Phase I Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-attenuated Respiratory Syncytial Virus Vaccine, RSV 6120/ΔNS1, Lot RSV#018A, or RSV 6120/F1/G2/ΔNS1, Lot RSV#016A, Delivered as Nose Drops to RSV-seropositive Children 12 to 59 Months of Age and RSV-seronegative Infants and Children 6 to 24 Months of Age

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Respiratory Syncytial Virus (RSV) 6120/ΔNS1

RSV 6120/F1/G2/ΔNS1

Provided by:
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LIST OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices (CDC)
AE	adverse event
AGM	African green monkey
cDNA	complementary deoxyribonucleic acid
CFR	Code of Federal Regulations
CI	confidence interval
CIR	Center for Immunization Research
CRF	case report form
CRL	Charles River Laboratories
CSO	Clinical Safety Office
CTM	clinical trial material
DCR	Division of Clinical Research
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ELISA	enzyme-linked immunosorbent assay
F protein	fusion protein
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMT	geometric mean titer
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	investigator's brochure
ICD	informed consent document
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IgA, IgG, IgE	immunoglobulin A, G, E
i.n.	intranasally
IND	investigational new drug
IRB	institutional review board

i.t.	intratracheally
JHSPH	Johns Hopkins Bloomberg School of Public Health
JHU	Johns Hopkins University
LID	Laboratory of Infectious Diseases
LRI	lower respiratory tract illness
LRT	lower respiratory tract
MOP	manual of procedures
mRNA	messenger ribonucleic acid
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
ORF	open reading frame
PE	physical examination
PFU	plaque-forming units
PI	principal investigator
PRNT	plaque reduction neutralization
r	recombinant
RE	regulatory entity
RNA	ribonucleic acid
rRT-PCR	reverse transcription polymerase chain reaction
RSV	respiratory syncytial virus
RT-qPCR	quantitative reverse transcription polymerase chain reaction
SAE	serious adverse event
SAM	synthetic absorptive matrix
SERF	Safety Expedited Report Form
SMM	sponsor medical monitor
SOP	standard operating procedure
SUSAR	serious, unexpected, suspected adverse reaction
TL	tracheal lavage

ts	temperature sensitivity
UP	unanticipated problem
URI	upper respiratory tract illness
URT	upper respiratory tract
US	United States
VAR	vaccine administration record
WIRB	Western Institutional Review Board
wt	wild-type

PROTOCOL SUMMARY

Phase I Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-attenuated Respiratory Syncytial Virus Vaccine, RSV 6120/ΔNS1, Lot RSV#018A, or RSV 6120/F1/G2/ΔNS1, Lot RSV#016A, Delivered as Nose Drops to RSV-seropositive Children 12 to 59 Months of Age and RSV-seronegative Infants and Children 6 to 24 Months of Age

SHORT TITLE RSV 6120/ΔNS1 or RSV 6120/F1/G2/ΔNS1

PURPOSE To assess whether the RSV 6120/ΔNS1 or RSV 6120/F1/G2/ΔNS1 vaccines will each be safe, infectious and immunogenic in this age group.

DESIGN A double blind, randomized, placebo-controlled study design will be used to evaluate the safety and immunogenicity of the study product in RSV-seropositive and RSV-seronegative participants. Participants will be randomized to receive RSV 6120/ΔNS1 vaccine, RSV 6120/F1/G2/ΔNS1 vaccine, or placebo in a 2:2:1 ratio

STUDY POPULATION Group 1: Healthy RSV-seropositive children ≥ 12 months to < 60 months
Group 2: Healthy RSV-seronegative infants and children ≥ 6 months to <25 months

SAMPLE SIZE Group 1: Approximately 25 RSV-seropositive children^
Group 2: Approximately 35-50 RSV-seronegative infants and children

STUDY PRODUCT Single intranasal dose of RSV 6120/ΔNS1, RSV 6120/F1/G2/ΔNS1, or placebo

Table 1: Inoculation schedule

Group #	N	Study Product	Dose
1	10	RSV 6120/ΔNS1	$10^{6.0}$ PFU*
	10	RSV 6120/F1/G2/ΔNS1	$10^{5.8}$ PFU*
	5	Placebo	0
2	14-20	RSV 6120/ΔNS1	$10^{5.0}$ PFU*
	14-20	RSV 6120/F1/G2/ΔNS1	$10^{5.0}$ PFU*
	7-10	Placebo	0

Group 1: RSV-seropositive: RSV serum neutralizing antibody titer ≥ 1:40; determined prior to inoculation and within the calendar year of inoculation.

^If 3 or more RSV-seropositive children (group 1) with the same treatment assignment have respiratory or febrile illness that coincides with shedding of > $10^{2.5}$ PFU of vaccine virus/mL of nasal wash as detected by culture, then we will evaluate the safety, infectivity, and immunogenicity of that vaccine virus in an additional 15 RSV-seropositive children before determining whether to continue its evaluation in group 2

Group 2: RSV-seronegative: RSV serum neutralizing antibody titer < 1:40; determined ≤ 42 days prior to inoculation

*plaque-forming units (PFU)

Our intent is to randomize (2:2:1; RSV 6120/ΔNS1: RSV 6120/F1/G2/ΔNS1: placebo) for evaluation of each vaccine and placebo. Evaluation of a vaccine will proceed to group 2 if there are not more than 2 solicited adverse events (AEs) and no lower respiratory tract illnesses (LRIs) associated with that vaccine in RSV-seropositive children. If 3 or more RSV-seropositive children (group 1) with the same treatment assignment have respiratory or febrile illness that coincides with shedding of > $10^{2.5}$ PFU of vaccine virus/mL of nasal wash as detected by culture, then we will evaluate the safety, infectivity, and immunogenicity of that vaccine virus in an additional 15 RSV-seropositive children before determining whether to continue its evaluation in group 2, or whether to discontinue the evaluation of this candidate.

STUDY DURATION

Participants will be enrolled in the study , outside of the RSV season. RSV-seropositive participants will be followed for 28 days after inoculation, and RSVseronegative participants will remain on the study until they complete the post–RSV season visit.

Figure 1.1-1: Group 1 – Study Overview

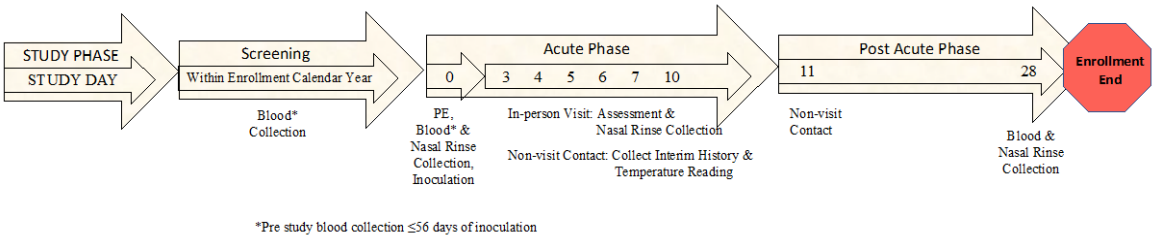
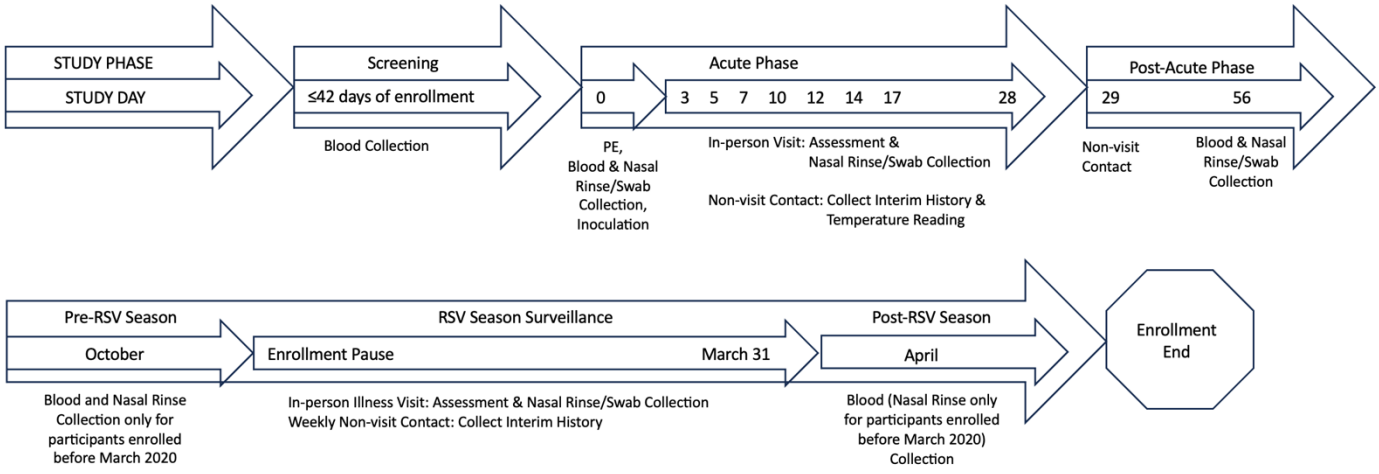


Figure 1.1-2: Group 2 – Study Overview



(nasal rinse or nasal swab may be obtained at all timepoints; post-surveillance specimens to be collected beginning April 1st)

1. BACKGROUND AND SCIENTIFIC RATIONALE

1.1. BACKGROUND

1.1.1. Epidemiology, Disease Burden, and the Need for a Vaccine

In the United States (US) alone, respiratory syncytial virus (RSV) is responsible for 75,000 to 125,000 hospitalizations of infants yearly (1), and in 2015, RSV was estimated to have caused at least 33 million cases of acute lower respiratory infection (ALRI) in children under 5 years old, resulting in an estimated 3.2 million RSV LRI hospitalizations and approximately 59,600 RSV-attributable deaths (2, 3). In temperate climates, annual RSV epidemics occur in late winter and early spring, and nearly all children are infected within the first 2 years of life. RSV illness can range from mild upper respiratory tract illness (URI), including rhinitis, pharyngitis, and coryza, to severe LRI, including bronchiolitis and pneumonia. Beyond the acute burden of disease caused by RSV, severe RSV disease in infancy may predispose to reactive airway disease during childhood (4, 5).

RSV is an enveloped ribonucleic acid (RNA) virus that is a member of the newly organized *Pneumoviridae* family, genus *Orthopneumovirus* (6). RSV has a single negative-sense strand RNA genome of 15.2 kilobases encoding 10 messenger ribonucleic acids (mRNAs). Each mRNA encodes a single protein, with the exception of the M2 mRNA, which contains 2 overlapping open reading frames (ORFs). The 11 RSV proteins are the viral RNA-binding nucleoprotein N, the phosphoprotein P, the large polymerase protein L, the attachment glycoprotein G, the fusion glycoprotein F, the small hydrophobic surface glycoprotein SH, the internal matrix protein M, the 2 nonstructural proteins NS1 and NS2, and the M2-1 and M2-2 proteins encoded by the M2 mRNA. The gene order is: 3'-NS1-NS2-N-P-M-SH-G-F-M2-L-5'. RSV transcription and genome replication take place exclusively in the cytoplasm, and virions form by budding from the apical plasma membrane of respiratory epithelial cells.

A formalin-inactivated vaccine against RSV was evaluated clinically in the 1960s and did not confer protection; instead, disease enhancement occurred at a high rate following natural infection of vaccinees with wild-type (wt) RSV (7). Studies in experimental animals established that disease enhancement was specific to non-replicating RSV vaccines and not seen with infectious RSV or replicating vaccine vectors (8, 9).

Currently, no licensed immunization against RSV is available to protect healthy older infants and toddlers. Passive immunoprophylaxis with the monoclonal RSV-neutralizing antibody palivizumab (Synagis®; MedImmune) is available for high-risk infants; this approach is not feasible for general use. Recently, the extended half-life RSV mAb nirsevimab was licensed by the FDA to provide passive immunity to healthy infants through their first RSV season and to high-risk infants through their second RSV season (https://www.fda.gov/news-events/press-announcements/fda-approves-new-drug-prevent-rsv-babies-and-toddlers). Additionally, licensure of the RSVpreF maternal vaccine is expected in Augut 2023. Both of these products are expected to provide passive immunity to younger infants but do not provide active, durable immunity and are not expected to protect older infants and toddlers against RSV disease.

Attempts at developing RSV vaccines at the National Institute of Allergy and Infectious Diseases (NIAID) have focused largely on live-attenuated approaches to provide active immunity to older inants and young children (10). Importantly, throughout a period of over 20 years, a number of live-

attenuated investigational RSV vaccines have been evaluated in RSV-naïve infants and children, and enhanced disease following wt RSV infection of vaccinees has not been observed (11). Apart from the absence of enhanced disease, live-attenuated RSV vaccines have a number of known advantages over non-replicating RSV vaccines. They can be administered intranasally (i.n.), induce protective mucosal immunity in the respiratory tract (as well as systemic immunity), infect in the presence of maternally-derived RSV serum antibody, and have been well tolerated and immunogenic when administered to infants as young as four weeks of age (12).

Human RSV has a single serotype with two antigenic subgroups, A and B. The two subgroups exhibit a 3- to 4-fold reciprocal difference in neutralization by polyclonal convalescent serum. Analysis of glycoprotein-specific responses in infants by enzyme-linked immunosorbent assay (ELISA) with purified F and G glycoproteins showed that the fusion proteins (F proteins) were 50% related antigenically, and the G proteins were 7% related (13). Consistent with this level of antigenic relatedness, F protein expressed by a recombinant (r) vaccinia virus was equally protective in cotton rats against challenge with either subgroup A or B, whereas the G protein was 13-fold less effective against the heterologous subgroup (14). Thus, the F protein is responsible for most of the observed cross-subgroup neutralization and protection, and a subgroup A vaccine virus is likely to induce a broad immune response against wt RSV of either subgroup. Antibodies to the F protein are one of the endpoints evaluated in this study.

The RSV vaccines to be evaluated in this study were derived using a recombinant deoxyribonucleic acid (rDNA)–based technique called reverse genetics (15). The technique of reverse genetics has been used to produce a number of licensed vaccines; among them is FluMist® (MedImmune). This technique allows *de novo* recovery of infectious virus entirely from complementary DNA (cDNA) in a qualified cell substrate under defined conditions. Reverse genetics provides a means to introduce predetermined mutations into the RSV genome via the cDNA intermediate. Derivation of vaccine virus from cDNA minimizes the risk of contamination with adventitious agents and helps to keep the passage history brief and well documented. Once recovered, the vaccine virus is propagated in the same manner as a biologically derived virus. As a result of repeated passage and amplification, the drug substance (clinical trial material [CTM]) does not contain any rDNA. The RSV vaccine candidates to be tested under this protocol are derivatives of strain A2, subgroup A.

The vaccines to be evaluated in this study, RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1, are cDNA derived live-attenuated RSV vaccine candidates that contain a deletion of the NS1 gene (ΔNS1) (16). The RSV NS1 protein functions as viral interferon antagonist and as an inhibitor of RSV induced apoptosis, and deletion of NS1 is attenuating (16-24). NS1 also has a suppressive effect on the maturation of dendritic cells. RSV with the NS1 deletion is more potent in activating CD8+ T cells and Th17 cells, and its activating effect on IL4-producing CD4+ T cells is decreased (25, 26). Genetic stability of live-attenuated RNA viruses is a concern in clinical vaccine development. The deletion of an entire gene, the NS1 gene, should be refractory to genetic or phenotypic reversion. The envelope glycoproteins F and G represent the major RSV neutralization and protective antigens. RSV 6120/F1/G2/ΔNS1 was designed for increased expression of the F and G proteins (27); to achieve this, both glycoprotein genes were moved in the RSV genome from their natural 7th (G) and 8th (F) position to the first (F1) and second (G2) genome promoter proximal position in RSV 6120/F1/G2/ΔNS1.

In historic studies in chimpanzees, the only experimental animal model in which replication and virulence of RSV approaches that in humans, replication of recombinant RSV lacking the NS1 gene was reduced compared to recombinant wt RSV by about 2,200 fold and 17,000 fold in the upper respiratory tract (URT) and lower respiratory tract (LRT), respectively, with low mean peak titers of 1.6 and 1.2 $\log_{10}$ PFU per mL in nasopharyngeal swabs and tracheal lavage (TL) fluid (16). Despite the low level of replication, the virus was immunogenic and, importantly, protective against wt RSV challenge infection in chimpanzees. In preclinical studies, both vaccine candidates were significantly restricted in replication compared to recombinant wt RSV in a primary human airway epithelial cell model. In preclinical studies in African green monkeys (AGM), replication in the upper and lower respiratory tract of both vaccine candidates was reduced compared to recombinant wt RSV. RSV 6120/F1/G2/ΔNS1, expressing the glycoprotein genes from promoter proximal positions, was further restricted in replication than RSV 6120/ΔNS1, but comparable to RSV 6120/ΔNS1 in its immunogenicity in the AGM model.

1.2. PRIOR RESEARCH

1.2.1. Experimental Vaccines against RSV

Efforts have been directed toward the development of a live-attenuated RSV vaccine because of the advantages of live-attenuated vaccines over inactivated or subunit vaccines. These advantages include the ability to (i) induce the full spectrum of protective immune responses including serum and local antibodies as well as CD4+ and CD8+ T cells and innate immunity; (ii) infect and replicate in the presence of maternal antibody, permitting immunization of young infants; and (iii) produce an acute, self-limited, attenuated infection that is well tolerated and readily eliminated from the respiratory tract. Another important advantage is the absence of vaccine-related enhanced disease, as has been confirmed in clinical studies (11).

The vaccines to be evaluated in this protocol, RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1, have not been previously evaluated in humans. However, over recent years, NIAID's ongoing RSV vaccine development program has evaluated several live-attenuated pediatric RSV vaccine candidates sequentially in adults, RSV seropositive children, and RSV seronegative infants and children. In one approach, RSV was attenuated by temperature sensitivity (*ts*) mutations, in combination with deletion of the SH gene (12, 28-30). In another approach, RSV was attenuated by deletion of the transcription processivity factor M2-2 (NCT02601612, NCT02952339, NCT03102034, NCT03099291, NCT02890381, NCT03227029). In addition, several live-attenuated RSV vaccine candidates with a deletion of another RSV interferon antagonist, NS2, were evaluated sequentially in adults, children, and infants. Results from the most recent study of the lead candidate with NS2 deletion (ref Luongo), RSVΔNS2/Δ1313/I1314L (NCT01893554), are summarized below.

In a recent Phase I trial (NCT01893554), the RSVΔNS2/Δ1313/I1314L candidate vaccine was evaluated in RSV-seropositive children 12-59 months of age at a dose of 10^6 PFU (10V, 5P), and no shedding or immune response was detected, indicative of attenuation. Next, it was evaluated in seronegative children 6-24 months of age at two sequential dose levels.

At the lower dose of 10^5 PFU (15V, 7P), the vaccine was poorly infectious (7% and 80% of recipients shed virus detected by culture and quantitative reverse transcription polymerase chain reaction [RT-qPCR], respectively) and immunogenicity was low. 73% of the vaccinees and 57% of placebo recipients experienced a respiratory or febrile illness during the first 28 days following

receipt of vaccine. Specifically, mild rhinorrhea was reported in 11 of 15 (73%) vaccinees and 4 of 7 (57%) of placebo recipients, frequently associated with shedding of rhinovirus. Mild cough was reported in two participants of each cohort, associated with shedding of adventitious viruses in all cases.

