 30 minutes after inoculation with appropriate medical equipment available in case of a rare, unanticipated, anaphylactic reaction.
6. Assess AEs.

Clinical Monitoring and Evaluation (Days 1 through 7)

See Appendix B for a tabular representation of study procedures.

Study Days 1-6:

1. Perform focused history and history-directed physical examination focusing on any acute complaints and record findings (daily).
2. Record vital signs (daily).
3. Assess AEs (daily).
4. Identify, provide, and record symptomatic clinical care as permitted per protocol.
5. Obtain NP wash for viral RNA detection, viral culture, and for cytokine assays. Qualitative RSV PCR by FilmArray will be performed on Days 1, 2, 3, and then

daily from Day 5 through day of discharge; quantitative RSV PCR. Viral culture will be performed daily starting on Day 2 through day of discharge. Nasal cytokine assays will be performed on Day 1 only.

6. Obtain cotton-tipped nasal swab for microbiome analysis (Days 2 and 4).
7. Subjects fill in FLU-PRO symptom questionnaire once daily in the evening.
8. On Day 1 only, obtain CBC with differential and T, B, and NK cell lymphocyte immunophenotyping; and research blood for immune cell phenotyping, transcriptome profiling, and cytokine analysis.
9. On Day 3 only, obtain CBC with differential, acute care panel, and hepatic panel.

Study Day 7:

1. Perform focused history and history-directed physical examination focusing on any acute complaints and record findings.
2. Record vital signs.
3. Assess AEs.
4. Subjects fill in FLU-PRO symptom questionnaire in the evening.
5. Obtain CBC with differential, acute care panel, and hepatic panel.
6. Obtain lymphocyte immunophenotyping, TBNK.
7. Obtain SAM strip for mucosal cytokines and/or antibody analysis
8. Obtain nasal swab for microbiome sequencing and culture.
9. Obtain NP wash for RSV fresh and quantitative culture, viral RNA detection by quantitative RT-PCR and FilmArray, nasal secretory IgA, and cytokine assays.
10. Obtain research blood for RSV antibody and neutralization assays.
11. Obtain research blood for B- and T-cell assays, immune cell phenotyping, transcriptome profiling and cytokine analysis.
12. Evaluation for discharge criteria:
    - The participant must have 2 consecutive negative NP washes for RSV RNA, as detected by FilmArray assay (participant and study team will remain blinded to the RSV PCR results and an honest broker, designated by the PI, will notify the study team when a participant's PCR results satisfy the discharge criteria)
    - The participant does not have any signs or symptoms of possible RSV-associated lower respiratory tract illness

We expect that a healthy volunteer who remained well throughout Day 7 after challenge and who was not shedding RSV by Day 7 would not proceed to develop a symptomatic

respiratory illness thereafter or present risk for virus transmission after discharge. In healthy adults, the duration of viral shedding associated with onset of symptomatic infection in the majority of adults lasts less than 7 days, with an average shedding duration (detected using viral culture) of 3.9 days.[19] For discharge, we rely on RT-PCR for documentation of clearance of viral shedding. Molecular detection of RSV by RT-PCR is more sensitive than by culture, as seen in prior RSV challenge studies (Appendix D). The mean duration of RSV shedding by RT-PCR is 8-10 days.

In the event that a participant who is no longer shedding RSV has persistent mild-to-moderate upper respiratory illness by Day 7, then we will continue to provide clinical evaluation and recommendations for treatment, according to the standard of care and discharge the subject. In the event that a participant who is no longer shedding RSV has lower respiratory tract symptoms by Day 7, we would evaluate for indications to escalate care or continue standard of care and evaluate for discharge only when the subject is clinically stable.

Subjects remaining on inpatient care after study Day 7:

1. Perform focused history and history-directed physical examination focusing on any acute complaints and record findings.
2. Record vital signs.
3. Assess AEs.
4. Subjects fill in FLU-PRO symptom questionnaire once daily in the evening up to and including Day 14.
5. Obtain SAM strip on Day 10.
6. Obtain nasal swabs for microbiome sequencing and culture on Day 10.
7. Obtain NP wash for RSV culture and viral RNA detection and research blood for mucosal cytokine and antibody and lymphocyte assays, as per table in Appendix B. For Day 10: Obtain research blood for B- and T-cell assays and quantitative and molecular immune cell phenotyping, serum for RSV-specific antibody testing and total serum IgG and IgA, serum for cytokine analysis. Day 11 onward, on a daily basis, nasal research procedures consist of nasal wash tested by FilmArray PCR for respiratory viruses, quantitative RT-PCR, quantitative viral culture, and fresh viral culture.
8. Evaluate volunteer for discharge criteria, as noted above, on a daily basis

6.2.1 Treatment Plan for Persistent Symptoms Associated with RSV Illness

Participants reporting persistent and significant symptoms associated with RSV illness may be offered symptomatic relief after clinical evaluation. If symptoms are refractory to single doses of over-the-counter acetaminophen (325-650 mg) and significantly, adversely impact daily activities (as assessed by the PI), such as the ability to sleep, then ibuprofen (200-600 mg), pseudoephedrine (30-60 mg), or throat lozenges may be provided, after clinical evaluation, for symptoms such as pain, persistent cough, nasal or sinus symptoms interfering with sleep, or fever when associated with significant discomfort.

Indications for offering symptomatic treatment to participants who have not yet met a study definition of RSV-associated upper respiratory illness and/or have not completed study evaluations (including research labs) through Day 7 will be determined by the PI applying Good Clinical Practice (GCP) principles.

6.2.2 Escalation of Care Plan

Subjects who continue to shed virus at Day 7 will be strongly encouraged to remain in the Clinical Center until they have 2 consecutive negative NP washes for RSV. A prolonged stay in the Clinical Center required as a result of extended shedding will not be considered a SAE. Should a subject elect to leave the inpatient unit at any point prior to meeting per-protocol discharge criteria, appropriate precautions will be discussed in detail with the subject to minimize risks to the subject and any contacts (e.g. hand hygiene, avoidance of persons at high risk for complication from RSV, return to clinic for nasal wash follow-up to determine if RSV shedding continues). The Clinical Safety Office (CSO)/Sponsor Medical Monitor will be notified immediately if this situation occurs.

6.2.2.1 Increased Severity of Clinical Illness

Any change in clinical status deemed important by the examining physician in consultation with the PI will initiate an escalation of care for that individual. This will be considered an SAE. Indicators for increased care may include the following:

1. Severe respiratory illness (Section 11.1).
2. Fever with rigors.

3. Any clinically significant deterioration of a participant's overall hemodynamic status.
4. Any other change noted in the physician's exam or symptoms/signs deemed significant by the responsible medical and/or study staff in conjunction with the PI. This includes any untoward signs or symptoms that are inconsistent with the expected course of RSV in a generally healthy individual.

Escalation of care will be individualized to address the specific needs of the subject and immediate steps will be taken. If deemed appropriate, standard-of-care treatments will be started immediately as deemed necessary by the PI. Appropriate laboratory, radiographic, and microbiological diagnostic testing will be performed to evaluate the participant and diagnose any complicating illness.