The vaccine was subsequently evaluated at the higher dose of 10^6 PFU in RSV seronegative infants and children 6-24 months of age (20V/10P). In this cohort, 80% and 90% of vaccine recipients shed vaccine detected by culture and RT-qPCR, respectively [median peak titers (MPT) 1.7 $\log_{10}$ PFU/mL; 3.6 $\log_{10}$ copies/mL]. During the first 28 days following receipt of vaccine, 55% of vaccinees and 70% of placebo recipients experienced a respiratory or febrile illness, mostly mild rhinorrhea [10 of 20 (50%) of vaccinees; 4 of 10 (40%) of placebo recipients]. Cough was reported in one vaccinee (5%) and 3 placebo recipients (30%), and a single case of otitis media was present in a placebo recipient. Fever was also less frequent in vaccinees [2 of 20 (10%), highest grade: Grade 2] than in placebo recipients [4 of 10 (40%), highest grade: Grade 3]. Other respiratory viruses (rhinovirus, parainfluenza virus type 3, adenovirus, enterovirus, bocavirus) were detected concurrently with respiratory symptoms in most cases. Among vaccine recipients, mild rhinorrhea without a detectable adventitious agent was observed in five participants (25%) during the time period of vaccine virus shedding. In placebo recipients, mild rhinorrhea without a detectable adventitious agent was observed in 2 of 10 participants (20%) during the same period. Overall, this shows that the vaccine was well tolerated. Ninety percent of the vaccine recipients developed an RSV F immunoglobulin G (IgG) antibody response, and 80% developed an RSV neutralizing antibody response, detected on Day 56 after receipt of vaccine. The serum antibody responses to RSV ΔNS2/Δ1313/I1314L were durable over the RSV surveillance season, and the vaccine candidate primed for strong anamnestic responses to wt RSV. Based on these results, RSV ΔNS2/Δ1313/I1314L was selected as an appropriate candidate for further clinical evaluation.

1.2.2. Preclinical Studies

1.2.2.1. Replication of RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 (Experimental Lots) in vitro

Multicycle replication of experimental lots of RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 was compared to that of recombinant RSV 6120, a control virus with a phenotype approaching that of wt RSV. Two experimental systems were used, namely Vero cells which do not express type I interferon, and a 3D mucociliary human tissue model consisting of normal differentiated primary human tracheal/bronchial airway epithelial cells (EpiAirway, Mattek, Inc.). To evaluate replication in Vero cells, triplicate 25 cm flasks of subconfluent Vero cells were infected at an MOI of 0.01 with RSV 6120/ΔNS1, RSV 6120/F1/G2/ΔNS1, or RSV 6120. After 2 hours of adsorption, the inoculum was gently removed, and the cultures were washed and incubated with fresh medium at 37°C. To evaluate replication in primary human airway cells, cultures of pseudostratified differentiated airway cells were incubated in an air-liquid interface at 37°C in 12-mm transwell inserts with 0.4 µM pore sizes in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with a proprietary mix of epidermal growth factors and other proprietary factors. Prior to infection, the apical surfaces of the cultures were washed with 500 µL PBS to remove any accumulated mucus. Triplicate wells were infected at an MOI of 0.1 with 100 µl per well of RSV 6120/ΔNS1, RSV 6120/F1/G2/ΔNS1, or RSV 6120. After 2 hours of adsorption, the inoculum was gently removed, and the apical surfaces of the cultures were washed 3 times with 300 µL PBS. Apical washes were collected daily on days 1-7 post-infection to assay viral replication. For these washes, 350 µL serum-free DMEM was applied to

the apical surface of the cultures. Plates were returned to the incubator for 30 minutes of incubation. Apical medium was then collected, snap frozen, and stored at -80°C. Apical medium supernatants, as well as the supernatants from the Vero replication study, were titered by plaque assay on Vero cells, incubated at 37°C, 5% CO_2, as described above. The lower limit of detection of the samples was 0.7 log_{10} PFU per mL.

Recombinant wt RSV 6120 replicated efficiently in Vero cells and in differentiated primary human tracheal/bronchial epithelial cells (Figure 1.2-1). In Vero cells, which do not produce type I interferon, peak titers of RSV 6120/ΔNS1 and the control virus RSV 6120/F1/G2/ΔNS1 were less than 10-fold lower than those of wt RSV. However, in interferon competent mucociliary normal human tracheal/bronchial epithelial cells, RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 were highly restricted in replication compared to the parental wt like virus, and titers were about 1000-fold (RSV 6120/ΔNS1) and 10,000-fold (RSV 6120/F1/G2/ΔNS1) lower than those of the control virus. Thus, in interferon competent cells, the deletion of the NS1 gene is much more restrictive than in Vero cells. RSV 6120/F1/G2/ΔNS1 with glycoprotein genes shifted to promoter proximal positions was further restricted than RSV 6120/ΔNS1 with NS1 deletion alone.

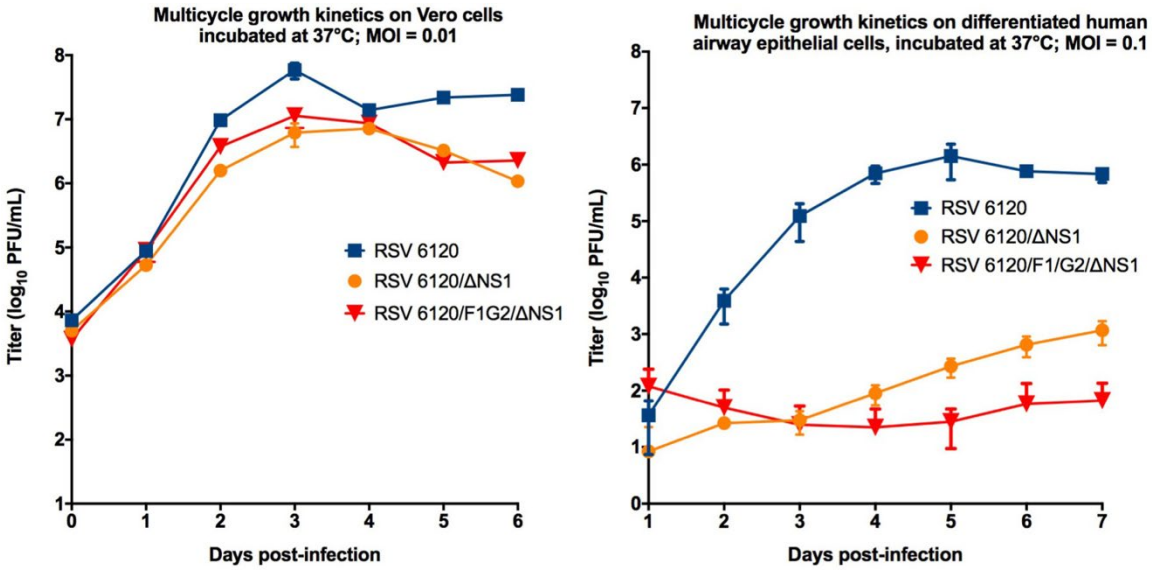


Figure 1.2-1. Multicycle replication of experimental lots in Vero cells (left) or in an in-vitro model of normal human bronchial/tracheal epithelial cells (right). Cultures were infected at a multiplicity of infection of 0.01 (Vero cells) or 0.1 (epithelial cells), and supernatants were harvested on indicated days, snap frozen, and titered later on Vero cells.

1.2.2.2. Replication of experimental lot and CTM of RSV 6120/ΔNS1 and RSV 6120/F1/G2/ ΔNS1 in African green monkeys (AGMs)

Replication and immunogenicity of RSV 6120/ΔNS1 was evaluated in nonhuman primates (NHP), in African green monkeys (AGMs). AGMs are semi-permissive for RSV infection. The first NHP study was done to evaluate an Experimental Lot of RSV 6120/ΔNS1, and a second study was done to evaluate the CTM of RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 (Lots RSV#016A and RSV#018A). Male and female AGMs which were seronegative for RSV were inoculated (i.n.) and intratracheally (i.t.) with RSV 6120/ΔNS1 or RSV 6120/F1/G2/ΔNS1. A total dose of 2 x 10^6 PFU (corresponding to $10^{6.3}$ PFU) per animal was used for all inoculations, except for CTM RSV 6120/F1/G2/ΔNS1, Lot RSV#018A, which was used at a dose of 1 x 10^6 PFU (corresponding to $10^{6.0}$ PFU per animal). Nasopharyngeal (NP) swabs were collected daily on Days 0 through 10 and on Days 12 and 14 (Table 26), TL samples were collected every other day from Day 2 through Day 14 (Table 27), and virus shedding was analyzed by immunoplaque assay (Table 28). Serum RSV neutralizing antibody titers were determined by a complement-enhanced 60% plaque reduction neutralization assay (Table 28). Results from studies following the same protocol, performed in animals from the same group and origin, inoculated with recombinant wt RSV A2 at the same dose, were included for comparison. Studies were approved by the Animal Care and Use Committee of NIAID, National Institutes of Health (NIH).

In both studies, shedding of RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 from the URT and LRT was detectable over several days (Table 26, Table 27). In both studies, the mean peak titers of RSV 6120/ΔNS1 in the NP samples were significantly reduced compared to that of wt RSV A2 (3.0 and 2.2 $\log_{10}$ PFU per mL for the Experimental lot and the CTM of RSV 6120/ΔNS1, compared to 4.2 $\log_{10}$ PFU per mL for RSV A2; significantly lower than RSV A2; $P<0.001$ and $P<0.01$, ANOVA, Tukey post-hoc analysis). The mean peak titer of RSV 6120/F1/G2/ΔNS1 was even further reduced to 1.5 $\log_{10}$ PFU per mL ($P<0.05$ compared to the mean peak titer of RSV in the CTM of RSV 6120/ΔNS1, $P<0.0001$ compared to RSV A2). The mean peak titers in the LRT, measured by titration of TL samples, were 3.3 and 3.5 $\log_{10}$ PFU per mL for the Experimental lot and the CTM of RSV 6120/ΔNS1, compared to 4.1 $\log_{10}$ PFU per mL for RSV A2. Replication of the CTM of RSV 6120/F1/G2/ΔNS1 was lower than that of RSV 6120/ΔNS1, at mean peak titers of 2.2 $\log_{10}$ PFU per mL ($P<0.05$; ANOVA, Tukey post-hoc analysis).

In both studies, the RSV 6120/ΔNS1 virus induced serum neutralizing antibodies that were comparable to or slightly higher than wt RSV A2 (Table 28). The titers induced by RSV 6120/F1/G2/ΔNS1 were slighly but not significantly lower than those of wt RSV. These results show that RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1, administered i.n. and i.t., are immunogenic in AGMs.

These results indicate that RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 are restricted for replication in the upper and lower respiratory tract of AGMs. At the dose of 1 x 10^6 PFU per site, replication of RSV 6120/ΔNS1 in the upper and lower respiratory tract was detected in all AGMs, and RSV 6120/ΔNS1 was immunogenic. At a dose of 5 x 10^5 PFU per site, replication of RSV 6120/F1/G2/ΔNS1 was significantly reduced compared to wt RSV in the upper and lower respiratory tract, but the difference in immunogenicity to wt RSV was not significant, despite the low level of replication. In summary, it is anticipated that the investigational live vaccine candidates RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 will be well-tolerated in RSV seropositive participants upon intranasal application, and well-tolerated, infectious, and immunogenic in RSV seronegative participants.

1.2.3. Previous Clinical Experience

To our knowledge, RSV vaccine viruses containing NS1 deletions have not been previously tested in humans. Data from recent clinical evaluations of other live-attenuated RSV vaccine candidates containing deletion mutations are described in Section 1.2.1.

1.3. RATIONALE

In previous Phase I studies of live-attenuated RSV vaccines in RSV-seronegative infants and children 6 to 24 months of age, RSV strains based on two different attenuation strategies have emerged as the most promising candidates: (1) candidates that are attenuated by deletion of the RSV interferon antagonist NS2, and (2) candidates attenuated by deletion of the RNA regulatory protein M2-2. Results from chimpanzee studies suggest that the deletion of the second RSV interferon antagonist, NS1, may represent a third powerful attenuation strategy for RSV vaccine candidates, yielding a level of attenuation similar to that of the deletion of M2-2. RSV 6120/ΔNS1 was designed to be attenuated by a 529 nucleotide deletion of the NS1 gene. In addition to its attenuating effect, the NS1 deletion also is expected to increase immunogenicity due to the immune-stimulatory effects of interferon. To further increase immunogenicity, RSV 6120/F1/G2/ΔNS1 was generated. This candidate has the RSV glycoprotein genes, encoding the major neutralizing antigens F and G, moved to the first and second genome promoter proximal position, increasing their level of expression. Based on preclinical results in primary human airway epithelial cells and African green monkeys, this genome rearrangement is expected to be further attenuating in the natural human host. This Phase 1 study will evaluate the suitability of the deletion of NS1 and of a genome rearrangement to generate safe and immunogenic live-attenuated RSV vaccines.

The rationale for sequential evaluation in RSV seropositive children, followed by evaluation in RSV seronegative children and infants is as follows. This will be the first-in-human testing of RSV vaccine candidates containing a deletion of the NS1 gene. Based on chimpanzee studies and pre-clinical testing results, the NS1 deletion is expected to be highly attenuating, similar to deletion of the M2-2 open reading frame (ORF), and it is expected that replication of RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 will be highly restricted or absent in RSV-seropositive children. If these candidates are found to be safe and restricted in RSV-seropositive children, testing will proceed to RSV-seronegative children.

This protocol will evaluate safety, infectivity, replication, and immunogenicity of both candidates, with particular attention to vaccine virus infectivity and replication (i.e., percentage of participants shedding virus, peak vaccine virus titer in nasal washes or nasal swabs, as well as duration of shedding), which are the most quantifiable metrics for the level of attenuation. Based on results from this study, a comparison of infectivity and immunogenicity of these candidates will be possible. It is anticipated that the lead candidate emerging from this study will move forward to expanded studies to further evaluate safety and immunogenicity.

The primary immunogenicity endpoints to be evaluated in this study are serum RSV neutralizing antibody titers and serum antibody titers to the RSV F protein (measured by ELISA). Neutralizing antibody is a well-established and important surrogate marker of protection from RSV disease. Antibodies to the F protein are also associated with cross-subgroup neutralization and protection. These assays will be performed at the Johns Hopkins University (JHU) Center for Immunization Research (CIR) laboratory.

1.4. CLINICAL DEVELOPMENT PLAN

The investigational RSV vaccines RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 will be evaluated sequentially in RSV-seropositive children followed by evaluation in RSV-seronegative infants and children. The main purpose of this study is to determine whether these vaccines are well tolerated and restricted in replication in RSV-seropositive children, and well tolerated, infectious, immunogenic, and genetically stable in RSV-seronegative infants and children.

The primary immunogenicity endpoints to be evaluated are RSV neutralizing antibody titer, and RSV F protein antibody (by ELISA). Neutralizing antibody is a well-established and important surrogate marker of effective immunity to RSV disease. Antibodies to the F protein are also associated with cross-subgroup neutralization and protection (14). These assays will be performed at the JHU CIR laboratory.

1.5. HYPOTHESES

RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 will be well-tolerated in RSV-seropositive and RSV-naïve participants. In RSV-seronegative participants, these vaccines are expected to be safe and result in infection, limited vaccine replication, and the induction of neutralizing antibody responses to RSV.

2. OBJECTIVES

2.1. PRIMARY OBJECTIVES

Safety

To estimate and compare (each vaccine group to placebo):

1. the frequency and severity of study product–related solicited and unsolicited AEs in RSV-seropositive participants (group 1) from Day 0 through the 10th day following inoculation
2. the frequency and severity of study product–related solicited and unsolicited AEs in RSV-seronegative participants (group 2) from Day 0 through the 28th day following inoculation
3. the frequency of study product–related serious adverse events (SAEs) and lower respiratory tract illness (LRIs) in RSV-seropositive participants (group 1) from Day 0 through the 28th day following inoculation
4. the frequency of study product–related SAEs in RSV-seronegative participants (group 2) from Day 0 through the 56th day following inoculation

Infectivity

1. To determine the peak titer of vaccine virus shed and duration of virus shedding by each participant
2. To assess the proportion of vaccinated participants infected* with study vaccine. For the seronegative cohort, the primary aim is to determine whether >90% of vaccinees are infected with vaccine virus

   * Infected with vaccine virus is defined as shedding vaccine virus, detected by RT-qPCR, and/or ≥4-fold rise in RSV-specific serum antibodies, detected by ELISA against the RSV F protein and/or RSV plaque reduction neutralization (RSV-PRNT) assay.

Immunogenicity

1. To characterize serum antibody responses to the study product in each study arm 28 days after inoculation of RSV-seropositive vaccine recipients (group 1)
2. To characterize serum antibody responses to the study product in each study arm 56 days after inoculation of RSV-seronegative vaccine recipients (group 2)

2.2. SECONDARY OBJECTIVES

1. To characterize the frequency and severity of symptomatic medically attended respiratory and febrile illness in RSV-seronegative vaccine and placebo recipients (group 2) who experience natural infection with wt RSV during the subsequent RSV season
2. To characterize serum antibody responses in RSV-seronegative vaccine and placebo recipients (group 2) who experience natural infection with wt RSV during the subsequent RSV season
3. To characterize mucosal antibody responses to each vaccine in RSV-seropositive (group 1) and a subset of RSV-seronegative (the first 13 group 2 enrolled prior to March 2020) participants
4. To identify differences in infectivity and immunogenicity between the vaccines.

2.3. EXPLORATORY OBJECTIVE

Study samples may be used in comparative assays with samples from other RSV vaccine studies initiated by the Laboratory of Infectious Diseases (LID), NIAID, NIH.

3. STUDY DESIGN

The study will be double-blind, randomized, and placebo-controlled. Participants in groups 1 and 2 will be randomized at a ratio of 2:2:1 to the three arms of the study; two active vaccine arms and one placebo arm. Placebo recipients are needed in pediatric studies to distinguish the background respiratory and febrile illnesses that occur in infants and children from those attributable to study vaccine. These numbers were chosen based upon experience with Phase I evaluation of other live-attenuated respiratory virus candidate vaccines (28-30) and statistical considerations (see Section 9).

The vaccine will be evaluated in a stepwise fashion beginning with RSV-seropositive children (group 1) and proceeding sequentially in RSV-seronegative children (group 2). For the purpose of this study, RSV-seropositive is defined as having a serum neutralizing antibody titer of ≥ 1:40, and RSV-seronegative is defined as having a serum neutralizing antibody titer of < 1:40. This definition has been used in previous evaluations of live-attenuated RSV vaccines (12, 29, 31). In these previous studies, live-attenuated RSV vaccines were highly restricted in replication and poorly immunogenic in children with titers ≥ 1:40 but were far less restricted in replication and highly immunogenic in children with titers < 1:40. These data suggest that this neutralizing antibody cutoff can distinguish effectively between RSV-experienced and RSV-naïve children.

Our intent is to randomize 2:2:1; RSV 6120/ΔNS1: RSV 6120/F1/G2/ΔNS1: placebo. Evaluation of each vaccine will proceed to group 2 if there are not more than 2 solicited adverse events (AEs) and no LRIs associated with that vaccine in RSV-seropositive children. If 3 or more RSV-seropositive children (group 1) with the same treatment assignment have respiratory or febrile illness that coincides with shedding of $>10^{2.5}$ PFU of vaccine virus/mL of nasal wash as detected by culture, then we will evaluate the safety, infectivity, and immunogenicity of the vaccine virus in an additional 15 RSV-seropositive children before determining whether to continue with group 2, or a decision will be made to discontinue the evaluation of this candidate.

Enrollment occurs at the time of inoculation with study product, Day 0, and will avoid the time during which wt RSV typically circulates in the community, through ongoing local and national (CDC NREVSS) surveillance(Appendix IV).

For group 1, RSV-seropositive children, the total duration of participation in the study is 28 days, consisting of an Acute Phase, from the day of inoculation (Study Day 0) through Study Day 10, and a Post-Acute Phase, from Study Day 11 through Study Day 28 (Table 29).

For group 2, RSV-seronegative children, the duration of participation in the initial phase of the study is 56 days. The initial phase consists of two components: an Acute Phase, from the day of inoculation (Study Day 0) through Study Day 28, and a Post-Acute Phase, from Study Day 29 through Study Day 56 (Table 30).

During the Acute Phase of the study, the participants' parents/guardians will be contacted daily. These contacts will consist either of an in-person evaluation of interim medical history, clinical assessment, and nasal rinse or swab, or an interim medical history conducted by a mutually agreed upon communication method. During the Acute Phase, the participants will be evaluated for AEs and SAEs. Participants who have a febrile or respiratory illness or otitis media will have a study visit to perform a clinical assessment and a nasal rinse or swab. Children will not be enrolled in this study while a Stay at Home Order is in place. However, in the event that a Stay at Home Order is initiated within the first 28 days following inoculation, if a child develops a respiratory illness, parents may be asked to obtain the nasal swabs, and clinical assessments may be completed via remote or in-person research visits. During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate. Parents will be informed of these possibilities prior to enrollment, and will be trained in the methods used to collect an anterior nasal swab specimen. The nasal rinse or swab will be tested for RSV and for other respiratory pathogens that are considered adventitious agents. During the Post-Acute Phase, the RSV-seropositive participants' parents/guardians will be instructed to contact the study staff if any SAEs or LRIs occur and will have a scheduled follow-up visit on the 28th day after inoculation, and the RSV-seronegative participants' parents/guardians will be instructed to contact the study staff if any SAEs occur and will have a scheduled follow-up visit on the 56th day after inoculation. The schedules of evaluations during the Acute Phase and Post-Acute Phase are shown in Appendix II, Table 29, and Table 30.

For the RSV-seronegative participants (group 2), the study has a third phase that assesses the incidence and severity of RSV-associated illness occurring during the RSV season following inoculation. During the RSV Season Surveillance Period, beginning on the date of seasonal enrollment pause and ending on March 31, study staff will make weekly contact with the participants' parents/guardians to identify medically attended episodes of fever, URI, LRI, or otitis media. Participants who have such an episode will have an in-person evaluation of interim medical history, clinical assessment, and a nasal rinse or swab to evaluate for RSV and other respiratory pathogens that are considered adventitious agents (Appendix III, Table 31). As noted above, if a Stay at Home Order is initiated during the RSV Surveillance Period, parents may be asked to obtain nasal swabs and clinical assessments may be completed via remote or in-person research visits.

The RSV-seronegative participants will also have a study visit during the post-RSV season to collect a blood sample to assess the durability of the vaccine response and to assess the immune response to naturally occurring wt RSV infection. For 13 participants enrolled prior to March 2020, a pre-RSV season blood draw and nasal rinse and/or nasosorption sample (using the synthetic absorptive matrix (SAM) strip) for immunological assays were collected. The samples will be used to assess the durability of the vaccine response. The RSV-seronegative participants may have an overlap between the initial phases and the RSV surveillance phase of the study, depending upon the date of inoculation.

4. STUDY POPULATION

In group 1, approximately 25 RSV-seropositive children ≥ 12 months to < 60 months of age will be enrolled. In group 2, approximately 35 to 50 RSV-seronegative infants and children ≥ 6 months to <25 months of age will be enrolled. These numbers were chosen based upon experience with phase I evaluation of other live attenuated respiratory virus candidate vaccines (11,23,25) and statistical considerations (Section 9). Placebo recipients are needed in pediatric studies to distinguish the

background respiratory illnesses that occur in infants and children from those attributable to study vaccine. Infants and children will be selected for participation according to the described study-specific inclusion and exclusion criteria.

4.1. INCLUSION CRITERIA FOR RSV-SEROPOSITIVE CHILDREN

Potential RSV-seropositive participants must meet all of the following inclusion criteria to be enrolled in this study:

4.1.1. ≥ 12 months of age and < 60 months of age at the time of inoculation

4.1.2. Screening serum specimen for RSV-neutralizing antibody is obtained within the calendar year of inoculation

4.1.3. Seropositive for RSV antibody, defined as serum RSV-neutralizing antibody titer ≥ 1:40

4.1.4. Pre-inoculation serum sample for RSV-neutralizing antibody specimen is obtained no more than 56 days prior to inoculation

4.1.5. In good health based on review of the medical record, history, and physical examination (PE) at the time of inoculation

4.1.6. Received routine immunizations appropriate for age based on the Advisory Committee on Immunization Practices (ACIP) Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger

4.1.7. Growing normally for age as demonstrated on a standard growth chart and has a current height and weight above the 3rd percentile for age

4.1.8. Expected to be available for the duration of the study

4.1.9. Parent/guardian is willing and able to provide written informed consent

4.2. EXCLUSION CRITERIA FOR RSV-SEROPOSITIVE CHILDREN

Potential RSV-seropositive participants who meet any of the following exclusion criteria will be excluded from participation in this study:

4.2.1. Born at less than 34 weeks gestation

4.2.2. Maternal history of positive human immunodeficiency virus (HIV) test

4.2.3. Evidence of chronic disease

4.2.4. Known or suspected impairment of immune function

4.2.5. Bone marrow/solid organ transplant recipient

4.2.6. Major congenital malformations, including congenital cleft palate or cytogenetic abnormalities

4.2.7. Suspected or documented developmental disorder, delay, or other developmental problem

4.2.8. Cardiac abnormality requiring treatment

4.2.9. Lung disease or reactive airway disease

4.2.10. More than one episode of wheezing in the first year of life

4.2.11. Wheezing episode or received bronchodilator therapy within the past 12 months

4.2.12. Wheezing episode or received bronchodilator therapy after the age of 12 months

4.2.13. Previous receipt of supplemental oxygen therapy in a home setting

4.2.14. Previous receipt of an investigational RSV vaccine

4.2.15. Previous receipt or planned administration of anti-RSV antibody product including ribavirin, RSV Ig or RSV mAb

4.2.16. Previous receipt of immunoglobulin or any antibody products within the past 6 months

4.2.17. Previous receipt of any other blood products within the past 6 months

4.2.18. Previous anaphylactic reaction

4.2.19. Previous vaccine-associated adverse reaction that was Grade 3 or above

4.2.20. Known hypersensitivity to any vaccine component

4.2.21. Member of a household that contains an infant who is less than 12 months of age at the date of inoculation through the 10^{th} day after inoculation

4.2.22. Member of a household that, at the date of inoculation through the 10^{th} day after inoculation, contains an immunocompromised individual including but not limited to:

- a person who is HIV-infected
- a person who has cancer and has received chemotherapy within the 12 months prior to enrollment
- a person living with a solid organ or bone marrow transplant

4.2.23. Will attend a daycare facility that does not separate children by age and contains an infant who is < 12 months of age at the date of inoculation through the 10^{th} day after inoculation

4.2.24. Receipt of any of the following prior to enrollment:

- any inactivated vaccine or live-attenuated rotavirus vaccine within the 14 days prior, or
- any live vaccine, other than rotavirus vaccine, within the 28 days prior, or
- another investigational vaccine or investigational drug within 28 days prior, or
- salicylate (aspirin) or salicylate-containing products within the past 28 days

4.2.25. Scheduled administration of any of the following after planned inoculation:

- inactivated vaccine or live-attenuated rotavirus vaccine within the 14 days after, or
- any live vaccine other than rotavirus within the 28 days after, or
- another investigational vaccine or investigational drug within the 28 days after

4.2.26. Receipt of any of the following medications within 3 days of study enrollment:

- systemic antibacterial, antiviral, antifungal, anti-parasitic, or antituberculous agents, whether for treatment or prophylaxis, or
- intranasal medications, or
- other prescription medications except the permitted concomitant medications listed in Section 5.0

4.2.27. Any of the following events at the time of enrollment:

- fever (temporal or rectal temperature of ≥100.4°F), or
- upper respiratory signs or symptoms (rhinorrhea, cough, or pharyngitis) or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media

4.3. INCLUSION CRITERIA FOR RSV-SERONEGATIVE INFANTS & CHILDREN

Potential RSV-seronegative participants must meet all of the following inclusion criteria to be enrolled in this study:

4.3.1. ≥ 6 months of age and < 25 months of age at the time of inoculation

4.3.2. Screening and pre-inoculation serum specimens for RSV-neutralizing antibody are obtained no more than 42 days prior to inoculation

4.3.3. Seronegative for RSV antibody, defined as serum RSV-neutralizing antibody titer < 1:40

4.3.4. In good health based on review of the medical record, history, and PE at the time of inoculation

4.3.5. Received routine immunizations appropriate for age based on the ACIP Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger

4.3.6. Growing normally for age as demonstrated on a standard growth chart, AND

- If < 1 year of age: has a current height and weight above the 5th percentile for age
- If ≥ 1 year of age: has a current height and weight above the 3rd percentile for age

4.3.7. Expected to be available for the duration of the study

4.3.8. Parent/guardian is willing and able to provide written informed consent

4.4. EXCLUSION CRITERIA FOR RSV-SERONEGATIVE INFANTS & CHILDREN

Potential RSV-seronegative participants who meet any of the following criteria will be excluded from this study:

4.4.1. Born at less than 34 weeks gestation

4.4.2. Born at less than 37 weeks gestation, and at the date of inoculation less than 1 year of age

4.4.3. Maternal history of a positive HIV test

4.4.4. Evidence of chronic disease

4.4.5. Known or suspected infection or impairment of immunological functions

4.4.6. Bone marrow/solid organ transplant recipient

4.4.7. Major congenital malformations, including congenital cleft palate or cytogenetic abnormalities

4.4.8. Suspected or documented developmental disorder, delay, or other developmental problem

4.4.9. Cardiac abnormality requiring treatment

4.4.10. Lung disease or reactive airway disease

4.4.11. More than one episode of wheezing in the first year of life

4.4.12. Wheezing episode or received bronchodilator therapy within the past 12 months

4.4.13. Wheezing episode or received bronchodilator therapy after the age of 12 months

4.4.14. Previous receipt of supplemental oxygen therapy in a home setting

4.4.15. Previous receipt of an investigational RSV vaccine

4.4.16. Previous receipt or planned administration of anti-RSV product including ribavirin, RSV Ig, or RSV mAb within the past 6 months

4.4.17. Previous receipt of immunoglobulin or any antibody products within the past 6 months

4.4.18. Previous receipt of any blood products within the past 6 months

4.4.19. Previous anaphylactic reaction

4.4.20. Previous vaccine-associated adverse reaction that was Grade 3 or above

4.4.21. Known hypersensitivity to any study product component

4.4.22. Member of a household that contains an infant who is less than 6 months of age at the date of inoculation through the 28th day after inoculation

4.4.23. Member of a household that, at the date of inoculation through the 28th day after inoculation, contains an immunocompromised individual including but not limited to:

- a person who is HIV-infected
- a person who has cancer and has received chemotherapy within the 12 months prior to enrollment
- a person living with a solid organ or bone marrow transplant

4.4.24. Attends a daycare facility that does not separate children by age and contains an infant < 6 months of age at the date of inoculation through the 28th day after inoculation

4.4.25. Receipt of any of the following prior to enrollment:

- any inactivated influenza vaccine within 3 days prior, or
- any inactivated vaccine or live-attenuated rotavirus vaccine within the 14 days prior, or
- any live vaccine, other than rotavirus vaccine, within the 28 days prior, or
- another investigational vaccine or investigational drug within 28 days prior, or
- salicylate (aspirin) or salicylate-containing products within the past 28 days

4.4.26. Scheduled administration of any of the following after planned inoculation

- inactivated vaccine or live-attenuated rotavirus vaccine within the 14 days after, or
- any live vaccine other than rotavirus within the 28 days after, or
- another investigational vaccine or investigational drug within the 56 days after

4.4.27. Receipt of any of the following medications within 3 days of study enrollment:

- systemic antibacterial, antiviral, antifungal, anti-parasitic, or antituberculous agents, whether for treatment or prophylaxis, or
- intranasal medications, or
- other prescription medications except the permitted concomitant medication listed below

Permitted concomitant medications (prescription or non-prescription) include nutritional supplements, medications for gastroesophageal reflux, eye drops, and topical medications, including (but not limited to) cutaneous (topical) steroids, topical antibiotics, and topical antifungal agents.

4.4.28. Any of the following events at the time of enrollment:

- fever (temporal or rectal temperature of ≥100.4°F), or
- upper respiratory signs or symptoms (rhinorrhea, cough, or pharyngitis) or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media

- contact with a person diagnosed with COVID-19 disease or active SARS-CoV-2 infection within the past 10 days

4.5. CO-ENROLLMENT CONSIDERATIONS

Co-enrollment in an investigational vaccine or investigational drug study is not allowed during this study.

4.6. RE-ENROLLMENT CONSIDERATIONS

RSV-seropositive participants who receive placebo may re-enroll in the study following completion of the Study Day 28 visit if they continue to meet all eligibility requirements. RSV-seronegative participants who receive placebo may re-enroll in the study following completion of the initial RSV surveillance period if they continue to meet all eligibility requirements. Subjects will not be allowed to enroll in the study more than twice.

4.7. RECRUITMENT PROCESS

CIR research staff will recruit participants from pediatric practices and clinics in the greater Baltimore/Washington area. Upon referral by the participants' primary care provider or the provider's staff, CIR staff will contact parents/guardians in person or remotely as appropriate,via telephone, email, or text message, send them IRB-approved informational brochures, and obtain contact information. Study staff will follow up with interested parents/guardians to elaborate upon the details and requirements of the screening and study process.

CIR research staff may also recruit potential participants by sending IRB-approved documents through mail, email, or electronic medical record messaging (e.g. MyChart) to children of local pediatric practices and clinics, and to households in local zip codes containing age-appropriate children. In addition, CIR staff may recruit participants through group gatherings such as health fairs and by social media posting IRB-approved recruitment materials.

If parents/guardians are interested in having their child participate and the child meets the minimum inclusion and exclusion criteria, then the study staff will schedule a screening visit to determine the child's eligibility.

4.8. PARTICIPANT RETENTION

Study staff will make every effort to retain participants in the study, thereby minimizing potential biases associated with loss to follow-up.

4.9. PARTICIPANT WITHDRAWAL OR TERMINATION FROM THE STUDY

Participants in this study may voluntarily withdraw from the study at any time. Any participant who has received study product will be encouraged to remain in the safety evaluation for the duration of the study even if sample collection is refused.

A participant may withdraw or terminate participation in the study early for any of the following reasons:

- Withdrawal of consent – applies to a parent/guardian who verbally or in writing withdraws consent for the participant to continue in the study for any reason.
- Noncompliant with protocol – applies to a parent/guardian who does not comply with protocol-specific visits or evaluations on a consistent basis, such that adequate follow-up is not possible and the participant's safety would be compromised by continuing in the study.
- Investigator discretion – participant withdrawal may occur if the investigator believes that it is in the best interest of the participant.
- Other – a category used when previous categories do not apply; requires an explanation.

The study may be ended for the following reasons:

- Research is terminated by sponsor or investigator – applies to the situation where the entire study is terminated by the sponsor or investigator for any reason.
- The study sponsor, CIR, the institutional review board (IRB), the Office for Human Research Protections (OHRP), NIAID, or the US Food and Drug Administration (FDA) may decide to end the study.

For any participant who withdraws or who is terminated from the study prior to completion of follow-up, study staff will document the reason for the withdrawal or termination. In the event that the circumstances that led to a participant's withdrawal or termination change, the study staff will contact the principal investigator (PI) to discuss options for resumption of follow-up. Withdrawn participants will not be replaced.

5. STUDY PRODUCT

The unblinded dispenser should consult standard operating procedures (SOPs) and the study manual of procedures (MOP). Refer to Figure 1.1-1 and Figure 1.1-2 for an overview of the study design and to the investigator's brochure (IB) for further information about the study product.

The products that will be administered in this study are:

- Group 1: Live Recombinant Respiratory Syncytial Virus RSV 6120/ΔNS1, approximately $10^{6.0}$ PFU or RSV 6120/F1/G2/ΔNS1 approximately $10^{5.8}$ per 1.0 mL vaccine
- Group 2: Live Recombinant Respiratory Syncytial Virus RSV 6120/ΔNS1 or RSV

6120/F1/G2/ΔNS1, approximately $10^{5.0}$ PFU per 0.5 mL vaccine

- Group 1: Placebo for the RSV vaccine will be Lactated Ringer's Solution for Injection, USP 1.0 mL
- Group 2: Placebo for the RSV vaccine will be Lactated Ringer's Solution for Injection, USP 0.5 mL

5.1. STUDY PRODUCT REGIMENS

Enrolled study participants will receive a single dose of the indicated vaccine or placebo, administered as nose drops.

5.2. STUDY PRODUCT FORMULATION

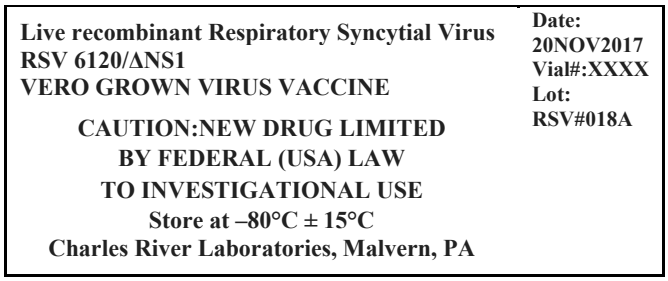
5.2.1. Vaccines

The RSV 6120/ΔNS1 vaccine is provided in a sterile 2.0-mL cryovial, each containing 0.6 mL of vaccine (Lot RSV#018A). The vaccine virus concentrate is diluted by trained research personnel to a dose of approximately $10^{6.0}$ PFU (group 1) in 1.0 mL or $10^{5.0}$ PFU (group 2) in a 0.5-mL volume. The vaccine vial is labeled as shown in Figure 5.2-1.

The RSV 6120/F1/G2/ΔNS1 vaccine is provided in a sterile 2.0-mL cryovial, each containing 0.6 mL of vaccine (Lot RSV#016A). The vaccine virus concentrate is used undiluted at a dose of approximately $10^{5.8}$PFU (group 1) in 1.0 mL or is diluted by trained research personnel to a dose of $10^{5.0}$ PFU (group 2) in a 0.5-mL volume. The vaccine vial is labeled as shown in Figure 5.2-2.

Figure 5.2-1: Investigational Product Label Sample

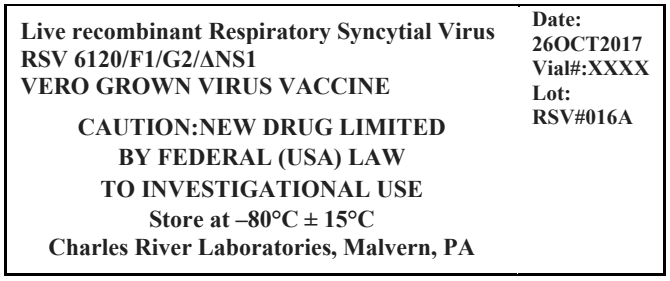
RSV 6120/ΔNS1: titer 6.2 $\log_{10}$ PFU per mL



(enlarged sample)

Figure 5.2-2: Investigational Product Label Sample

RSV 6120/F1/G2/ΔNS1: titer 5.8 $\log_{10}$ PFU per mL



(enlarged sample)

5.2.2. Diluent for RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1

The diluent for RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1is Lactated Ringer's Solution for Injection, USP.