6.3 Follow-up Visit (Day 28)

A follow-up outpatient visit will be scheduled for Day 28 (-1 to +10 days). The following procedures will be conducted:

1. Obtain interim history and physical to assess for AEs.
2. Obtain and record vital signs.
3. Obtain diary card. Subjects will record symptoms starting the day after discharge and leading up to this visit.
4. Obtain SAM strip for mucosal cytokines and/or antibody analysis.
5. Obtain nasal swab for microbiome sequencing and culture.
6. Obtain NP wash for immunoglobulins, secretory IgA, and cytokine analysis.
7. Obtain fasting, research blood for T-cell assays, immune cell phenotyping, transcriptome profiling, cytokine analysis, RSV antibody and neutralization assays.
8. Obtain T, B, and NK cell flow cytometry test.
9. Obtain blood for CBC with differential, acute care panel, and hepatic panel.
10. Assess for treatments that could potentially interfere with RSV-induced immunity (Section 5.6).

If a close contact of the subject becomes ill with a respiratory tract infection during Days 7-28, then the subject will be asked to request that the close contact inform the study team and enroll in the NIH protocol 11-I-0109, "Viral Infections in Healthy and Immunocompromised Hosts," to determine if their illness is being caused by RSV, and

then to identify the virus. All efforts will be made to document transmission of virus that may have occurred after discharge.

6.4 Final Study Visit (Day 56)

A final study visit will be scheduled for Day 56 (-5 to +15 days). The following procedures will be conducted:

1. Obtain interim history and physical to assess for AEs.
2. Obtain and record vital signs.
3. Obtain diary card.
4. Obtain SAM strip for mucosal cytokines and/or antibody analysis.
5. Obtain nasal swab for microbiome sequencing and culture.
6. Obtain NP wash for immunoglobulins, secretory IgA, and cytokine analysis, RSV antibody and neutralization assays, immune cell phenotyping, transcriptome profiling .
7. Obtain blood for CBC with differential, acute care panel, and hepatic panel.
8. Obtain T, B, and NK cell flow cytometry test.
9. Assess for treatments that could potentially interfere with RSV-induced immunity (Section 5.6).

6.5 Interim Safety Visit

Interim safety visit(s) will be scheduled for any participant who has a clinically significant AE for which follow-up is required prior to Day 28 or Day 56 or for a participant who withdraws from the inpatient phase of the study prior to meeting discharge criteria and is known to have a NP wash positive for RSV on the day of discharge.

The following procedures may be performed:

1. Obtain interim history and physical to assess AEs.
2. If a prior respiratory pathogen panel result was abnormal, an NP wash may be obtained for respiratory pathogen panel, RSV culture, and quantitative viral RNA detection. Surplus NP wash will become a stored research specimen (Section 9).
3. If a prior laboratory result was abnormal, this abnormality may be re-evaluated with a repeat blood draw.
4. Any other event that the PI deems to require follow-up will be evaluated during the interim safety visits.

6.6 Early Termination Visit

A termination visit will be performed at the time of subject withdrawal. Interim safety visits or safety visits on Day 28 and/or Day 56 will be recommended to any subject who has received study virus and withdraws from the inpatient phase of the study prematurely or who has significant AEs that require follow-up. Study discontinuation will be determined on a case-by-case basis.

An individual may be withdrawn for any of the following reasons:

1. An individual subject's decision.
2. A clinical AE, lab abnormality, or medical condition for which continued participation in the study would not be in the best interest of the subject.
3. Non-compliance with study procedures to the extent that it is harmful to the subject or integrity of study data.
4. Use of any investigational drug or investigational vaccine other than the study agent within 60 days after inoculation with RSV A2.
5. Receipt of a licensed vaccine within 60 days after inoculation with RSV A2.
6. Receipt of immunoglobulins and/or blood-derived products during the study period, with exception that packed red blood cells are not prohibited if/when given with emergent indications and not needed for ongoing therapy.
7. Chronic administration of immunosuppressant(s), defined as > 14 days (if corticosteroid, then dose is ≥ 10 mg/day of prednisone equivalent), or other immune modifying drug(s) taken during the study period. (Nasal corticosteroids are prohibited after inoculation with RSV A2, but may be used after the Day 28 study visit.)
8. Any confirmed or suspected immunosuppressive condition or immunodeficient condition.

6.7 Pregnancy and Follow-up Visit

If pregnancy is discovered before virus inoculation, then the subject will be released before participation and no further follow-up is needed. If pregnancy is discovered after virus inoculation, then the subject will be advised to notify obstetrician of study agent exposure, and we will continue to follow the participant for safety and pregnancy outcomes. Pregnancy events will also be reported to the sponsor medical monitor, the Safety Monitoring Committee (SMC), and/or the institutional review board (IRB).

7 Laboratory Evaluations

7.1 **Clinical Laboratory Evaluations** (all performed in the NIH Clinical Center):

1. CBC with differential.
2. Acute care panel that includes sodium, potassium, chloride, bicarbonate, serum creatinine, and urea.
3. Mineral panel that includes albumin, calcium, magnesium, and phosphorus.
4. Hepatic panel that includes alkaline phosphatase, ALT, AST, and total and direct bilirubin.
5. Urine or serum pregnancy test.
6. T, B, and NK cell flow cytometry test.
7. NP wash for qualitative RSV PCR. Before viral challenge and during the specified time points post-inoculation, NP wash specimens will be tested with the FilmArray® Respiratory Panel (BioFire Diagnostics, Inc. [Salt Lake City, UT, USA]). The assay will be performed by the NIH Clinical Center microbiology lab. This assay is a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory diagnostic test and will be used to determine discharge eligibility.

7.2 Research Laboratory Evaluations:

NP washes/swabs will be conducted as described in Section 6. Samples will be tested for the following:

1. RSV detection by quantitative RSV RT-PCR and viral culture. Starting on Day 1, daily nasal wash specimens will be tested by FilmArray assay in the DLM. Starting on Day 1, nasal wash specimens will be tested for RSV by quantitative viral titer and culture (CIR at JHU; Baltimore, MD, USA).
2. Cytokine and chemokine assays will be conducted at the LID (NIAID) and the Center for Human Immunology, Autoimmunity, and Inflammation (CHI, National Heart, Lung, and Blood Institute [NHLBI]).
3. RSV mucosal immunity will be done by measuring secretory IgA antibody levels.
4. Nasal microbiome analysis (NIAID Microbiome Initiative).

Blood samples will be analyzed as follows:

1. Serum RSV neutralization assays for RSV (CIR/JHU).
2. RSV-specific T-cell responses/T-cell repertoire to be conducted by Dr. Cyril Le Nouen, PhD (LID/NIAID).
3. Molecular and quantitative immune cell phenotyping to include FACS analysis to be conducted by CHI.
4. Serum cytokine analysis to be conducted by CHI.
5. Transcriptome profiling using PAXgene (CHI/NHLBI).

The amount of blood drawn for research purposes will be within the limits allowed for adult research subjects by the NIH Clinical Center (Medical Administrative Policy 95-9, Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf).

8 Potential Risks and Benefits

8.1 Potential Risks

Study agent inoculation

Complications or severe illnesses associated with RSV A2 challenge inoculation are rare and include:

- Pneumonia
- Secondary bacterial infections
- Hypoxemia/respiratory failure
- Anaphylaxis

Also, as with any investigational product, there is a theoretical possibility of risks about which we have no present knowledge. Subjects will be informed of any such risks should further data become available.

We expect that subjects will develop RSV illness. These expected events are listed in Section 11.1. Subjects will not be allowed to self-medicate for symptom relief; symptomatic treatment may be provided by study staff after clinical evaluation as needed for pain, persistent cough, nasal symptoms interfering with sleep, and/or fever when associated with significant discomfort.

Subjects may also be inconvenienced by being confined to the isolation unit for the duration of the hospital stay.

Acetaminophen

Acetaminophen is well tolerated and safe in recommended doses. Potential acetaminophen toxicity may occur at high doses exceeding the therapeutic limit.

NP washes/swabs

Potential risks associated with NP washes and/or swabs include pain or discomfort, a gag reflex, and very rarely, epistaxis.

Venipuncture

Risks occasionally associated with venipuncture include pain and bruising at the site of venipuncture, bleeding, lightheadedness, and, rarely, infection or syncope.

Pulmonary function tests

There are minimal risks associated with PFTs for healthy volunteers. It may cause mild shortness of breath and fatigue, but is otherwise noninvasive and safe.

8.2 Potential Benefits

Subjects will not receive any direct benefit from participation in this study.

Through a healthy volunteer's participation in this study, the scientific and medical community may gain new knowledge and insights to guide development and testing of RSV antivirals, vaccine candidates, and potentially novel treatment strategies. This knowledge may advance efforts to bring benefit to populations of patients most vulnerable to severe RSV complications.

9 Research Use of Stored Human Samples, Specimens or Data

- **Intended Use:** Samples and data collected under this protocol may be used to study RSV infection and response in healthy adults. Human genetic testing will

include HLA typing. Also, human DNA may be isolated from stored research samples in the future for genetic testing.

- **Storage:** Access to stored samples will be limited using a locked freezer in a locked laboratory. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.
- **Tracking:** Samples will be stored at the National Laboratory for Cancer Research in Frederick, MD. Samples will be tracked using a database located on a password-protected computer, which will be maintained by the investigators and their designees. Only investigators and their designees will have access to this database.
- **Disposition at the Completion of the Protocol:**
  - In the future, other investigators (both at NIH and outside) may wish to use these samples and/or data for research purposes. If the planned research falls within the category of "human subjects research" on the part of the NIH researchers, NIH IRB review and approval will be obtained. This includes the NIH researchers sending out coded and linked samples or data and getting results that they can link back to their subjects.
- **Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:**
  - Any loss or unanticipated destruction of samples or data (for example, due to freezer malfunction) that meets the definition of a reportable event will be reported to the NIH IRB according to NIH Human Research Protection Program (HRPP) Policy 801.
  - Additionally, subjects may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the subject and to the IRB. This decision will not affect the subject's participation in this protocol or any other protocols at NIH.

10 Remuneration Plan for Subjects

Participants will be compensated according to Table 4.

Table 4 Participant remuneration

Study Day	Remuneration ($)
Inpatient Study Phase	
−1	220
0	225
1	225
2	225
3	225
4	225
5	225
6	225
7	225
Initial Inpatient Subtotal	2020
Each Additional Inpatient Day[a]	325
Inpatient Completion Bonus	425
Outpatient Study Phase	
Follow-up Visit	200
Final Study Visit	225
Maximum Follow-up Subtotal [b]	*425*
Total Compensation for Full Study Participation[c]	2870

a Subjects will continue inpatient care if they continue to display RSV-related symptoms and are shedding virus after Day 7. Inpatient care will continue until discharge criteria are met (see Section 6.2).
b If an interim visit is required, compensation for this visit will be $200
c Full participation is defined as the initial inpatient phase (Days −1 to 7), 1 follow-up visit (Day 28), and a final study visit (Day 56).

11 Assessment of Safety

11.1 Definitions for Sponsor Reporting

The NIAID CSO is responsible for sponsor safety oversight of this study, and the definitions below comply with CSO requirements.

Adverse Event (AE): An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Expected Events: The following signs or symptoms are induced by or associated with RSV illness, and any combination of these (within the range from Grade 1 to Grade 3) is expected to occur with virus challenge. These symptoms will not be reported as unexpected AEs; they will be recorded as Expected Events of RSV infection in CRIMSON per protocol. Each individual with an expected AE consistent with RSV illness but of atypical duration or severity will be evaluated with good clinical judgment

for appropriate further management. In addition, the PI will monitor all subjects in aggregate, and any pattern of severity, or unexpectedly high frequency of a particular event, will be treated as a safety signal and potential safety concern and will prompt consultation with the sponsor.

- Nasal congestion or rhinorrhea
- Sore throat
- Cough
- Headache
- Sinus congestion or pain
- Fever, with temperature ranging from 38°C (100.4°F) to 39.4°C (103°F)
- Ear pain

Adverse Reaction (AR): An AE that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR): An AE for which there is a reasonable possibility that the investigational agent caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. A SAR implies a lesser degree of certainty about causality than AR, which implies a high degree of certainty.

Serious Adverse Event (SAE): An SAE is an AE that results in one or more of the following outcomes:

- death
- a life threatening event (places the subject at immediate risk of death from the event as it occurred)
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event

Medical and scientific judgment should be exercised in deciding events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event: An AE is unexpected if it is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is a Suspected Adverse Reaction that is both serious and unexpected.

Unanticipated Problem (UP): A UP is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document or other study documents; and
    b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

Serious Unanticipated Problem (UP): A UP that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others. Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. These events may involve a greater risk of social or economic harm to subjects or others rather than physical/psychological harm. Such events would be considered a non-serious UP. Examples of a UPnonAE include a breach of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Unanticipated Problem that is not an Adverse Event (UPnonAE): An UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered non-serious UPs. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation: Any change, divergence, or departure from the IRB-approved research protocol.

Major Deviations: Deviations from the IRB-approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.

Minor Deviations: Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Non-compliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

Serious Non-compliance: Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially affects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.

Continuing Non-compliance: A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

11.2 Documenting, Recording, and Reporting Adverse Events

All AEs occurring from the time of viral challenge through the final study visit will be documented, recorded, and reported.

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the subject’s medical record/source document,

- recorded in CRIMSON, and
- reported as outlined below (e.g., IND Sponsor, IRB, and FDA).