5.2.3 Placebo for RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1

The placebo for RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1is is Lactated Ringer's Solution for Injection, USP.

5.3. STUDY PRODUCT STORAGE

Vaccine will be stored in a secure freezer at -80°C ± 15°C. It must remain frozen until the time of use. Once the vaccine is thawed, it should never be refrozen for reuse. Vaccine will be prepared from new, unopened containers for each use.

Lactated Ringer's Solution for Injection, USP should be stored at room temperature as recommended by the supplier until the day before study product preparation. Vaccine diluent/placebo must be transferred to a secure 2°C to 8°C refrigerator at least 24 hours before use.

Procedures for managing the vaccine and diluent/placebo shipment are in the CIR JHU Pharmacy SOP.

5.4. STUDY PRODUCT PREPARATION

The diluent for the vaccine, the placebo for the vaccine, and the RSV vaccine must be prepared by following the detailed instruction in the Pharmacy SOP.

The unblinded dispenser will prepare the correct dose of study product for each participant in a biological safety cabinet (BSC) or compounding aseptic containment isolator (CACI) using aseptic technique. If necessary to preserve blinding, all prepared syringes will be as described in the MOP.

5.4.1. Diluent

The diluent is Lactated Ringer's Solution for Injection, USP.

5.4.2. Placebo

Placebo is Lactated Ringer's Solution for Injection, USP.

Placebo will be drawn up in a sterile syringe to a volume of 1.0 mL for group 1 and to a volume of 0.5 mL for group 2 and labeled per instructions in the MOP. If necessary to preserve blinding, all prepared syringes will be as described in the MOP.

5.4.3. Live RSV 6120/ΔNS1 or RSV 6120/F1/G2/ΔNS1

Diluent will be prepared prior to removal of vaccine from the freezer. The MOP will be followed for proper handling of the study product.

When manipulating the undiluted study product, the smallest gauge needle possible will be used to avoid loss of study product in the needle and syringe hub. For participants in group 1, the frozen study product will be thawed and diluted prior to administration with Lactated Ringer's Solution for Injection, USP to a dose of approximately $10^{6.0}$ PFU (RSV 6120/ΔNS1) or administered undiluted at the indicated dose of $10^{5.8}$ PFU (RSV 6120/ F1/G2/ΔNS1) in 1.0 mL. For participants in group 2, the frozen study product will be thawed and diluted prior to administration with Lactated Ringer's Solution for Injection, USP to a dose of approximately $10^{5.0}$ PFU in 0.5mL.

The diluted study product will be drawn up to a volume of 1.0 mL (group 1) or 0.5 mL (group 2) in a sterile syringe and labeled per instructions in the MOP. The labeled filled syringes will be transported to the clinical site for administration in a Credo medical transport cooler or cooler with ice or cold packs with the lid closed, to maintain temperature at 2ºC to 8ºC. Vaccine must be administered within 4 hours of being removed from the freezer. Placebo must be administered within 4 hours of being removed from the refrigerator.

Samples of undiluted (if available) and diluted study product will be aliquoted from the remaining vaccine that has been prepared. The samples will be snap-frozen as per the MOP and stored at -80°C ± 15°C separate from the concentrated study product. Titration of vaccine will be completed to confirm the titer of the vaccine administered to the participants.

The CIR JHU Pharmacy SOP provides detailed instructions on study product storage, handling, and preparation. Details on labeling study product and transporting it to the clinic can be found in the MOP.

5.5. STUDY PRODUCT INOCULATION PROCEDURE

All study participants will receive a single dose of study product, administered as nose drops. There is no nasal preparation prior to administration. While the participant is supine, a volume of 1.0 mL (group 1) or 0.5 mL (group 2) of study product will be delivered as nose drops (approximately 0.5 mL [group 1] or 0.25 mL [group 2] per nostril) using a sterile, needle-less, masked syringe. Participant will remain supine for approximately 60 seconds following inoculation.

5.6. STUDY PRODUCT ACQUISITION

The clinical lots of RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 were generated by Charles River Laboratories (CRL) using the seed virus provided by the NIH.

Lactated Ringer's Solution for Injection, USP will be used as diluent and placebo.

Vaccine virus will be stored at a NIAID designated commercial repository until formally requested by the PI/designee. Prior to IRB approval and FDA safe to proceed determination, the PI/designee at JHU CIR may request that vials of vaccine be transferred from the sponsor to the CIR laboratories or Investigational Drug Service pharmacy. However, only after receipt of IRB approval and FDA safe to proceed determination will vials of vaccine be used for administration to study participants. The initial shipment may contain the full number of vials needed for implementation of the protocol. Procedures for ordering and shipping of the study product are in the MOP.

5.7. STUDY PRODUCT ACCOUNTABILITY

The unblinded dispenser is responsible for maintaining an accurate inventory and accountability record of study-product and Lactated Ringer's Solution for Injection, USP for this study. A copy of the randomization code will be retained by the unblinded dispenser as outlined in the MOP. Without written request to unblind, the randomization code may not be released. The unblinded dispenser will be responsible for maintaining the blind.

5.8. DISPOSITION OF USED/UNUSED STUDY PRODUCT

After the unblinded dispenser dilutes the vaccine and draws up the vaccine into a syringe for administration, the label will be removed from the vaccine vials used for preparation and placed in the accountability log. In this manner, monitoring personnel will be able to verify the accountability of all vaccine vials used for the study. If there is any vaccine left after the syringes have been drawn up and aliquots have been removed for titering, it will be destroyed by research personnel as per the MOP.

5.9. FINAL DISPOSITION OF STUDY PRODUCTS

After study completion or termination, all unused study product will be disposed of per the sponsor's instructions.

5.10. CONCOMITANT MEDICATIONS

Permitted concomitant medications at enrollment (prescription or non-prescription) include nutritional supplements, medications for gastroesophageal reflux, eye drops, and topical medications, including (but not limited to) cutaneous (topical) steroids, topical antibiotics, and topical antifungal agents.

The use of prophylactic antipyretics, decongestants, or antihistamines is discouraged during the Acute Phase: day of inoculation day through the 10^{th} day after inoculation for group 1; and day of inoculation through the 28^{th} day following inoculation for group 2. However, use of these medications for treatment of symptoms is allowed.

Due to the potentially confounding effect on study immunogenicity results, the following concomitant medications should be avoided after inoculation unless deemed clinically necessary.

- Licensed inactivated vaccine or live-attenuated rotavirus vaccine within 14 days of inoculation, with the exception of inactivated influenza vaccine
- Licensed live virus vaccine, other than rotavirus vaccine, within 28 days of inoculation
- Systemic corticosteroids for more than 14 days at a dosage equivalent to prednisone at $\geq$ 2 mg/kg or 20 mg daily, or other immune-modifying drugs within 28 days of inoculation
- Immunoglobulins and/or any blood products within 28 days of inoculation
- Investigational drug or investigational vaccine within 28 days of inoculation for group 1 and within 56 days of inoculation for group 2

6. STUDY VISITS AND PROCEDURES

An overview of the study visits, evaluation schedule, and specimen collection is provided in Appendix II and Appendix III. This section contains additional information on visit-specific study procedures.

Study visits, except inoculation, may be performed at one of the clinical sites or at a mutually agreed-upon location. Inoculation must be performed at one of the CIR clinical sites or pediatric offices where emergency supplies are available. All clinical tasks will be performed by a medical professional. The PE will include temperature, heart rate, respiratory rate, assessment of ears, eyes, nose, and throat (EENT), lungs, heart, and skin, as well as abdominal, musculoskeletal, and, as appropriate, developmental exams. A focused clinical assessment will include temperature, heart rate, respiratory rate, EENT, lung, heart and lymph nodes assessment.

All specimens will be obtained, processed, stored and transferred per the MOP.

In addition to the protocol-specified procedures listed in this section, study staff may complete other tasks consistent with SOPs, including but not limited to collecting, reviewing, and updating demographic and locator information; reviewing elements of informed consent; scheduling telephone contacts and visits; providing instructions for contacting study staff between visits; providing visit reminders; and following up on missed visits. All visit procedures will be documented in accordance with the MOP. Refer to Table 18 and Table 19 and Section 10 for more information on documentation requirements and completion of case report forms (CRFs).

The study's medical professional will inform parent/guardian of any significant abnormal physical findings and, after obtaining parental release of information, will make appropriate referrals to the child's primary caregiver, if necessary.

6.1. CONSENTING PROCESS

The screening process may be completed under a separate screening protocol and consent or under the study protocol and consent. The parent/guardian must complete the informed consent process and sign the screening or study informed consent document (ICD) before procedures are performed. The consenting process for the study may take place during the screening visit or may be conducted at a separate visit prior to inoculation. During the consenting process, the child's parent will review the ICD, be encouraged to ask questions, and complete a comprehension assessment to evaluate consent understanding. Study staff will use incorrect answers from the comprehension assessment to identify areas of the ICD that need further review with the parent/guardian. This will help ensure that the parent/guardian has sufficient understanding of the process before the ICD is signed. Parent/guardian will be offered a copy of the ICD.

During the consenting process, the parent will be given the opportunity to provide permission for use of their child's picture in recruitment flyers, articles, or presentations. In addition, the parent will be given the opportunity to provide permission for storing their child's specimens for future respiratory virus vaccine studies and for research purposes.

As needed, a parent/guardian will complete a Health Insurance Portability and Accountability Act (HIPAA) medical record release to allow study staff to review medical history and immunization records, and to allow review of AEs during the study. Only those portions of the medical record that

are pertinent to the study will be maintained in the study chart.

6.2. SCREENING VISIT

The screening process will include reviewing the medical record, obtaining a medical history from parent/guardian, and conducting a PE. The PE will occur on the day of screening or the day of inoculation. The medical record review should include review of history related to the study eligibility criteria, the immunization record, and growth chart data. The medical history from the parent should include demographics, prior diagnoses, current medications, signs and symptoms, developmental status, age of household members, day care attendance, and use of medications prior to inoculation.

The screening visit will also include obtaining a serum sample to test for the presence of RSV antibodies. Approximately 5 mL of blood is sufficient quantity for the screening and pre-inoculation assays. The procedure will be performed and documented per the MOP. As in previous phase I trials of other live-attenuated RSV vaccines (12, 28-30), other screening laboratory tests will not be performed on the participant. Such tests are not routinely performed as part of well-child care, given that the risk of undiagnosed hepatic, metabolic, and renal diseases is much lower in children than in adults (32).

For the RSV-seropositive participants, the screening serum sample must be obtained in the same calendar year as study inoculation. The pre-inoculation serum antibody sample must be obtained no more than 56 days prior to inoculation.

For the RSV-seronegative participants, the screening serum sample must be obtained no more than 42 days prior to inoculation. Study staff should consider potential randomization and inoculation dates when scheduling the participant screening visits.

Table 2: Screening Visit Procedures

Screening Visit Procedures		
Administrative Tasks		• Conduct and document consenting process • Confirm parent/guardian's informed consent comprehension • Obtain release of medical records as required per HIPAA • Review and document medical history • Assess eligibility
Clinical Tasks		• Obtain, review, and document medical history • Perform complete PE, or PE may be deferred until study Day 0 • Address any concerns • Document findings • Review participant's immunization record • Review participant's medical records to determine age-appropriate developmental assessment, and participant's weight and length
Laboratory Tasks	**Blood**	*Collect blood for*: screening[1] and pre-inoculation[2] sample for RSV serum antibody testing
Follow-up Preparation		• Review enrollment visit

1. Obtained within the same calendar year as inoculation for RSV-seropositive participants and no more than 42 days prior to inoculation for RSV-seronegative participants

2. Obtained no more than 56 days prior to inoculation for RSV-seropositive participants and no more than 42 days prior to inoculation for RSV-seronegative participants

6.3. RANDOMIZATION

Randomization numbers will be assigned by the unblinded study personnel and will be forwarded to the Data Safety Monitoring Board (DSMB) Executive Secretary. Unblinded study personnel will label the syringes per the MOP. The vaccine administrator and verifier will initial the label and place the label in the participant's study chart. The vaccination time will be documented on the vaccine administration record (VAR). All syringes will be disposed of by the study staff following the enrollment visit.

A copy of the randomization code will be retained by the unblinded dispenser. Without the PI's written request to unblind, the randomization code will not be released to the clinical staff until the acute monitoring phase is complete for each participant within the randomization group. Pediatric participants will be permuted block-randomized, in groups of five, to ensure that the 2:2:1 ratio of treatment arm 1, to treatment arm 2, to control participants will be maintained across time. This allows for maintenance of the blind during each participant's acute monitoring phase but also provides early information regarding safety, which is appropriate and common practice for phase 1 respiratory virus vaccine studies (11, 23, 25). Detailed procedures for randomization and unblinding will be placed in the pharmacy study files and are included in the MOP.

If the need arises to unblind a specific participant's assignment in the event of a serious illness prior to completion of the acute monitoring phase, procedures for early unblinding will be followed. In the event that unblinding is required, only that specific participant's assignment will be unblinded. Whenever possible, the PI and sponsor will make a decision regarding early unblinding in collaboration with the Data and Safety Monitoring Board (DSMB). The sponsor and the DSMB Executive Secretary will be notified of the event in real time.

In group 1, approximately 25 RSV-seropositive infants and children will be enrolled in the study and will receive RSV 6120/ΔNS1 ($10^{6.0}$ PFU), RSV 6120/F1/G2/ΔNS1 ($10^{5.8}$ PFU), or placebo in a 2:2:1 ratio (10:10:5). As noted in Section 3.0, each vaccine may be evaluated in up to 15 additional seropositive children if 3 or more RSV-seropositive children (group 1) with the same treatment assignment have respiratory or febrile illness that coincides with shedding of > $10^{2.5}$ PFU of vaccine virus/mL of nasal wash as detected by culture. In group 2, approximately 35 to 50 RSV-seronegative infants and children will be enrolled in the study and will receive RSV 6120/ΔNS1 ($10^{5.0}$ PFU), RSV 6120/F1/G2/ΔNS1 ($10^{5.0}$ PFU), or placebo in a 2:2:1 ratio (20:20:10).

In the case of children from the same household, all eligible children will be assigned to the same study product regimen. This will be done to reduce the potential cross-contamination that could result if children living together were to receive different study products. All enrolled children will be included in safety analyses.

The PI, and the Scientific Investigator(s) of the Laboratory of Infectious Diseases will be unblinded to all data at the completion of the Post-Acute Phase of follow-up (Day 28 for RSV-seropositive and Day 56 for RSV-seronegative) for the last participant enrolled in each calendar year to enable more efficient and timely study evaluation and planning for appropriate next steps with respect to RSV candidate vaccine development.

6.4. ENROLLMENT

6.4.1. Enrollment - Inoculation (Day 0)

Inoculation with study product will avoid the time during which wt RSV circulates in the community, through ongoing local and national (CDC NREVSS) surveillance.. For the purpose of this protocol, enrollment will correspond to inoculation with investigational product, which occurs on study Day 0, and must be completed at a CIR office site or pediatric practice where emergency supplies are available. Prior to inoculation, the clinical staff will provide an authorized request for study product to the unblinded dispenser. The request must include the information outlined in the MOP.

The ICD will be obtained from the parent/guardian of each child who participates in this study prior to the performance of any study procedures or inoculation. If not previously obtained, then the ICD will be completed on the day of enrollment. The child's parent/guardian will be encouraged to ask questions and complete a comprehension assessment to evaluate understanding of the study. This will help ensure that the parent/guardian has sufficient understanding of the study process before the ICD is signed.

If the participant is noted to have any of the following on enrollment day, inoculation must be deferred:

- fever (temporal or rectal temperature of ≥ 100.4°F), or
- URI or LRI symptoms or signs (including but not limited to rhinorrhea, cough, pharyngitis), or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media
- contact with a person diagnosed with COVID-19 disease or SARS-CoV-2 infection within the preceding 10 days

If the inoculation for an RSV-seropositive participant has to be deferred to the following calendar year, or if the 42-day window from screening to inoculation is exceeded for an RSV-seronegative participant, then the infant or child must be rescreened.

Table 3: Enrollment Visit Procedures

Enrollment Visit Procedures *(Day 0)*		
Administrative Tasks		• Confirm study ICD is completed and signed and dated by both parent/guardian and study staff who completed the consenting process • Complete eligibility determination and confirmation • Complete paper-based eligibility checklist • Obtain release of medical records as required per HIPAA
Clinical Tasks		• Obtain interim history from parent/guardian • Perform PE: o Complete PE if deferred at screening, OR o Focused PE if physical completed at screening • Address any concerns • Document findings • Confirm eligibility
Laboratory Tasks	**Blood**	*If insufficient volume obtained at screening, collect blood for*: • Pre-inoculation RSV antibody titer*
	Nasal Wash or Swab and/or Nasosorption SAM Strip	*Collect nasosorption strip and/or nasal wash for Group 1 and the first 13 participants enrolled in Group 2:* • RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash or swab.
		The nasal wash or swab must be obtained prior to administering the study product. *Collect nasal wash or swab for:* • RSV viral detection and quantification

Study Product Administration	• Administer study product and maintain participant in a supine position for a minimum of 1 minute • Observe for a minimum of 30 minutes after inoculation to evaluate for immediate hypersensitivity reactions
Follow-up Preparation	• Provide the following: temperature card with explanation, temporal and rectal thermometers with instructions for use, illness criteria explanation, and study personnel contact information • Schedule non-visit day contact and schedule next in-person visit • Offer parent/guardian safety seat education materials, and a safety seat educator appointment • Offer parent lactation services by a certified lactation counselor, if appropriate

*Within same calendar year as screening for RSV-seropositive participants, and no more than 42 days from screening for RSV-seronegative participants

The parent/guardian will record the infant/child's temperatures and signs of illness on the temperature card and will report these to study personnel during an in-person or remote research visit or non-visit day contact. New rectal thermometers will be given and temporal artery thermometers will be provided to parent/guardian for use during the study. For temperature measurements, parent/guardian will be instructed to use the study-provided temporal artery thermometer to screen for elevated temporal artery temperatures. This device is used to minimize the number of rectal temperature measurements and has been shown to be an effective screening tool for rectal fever (33). The parent/guardian will measure temporal artery temperatures following the manufacturer's directions. If any temporal artery temperature is ≥ 100.0°F, parent/guardian will be asked to measure a rectal temperature within 20 minutes (33). For study-specific management and grading of temperatures, see Section 8, Table 21.

6.5. STUDY PHASES

Refer to Figure 1.1-1 and Figure 1.1-2 for timelines of study visits. The Acute Phase begins with inoculation and ends at midnight on the 10th day after inoculation for RSV-seropositive participants, and at midnight on the 28th day after inoculation for RSV-seronegative participants. During the Acute Phase of the study, a study healthcare professional will be available by telephone 24 hours a day for consultation with parent/guardian regarding any illnesses that may occur.

Study personnel will have daily contact with RSV-seropositive participants' parents/guardians for the first 10 days after inoculation, and for the RSV-seronegative participants during the first 28 days after inoculation. This 28-day period is consistent with the duration of shedding of live-attenuated respiratory virus vaccines in RSV-seronegative participants (33-36).

On non-visit days, study staff will contact the parent/guardian and will record the parent/guardian-provided temperatures and signs of illness. Participants with illness may have additional visits to assess the illness (Section 6.7, Illness Visit).

6.5.1. RSV-Seropositive Children (Group 1)

6.5.1.1. Acute Phase: In-person Study Visit Days

An in-person study visit and clinical assessment performed by a healthcare professional will be completed during visits on Days 3, 4, 5, 6, 7, and 10 after inoculation, with a visit window of ± 1 day. If an in-person visit is moved by ± 1 day, then the non-visit day contact will be completed in place of the original interim visit date.

Table 4: Acute Phase In-Person Visit Procedures – RSV-Seropositive Children

Days 3, 4, 5, 6, 7 and 10 In-person Visit Procedures *(each visit ± 1 days)*		
Clinical Tasks		• Document the participant's previous days' interim history as reported from the parent/guardian, include: ▪ medications and/or immunizations ▪ signs and symptoms of illness ▪ highest temperature reading, indicate temperature method • Perform focused clinical examination • Address any concerns • Review safety data • Document findings
Laboratory Tasks	**Nasal wash**	*Collect nasal wash for:* • RSV viral detection and quantification
Follow-up Preparation		• Schedule non-visit day contact and follow-up, in-person visits ***Day 10 only**:* • Review SAE and LRI criteria with participants and how to contact study personnel during Post-Acute Phase

During an Acute Phase study visit, if the participant is diagnosed with or suspected of having URI, LRI, otitis media or fever (as defined in Appendix IV), testing of the nasal wash specimen for adventitious agents will be performed as described in the MOP.

6.5.1.2. Acute Phase: Non-Visit Study Day Contacts

In the 10 days following inoculation, parental contact will be made on days that an in-person visit is not completed: Days 1, 2, 8, and 9 (each visit ± 1 day). On non-visit days, study staff will contact the parent/guardian and will record the parent/guardian-provided temperatures and signs of illness. Participants with illness may have additional visits to assess the severity of the illness (Section 6.8).

Table 5: Acute Phase Non-Visit Contact Procedures – RSV-Seropositive Children

Days 1, 2, 8, and 9 Contact Procedures *(each visit ± 1 day)*	

Clinical Tasks	• Document the participant's previous days' interim history as reported from the parent/guardian, include:
	  • medications and/or immunizations
	  • signs and symptoms of illness
	  • highest temperature reading, indicate temperature method
	• Address any concerns
	• Review safety data
	• Document findings

Follow-up Preparation	• Schedule an illness appointment if necessary

6.5.1.3. Study Day 11

There will be a non-visit contact on Day 11 to obtain interim history through midnight on the 10th day following inoculation. If the Day 10 Visit takes place on Day 11, it is not necessary to have an additional contact with the family on Day 11.

Table 6: Day 11 Non-Visit Procedures – RSV-Seropositive Children

Day 11 Non-Visit Procedures	

Clinical Tasks	• Document the participant's previous days' interim history as reported from the parent/guardian, include:
	  ▪ medications and/or immunizations
	  ▪ signs and symptoms of illness
	  ▪ highest temperature reading, indicate temperature method
	• Address any concerns
	• Review safety data
	• Document findings

Follow-up Preparation	• Review SAE and LRI criteria with participants and how to contact study personnel during Post-Acute Phase

6.5.1.4. Post-Acute Phase

The Post-Acute Phase begins on the 11th day after inoculation and ends on the 28th day after inoculation. During the Post-Acute Phase, parent/guardian will be instructed to monitor for and contact the study staff if their child has symptoms that are suggestive of a SAE or LRI. If the parent reports an SAE or LRI that may meet the study pause or stop criteria (Section 8.3), then an Illness Visit will be scheduled (Section 6.7).

6.5.1.5. Study Day 28 Visit

The Day 28 Visit should be conducted between 28 and 35 days following inoculation. Because the Post-Acute Phase ends as the 28th day following inoculation, only events through that time should be evaluated as having occurred during the Post-Acute Phase.

Table 7: Day 28 Visit Procedures – RSV-Seropositive Children

Day 28 Visit *(+7 Days)*		
Administrative Tasks		• Provide study compensation
Clinical Tasks		• Document the participant's interim history as reported from the parent/guardian from midnight of the 10th day through the 28th day following inoculation, including: ▪ visits to medical provider/hospitalizations ▪ medications and/or immunizations ▪ signs and symptoms of illness meeting SAE or LRI criteria • Address any concerns • Review safety data • Document findings
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to RSV
	Nasal Wash and/or Nasosorption SAM Strip	*Collect nasosorption strip for:* • RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash.
		Collect nasal wash for: • RSV antibody assays

6.5.2. RSV-Seronegative Infants and Children

6.5.2.1. Acute Phase: In-person Study Visit Days

An in-person study visit and clinical assessment performed by a healthcare professional will be completed during visits on Study Days 3, 5, 7, 10, 12, 14, 17 and 28 after inoculation with a visit window of ± 1 day. If an in-person visit is moved by ± 1 day, then the non-visit day contact is completed in place of the original interim visit date. The relevant CDC and JHU institutional guidance regarding in-person visits and use of personal proctective equipment will be followed. If a Stay at Home Order is initiated following inoculation, or if the child has a respiratory or febrile

illness, the parent may be asked to obtain the nasal swab and the clinical assessment may be done via a remote or in-person research visit. Transport of specimens from the home to the research laboratory will be arranged as needed.

Table 8: Acute Phase In-Person Visit Procedures – RSV-Seronegative Children

Days 3, 5, 7, 10, 12, 14, 17, and 28 In-person Visit Procedures *(each visit ± 1 days)*		
Clinical Tasks		• Document the participant's previous days' interim history as reported from the parent/guardian, include: ▪ medications and/or immunizations ▪ signs and symptoms of illness ▪ highest temperature reading, indicate temperature method • Perform focused clinical examination • Address any concerns • Review safety data • Document findings
Laboratory Tasks	**Nasal Wash or Swab** **Nasosorption SAM Strip**	*Collect nasal wash or swab for:* • RSV viral detection and quantification ***In addition to above, Day 28 only for the first 13 participants enrolled prior to March 2020****: Collect nasosoption strip and/or nasal wash for:* • RSV antibody assays
Follow-up Preparation		• Schedule non-visit day contact and follow-up, in-person visits ***Day 28 only****:* • Review SAE criteria with participants and how to contact study personnel during Post-Acute Phase • If Day 28 visit is conducted on or after seasonal enrollment pause: • Review plans for weekly contact during the RSV-season surveillance period (Section 6.6.2)

6.5.2.2. Acute Phase: Non-Visit Study Day Contacts

In the 28 days following inoculation, parental contact will be made on days that an in-person visit is not completed: Days 1, 2, 4, 6, 8, 9, 11, 13, 15, 16, and 18–27 (each visit ± 1 day). On non-visit days, study staff will contact the parent/guardian and will document the parent/guardian-provided temperatures and signs of illness. Participants with illness may have additional visits to assess the severity of the illness (Section 6.7).

Table 9: Acute Phase Non-Visit Contact Procedures – RSV-Seronegative Children

Days 1, 2, 4, 6, 8, 9, 11, 13, 15, 16, 18 , 19, 20, 21, 22, 23, 24, 25, 26 and 27 Contact Procedures *(each visit ± 1 day)*	
Clinical Tasks	• Document the participant's previous days' interim history as reported from the parent/guardian, include: ▪ medications and/or immunizations ▪ signs and symptoms of illness ▪ highest temperature reading, indicate temperature method • Address any concerns • Review safety data • Document findings
Follow-up Preparation	• Schedule an illness appointment if necessary

6.5.2.3. Study Day 29

There will be a non-visit contact on Day 29 to obtain interim history through midnight on the 28th day following inoculation. If the Day 28 Visit takes place on Day 29, it is not necessary to have an additional contact with the family on Day 29.

Table 10: Day 29 Non-Visit Procedures – RSV-Seronegative Children

Day 29 Non-Visit Procedures	
Clinical Tasks	• Document the participant's previous days' interim history as reported from the parent/guardian, include: ▪ medications and/or immunizations ▪ signs and symptoms of illness ▪ highest temperature reading, indicate temperature method • Address any concerns • Review safety data • Document findings
Follow-up Preparation	• Review SAE criteria with participants and how to contact study personnel during Post-Acute Phase

6.5.2.4. Post-Acute Phase

The Post-Acute Phase begins on the 29th day after inoculation and ends on the 56th day after inoculation. During the Post-Acute Phase, parent/guardian will be instructed to monitor for and contact the study staff if their infant or child has symptoms that are suggestive of a SAE. If the

parent reports an SAE that may meet the study pause or stop criteria (Section 8.3) then schedule an Illness Visit (Section 6.7).

6.5.2.5. Study Day 56 Visit

The Day 56 Visit should be conducted between 56 and 63 days following inoculation. Because the Post-Acute Phase ends on the 56th day following inoculation, only events through that time should be evaluated as having occurred during the Post-Acute Phase. If a Stay at Home Order is put in place, any required blood draws will be collected in-person as soon as possible after the Stay at Home Order is lifted.

Table 11: Day 56 Visit Procedures – RSV-Seronegative Children

Day 56 Visit *(+7 Days)*		
Administrative Tasks		• Provide first study compensation payment
Clinical Tasks		• Document the participant's interim history as reported from the parent/guardian from midnight of the 28th day through the 56th day following inoculation, including: ▪ visits to medical provider/hospitalizations ▪ medications and/or immunizations ▪ signs and symptoms of illness meeting SAE criteria • Address any concerns • Review safety data • Document findings
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to RSV
	Nasal Wash and/or Nasosorption SAM Strip	*Collect nasosorption strip and/or nasal wash for13 participants enrolled prior to March 2020:* • RSV antibody assays • Note: The nasosorption must be performed prior to the nasal wash
Follow-up Preparation		Review plans for weekly contact during the RSV Season Surveillance Period (Section 6.6.2). For participants enrolled prior to March 2020, if Day 56 Visit is conducted: • Prior to October 1 ▪ schedule Pre-RSV Season Visit (Section 6.6.1) • On or after October 1 ▪ Day 56 Visit will also be the Pre-RSV Season Visit ▪ Review plans for weekly contact during the RSV Season Surveillance Period (Section 6.6.2)

6.5.2.6. Period after Day 56 through Start of RSV Surveillance Phase

During this period, contact with the participant is not required. No clinical data will be recorded on CRFs or reported under this protocol except for data as outlined in Table 19 and in Section 7.2. For participants enrolled prior to March 2020, contact with the participant may have occurred for the Pre-RSV Season Study Visit described in Section 6.6.1.

6.6. RSV SURVEILLANCE SEASON (RSV-SERONEGATIVE SUBJECTS)

6.6.1. Pre-RSV Season Surveillance Study Visit Only for Participants Enrolled Prior to March 2020

For the 13 participants enrolled prior to March 2020, an in-person Pre-RSV Season Study Visit was completed between October 1- 31 for collection of a blood sample, nasal wash samples, and/or nasosorption strips for RSV antibody assays.The Pre-RSV Season Study Visit is not required if the Day 56 Visit is conducted on or after October 1; the samples collected at the Day 56 Visit are sufficient for the Pre-RSV Season Study Visit. If a Stay at Home Order is put in place, any required blood draws will be collected in-person as soon as possible after the Stay at Home Order is lifted.

Table 12: Pre-RSV Season Surveillance Study Visit Procedures: for 1st 13 participants

Pre-RSV Season Study Visit (October 1 through October 31 of enrollment year)		
Clinical Tasks		• Document findings related to study procedure
Laboratory Tasks	**Blood**	*Collect blood for 13 participants enrolled prior to March 2020:* • Serum antibodies to RSV
	Nasal Wash and/or Nasosorption SAM Strip	*Collect nasosorption strip and/or nasal wash for 13 participants enrolled prior to March 2020:* • RSV antibody assays • Note: The nasosorption must be performed prior to the nasal wash
Follow-up Preparation		• Review plans for weekly contact during the RSV Season Surveillance Period (see Section 6.6.2)

6.6.2. Weekly Contact for Surveillance during the RSV Season

Based on previous data regarding the seasonality of RSV in the Baltimore, MD, area (Appendix IV, Figure 6.6-1), JHU will seasonally pause enrollment and start the RSV Surveillance Phase of the study based on local and/or national (CDC NREVSS) surveillance for RSV positivity locally in the Baltimore, MD area, as detailed in the MOP. The surveillance for RSV-associated disease will be conducted between pause of enrollment and March 31 during the first RSV season following receipt of study product. For some RSV-seronegative participants, surveillance during the RSV season may overlap with the Acute and/or Post-Acute study phases. In this case, all evaluations required for the RSV season surveillance and the relevant phases of the study will be conducted.

During the RSV season following receipt of study product, participants enrolled in this study will be monitored for symptomatic, medically attended, RSV-like illnesses listed below via weekly telephone or email communication or an in-person or remote visit. (Rhinorrhea and cough need not meet the Appendix IV criteria if they are reported as the primary reason for seeking medical attention):

- Medically attended fever
- Medically attended URI
- Medically attended otitis media
- Medically attended LRI

An Illness Visit will be scheduled within 3 days of study staff notification of any of these events (Section 6.7). Relevant CDC and JHU institutional guidelines regarding in-person visits and use of personal protective equipment to ensure protection of subjects, families, and staff will be followed in the context of the SARS-CoV-2 pandemic. If a Stay at Home Order is initiated, the parent may be asked to obtain the nasal swab for the RSV Surveillance illness, and the clinical assessment may be completed via a remote or in-person research visit.

Table 13: RSV Season Surveillance Procedures

RSV Season Surveillance following inoculation	
Clinical Tasks	• Obtain interim history • Review safety data • Document findings
Follow-up Preparation	• Continue with weekly contacts through March 31 • Schedule an Illness Visit if warranted • Schedule the Post-RSV Season Study Visit (targeted April 1-April 30th, with allowable window through September 30th if Stay at Home Orders require that this study visit be delayed)

6.6.3. Post-RSV Season Surveillance Study Visit

The Post-RSV Season Surveillance Visit will occur in the calendar year following receipt of study product, ideally between April 1 and April 30, with an allowable window through September 30th if Stay at Home Orders require that this study visit be delayed.

Table 14: Post-RSV Seasonal Surveillance Study Visit Procedures

Post-RSV Season Study Visit		
Administrative Tasks		• Provide study surveillance compensation
Clinical Tasks		• Document findings related to study procedures
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to RSV
	Nasal Wash and/or Nasosorption SAM Strip	*Collect nasosorption strip and/or nasal wash for 13 participants enrolled prior to March 2020:* • RSV antibody assays • Note: The nasosorption must be performed prior to the nasal wash

6.7. ILLNESS VISIT

The timeframe after staff notification in which the Illness Visit must occur depends on the study phase and the grading of the fever and respiratory symptoms per Section 8.2 and Table 20. If the Illness Visit occurs on a day concurrent with an in-person or remote study visit, a single nasal wash or swab collection is required and adventitious agent testing will be requested. All symptoms will be followed until resolution or deemed stable or chronic by appropriate medical personnel (e.g., medical doctor or nurse practitioner). Illness Visits may occur during any of the study phases. The relevant CDC and JHU institutional guidelines regarding in-person visits and use of personal protective equipment to ensure protection of subjects, families, and staff will be followed in the context of the SARS-CoV-2 pandemic. For illness visits, the parent may be asked to obtain the nasal swab. During remote research visits parents be asked to measure the child's temperature, heart rate, and respiratory rate. As otitis media and LRI cannot be detected by remote study visit, medical records from provider visits will be requested to document these events if a remote visit is completed or an in-person visit cannot be completed in a timely fashion.

If the acute or post-acute phases overlap the surveillance period (between the date of seasonal pause of enrollment and March 31), then the timelines for the acute and post-acute phases will be used.

Table 15: Illness Visit Timeframe

Illness Visit Timeframe			
Phase	**Symptoms**	**Grade**	**Visit Timeframe**
Acute	Fever, otitis media or URI	1	Within 3 days
Acute	Fever, otitis media or URI	≥ 2	Within 2 days
Acute; Post-acute seropositive*	LRI	Any	Within 1 day
Post-Acute	SAE that meets study pause or stop criteria (Section 8.3)	≥ 2	Within 3 days
RSV Season Surveillance	Medically attended fever, otitis media, URI or LRI	≥ 2	Within 3 days

*Days 0-28 for RSV-seropositive and RSV-seronegative children

Table 16: Illness Visit Procedures

Illness Visit Procedures		
Administrative Tasks		• Complete Adventitious Agent Assay Request for reverse transcription polymerase chain reaction (rRT-PCR) on nasal swab for adventitious agents • Complete medical record release if needed
Clinical Tasks		• Document the participant's interim history as reported from the parent/guardian, include: ▪ medications and/or immunizations ▪ signs and symptoms of illness ▪ highest temperature reading, indicate temperature method • Perform focused clinical examination • Address any concerns • Review safety data • Document findings
Laboratory Tasks	**Nasal Wash or Swab**	*Collect nasal wash or swab for:* • Viral detection and quantification
Follow-up Preparation		• Schedule follow-up as appropriate

6.8. EARLY DISCONTINUATION STUDY VISIT

In the event that a child is unable to continue participation in the study, parent/guardian will be encouraged to allow the participant to complete safety monitoring through Day 28 for RSV-seropositive participants and through Day 56 for RSV-seronegative participants. Every effort should be made to schedule a final Early Discontinuation Visit.

Table 17: Early Discontinuation Procedures

Early Discontinuation		
Administrative Tasks		• Record data on CRF
Clinical Tasks		• Document interim history • Address any concerns • Review safety data • Encourage parent/guardian to allow the participant to complete safety monitoring through Day 28 for RSV-seropositive participants and through Day 56 for RSV-seronegative participants • Document reason for early discontinuation
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to RSV
	Nasal Wash or Swab and/or Nasosorption SAM Strip	*If Early Discontinuation Visit is within 28 days of inoculation for RSV-seropositive participants or 56 days for 13 RSV-seronegative participants enrolled prior to March 2020 (Appendix II), collect nasosorption strip and/or nasal wash for:* • RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash or swab.
		Collect nasal wash or swab for: • Viral detection and quantification

6.9. LABORATORY PROCEDURES

6.9.1. Specimen Collection

Specimens will be collected for this study as indicated in the Schedule of Events (Appendix II and Appendix III). Further information on collection of blood, nasosorption and nasal wash or swab specimens will also be provided in the MOP.

6.9.2. Virus Detection

Specimens for quantification of vaccine virus shedding by viral culture and/or rRT-PCR will be obtained by nasal wash or swab once before and approximately 6 times after inoculation for RSV-seropositive participants and approximately 8 times after inoculation RSV-seronegative participants as shown in Appendix II & III. These specimens may also be tested for adventitious respiratory viruses if needed. Additional nasal wash or swab specimens for detection of RSV and adventitious respiratory viruses by culture and/or rRT-PCR will also be obtained from participants who meet illness criteria during the initial phase (Day 0 through Day 28 for RSV-seropositive participants; Day 0 through Day 56 for RSV-seronegative participants) as well as during the RSV Season Surveillance Period (from seasonal pause in enrollment – March 31) for all RSV-seronegative participants. If a participant becomes ill, then up to 10 additional nasal washes or swabs may be obtained to exclude infection with an adventitious virus. Laboratory testing will be performed by personnel who are not involved with clinical assessment to maintain the blinding status.