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

A laboratory abnormality will not be reported as an adverse event if ALL of the following criteria are met:

- It is no more than “Grade 1” or “Mild” per the protocol specified toxicity table (or investigator assessment if not listed on the table); AND
- It does NOT require an intervention (e.g., discontinuation of treatment, dose reduction/delay, additional assessments, or treatment); AND
- It is assessed by the PI as NOT related to the study agent(s) or study procedure(s); AND
- It is assessed by the PI as NOT clinically significant (e.g., the abnormal value does NOT suggest a disease or organ toxicity).

All abnormal laboratory findings will be reviewed on a routine bases by the PI to identify potential safety signals. An abnormal lab not included on the toxicity table should be assessed in a similar fashion to the criteria above.

All clinically relevant AEs that are not resolved by the end of the final study visit are followed until resolution or until stability is achieved (unless the subject is lost to follow-up).

11.3 Investigator Assessment of Adverse Events

The investigator will assess all AEs with respect to seriousness (criteria listed above), severity (intensity or grade), and causality (relationship to study agent and relationship to research) according to the following guidelines.

11.3.1 Severity

The Investigator will grade the severity of each AE according to toxicity table provided in Appendix E, and supplemented by the “Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events” Version 2.1, July 2017, which can be found at: https://rsc.tech-res.com/docs/default-source/safety/division-of-aids-(daids)-table-for-grading-the-severity-of-adult-and-pediatric-adverse-events-corrected-v-2-1.pdf

Some Grade 1 lab parameters on the DAIDS Toxicity Table (fibrinogen, potassium [low], uric acid [males only, elevated]) fall within the NIH lab reference range for normal

values. These normal values will not be reported as Grade 1 AEs. The Grade 1 values for these tests will be reported as follows:

- Fibrinogen: 100-176 mg/dL
- Potassium (low): 3.0-3.3 mmol/L
- Uric acid (males): 8.7-10.0 mg/dL
- Magnesium (low): 0.60-0.65 mmol/L

11.3.2 Causality

Causality (likelihood that the event is caused by the study agent(s)) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

OR

- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

OR

- definitely due to an alternative etiology

Note: Other factors should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

11.4 Investigator Reporting Responsibilities to the Sponsor

11.4.1 Adverse Events

AE data will be submitted to the IND Sponsor when requested for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

11.4.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) and sent to the CSO by fax or e-mail attachment. Deaths and immediately life threatening SAEs must be reported to the CSO within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

CSO CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
E-mail: rchspsafety@mail.nih.gov

SAEs that have not resolved by the end of the final study visit are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF and the SERF.

SAEs that occur after the study follow-up period that are reported to and are assessed by the investigator to be possibly, probably, or definitely related to study drug must be reported to the CSO.

11.4.3 Unanticipated Problems

UPs that are also AEs must be reported to the CSO and sent by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the CSO.

11.4.4 Pregnancy

All pregnancies will be reported on the Pregnancy Notification/Outcome Form to the CSO within 1 business day from site awareness.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness.

Although pregnancy itself is not an AE, events that meet SAE criteria during pregnancy, delivery, or in the neonate (e.g., congenital anomaly/birth defect) are reportable on the SERF.

In the event of pregnancy:

- Keep the subject in the study and follow for safety through delivery, including maternal and fetal outcomes.
- Report to safety oversight committee (SMC).
- Advise research subject to notify the obstetrician of study participation and study agent exposure.

11.5 Additional Investigator Reporting Procedures

11.5.1 Reporting Procedures to the IRB

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801.

11.5.2 Reporting to the NIAID Clinical Director

The PI will report UPs, major protocol deviations, and deaths to the NIAID Clinical Director according to institutional timelines.

11.6 Sponsor's Reporting Responsibilities

SUSARs as defined in 21 Code of Federal Regulations (CFR) 312.32 and determined by the IND Sponsor will be reported to FDA and all participating investigators as IND Safety Reports.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

11.7 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administration for all subjects and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

The halting rules are:

One or more subjects experience the same or similar SAEs that are possibly, probably, or definitely related to the study agent or procedures;

OR

Any safety issue that the PI and/or the CSO determines should halt the study.

The PI and/or CSO will determine if the study should be halted. In addition, the FDA may halt the study at any time following review of any safety concerns.

11.7.1 Reporting a Study Halt

If a halting rule is met, a description of the AE(s) or safety issue must be reported by the PI, within 1 business day, to the CSO, the IRB, and the SMC by fax or email.

11.7.2 Resumption of a Halted Study

The IND Sponsor, in collaboration with the PI, the lead associate investigators, and the SMC, will determine if it is safe to resume the study.

The PI will notify the IRB of the decision on resumption of the study.

11.8 Withdrawal Criteria for an Individual Subject

An individual subject will be withdrawn for any of the following:

- An individual subject's decision. (The investigator should attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- A confirmed diagnosis of another respiratory infection (other than RSV) during the inpatient portion of the study.
- The investigator determines that continued participation in the study would not be in the best interest of the subject.

If a participant is withdrawn before inoculation with the challenge virus, then no further testing or follow-up will be performed. Also, if a participant is admitted to the study unit and then withdrawn on day of admission (Day -1) prior to receiving study virus (for any reason), they may be replaced with an eligible and available "stand-by" participant on Day -1 (Section 3.1). If a participant withdraws after Day -1, then they will not be replaced.

Subjects will be strongly discouraged from withdrawing from the study after inoculation. We would encourage any participants who are shedding RSV to remain as an inpatient until viral shedding resolves completely. We will offer inpatient care with isolation conditions and use of any over-the-counter cold remedies as needed, and no further testing will be performed. If the participant insists on withdrawing completely and leaving the hospital after inoculation, then no further research testing will be performed. However, the participant will be encouraged to contact the PI or study coordinator after withdrawing if concerns or issues arise that are thought to be related to participation in this study. They will be offered the opportunity to present for a follow-up visit as well.

11.9 Safety Oversight

11.9.1 Safety Review and Communications Plan

A Safety Review and Communications Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the CSO, which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

11.9.2 Sponsor Medical Monitor

A medical monitor, representing the IND Sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The sponsor medical monitor will be responsible for performing safety assessments as outlined in an SRCP.

11.9.3 Safety Monitoring Committee

The NIAID Intramural SMC includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The Committee will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. The Committee may convene additional reviews as necessary. These reviews will occur after each cohort has completed the inpatient phase and prior to enrolling the subsequent cohorts. All SAEs, all UPs, and all IND Safety Reports will be reported by the PI to the SMC at the same time they are submitted to the IRB or IND Sponsor. Please see Table 5 and Table 6 in Section 13.1 for criteria pertaining to continuing the study after cohort 3 and for criteria for stopping the study it too low of a rate of infectivity is encountered. The PI will notify the SMC when halting criteria are met. The PI will submit the written SMC summary reports with recommendations to the IRB.

12 Clinical Monitoring Structure

12.1 Site Monitoring Plan

As per International Conference on Harmonisation (ICH) GCP 5.18 and 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the “NIAID Intramural Clinical

Monitoring Guidelines. Monitors under contract to NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points in CRIMSON, and prompt reporting of all SAEs; 3) to compare abstracted information entered into CRIMSON with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections [OHRP], FDA) and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator will make study documents (e.g., consent forms) CRIMSON data abstracts and pertinent hospital or clinical records (including CRIMSON) readily available for inspection by the IRB, the FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

13 Statistical Considerations

This study is a phase 1 study to primarily assess the safety and infectability of a high dose, recombinant RSV A2 challenge virus administered by nasal atomizer to healthy adults and secondarily assess RSV illness following experimental infection. We will assess RSV shedding and illness as primary and secondary outcomes, respectively. The sample size calculations are based on a specified frequency of RSV shedding. The analyses performed will be descriptive.

13.1 Sample Size Justification

Sample size calculations are based on ensuring a high probability of correctly concluding the shedding rate is above 55% for the 10^7 PFU dose of RSV A2 using a nasal atomizer. We also will have a high probability of observing an SAE if the true SAE rate is greater than 9%. The total sample size is 25 participants with the first cohort of size 4 ($10^{6.3}$ PFU dose) and 3 cohorts of size 7. After cohorts 2 and 3 are completed, the number of participants who had viral shedding will be evaluated and if the number is too low, the study will be stopped for inactivity (in Table 5 below, row 2 gives the criteria for continuing the study after each evaluation time and the number of participants with shedding at the end of the study that will be used to conclude activity is sufficiently high). The study will have a high probability of correctly concluding the 10^7 PFU RSV A2 dose results in activity above 55% when the true rate is 77% (80% power). The probability of incorrectly concluding the RSV A2 dose results in activity above 55% when the true rate is 55% or less is smaller than 0.10. Table 6 shows the probability of stopping early under uninteresting rates of activity. Row 2 shows the probability of stopping after 7 patients is 0.153 and the probability of stopping after 14 patients is 0.512 (assuming you didn't stop after 7 patients). Overall there is a 0.665 probability of stopping early if the true activity rate is 0.55.

Table 5 Criteria for continuing the study after cohort 3 and the final criteria for interesting shedding rate at the end of the study based on 3 different null and alternative shedding rates

Uninteresting shedding rate (null rate)	True rate that provides at least 80% power (alternative rate)	Number of participants with shedding needed to continue study after first 7 participants (cohort 2)	Number of participants with shedding needed to continue study after 14 participants (cohorts 2 plus 3)	Number of participants with shedding needed to conclude high activity at the end of the study (n=21; cohorts 2-4)
.5	.73	3	7	14
***.55**	**.77**	**3**	**9**	**15**
.6	.81	3	9	16

* The null and alternative rates that best meet the needs of this study are .55 and .77 and are shown in the middle row.

Table 6 Probability of stopping early under the specified criteria in Table 5 for uninteresting rates of activity

Uninteresting shedding rate (null rate)	Probability of stopping after 7 participants	*Probability of stopping after 14 participants	Overall probability of stopping early
.5	.227	.209	.436
.55	**.153**	**.512**	**.665**
.6	.096	.540	.636

*This probability is assuming the study doesn't stop after 7 participants.

For the safety endpoint of SAE there is a high probability (.89) of observing at least 1 SAE out of 21 participants if the true SAE rate is 10%, and a probability of .65 of observing at least 1 event if the true SAE rate is 5%.

13.2 Analysis Plan

At the end of the study, we will conclude whether the dose is active based on the criteria above. Confidence intervals (CIs) around the shedding rate will also be calculated.

Key characteristics of the study analyses are described here. A more comprehensive and detailed (separate) analysis plan will be developed prior to the conclusion of the trial.

Description of the Statistical Methods to Be Employed:

- Where applicable, safety events based on continuous outcomes such as laboratory values will be reported as actual test values, which for study group summaries may also be depicted in dot plots or other descriptive displays.
- Differences in quantitative variables, such as shedding frequency in this challenge protocol compared to the first challenge protocol, will be assessed by using a Wilcoxon test or perhaps a t or F test (if normality of the responses is a credible assumption).

- Correlations will be assessed by Spearman's rank correlation, but Pearson's correlation as well as linear regression may be employed for data that are sufficiently normal.
- CIs will be calculated for all summary means (of raw values or of their logs, where appropriate) as well as proportions.
- Methods to adjust p-values to account for multiple comparisons (other than standard repeated measures analysis of variables that change over time) are not planned for use.

Level of Significance to be Used*:* A two-sided p-value of 0.05 will be used to denote conventional statistical significance.

Subpopulation Analyses of Intervention Effect: Symptoms among those who become infected.

Handling of Missing and Spurious Data: Missing data will be assumed to be at random, unless there is evidence to the contrary (e.g., drop-outs differ by dose or other variable(s)). The potential effects of missing data will be handled by sensitivity analysis for critical conclusions.

14 Ethics/Protection of Human Subjects

14.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject's medical record. The rights and welfare of the subjects will be protected by emphasizing

to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Informed consent will be obtained during the Screen 2 Visit (between days −30 and −2). Alternatively, informed consent may also be obtained on Day −1 prior to any protocol-specific procedures, if it was not obtained during screening.

14.1.1 Non–English-Speaking Participants

If a non–English-speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the Clinical Center Short Written Consent Form for Non–English-Speaking Research Participants in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study as described in Medical Administrative Series Policy M77-2, NIH HRPP Standard Operating Procedure 12, and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the Clinical Center will be used whenever possible. The interpreter will interpret all oral communications (English to target language and conversely) between the investigator and a limited English-proficient participant, facilitate discussions, and clarify information as necessary.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The Clinical Center Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record (CRIMSON), including the name of the interpreter. Further, all instances of use of the Clinical Center Short Written Consent Form will be reported to the IRB at the time of annual review. If the Clinical Center Short Written Consent Form is used three times or more for the same language, this will be reported to the IRB immediately.

14.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and 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. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, NIAID, and OHRP.

15 Data Handling and Record Keeping

15.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

15.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH-GCP guidelines. Study records will be maintained by the PI for a minimum of 3 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from NIAID/OCRPRO.

Appendix A: Scientific References

1. Collins P, Karron R. Respiratory Syncytial virus and Metapneumovirus. In: Knipe DM, Howley PM, eds. *Fields virology*. 6th ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2013:1086-1129.

2. Haynes AK, Prill MM, Iwane MK, Gerber SI, Centers for Disease C, Prevention. Respiratory syncytial virus--United States, July 2012-June 2014. *MMWR. Morbidity and mortality weekly report.* 2014;63(48):1133-1136.

3. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet.* 2010;375(9725):1545-1555.

4. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *American journal of diseases of children.* 1986;140(6):543-546.

5. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. *The New England journal of medicine.* 2005;352(17):1749-1759.

6. Falsey AR, McElhaney JE, Beran J, et al. Respiratory syncytial virus and other respiratory viral infections in older adults with moderate to severe influenza-like illness. *The Journal of infectious diseases.* 2014;209(12):1873-1881.