6.9.3. Specimen Collection

For measurement of RSV serum antibodies, serum specimens will be obtained in RSV-seropositive children not more than 56 days prior to inoculation and on Day 28 (+7) post-inoculation; and in RSV-seronegative infants and children not more than 42 days prior to inoculation and on Day 56 (+7) post inoculation. In addition, pre-RSV season serum specimens will be collected for the the first 13 RSV-seronegative participants enrolled prior to March 2020, and post-RSV season serum specimens will be collected in all RSV-seronegative infants and children. These samples will be used to determine whether a four-fold or greater rise in antibody titer has occurred during the RSV season, which would signify infection with wt RSV. This will allow comparison of the rate and severity of significant RSV illness following infection with wt virus, as well as comparison of the antibody responses, in vaccine and placebo recipients.

Specimens will be obtained by venipuncture, finger-stick, or heel-stick. No more than 5 mL of blood will be drawn at each blood draw visit. A maximum of 10 mL of blood will be taken from RSV-seropositive participants for study purposes. A maximum of 15 mL of blood will be taken from RSV-seronegative participants for study purposes from screening through surveillance.

For RSV-seropositive participants, nasal wash specimens and/or nasosorption strips for measurement of secretory immunity will be obtained before inoculation and 28 days after inoculation. For 13 RSV-seronegative participants enrolled prior to March 2020, nasal wash specimens and/or nasosorption strips for measurement of secretory immunity will be obtained before inoculation, 28 and 56 days after inoculation, and at the pre- and post-RSV season surveillance visits. These specimens may be generated from the same nasal wash obtained for viral culture, but specimen processing is different as described in the MOP.

6.9.4. Specimen Preparation, Testing, Storage, and Shipping

All specimens collected for this study will be labeled, transported, processed, tested, stored and/or shipped in accordance with the MOP and the JHU CIR SOPs. The frequency of specimen collection and testing will be directed by the Schedule of Events (Appendix II and Appendix III).

6.9.5. Research Laboratories

Quantitation of the amount of vaccine virus shed, assays to measure immune responses before and after inoculation, and assessment of nasal washes or swabs for adventitious viral agents will be performed at the CIR. Nasosorption assays will be performed at the LID, NIAID. Cytokine/chemokine assays may also be performed on nasal washes or swabs from participants infected with vaccine virus if sufficient material is available. Selected specimens may be sent to LID, NIAID for confirmatory testing.

6.9.6. Plan for Use and Storage of Biological Samples

All specimens collected as part of this study may, with the parent/guardian's permission, be stored for future research as part of CIR's approved biospecimen repository for vaccine research. These samples and data may be used for future screening for respiratory virus vaccine studies and to learn more about RSV infection and other diseases. The parent/guardian or child will not own the blood specimens, nasal specimens, or data after it is given to the study. No financial benefit will be provided to the parent/guardian or child from any product or idea created by the investigators using the data or materials. These samples will not be sold or used to make commercial products. Genetic tests will not be performed on these samples unless a separate informed consent is obtained.

Samples stored in the repository will be labeled with the study identification number of the participants that, by themselves, cannot identify study participants but are linkable to the study databases generated by the main study. The repository database will contain only the study participants' numbers. A master log linking the study participants' identification numbers to their names is maintained by the study's clinical staff in a password-protected computer system with limited access to authorized research team members and will not be shared with the laboratory. Study participants, or their parents/guardians, may withdraw consent for future testing of their specimens at any time.

6.9.7. Biohazard Containment

As the transmission of blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel. The procedures for obtaining, shipping, and handling of all specimens for this study will follow the current recommendation of the Centers for Disease Control and Prevention (CDC) and the NIH. All infectious specimens will be transported using packaging mandated in Title 42 of the Code of Federal Regulations (CFR), Part 72 (42 CFR 72) and in accordance with individual carrier guidelines (e.g., Federal Express).

7. SAFETY ASSESSMENT, MONITORING, AND REPORTING

Participant safety will be carefully assessed, monitored, and reported at multiple levels throughout this study. Sections 7.1-7.4 describe safety-related roles, responsibilities, and procedures. The safety monitoring roles of the NIAID Intramural Data Safety and Monitoring Board (DSMB) are briefly referenced in Section 7.1.4 and described in detail in Section 9.4.2.

7.1. SAFETY-RELATED ROLES AND RESPONSIBILITIES

7.1.1. Principal Investigator

The PI is responsible for continuous monitoring of the CIR's study participants and for alerting the Scientific Investigators if unexpected concerns arise. Trained study staff will record safety-related data on CRFs as indicated in Section 7.2. The PI is also responsible for prompt reporting to the IRBs and other applicable review bodies of any unanticipated problems (UPs) involving risks to participants or others.

7.1.2. Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plan (SRCP) will be developed for the protocol. The SRCP is an internal communications document between the PI and the IND sponsor (OCRPRO) Clinical Safety Office, 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.

7.1.3. 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 a Safety Review and Communications Plan (SRCP).

7.1.4. Data Safety Monitoring Board

An independent DSMB will monitor participant safety through routine and as-needed reviews of study data. Refer to Section 9.4.2 for more information on the composition and role of the DSMB in the monitoring of this study.

7.1.5. Sponsor Reporting

A SUSAR is a suspected adverse reaction that is both serious and unexpected, as defined in 21 CFR 312.32. Events that meet SUSAR criteria will be reported promptly by the PI to the CSO and will be evaluated by the PI and CSO as a possible protocol Stop trigger. SUSARs will be reported to the FDA and all participating investigators as IND Safety Reports. The IND Sponsor will also submit a

brief report of the progress of the investigation to the FDA on an annual basis as defined in 21 CFR 312.33.

Following notification from the PI, OCRPRO, as the IND Sponsor, will report all SAEs to the FDA within the required timelines. Fatal and life-threatening events will be reported within 7 calendar days of the sponsor's awareness and all other SAEs will be reported within 15 calendar days of the sponsor's awareness.

7.2. SAFETY-RELATED RECORDING ON CRFS

AEs that occur during protocol-specified AE reporting periods (see Table 18 and Table 19) following inoculation of study product should be considered AEs. The current section outlines which events should be collected on source documents and which should be recorded on CRFs for inclusion in the database.

AEs may be observed by the study investigator or designee, elicited or volunteered from the parent/guardian or participant, or captured on participant's temperature cards. Assessment of safety will include clinical observation and monitoring of laboratory parameters as necessary. Follow-up measures such as history, clinical assessment, and laboratory testing and/or treatment may be necessary if a participant experiences an AE. Details of AEs will be properly documented on the source documents, recorded on CRFs, and reported to LID investigators and the DSMB in separate semi-annual and annual reports. AEs will be provided to the IRB as defined by the IRB policy.

This study has several periods of AE observation that have different AE CRF recording requirements. In addition, for RSV-seronegative participants, there may be a period when no AEs are recorded on CRFs if the Day 56 Visit occurs in advance of the start of the RSV Season Surveillance Period (date of seasonal enrollment pause). The AEs (solicited and unsolicited; and SAEs) to be recorded on CRFs and the study phase and the calendar dates during which they are to be reported are defined in Table 18 and Table 19.

Concomitant medications and AEs identified during the acute and post-acute phase will be recorded on CRFs. Medically-attended febrile, respiratory or OM during RSV season surveillance and concomitant medications related to SAEs identified during the RSV season surveillance period will be recorded on CRFs. AEs will be recorded as signs, symptoms, laboratory test results, and diagnoses, as shown in Table 18 and Table 19. In addition, the following concomitant medications will be recorded on CRFs regardless of whether the identified AE is recorded on the CRF:

- All cough and cold remedies including decongestants, cough suppressants, expectorants
- All nasal sprays (except saline spray)
- All antihistamines
- All antipyretics
- All prescription medications

Table 18: AE CRF Recording Requirements for RSV-Seropositive Participants

Study Phase at the Time of Event Onset	Calendar Date	AEs to Record on CRFs	Concomitant Medications to Record on CRFs
Day 0 through midnight of 10th day following inoculation (Acute Phase)	ANY	• All SAEs • All solicited AEs that meet Appendix IV, Table 32 criteria • All unsolicited AEs (Grades 1 to 4) • Exception of diaper rash, teething pain, and spitting up unless treated with prescription medication or non-prescription medications with antipyretic properties	Record these medications on the CRFs regardless of whether the related event is recorded on the CRFs: • All cough and cold remedies including decongestants, cough suppressants, expectorants • All nasal sprays (except saline spray) • All antihistamines • All antipyretics • All prescription medications For SAEs and LRIs: • All medications related to the recorded event
From the 11th day after inoculation to the 28th day after inoculation (Post-Acute Phase)	ANY	• All SAEs and LRIs	• All medications related to the recorded event
Throughout study	ANY	• Unresolved AEs or SAEs with onset date from Day 0 to 28th day after inoculation	• All medications related to the recorded event

Table 19: AE CRF Recording Requirements for RSV-Seronegative Participants

Study Phase at the Time of Event Onset	Calendar Date	AEs to Record on CRFs	Concomitant Medications to Record on CRFs
Day 0 through midnight of 28th day following inoculation (Acute Phase)	ANY	• All SAEs • All solicited AEs that meet Appendix IV, Table 32 criteria • All unsolicited AEs (Grades 1 to 4) • Exception of diaper rash, teething pain, and spitting up, unless treated with prescription medication or non-prescription medications with antipyretic properties	Record these medications on the CRFs regardless of whether the related event is recorded on the CRFs: • All cough and cold remedies including decongestants, cough suppressants, expectorants • All nasal sprays (except saline spray) • All antihistamines • All antipyretics • All prescription medications For SAEs and LRIs: • All medications related to the recorded event
From the 29th day after inoculation to the 56th day after inoculation (Post-Acute Phase)	ANY	• All SAEs	• All medications related to the recorded event
From Day 56 Visit through start of RSV Season Surveillance Period	Up to date of seasonal pause in enrollment in year of inoculation	• Grade ≥ 3 AEs or SAEs that is deemed related to Pre-RSV Season Study Visit procedures	• All medications related to the recorded event
RSV Season Surveillance Period	Date of seasonal pause in enrollment to March 31	• Fevers, LRIs, URIs, and/or otitis media that are medically attended • All SAEs Note: These events do not need to meet the Appendix IV, Table 32 criteria	For SAEs and LRIs (all grades): • All medications related to the recorded event Medications related to recorded medically attended illness should be documented in source notes
Post-RSV Season	Ideally April 1 to April 30; allowable through Sept 30th in the year after the inoculation	• Grade ≥ 3 AEs or SAEs that are deemed related to Post-RSV Season Study Visit procedures	• All medications related to the recorded event

Table 19: AE CRF Recording Requirements for RSV-Seronegative Participants

Study Phase at the Time of Event Onset	Calendar Date	AEs to Record on CRFs	Concomitant Medications to Record on CRFs
Throughout study	ANY	• Unresolved AEs or SAEs with onset from Day 0 to midnight on the 28th day after inoculation • Unresolved SAEs with onset prior to the 56th day following inoculation • Unresolved SAEs with onset during RSV Surveillance Period or related to the Pre- or Post-RSV Season Study Visit	• All medications related to the recorded event

7.3. SERIOUS ADVERSE EVENT REPORTING

Serious Adverse Events (SAEs) are described in Section 8.1.2. All SAEs will be reviewed by a study physician, recorded on the Safety Expedited Report Form (SERF) and followed through to resolution. SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the participant 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. In addition to the SAE Reporting Category identified below, other AEs that must be reported in an expedited manner are LRIs as defined in Appendix IV if they occur during study Days 0 to 28 in group 1 and group 2.

Deaths and immediately life-threatening SAEs will be reported within 1 business day after the site becomes aware of the event. All other SAEs will be reported within 3 business days of site awareness. SAEs will be reported either by fax or e-mail to all of the following:

- Sponsor: Office of Clinical Research Policy and Regulatory Operations, NIAID, NIH: Clinical Safety Office (CSO): Phone: 301-846-5301, Fax: 301-846-6224, rchspsafety@mail.nih.gov
- DSMB Executive Secretary: Phone 301-846-5301, Fax: 301-846-6224, niaiddsmbia@niaid.nih.gov
- LID, NIAID: Ursula Buchholz, PhD, NIAID/NIH, 301-594-1533, Fax: 301-480-1268, Email: ubuchholz@niaid.nih.gov

SAEs will also be reported to Western Copernicus Group Institutional Review Board (WCGIRB contact information below) based on its reporting requirements:

- Western Copernicus Group Institutional Review Board (WCG IRB), 1019 39th Ave SE, Puyallup, WA 98374, Phone: 1-855-818-2289

7.4. REPORTING OF UNANTICIPATED PROBLEMS TO OFFICE OF CLINICAL RESEARCH POLICY AND REGULATORY OPERATIONS (OCRPRO)

An unanticipated problem (UP) is defined as any event, incident, experience, or outcome that is:

1. Unexpected in terms of nature, severity, or frequency in relation to
   a. The research procedures that are described in the IRB-approved research protocol and informed consent or other study documents; and
   b. The characteristics of the participant population being studied; and
2. Related to participation in the research; and
3. Places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. Per the investigational new drug (IND) sponsor, an AE with a serious outcome will be considered increased risk.

UPs must be reported to the local IRB per their requirements. Non-Serious AEs that are UPs must also be reported to the sponsor CSO. Submit the local IRB UP report form to the CSO at the following address no later than 7 calendar days of the PI awareness of the event.

SPONSOR CLINICAL SAFETY OFFICE 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

UPs that are not AEs (UPnonAE) are not routinely reported to the CSO. However, an UPnonAE that may, in the opinion of the investigator, involve risk to the participant, affect others in the research study, or significantly impact the integrity of research data would be considered a non-serious UP and would be reported to the CSO. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

8. PARTICIPANT MANAGEMENT

8.1. MANAGEMENT OF ADVERSE EVENTS

An AE is any untoward medical occurrence in a participant administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the subject's participation in the research, whether or not related to the subject's participation in the research. This includes exacerbation of pre-existing conditions and intercurrent illnesses.

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.

All AEs identified in this study will be source documented, consistent with the policies and procedures referenced in Section 10. Among other details, source documentation will include the severity of each event (graded as described in Sections 8.2.1 and 8.2.2 and its relationship to study product, assessed by the MD or NP according to the following categories and definitions:

Related — There is a reasonable possibility that the adverse event may be related to the study drug.

Not related — There is not a reasonable possibility that the adverse event may be related to the study drug.

There are two categories of AEs specific to CIR 330: solicited and unsolicited. Solicited AEs are described in Section 8.1.1. Unsolicited AEs are all other AEs. However, for infants and children, the following common events will not be recorded as AEs unless a prescribed concomitant medication is used to treat them: non-infectious rashes, teething pain, and spitting up. The use of medications will only be captured in the event of an AE or pre-existing condition.

Serious Adverse Events (SAEs) are described in Section 8.1.2.

8.1.1. Solicited Adverse Events

Solicited AEs are predefined AEs that can occur after study product administration, may be expected to occur if the study product is insufficiently attenuated, and have protocol-specific criteria for reporting.

Solicited AEs, whether identified by a parent/guardian or clinician, are only recorded on CRFs if they meet the definitions per Appendix IV, Table 32 Individual symptoms listed in the "events" column that fail to meet the criteria in the "definition" column in Appendix IV, Table 32 are recorded in source documents but are not recorded on the CRFs. During the Acute Phase of this study, Days 0 through 10 for RSV-seropositive participants and Days 0 through 28 for RSV-seronegative participants, solicited AEs meeting the criteria for reporting will be recorded on CRFs, assigned a severity grade (Section 8.2), and assessed for relationship to study product (see Section 8.1). Solicited AEs are defined in Appendix IV and include the following:

1. Fever
2. URI
    a. Rhinorrhea,
    b. Pharyngitis,
    c. Cough without LRI, or
    d. Hoarseness
3. Otitis Media
4. LRI
    a. Wheezing,
    b. Pneumonia,
    c. Laryngotracheobronchitis (croup),
    d. Rhonchi, or
    e. Rales

Solicited AEs Elicited by History Unconfirmed by Clinical Assessment

With the exception of fever, solicited AEs reported by parents/guardians are NOT recorded on CRFs if a clinical assessment done on the day of the event(s) does not/did not confirm their presence. For example, if a parent/guardian reports rhinorrhea on the day of visit, and there is/was no rhinorrhea upon exam, then the participant is considered to not have rhinorrhea that day

If the parent/guardian report of a fever meets the "definition" column criteria in Appendix IV on a day on which there was a clinical assessment, the fever will be recorded on CRFs regardless of whether the clinical assessment confirmed its presence.

Events elicited by parent/guardian history for days on which there was no clinical exam will be:

1) Recorded on the CRFs as AEs if the parent/guardian description meets the "definition" column criteria in Appendix IV
2) Recorded only on the source document, and NOT on the CRF, if the parent/guardian description fails to meet the "definition" column criteria in Appendix IV. For example, both rhinorrhea and cough must each occur on 2 consecutive days to meet the definition required for reporting per Appendix IV

8.1.2. Serious Adverse Events

A SAE is an AE, whether considered related to the study product or not, that meets one or more of the following criteria:

1. Results in death during the period of protocol-defined surveillance.
2. Is life threatening: defined as an event in which the participant was at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death were it more severe.
3. Requires inpatient hospitalization (or prolongation of existing hospitalization): defined as at least an overnight stay in the hospital or emergency ward for treatment that would have been inappropriate if administered in the outpatient setting.
4. Results in a persistent or significant disability/incapacity.
5. Is a congenital anomaly or birth defect.
6. Is an important medical event that may not be immediately life threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the outcomes listed above.

8.1.3. Unexpected Adverse Event

An AE is unexpected if it is not listed in the IB or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed.

It is the responsibility of the IND Sponsor to make this determination.

8.2. GRADING THE SEVERITY 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). All AEs and fever will be graded using the protocol-defined grading system outlined in Table 20 and Table 21.

8.2.1. AE Grading

Table 20: Grading for Adverse Events

Severity	Defined
Grade 1 Mild	No medical intervention required; may include over-the-counter medications managed by the participant or caregiver for treatment of symptoms
Grade 2 Moderate	Moderate symptoms, i.e., symptoms interfering to some degree with usual activities. In most cases, symptoms severe enough to necessitate a medical care visit would likely meet this criterion; however, if medical care is sought and symptoms are assessed as only mild, the event may remain Grade 1. If prescription medication is used or recommended for symptoms, the event automatically moves to at least Grade 2.
Grade 3 Severe	Prolonged medical intervention and/or hospitalization required
Grade 4 Life threatening	Illness requiring hospitalization with intensive care
Grade 5 Death	Event resulting in fatal outcome to the participant

8.2.2. Fever Grading

Table 21: Grading for Fever

Severity	Defined
Grade 1	≥100.4ºF but ≤101.4ºF
Grade 2	≥101.5ºF but ≤102.4ºF
Grade 3	≥102.5ºF but ≤104.8ºF
Grade 4	≥104.9ºF

Applies to any modality of temperature measurement

8.3. PAUSING AND STOPPING RULES

If any of the following occur in a participant during the specified period after receiving the study product, additional enrollment/inoculations will be temporarily suspended (paused) (Table 22).

Table 22: Pausing Rules

Specified Phase	Event	Reporting
Acute and Post-Acute Days 0 through 28 following inoculation for RSV-seropositive participants and Days 0 through 56 following inoculation for RSV-seronegative participants	An SAE that cannot be attributed to an etiology or cannot be attributed to a cause unrelated to the study product.	A description of the vaccine-associated AE(s) or safety issue must be reported by the PI or study staff, within one business day of the PI's awareness, to the CSO and the DSMB by fax or email and the IRB, as applicable
Acute Only Days 0 through 10 following inoculation for RSV-seropositive participants and Days 0 through 28 following inoculation for RSV-seronegative participants	An LRI per Appendix IV, OR A fever of Grade 4 OR Any Grade 3 or above solicited AE (other than fever)	
Post-Acute Days 11 through 28 following inoculation for RSV-seropositive participants	An LRI per Appendix IV	A description of the vaccine-associated AE(s) or safety issue must be reported by the PI or study staff, within three business days of the PI's awareness, to the CSO and the DSMB by fax or email

The PI and/or CSO will determine if the study should be paused or stopped. Pausing the study requires suspension of enrollment until a decision is made whether or not to continue enrollment and study vaccine administration or permanently stop the study.

The IND sponsor (OCRPRO) will determine if the FDA needs to be notified. The FDA may stop the study at any time following review of any safety concerns. The DSMB and IND sponsor will be informed and receive pertinent data of the event by the PI. Follow-up visits for participants already inoculated will continue as outlined.

If the event is determined to have occurred in a participant who received active agent (vaccine), and the event meets one of the following pausing rule criteria, then the event will be reviewed by the DSMB prior to resuming enrollment or permanently stopping the study.

1. One or more participants experience an SAE that cannot be attributed to an etiology or cannot be attributed to a cause unrelated to the study vaccine, OR
2. One or more participants develop LRI associated with shedding of vaccine virus at the time of the LRI (even if another pathogen is identified, unless the RSV is confirmed to be wt RSV), OR
3. One or more participants develop LRI that is not explained by a diagnosis unrelated to the vaccine virus, OR
4. One or more participants experiences a Grade 4 fever or any Grade 3 or Grade 4 solicited AE other than fever associated with shedding of vaccine virus, OR
5. Any pattern of research laboratory values or clinical symptoms is observed that the study team considers a significant safety issue for participants.

The IND Sponsor, in collaboration with the PI, the DSMB and the Safety Review Team will determine if it is safe to resume the study or if the study needs to be stopped. The PI will notify the

IRB of the decision on whether the study will be resumed or stopped. In the event of an SAE, the study may be resumed if it can be demonstrated to the DSMB that there is no proven causal relationship with the vaccine.

9. STATISTICAL CONSIDERATIONS

9.1. GENERAL DESIGN ISSUES

9.1.1. General Design

The goal of this Phase I, double-blinded, randomized, placebo-controlled vaccine trial is to assess the safety, infectivity, and immunogenicity of the RSV 1620/ΔNS1 and RSV 1620/F1/G2/ΔNS1 vaccine candidates in RSV-seropositive and RSV-seronegative pediatric participants and to assess whether these two vaccines are good candidates to move forward into a Phase IB study. In Group 1, approximately 25 RSV-seropositive infants and children will be enrolled in the study and will receive RSV 6120/ΔNS1 ($10^{6.0}$ PFU), RSV 6120/F1/G2/ΔNS1 ($10^{5.8}$ PFU), or placebo in a 2:2:1 ratio (10:10:5). In Group 2, approximately 35-50 RSV-seronegative infants and children will be enrolled in the study and will receive RSV 6120/ΔNS1 (10^{5} PFU), RSV 6120/F1/G2/ΔNS1 (10^{5} PFU), or placebo in a 2:2:1 ratio (20:20:10).

9.1.2. Description of the Statistical Methods to be Employed

This study, like other phase I studies, is exploratory, rather than confirmatory; its purpose is to assess frequencies of AEs and patterns of immune responses. Descriptive approaches will be used to meet the protocol objectives as stated in Section 2 of this protocol, as well as formal statistical tests as outlined in Section 9.5.

The Study Team is interested in whether the vaccines are better than placebo with respect to infectivity and immunogenicity; thus, the statistical comparisons between vaccine and placebo groups will consist of one-tailed tests. The study will not be powered for the modest difference expected between the two vaccines; thus, the groups receiving vaccine will be compared by means of descriptive analyses. Both vaccines may be used in a subsequent trial aimed at assessing efficacy, provided that this is not counter indicated by safety results.

9.2. OUTCOME MEASURES

9.2.1. Primary Outcome Measures

Safety: Types and grades of study product-related:

- Solicited AEs as defined in Appendix IV from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative participants
- Unsolicited AEs from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative participants

- SAE (Section 8.1.2) from Study Days 0-28 for RSV-seropositive and Study Days 0-56 for RSV-seronegative participants

Infectivity:
- Infection with RSV as defined as:
    1) vaccine virus identified in a nasal wash or swab from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative (a binary outcome based on nasal washes or swabs done throughout the study period; Day 0 nasal wash or swab will be counted as baseline); or
    2) ≥4-fold rise in serum RSV neutralizing antibody titer or serum RSV F (IgG) titer from Study Days 0-28 for RSV-seropositive and Study Days 0-56 for RSV-seronegative participants
- Peak titer of vaccine virus shed from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative participants
- Duration of virus shedding in nasal washes or swabs as determined by a) culture and/or b) rRT-PCR from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative participants

Immunogenicity:
- ≥4-fold rise in RSV-neutralizing antibody titer from study entry to Study Day 28 for RSV-seropositive and from study entry to Study Day 56 for RSV-seronegative participants
- ≥4-fold rise in IgG antibody responses to RSV F glycoprotein (by ELISA) from study entry to Study Day 28 for RSV-seropositive and from study entry and Study Day 56 for RSV-seronegative participants

9.2.2. Secondary Outcome Measures

- The frequency and severity of symptomatic, medically attended respiratory and febrile illness in the RSV-seronegative (group 2) vaccine and placebo recipients who experience natural infection with wt RSV during the RSV season.
- The antibody responses in the RSV-seronegative vaccine and placebo recipients who experience natural infection with wt RSV during the RSV season.
- Mucosal antibody titers to vaccine, in nasal wash or nasosorption samples in Group 1 and the first 13 Group 2 participants enrolled prior to March 2020.

9.3. SAMPLE SIZE AND ACCRUAL

9.3.1. Sample Size and Randomization

In group 1, approximately 25 RSV-seropositive infants and children will be enrolled in the study and will receive RSV 6120/ΔNS1 ($10^{6.0}$ PFU), RSV 6120/F1/G2/ΔNS1 ($10^{5.8}$ PFU), or placebo in a 2:2:1 ratio (10:10:5), and in Group 2, approximately 35-50 RSV-seronegative infants and children will be enrolled in the study and will receive RSV 6120/ΔNS1 (10^5 PFU), RSV 6120/F1/G2/ΔNS1 (10^5 PFU), or placebo in a 2:2:1 ratio (20:20:10). In total, approximately 30 vaccine recipients per vaccine arm and 15 placebo recipients will provide data for the primary objectives. The sample size was chosen based upon past experience with phase I evaluation of other live-attenuated respiratory virus candidate vaccines (29, 30, 37). The 2:2:1 randomization ratio will be used to maximize the

information obtained regarding the response of children to the RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 vaccines. In the event that a participant is discontinued early from the study the participant will not be replaced.

Block randomization, in groups of five, will be used to ensure that the 2:2:1 ratio of treated to control participants will be maintained across time. All enrolled children will be included in safety analyses.

Given the small sample size, the study will have limitations with respect to detecting AEs and estimating the rates of such events in the population represented by the study sample.

The following calculations focus on the assessment of the tolerability of the vaccine and, in particular, occurrence of LRI, which occurs very infrequently in children who participate in these types of studies but would be considered a sentinel safety event if observed in children infected with vaccine virus.

Table 23 shows the probability of observing 0 events of LRI within the sample of 20 seronegative vaccinees, as well as the probability of observing 1 or more events or 2 or more events, under a range of assumptions concerning the true rate of such events in the participant population represented by this sample. From this table, it can be seen that if the true proportion of LRI (or other AE) is at least 10%, there is a 61% chance of observing 2 or more events in a group of size 20, and an 88% chance of observing at least a single event.

Table 23: The Probability of Observing LRI Events in Seronegative Vaccine Recipients

	N = 20		
True underlying probability of LRI or AEs	Pr (0 events)	Pr (1+ events)	Pr (2+ events)
.01	0.82	0.18	0.02
.03	0.54	0.46	0.12
.05	0.36	0.64	0.26
.1	0.12	0.88	0.61
.15	0.04	0.96	0.82

Table 24 presents 90% confidence intervals (CIs) around potential rates of LRI or AEs that might be observed in the sample of 20 vaccine recipients. The CIs around similar rates in a sample of 10 placebo recipients are also presented. Note that if no LRI or AEs are detected among the 20 vaccine recipients, we are 90% confident that the true probability of AEs in the population from which the sample is drawn is between 0 and 14%.

Table 24: Percent of Participants Experiencing LRI or AEs with Exact 90% CI

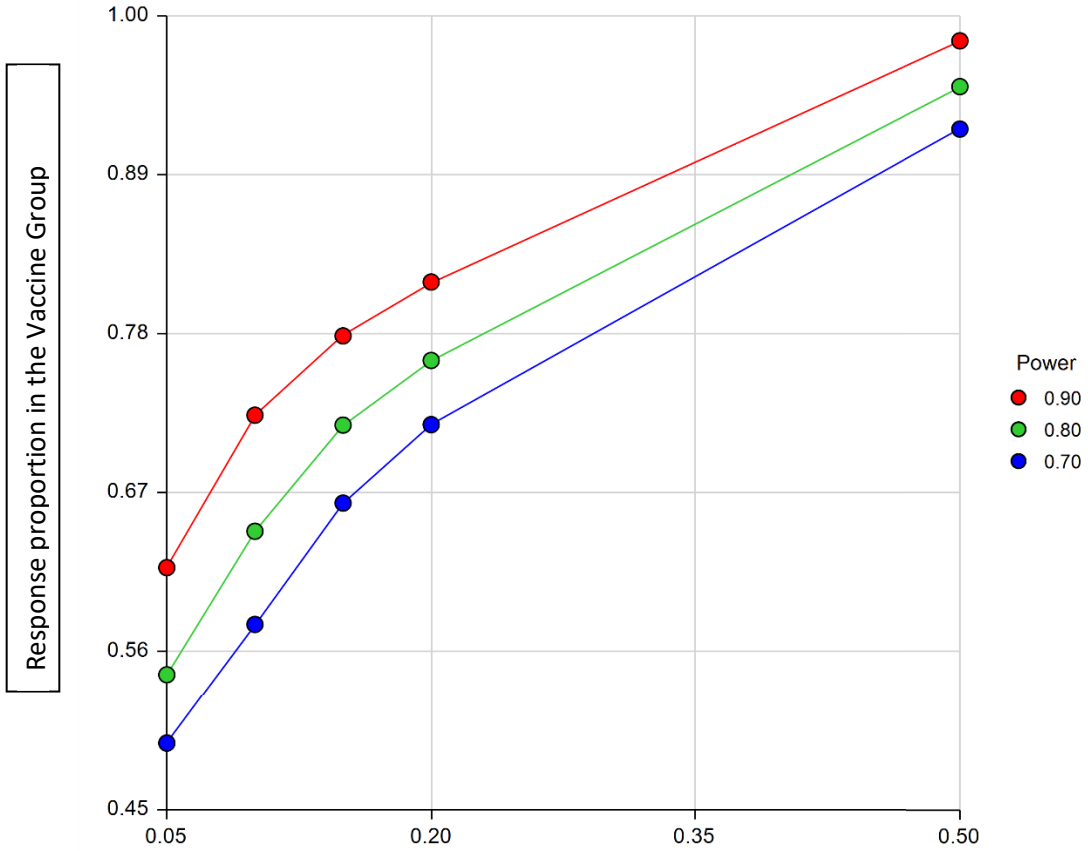
N	% LRI or AEs	90% CI
10	0%	0% -- 26%
20	0%	0% -- 14%
10	10%	1% -- 39%
20	10%	2% -- 28%
10	20%	4% -- 51%
20	20%	7% -- 40%
10	30%	9% -- 61%
20	30%	14% -- 51%

Group sample sizes of 20 in each vaccinated group and 10 in the placebo group would achieve 80% power to detect a difference between each vaccine group and the placebo group proportions of about 0.45. The test statistic used is the one-sided Fisher's exact test. The alpha level of the test was targeted at 0.05. Table 25 presents examples of true group differences which can be detected with 80% power, and Figure 9.3-1 graphically shows power curves for 90% power, 80% power, and 70% power given the sample sizes.

Table 25: Magnitude of Difference in Responses Detectable with 80% Power

Response Proportion in the Placebo Group	Response Proportion in the Vaccinated Group	Difference
0.05	0.52	0.47
0.1	0.59	0.49
0.15	0.65	0.50
0.2	0.71	0.51
0.5	0.95	0.45

Figure 9.3-1: Power Curves for Assessment of Vaccine Virus Shedding in RSV seronegative Vaccinees



(Alpha=.05; N Vaccine=20; N Placebo=10; 1-Sided Exact Test)

With a sample size of 20 seronegative vaccine recipients (for each vaccine), the 90% CI around a sample mean peak titer of 2.5 $\log_{10}$, with a SD of 1.5 (from CIR 291/IMPAACT 2000) is (1.92, 3.08). This ensures with 90% confidence that the true population mean peak titer is between 1.92 and 3.08 $\log_{10}$, and with 95% confidence that the true population mean is not lower than 1.92 $\log_{10}$.

With the same sample size of 20, the 90% CI around a proportion of 18/20 (90%) vaccine recipients who shed vaccine virus is (72%-98%). For a proportion of 19/20 (95%) the 90% CI is (78%-99.7%), and for 20/20 (100%) the 90% CI is (86%-100%). For the target proportion of 95%, this ensures with 95% confidence that the true proportion of vaccine recipients who shed vaccine virus is not lower than 78%.

9.4. MONITORING

9.4.1. Site Monitoring Plan

As per International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use - Good Clinical Practice (ICH-GCP) 5.18 and FDA 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 the 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 ICF process for each monitored participant; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare CRIMSON data abstracts with individual participants' records and source documents (participants' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original participant 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 (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 (and/or designee) will make study documents (e.g., consent forms, CRIMSON data abstracts) and pertinent hospital or clinical records readily available for inspection by the local 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.

9.4.2. Monitoring by the NIAID Intramural Data and Safety Monitoring Board

The NIAID Intramural DSMB is constituted to review the safety data of Intramural NIAID clinical studies that require DSMB oversight. The NIAID Intramural DSMB includes independent experts in infectious diseases, biostatistics, and clinical research 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 DSMB will review the protocol prior to opening the study to enrollment. The DSMB will meet at least twice a year or on a schedule specified by the DSMB to review the completeness of the study data, the adherence to the protocol, and AE data. Cumulative safety data (pooled across arms, with the vaccine and placebo arms presented together) will be submitted to the DSMB Executive Secretary for DSMB review. The DSMB Executive Secretary will provide the PI with DSMB recommendations promptly, and the official DSMB Report will then be provided in a timely fashion through the office of the NIAID Clinical Director. The PI will submit the written DSMB recommendations to the IRB upon receipt. All SAEs, LRIs, UPs and IND safety reports will be reported by the PI to the DSMB at the same time that they are submitted to the IRB and/or IND sponsor (OCRPRO). The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the Board at the time a pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study.

9.5. ANALYSES

9.5.1. Assessment of Primary Objectives

Safety data from all participants who have been inoculated will be summarized together, including data from participants who discontinue study early or have some missed visits. In the immunogenicity analyses, those who do not provide data for the Day 28 Visit follow-up for RSV-seropositive participants and for the Day 56 Visit follow-up for RSV-seronegative participants (due to early discontinuation or missed visit) will be treated as "failures." Sensitivity analyses will be performed to check if the results are consistent with those when these participants are excluded. Participants who receive any of the disallowed treatments listed in Section 5.10 during the first 28 days after inoculation for RSV-seropositive participants and during the first 56 days after inoculation for RSV-seronegative participants may be excluded from the immunogenicity evaluations after the time of the treatment. These participants, however, will be included in the safety evaluations for the duration of the study. These participants will not be replaced. Details of the analyses listed below will be included in the statistical analysis plan.

The frequency of solicited AEs and unsolicited AEs, along with 90% CIs, during Study Days 0 to 10 in RSV-seropositive participants and during Study Days 0 to 28 in RSV-seronegative participants and of vaccine-related SAE during Study Days 0 to 28 in RSV-seropositive participants and during Study Days 0 to 56 in RSV-seronegative participants will be summarized. In addition, line listing of individual clinical solicited AEs and unsolicited AEs, graded by severity, will be prepared for events occurring during Study Days 0 to 10 in RSV-seropositive participants and during Study Days 0 to 28 in RSV-seronegative participants and vaccine-related SAE during Study Days 0 to 28 in RSV-seropositive participants and during Study Days 0 to 56 in RSV-seronegative participants.

The proportion of participants with infection defined as recovery of vaccine virus from a nasal wash or swab as determined by rRT-PCR, and/or a $\geq$4-fold rise in neutralizing antibody titer to RSV, will be summarized. A line listing of the individual peak titer of vaccine virus shed and duration of virus shedding in nasal washes or swabs by each individual will be prepared. In addition, the geometric mean peak titer and mean duration of virus shed will be provided for each study group.

The proportion of participants that develop 4-fold or greater rises in RSV-neutralizing antibody titer following vaccination will be summarized. A line listing of the individual RSV antibody titer pre- and post-vaccination will be prepared. In addition, the geometric mean titer (GMT) and median antibody titers will be provided for each treatment group. Line listings of individual RSV-neutralizing antibody responses as well as of antibody responses to the RSV F glycoprotein will be prepared as well.

The study results will be compared with the criteria listed in Section 2.1 to determine if these vaccines are promising candidates for further evaluation in expanded phase I studies or phase II studies.

9.5.2. Assessment of Secondary Objectives

The summary of the frequency and severity of symptomatic, medically attended respiratory and febrile illness in the RSV-seronegative vaccine and placebo recipients who experience natural infection with wt RSV during the RSV season will be presented. In addition, a line listing of the individual RSV antibody titers before and after the surveillance period will be prepared, and the GMT and median antibody titers will be provided for each study group. The adaptive immune responses to vaccine will be summarized for each study group.

The two vaccine groups will be compared descriptively with respect to peak viral titers and antibody titers following vaccination.

10 DATA HANDLING AND RECORD KEEPING

10.1. DATA MANAGEMENT RESPONSIBILITIES

The CIR will maintain adequate and accurate research records containing all information pertinent to the study for all screened and enrolled participants, including CRFs and supporting source data per the MOP.

Study data will be maintained on source documents with key data points abstracted and entered into CRIMSON. 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.

Source documents include all recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents may include the participant's medical records and laboratory reports per the MOP. Data will be collected directly from participants during study visits and contacts.

10.2. ESSENTIAL AND SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA

Study-related documentation will be completed as required by the IRB, the sponsor, and regulatory authorities. Continuing review documentation will be submitted to the IRB as specified by the IRB. An annual report will be submitted by the sponsor to the FDA based on the anniversary date that the IND for the RSV 6120/ΔNS1 and RSV 6120/F1/G2/ΔNS1 vaccines went into effect. These reports will provide a brief description of the progress of the investigation as outlined in 21 CFR 312.33 and will include any revisions of the protocol not previously submitted to the FDA.

All study records must be accessible for inspection, monitoring, and/or auditing during and after the conduct of the study by authorized representatives of the study sponsors and their contracted monitors, the FDA, regulatory authorities, IRB, OHRP, and other applicable regulatory entities. Records must be kept on-site throughout the period of study implementation; thereafter, instructions for off-site storage may be provided by NIH. No study records may be removed to an off-site location or destroyed prior to receiving approval from NIH.