7. Lewis FA, Rae ML, Lehman NI, Ferris AA. A syncytial virus associated with epidemic disease of lower respiratory tract in infants and young children. *The Medical Journal of Australia.* 1961;2:932-933.

8. Collins PL, Hill MG, Camargo E, Grosfeld H, Chanock RM, Murphy BR. Production of infectious human respiratory syncytial virus from cloned cDNA confirms an essential role for the transcription elongation factor from the 5' proximal open reading frame of the M2 mRNA in gene expression and provides a capability for vaccine development. *Proceedings of the National Academy of Sciences of the United States of America.* 1995;92(25):11563-11567.

9. Connors M, Crowe JE, Jr., Firestone CY, Murphy BR, Collins PL. A cold-passaged, attenuated strain of human respiratory syncytial virus contains mutations in the F and L genes. *Virology.* 1995;208(2):478-484.

10. Whitehead SS, Bukreyev A, Teng MN, et al. Recombinant respiratory syncytial virus bearing a deletion of either the NS2 or SH gene is attenuated in chimpanzees. *Journal of virology.* 1999;73(4):3438-3442.

11. Whitehead SS, Juhasz K, Firestone CY, Collins PL, Murphy BR. Recombinant respiratory syncytial virus (RSV) bearing a set of mutations from

cold-passaged RSV is attenuated in chimpanzees. *Journal of virology.* 1998;72(5):4467-4471.

12. Hall CB, Douglas RG, Jr., Schnabel KC, Geiman JM. Infectivity of respiratory syncytial virus by various routes of inoculation. *Infection and immunity.* 1981;33(3):779-783.

13. Lee FE, Walsh EE, Falsey AR, Betts RF, Treanor JJ. Experimental infection of humans with A2 respiratory syncytial virus. *Antiviral research.* 2004;63(3):191-196.

14. Noah TL, Becker S. Chemokines in nasal secretions of normal adults experimentally infected with respiratory syncytial virus. *Clinical immunology.* 2000;97(1):43-49.

15. Kravetz HM, Knight V, Chanock RM, et al. Respiratory syncytial virus. III. Production of illness and clinical observations in adult volunteers. *Jama.* 1961;176:657-663.

16. Hall CB, McBride JT. Respiratory syncytial virus--from chimps with colds to conundrums and cures. *The New England journal of medicine.* 1991;325(1):57-58.

17. McKay E, Higgins P, Tyrrell D, Pringle C. Immunogenicity and pathogenicity of temperature-sensitive modified respiratory syncytial virus in adult volunteers. *Journal of medical virology.* 1988;25(4):411-421.

18. Hall CB, Walsh EE, Hruska JF, Betts RF, Hall WJ. Ribavirin treatment of experimental respiratory syncytial viral infection. A controlled double-blind study in young adults. *Jama.* 1983;249(19):2666-2670.

19. Hall CB, Long CE, Schnabel KC. Respiratory syncytial virus infections in previously healthy working adults. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2001;33(6):792-796.

20. DeVincenzo JP, Whitley RJ, Mackman RL, et al. Oral GS-5806 activity in a respiratory syncytial virus challenge study. *The New England journal of medicine.* 2014;371(8):711-722.

21. Buchman CA, Doyle WJ, Pilcher O, Gentile DA, Skoner DP. Nasal and otologic effects of experimental respiratory syncytial virus infection in adults. *American journal of otolaryngology.* 2002;23(2):70-75.

22. Simoes EA, DeVincenzo JP, Boeckh M, et al. Challenges and opportunities in developing respiratory syncytial virus therapeutics. *The Journal of infectious diseases.* 2015;211 Suppl 1:S1-S20.

23. Habibi MS, Jozwik A, Makris S, et al. Impaired Antibody-mediated Protection and Defective IgA B Cell Memory in Experimental Infection of Adults with Respiratory Syncytial Virus. *American journal of respiratory and critical care medicine.* 2015.

24. DeVincenzo J, Lambkin-Williams R, Wilkinson T, et al. A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus. *Proceedings of the National Academy of Sciences of the United States of America.* 2010;107(19):8800-8805.

25. DeVincenzo JP, Wilkinson T, Vaishnaw A, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. *American journal of respiratory and critical care medicine.* 2010;182(10):1305-1314.

26. R Wolfe, D Braude. “Intranasal Medication Delivery for Children: A Brief Review and Update.” Pediatrics. 2010

27. Powers JH et al. Development of the Flu-PRO: a patient-reported outcome (PRO) instrument to evaluate symptoms of influenza. BMC Infect Dis. 2016;16: 1-11.

28. Memoli MJ, Czajkowski L, Reed S, et al. Validation of the wild-type influenza A human challenge model H1N1pdMIST: an A(H1N1)pdm09 dose-finding investigational new drug study. Clin Infect Dis. 2015;60: 693-702.

Appendix B: Schedule of Procedures/Evaluations

	Inpatient												Outpatient	
	Study Day													
Clinical Evaluations	–1	0	1	2	3	4	5	6	7 [a]	8-9	10	11+	28 (-1 to +10)	56 (-5 to +15)
Informed consent, if not signed during screen 2 visit or after	X													
Height and weight	X													
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FLU-PRO symptom questionnaire[f]	X		X	X	X	X	X	X	X	X	X	X		
Interim medical history and physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review/sign reproductive counseling form	X													
Diary card													X	X
Assessment of AEs		X	X	X	X	X	X	X	X	X	X	X	X	X
CBC with differential	X		X		X				X				X	X
Acute care panel	X[b]				X				X				X	X
Hepatic panel	X[b]				X				X				X	X
Urine qualitative drug screen	X													
Pregnancy testing, serum	X[c]													
HIV 1/2 rapid antibody test	X													
FilmArray PCR[d] , respiratory virus panel	X		X	X	X		X	X	X	X	X	X		
HLA typing	X													
EBV IgG	X													
Quantitative immunoglobulins	X													
Lymphocyte phenotyping, TBNK	X		X						X				X	X
Hepatitis B and C serology	X[g]													
EKG	X[g]													
CXR	X[g]													
PFT	X[b]													
Evaluate discharge criteria[e]									X	X	X	X		
Research Evaluations														
RSV A2 inoculation (0.5 ml per nare)		X												
Nasosorption using SAM strips (LID RSV lab)	X								X		X		X	X
Nasal swab – microbiome (sequencing) (LID RSV lab)	X			X		X			X		X		X	X
Nasal swab – microbiome (culture) (LID RSV lab)	X					X			X		X		X	X
NP wash – research lab–based quantitative RT-PCR, quantitative viral culture (JHU RSV lab)			X	X	X	X	X	X	X	X	X	X		
NP wash – fresh viral culture (LID RSV lab)			X	X	X	X	X	X	X	X	X	X		
NP wash – cytokines/chemokines (LID RSV lab)	X		X						X		X		X	X

NP wash – immunoglobulins (e.g.secretory IgA) (LID RSV lab)	X								X		X		X	X
Serum for total IgA or IgG (LID RSV lab)	X								X				X	
Serum for neutralizing antibody titer (PRNT), anti-RSV IgG, anti-F and/or anti-G (JHU RSV lab)	X								X				X	X
Cellular immune response T- and B-cell assays (Frederick IML PBMCs)	X	X							X		X		X	
Quantitative & molecular immune cell phenotyping (Frederick IML)	X	X	X						X				X	X
Serum cytokine analysis (Frederick IML)	X	X	X						X				X	
Transcriptome PAXgene (LID RSV lab)	X	X	X						X				X	X

a First possible discharge day. If the subject is still shedding virus at this time, then inpatient hospitalization will continue until they have 2 consecutive negative NP washes for RSV by FilmArray assay (Respiratory Virus Panel).
b Repeat labs and PFT on Day -1 if performed during screening period at >56 days prior to Day -1.
c Subjects of child-bearing potential must have a negative result prior to proceeding with virus inoculation.
d Multiplex PCR for respiratory viruses and bacteria (FilmArray, BioFire Diagnostics, Inc. [Salt Lake City, UT, USA]).
e Discharge criteria fulfilled if subject has 2 consecutive negative NP washes for RSV RNA (Film Array) and no evidence for RSV-associated lower respiratory tract disease.
f Administered using RED CAP; if RED CAP not working for participant, paper questionnaires will be administered once daily
g Perform on Day -1 if performed during screening at >120 days prior to Day -1

AE = adverse event; CBC = complete blood count; CHI = Center for Human Immunology, Autoimmunity, and Inflammation; CXR = chest x-ray; EBV = Epstein-Barr virus; EKG = electrocardiogram; HLA = human leukocyte antigen; Ig = immunoglobulin; LID = Laboratory of Infectious Disease; JHU = Johns Hopkins University; IML = Immunologic Monitoring Lab; NP = nasopharyngeal; PCR = polymerase chain reaction; PFT = pulmonary function tests; PRNT = plaque reduction neutralizing titer; RSV = respiratory syncytial virus; SAM = synthetic absorptive matrix; X = to be performed.

Appendix C: Blood Volumes for Specimen Collection

	Blood volume collected (ml)											
Evaluation	**Day −1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**28 (-1 to +10)**	**56 (-5 to +15)**
CBC with differential	2		2		2				2		2	2
Acute care/hepatic panel	4				4				4		4	4
Pregnancy testing, females	4											
HIV 1/2 rapid antibody test	4											
HLA typing	10											
EBV IgG	4											
Quantitative immunoglobulins	4											
Serum for total IgA/IgG; Neutralizing antibody titer: anti-RSV IgG, anti-F and/or anti-G	4								4		4	4
T, B, NK cell flow cytometry test	3		3						3		3	3
Cellular immune response assays	30	30							30	30	30	
Quantitative and molecular immune cell phenotyping	30	30	35						30		30	35
Cytokine analysis	8	8	8						8		8	
Transcriptome PAXgene	2.5	2.5	2.5						2.5		2.5	2.5
Daily volume	109.5	70.5	50.5	0	6	0	0	0	79.5	30	83.5	50.5
Cumulative volume	109.5	180	231	231	237	237	237	237	320	350	433.5	485

Appendix D: Summary of Prior Clinical Studies (2000-present)

Study (reference)	RSV subtype (strain)	Dose [a]	Pre-challenge serum neutralizing titer [b]	N [c]	% Shedding [d]	% Symptomatic of infected subjects [e]	Mean peak viral titer (log_{10} per ml)	Mean duration of shedding (days)
Lee et al, 2004[13]	A (A2)	4.7 ($TCID_{50}$)	<9.36	13	92 (cx)	100 [f]	2.2 (cx)	4.4 (cx)
		4.7 ($TCID_{50}$)	NA	14	50 (cx)	38 [g]	NA	NA
		3.7 ($TCID_{50}$)	NA	14	21 (cx)	40 [g]	NA	NA
Noah and Becker, 2000[14]	A (A2)	3.0 (PFU)	NA	9	33 (cx)	100 [f]	NA	NA
DeVincenzo et al, 2014[20,k]	A (Memphis-37)	4.0 (PFU)	NA	53	67.9 (PCR)	NA	6.0 - 6.4 (PCR) [h]	8 (PCR) [i]
DeVincenzo et al, 2010[24,k]	A (Memphis-37)	4.7 (PFU)	NA	42	71.4 (cx) 85.7 (PCR)	NA	3.2 (cx) [i] 4.5 (PCR) [i]	7 (cx) [i] 9 (PCR) [i]
DeVincenzo et al, 2010[25]	A (Memphis-37)	3.0 to 5.4 (PFU)	NA	35	60 (cx) 77 (PCR)	NA	4.5 (cx) [i] 6.5 (PCR) [i]	6.5 (cx) [i] 10 (PCR) [i]
Habibi et al, 2015[23]	A (Memphis-37)	4.0 (PFU)	NA	61	56 (PCR)	68 [f]	NA	NA
Buchman et al, 2002[21]	B	6.0 (PFU)	NA	32	47 (cx)	NA	NA	NA

[a] RSV dose administered (log_{10}); PFU, plaque-forming units; $TCID_{50}$, 50% cell culture infectious dose.
[b] microneutralization titer, reciprocal log_2; NA, data not available.
[c] N, number enrolled.
[d] % of subjects shedding RSV challenge virus; cx, determined by quantitative viral culture; PCR, determined by quantitative reverse transcription polymerase chain reaction.

[e] % of RSV challenge virus infected subjects exhibiting symptoms of upper respiratory tract infection. Lower respiratory tract symptoms were not detected in any study.
[f] infected defined as: RSV shedding detected.
[g] infected defined as: 4-fold or greater rise in RSV serum antibody titer and/or RSV shedding detected.
[h] Separate models were used to compare with 3 different antiviral treatment cohorts, resulting in slight differences in adjusted means in each model.
[i] approximate values, obtained from graphical data.
[k] Data from RSV challenge virus recipients treated with investigational antiviral drug were excluded.

Appendix E: Toxicity Table

The functional table below should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as Grade 5.

Parameter	Definition	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life-threatening)
Fever	Sustained rise in body temperature	37.7°C-38.6°C	38.7°C-39.3°C	39.4°C-40.5°C	>40.5°C
Nasal congestion or rhinorrhea	**Congestion**: stuffy or blocked nasal passages. May be observed on exam and/or cause changes in phonation **Rhinorrhea**: nasal drainage not associated with NP washes. May be observed on exam and/or cause nose blowing ≥2x/hr	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities and significant interference with sleep	N/A
Ear pain	Pain in the ear, may be associated with abnormal tympanic membrane landmarks	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities and requiring prescription strength medications	N/A
Sinus congestion or pain	A sensation of fullness, pressure or pain on the face overlying a sinus cavity	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities and significant interference with sleep	N/A
Sore throat	Pain in the throat, and/or difficulty swallowing. May also be accompanied by pharyngeal exudate, and/or enlarged tender lymph nodes.	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities and requiring prescription strength medications	Hospitalization

Parameter	Definition	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life-threatening)
				and/or IV hydration	
Epistaxis	Bleeding from any part of the nose or nasopharynx	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities and requiring intervention such as nasal packing	Hospitalization or operative intervention indicated
Headache	Pain located in any part of the head that can arise from different causes lasting ≥1 hour, as reported by subject	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities and requiring prescription strength medications	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache with significant impairment of alertness or other neurologic function
Cough	Episodes of forcefully expelling air from the lungs, often to clear irritants or secretions	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities and requiring prescription strength medications	Hospitalization
Hoarseness	Sustained rough or harsh changes in usual voice, often with throat irritation, noted by subject or health professional	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities	Hospitalization or operative intervention indicated to maintain patent airway

Parameter	Definition	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life-threatening)
Malaise	Generalized feeling of discomfort, illness, or lack of well-being.	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities	Incapacitating malaise causing inability to perform basic self-care functions
Chills	Feeling a sense of coldness accompanied by shivering	no or minimal interference with usual daily activities	greater than minimal interference with usual daily activities	inability to perform usual daily activities	N/A

Usual daily activities: Everyday tasks, performance of which is required for personal self-care and independent living, such as eating, bathing, dressing, toileting, and transferring.

Grading of AEs related to Vital Signs and Laboratory Values: Adapted from FDA Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007. Changes to the FDA tables are annotated as footnotes.

[1]Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	[4]Potentially Life Threatening (Grade 4)
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	Hospitalization for arrhythmia
[2] Bradycardia - beats per Minute	50 – 54	45 – 49	< 45	Hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	Hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	Hospitalization for malignant hypertension
[3] Hypotension (systolic) – mm Hg	85 – 89 and symptomatic	80 – 84 and symptomatic and requiring oral fluids	< 80 and symptomatic and requiring IV fluids	Hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

[1] Subject should be at rest for all vital sign measurements.

[2] Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

[3] Severity grading definition for hypotension includes added clarifications such that an asymptomatic low blood pressure reading is not an adverse event.

[4] Removed ER visit as necessary criteria for Grade 4 AE

	Mild (Grade 1)	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25–1.5 xULN**	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
[1] Hemoglobin (Female) decrease from baseline value - gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
[1] Hemoglobin (Male) decrease from baseline value – gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 – 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time) #	1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.10 – 1.20 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

[1] Severity grading for hemoglobin decrease on the basis of the magnitude of decrease from baseline is not applicable at

the Grade 1 level; only absolute hemoglobin will be used to define Grade 1 decrease. Increases in hemoglobin are AEs only for values above the upper limit of normal and are graded by the systemic illness clinical criteria.

1 X ULN was removed from the definition for PT increase.

* Any laboratory value shown as a “graded” value in the table that is within the institutional normal range will not be severity graded or recorded as an adverse event.

**”ULN” is the upper limit of the normal range.

Appendix F: Patient-Reported FLU-PRO Symptom Questionnaire

Participant ID : _____ Participant Initials :_____Date : ____/____/___

RSV Illness Symptom Questionnaire

People experience the RSV illness in different ways. We would like to know about the symptoms you have been experiencing during the past 24 hours. For each symptom, please mark one box ☐ under the response that best matches your experience. Mark the "Not at all" box, if you did not have that symptom in the past 24 hours.

What time is it? _____ AM / PM (please circle)

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Runny or dripping nose	☐	☐	☐	☐	☐
Congested or stuffy nose	☐	☐	☐	☐	☐
Sinus pressure	☐	☐	☐	☐	☐
Scratchy or itchy throat	☐	☐	☐	☐	☐
Sore or painful throat	☐	☐	☐	☐	☐
Difficulty swallowing	☐	☐	☐	☐	☐
Teary or watery eyes	☐	☐	☐	☐	☐
Sore or painful eyes	☐	☐	☐	☐	☐
Eyes sensitive to light	☐	☐	☐	☐	☐
Trouble breathing	☐	☐	☐	☐	☐
Chest congestion	☐	☐	☐	☐	☐

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Chest tightness	☐	☐	☐	☐	☐
Dry or hacking cough	☐	☐	☐	☐	☐
Wet or loose cough	☐	☐	☐	☐	☐
Felt nauseous (feeling like you wanted to throw-up)	☐	☐	☐	☐	☐
Stomach ache	☐	☐	☐	☐	☐
Felt dizzy	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Lack of appetite	☐	☐	☐	☐	☐
Sleeping more than usual	☐	☐	☐	☐	☐
Body aches or pains	☐	☐	☐	☐	☐
Weak or tired	☐	☐	☐	☐	☐
Chills or shivering	☐	☐	☐	☐	☐
Felt cold	☐	☐	☐	☐	☐
Felt hot	☐	☐	☐	☐	☐
Sweating	☐	☐	☐	☐	☐

In the past 24 hours, how often have you had any of the following symptoms?

	Never	Rarely	Sometimes	Often	Always

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Sneezing	☐	☐	☐	☐	☐
Coughing	☐	☐	☐	☐	☐
Coughed up mucus or phlegm	☐	☐	☐	☐	☐

	0 times	1 time	2 times	3 times	4 or more times
How many times did you vomit?	☐	☐	☐	☐	☐
How many times did you have diarrhea?	☐	☐	☐	☐	☐

Items to be asked in the daily diary through to Day 14 along with the FLU-PRO items.

1. Did you take any medication for your RSV illness symptoms today? (Please select one response only)

   ☐$_0$ No

   ☐$_1$ Yes

2. Do you have asthma, COPD (chronic obstructive pulmonary disease) or both?

   ☐$_0$ No

   ☐$_1$ Yes

3. [Only asked if answer to the question above is "yes"]. Did you use any rescue medication today for your asthma or COPD? (Please select one response only)

   ☐$_0$ No

   ☐$_1$ Yes

4. Overall, how severe were your RSV illness symptoms today? (Please select one response only)

☐$_0$ No flu symptoms today

☐$_1$ Mild

☐$_2$ Moderate

☐$_3$ Severe

☐$_4$ Very severe

5. Overall, how were your RSV illness symptoms today compared to yesterday? (Please select one response only)

☐$_1$ Much better

☐$_2$ Somewhat better

☐$_3$ A little better

☐$_4$ About the same

☐$_5$ A little worse

☐$_6$ Somewhat worse

☐$_7$ Much worse

6. How much did your RSV illness symptoms interfere with your usual activities today? (Please select one response only)

☐$_1$ Not at all

☐$_2$ A little bit

☐$_3$ Somewhat

☐$_4$ Quite a bit

☐$_5$ Very much

7. Have you returned to your usual activities today?

☐$_0$ No

☐$_1$ Yes

8. In general, how would you rate your physical health today? (Please select one response only)

☐$_1$ Poor

☐$_2$ Fair

☐$_3$ Good

☐$_4$ Very Good

☐$_5$ Excellent

9. Have you returned to your usual health today?

☐$_0$ No

☐$_1$ Yes