Study-related documents will be maintained for a period of at least 2 years after final marketing approval of the vaccine, or at least 2 years after the formal discontinuation of clinical development of the product (or longer based upon local laws). The sponsor is required to inform the PI as to when such documents need no longer be retained. No study document should be destroyed without prior written agreement between the sponsor and the PI. Storage of all study-related documents will be such that confidentiality will be strictly maintained. These records are also to be maintained in compliance with IRB, state, and federal medical records retention requirements, whichever are longest. Should the PI wish to assign the study records to another party and/or move them to another location, the PI must provide written notification of such intent to the sponsor with the name of the person who will accept responsibility for the transferred records and/or their new location. The sponsor must be notified in writing, and written permission must be received from the sponsor prior to destruction or relocation of research records.

10.3. CLINICAL INVESTIGATOR'S BROCHURES

The IBs comprehensively describe all the available preclinical experience with the experimental vaccines. If relevant new information becomes available during the course of the trial, the PI will receive revised IBs or amendments to the current versions.

10.4. QUALITY CONTROL AND QUALITY ASSURANCE

Essential documents and participant research records are participant to continuous quality control and quality assurance procedures consistent with the MOP.

11 CLINICAL SITE MONITORING

As per ICH-GCP 5.18 and FDA 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 the 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 ICF 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 (and/or designee) will make study documents (e.g., consent forms, CRIMSON data abstracts) and pertinent hospital, including CRIMSON, or clinical records readily available for

inspection by the local IRB, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator 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.

The sponsor will retain originals of the Form FDA 1572 and copies of other study documents as deemed necessary.

Statement of Compliance

The trial will be conducted in compliance with this protocol, ICH-GCP guidelines, FDA guidelines and applicable regulatory requirements. The CIR monitoring will be conducted according to the OCRPRO Clinical Trial Management Group's Monitoring Plan.

12 HUMAN SUBJECTS PROTECTIONS

12.1. INSTITUTIONAL REVIEW BOARD/ETHICS COMMITTEE REVIEW AND APPROVAL

Prior to study initiation, the PI will obtain IRB review and approval of this protocol and ICDs in accordance with 45 CRF 46. In addition to the initial review and approval, the IRB must review the study at least annually. The PI must also promptly report to the IRB any changes in the study and any UPs involving risks to participants or others.

All IRB policies and procedures must be followed, and complete documentation of all correspondence to and from the IRBs must be maintained in the essential document files.

A copy of the study approval (including approval of the ICD) is to be maintained in the investigator's study document binder, and a copy will be supplied to the sponsor.

During the study, the PI is responsible for providing the IRB with all documents subject to review (i.e., protocol amendments, ICD updates, advertisements, and any written information that may be provided to the participant's parents/guardians). Study progress reports will be made to the IRB by the investigator in accordance with IRB guidelines and government regulations.

12.2. VULNERABLE PARTICIPANTS

The NIH is mandated by law to ensure that children be included in clinical research when appropriate (38, 39). This study responds to that mandate and will provide clinical research data to inform RSV vaccine infectivity, safety and immunogenicity in children. Nonetheless, the infants and children who take part in this study are considered vulnerable participants per the US CFR, and site IRBs/IBCs must consider the potential risks and benefits to child participants as described in 45 CFR 46 Subpart D (for children).

12.3. INFORMED CONSENT

In obtaining and documenting informed consent, the PI must comply with the applicable regulatory requirements, ICH-GCP guidelines, and ethical principles. The written ICD must be approved by the IRB prior to its use.

Written informed consent for study participation will be obtained before any study-specific procedures are performed. The informed consent process will include information exchange, detailed discussion, and assessment of understanding of all required elements of informed consent, including the potential risks, benefits, and alternatives to study participation.

As part of the informed consent process, parents/guardians will also be asked whether they agree to storage and future research testing of the biological specimens that remaining after all protocol-specified testing has been completed. Future research testing of residual specimens may be declined with no impact on other aspects of study participation.

12.4. POTENTIAL BENEFITS

Participants may not receive any direct vaccine-related benefit from enrollment in this study. Some children who receive vaccine may be protected against infections with wt RSV that circulates in the community. It is hoped that information gained in this study will contribute to the development of a safe and effective vaccine for the prevention of illness associated with RSV infection.

Parents/guardian may be offered child safety seat educational material and referral to community inspection stations by study staff, and may be offered certified lactation counseling services, if appropriate.

12.5. POTENTIAL RISKS

12.5.1. Venipuncture

Risks occasionally associated with venipuncture include pain, bleeding, and bruising at the site of venipuncture, lightheadedness, infection, and rarely syncope. Before each blood draw, we will offer to use anesthetic skin cream to decrease the pain.

12.5.2. Nasal Wash or Nasal Swab

Risks occasionally associated with nasal wash or swab include pain or discomfort, and occasionally epistaxis. Nasal washes are not standard care in well children and are not usually performed on ill children, although many parents are advised to use over-the-counter saline solutions and/or nasal bulb suction to clear a young child's congested nostrils during an URI. The nasal bulb suction and saline-like solutions are 2 components of our nasal wash procedure.

12.5.3. Nasosorption SAM Strips

The risk associated with the Nasosorption SAM Strips is a nasolacrimal reflex which involves slight watering of the eyes.

12.5.4. Topical Anesthetic Cream

Risks occasionally associated with the use of topical anesthetic cream include temporary skin discoloration, skin irritation, rash, hives, and rarely, dizziness or drowsiness.

12.5.5. Receipt of Study Product

If a study vaccine (RSV 6120/ΔNS1 or RSV 6120/F1/G2/ΔNS1) is insufficiently attenuated, participants could experience rhinorrhea, cough, fever, otitis media, or LRI. Immediate hypersensitivity reactions including urticaria, anaphylaxis, or other immunoglobulin E (IgE)-mediated responses are possible, as with any vaccine. With any investigational vaccine, there is a theoretical possibility of risks about which we have no present knowledge. Parents/guardians will be informed of any such risks should further data become available.

The study participant's family does not pay for the study product or research visits including examinations and laboratory tests that are part of this study, including evaluation of illness, if any. NIAID agrees that the funding for appropriate acute care will be provided through the NIAID contract (HHS272200900010C) for any side effects that are determined to be related to the administration of vaccine.

12.6. REIMBURSEMENT/COMPENSATION

Compensation will be in the form of either check or pre-paid card. Participants' parent/guardian will receive compensation at the rate of $50 for the inoculation visit, $30 per each scheduled or unscheduled study visit, $5 per each scheduled non-visit day report and weekly surveillance report, and a $50 bonus for study completion. In addition, the parent/guardian will receive bus tokens, taxi fare, or parking passes as needed for study visits. Parent/guardian will be compensated only for those portions of the study that are completed. Compensation will be in accordance with IRB policies and procedures and will be subject to IRB approval.

Participants may receive age-appropriate books or small toys. The total value of the books or toys will not exceed $10 per participant.

12.7. PRIVACY AND CONFIDENTIALITY

All study procedures will be conducted in private and every effort will be made to protect participant privacy and confidentiality to the extent possible. Participant information will not be released without written permission to do so except as necessary for review, monitoring, and/or auditing as described in Section 9.4 and Section 10.2.

All study-related information will be stored securely. Participant research records will be stored in locked areas with access limited to study staff. All laboratory specimens, CRFs, and other documents that may be transmitted off-site will be identified by coded number only.

All local databases must be secured with password protected access systems. Lists, logbooks, appointment books, and any other documents that link participant identification numbers to personal identifying information should be stored in a separate, locked location in an area with limited access.

12.8. MANAGEMENT OF INCIDENTAL FINDINGS

Study clinicians will inform parents/guardians of all clinically meaningful PE findings and laboratory tests. When applicable, study clinicians will provide referrals to non-study sources of medical care for further evaluation and/or treatment of these findings.

13 ADMINISTRATIVE PROCEDURES

13.1. REGULATORY OVERSIGHT

CIR 330 is sponsored by the OCRPRO, Division of Clinical Research (DCR), NIAID, NIH.

OCRPRO is responsible for regulatory oversight of this study. Safety-related information pertaining to the study product will be distributed prior to and during the conduct of the study, in accordance with its sponsor obligations.

NIAID provides funding to the clinical research sites at which this study will be conducted. Each institute contracts with independent clinical site monitors who will perform monitoring visits as described in Section 9.4. As part of these visits, monitors will inspect study-related documentation to ensure compliance with all applicable local and US regulatory requirements.

13.2. PROTOCOL REGISTRATION

Prior to implementation of this protocol, and any subsequent full version amendments, the CIR must have the protocol and the protocol ICDs approved, as appropriate, by their local IRB/IBC, local IBC, and any other applicable regulatory entity (RE).

For any future protocol amendments, upon receiving final IRB/IBC and any other applicable RE approvals, CIR should implement the amendment immediately.

13.3. STUDY IMPLEMENTATION

This study will be conducted in accordance with the protocol, ICH guidelines, and all applicable local and US regulations. Study implementation will also be guided by the study-specific MOP and other study implementation materials.

13.4. PROTOCOL DEVIATION REPORTING

Protocol deviations must be documented in participant research records. Reasons for the deviations and corrective and preventive actions taken in response to the deviations should also be documented. See MOP for further instructions.

Deviations will be reported to the IRB and other applicable review bodies in accordance with the policies and procedures of these review bodies. Serious deviations that are associated with increased risk to one or more study participants and/or significant impacts on the integrity of study data must also be reported following procedures specified in the MOP.

13.5. CLINICALTRIALS.GOV

This protocol is subject to the US Food and Drug Administration Amendments Act of 2007 (FDAAA), including registration in ClinicalTrials.gov.

14 PUBLICATIONS

Publication of the results of this trial will be governed by NIAID policies. Any presentation, abstract, or manuscript will be made available for review by the pharmaceutical and NIAID sponsors prior to submission. Publication or presentation approval will conform to any Cooperative Research and Development Agreement (CRADA) or other collaborative agreement in place.

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APPENDICES

Appendix I: Tables Referenced in the Background and PRECLINICAL Sections

Table 26: Viral titers of nasopharyngeal swab samples from AGMs inoculated with RSV ΔNS1 mutants, or with recombinant wt RSV rA2

Virus Test Article[a]	AGM ID	NP virus titer (log_{10} PFU/mL) on indicated days[b]											Peak virus titer	Sum of daily titers[c]
		1	2	3	4	5	6	7	8	9	10	12/14		
RSV A2	7209	-	-	3.1	4.4	4.4	5.2	3.6	4.1	3.4	2.0	-	5.2	31.3
CTM [d]	7467	-	0.7	2.8	3.8	2.1	3.9	3.4	3.2	1.9	2.0	-	3.9	24.5
RSV#004A	7468	-	1.7	2.6	4.0	3.9	3.7	3.6	2.7	3.6	2.6	-	4.0	29.1
12RSV01	7492	0.7	1.2	2.3	3.9	2.9	3.9	3.9	2.4	1.7	1.0	-	3.9	24.3
	Mean:												***4.2***	***27.2***
	8960	-	0.7	-	1.7	2.2	2.7	2.8	1.8	-	-	-	2.8	13.7
6120/ΔNS1	8992	-	0.7	-	0.7	2.6	2.7	2.9	2.6	1.9	0.7	-	2.9	15.8
Exp. lot	8904	-	-	-	1.7	1.2	2.4	2.8	1.3	-	1.2	-	2.8	12.3
16RSV08-nontox	8951	-	-	-	2.3	2.3	3.1	3.5	2.6	1.5	-	-	3.5	17.0
	***Mean*:**												***3.0***	***14.7***
	9140	-	-	1.3	-	1.0	1.4	1.7	-	-	-	-	1.7	7.9
6120/ΔNS1	9147	-	0.7	1.3	1.9	2.5	2.5	2.8	2.7	1.8	1.7	-	2.8	17.1
CTM [e]	9116	-	-	-	-	-	0.7	-	1.6	-	-	-	1.6	5.8
RSV#018A	9136	-	-	0.7	1.4	1.9	2.6	2.0	2.8	1.3	-	-	2.8	14.1
18RSV01-tox														
	***Mean*:**												***2.2***	***12.3***
	9131	-	-	-	-	-	-	-	0.7	0.7	-	-	0.7	4.6
6120/F1/G2/	9084	-	-	-	-	-	1.0	-	1.4	-	1.7	-	1.7	6.9
ΔNS1 CTM [f]	9135	-	-	-	1.0	1.0	1.3	1.6	-	-	-	-	1.6	6.8
RSV#016A	9128	-	-	1.0	-	-	2.1	-	-	-	-	-	2.1	6.3
18RSV01-tox														
	***Mean*:**												***1.5***	***6.1***

[a] *Monkeys were inoculated i.n. and i.t. with 10^6 PFU of the indicated virus in a 1 mL inoculum per site (total dose =$2x10^6$ PFU/AGM), except for RSV#016A, which was used at a dose of 5 x 10^5 PFU in a 1 mL inoculum per site (total dose = 1 x 10^6 PFU).*

[b] *Virus titrations were performed on Vero cells. The lower limit of detection was 1.0 log_{10} PFU/mL. Samples with no detectable virus are represented as "-". Peak titers for each animal are underlined.*

[c] *The sum of daily titers is used as an estimate for the magnitude of shedding (area under the curve). A value of 0.35 was use for samples with no detectable virus.*

[d] *Lot RSV#004A; vial numbers 263, 886, 1108, 1567.*

[e] *Lot RSV#018A, vial numbers 0021-0026, 1267-1272, 2482-2485*

[f] *Lot RSV#016A, vial numbers .0020-0026, 1267-1274, 2482-2487.*

Table 27: Viral titers of tracheal lavage samples from AGMs inoculated with RSV ΔNS1 mutants, or with Recombinant wt RSV rA2

Virus Test Article[a]	AGM ID	TL virus titer (log_{10} PFU/mL) on indicated day[b]						Peak virus titer	Sum of daily titers[c]
		2	4	6	8	10	12/14		
RSV A2 CTM RSV#004A 12RSV01[d]	7209	3.0	3.8	4.3	4.5	2.6	-	4.5	18.9
	7467	3.0	3.0	4.3	3.4	2.8	-	4.3	17.2
	7468	2.5	1.7	2.9	3.0	2.9	-	3.0	13.7
	7492	1.9	3.5	4.7	3.3	-	-	4.7	14.8
	***Mean*:**							***4.1***	***16.2***
6120/ΔNS1 Exp. lot 16RSV08-nontox	8960	-	-	1.5	2.7	-	-	2.7	7.0
	8992	-	2.9	3.6	2.7	1.0	-	3.6	11.6
	8904	2.3	2.7	3.8	3.1	-	-	3.8	13.3
	8951	-	3.0	1.5	3.3	-	-	3.3	9.9
	***Mean*:**							***3.3***	***10.5***
6120/ΔNS1 CTM[e] **RSV#018A 18RSV01-tox**	9140	1.0	2.9	4.1	4.0	1.3	-	4.1	14.0
	9147	2.6	1.6	2.7	3.0	1.5	-	3.0	12.1
	9116	2.0	1.0	2.8	2.1	-	-	2.8	9.2
	9136	2.7	2.1	3.9	2.7	1.5	-	3.9	13.7
	***Mean*:**							***3.5***	***12.2***
6120/F1/G2/ ΔNS1 CTM[f] **RSV#016A 18RSV01-tox**	9131	-	-	1.0	-	-	-	1.0	4.5
	9084	-	1.8	2.3	2.3	1.8	1.0	2.3	9.8
	9135	1.3	1.5	2.4	2.1	-	-	2.4	8.7
	9128	2.4	1.5	3.0	2.4	1.3	-	3.0	11.3
	***Mean*:**							***2.2***	***8.6***

[a] *Monkeys were inoculated i.n. and i.t. with 10^6 PFU of the indicated virus in a 1 mL inoculum per site (total dose =$2x10^6$ PFU/AGM), except for RSV#016A, which was used at a dose of 5 x 10^5 PFU in a 1 mL inoculum per site (total dose = 1 x 10^6 PFU).*

[b] *Virus titrations were performed on Vero cells. The lower limit of detection was 1.0 log_{10} PFU/mL. Samples with no detectable virus are represented as "-". Underlined value indicates maximum titer for each animal.*

[c] *The sum of daily titers is used as an estimate for the magnitude of shedding (area under the curve). Values of 0.7 are used for samples with no detectable virus.*

[d] *Lot RSV#004A; vial numbers 263, 886, 1108, 1567.*

[e] *Lot RSV#018A, vial numbers 0021-0026, 1267-1272, 2482-2485*

[f] *Lot RSV#016A, vial numbers .0020-0026, 1267-1274, 2482-2487.*

Table 28: Serum $PRNT_{60}$ Titers in AGMs Inoculated with RSV ΔNS1 mutants, or with Recombinant wt RSV rA2

Virus Test Article	AGM ID	RSV Neutralization Titer (Log2 of reciprocal) on days		
		0	**21**	**28**
RSV rA2 wt RSV CTM RSV#004A [a] 12RSV01	7209	<3.3	7.1	6.7
	7467	<3.3	8.1	8.7
	7468	<3.3	5.9	5.9
	7492	<3.3	7.7	8.8
	***Mean*:**		**7.2**	**7.5**
6120/ΔNS1 Experimental lot 16RSV08-nontox	8960	<3.3	7.9	8.3
	8992	<3.3	8.5	8.8
	8904	<3.3	8.6	7.3
	8951	<3.3	8.4	8.4
	***Mean*:**		**8.4**	**8.2**
6120/ΔNS1 CTM [e] RSV#018A 18RSV01-tox	9140	<3.3	8.6	8.1
	9147	<3.3	7.1	7.4
	9116	<3.3	6.6	7.0
	9136	<3.3	9.3	9.2
	***Mean*:**		**7.9**	**7.9**
6120/F1/G2/ ΔNS1 CTM [f] RSV#016A 18RSV01-tox	9131	<3.3	4.3	5.4
	9084	<3.3	7.6	7.6
	9135	<3.3	6.4	7.1
	9128	<3.3	8.5	8.0
	***Mean*:**		**6.7**	**7.0**

[a] *Lot RSV#004A; vial numbers 263, 886, 1108, 1567.*

[b] *Lot RSV#018A, vial numbers 0021-0026, 1267-1272, 2482-2485*

[c] *Lot RSV#016A, vial numbers .0020-0026, 1267-1274, 2482-2487.The lower limit of detection of the 60% Plaque Reduction assay is 3.3 (Log_2 of the dilution reciprocal). Samples below the lower limit of detection are recorded as "-".*

Appendix II: Schedule of Events: Screening, Acute Phase, and Post-Acute Phase

Table 29: Schedule of Events: Group 1: RSV-Seropositive Participants

	ACUTE PHASE												POST-ACUTE PHASE				
	Screening	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12-27	Day 28	Illness Visit	Early DC
Study window			± 1 day												+7 days		
In person visit	X	X			X	X	X	X	X			X		*	X	X	X
Non-visit contact			X	X						X	X		X	*			
Informed consent	X																
History	X																
Interim history		X	X	X	X	X	X	X	X	X	X	X	X	*	X	X	X
Physical exam (full)	(X)	X															
Clinical assessment (focused PE)		(X)			X	X	X	X	X			X		*		X	
Administer study product		X															
Blood for: immunologic assays	5mL[a]														5mL		5mL
Nasal wash and/or nasosorption SAM strip for: RSV antibody		X													X		X
Nasal wash for: viral detection & quantification		X			X	X	X	X	X			X		*		X	X
Request adventitious agent assay														*		X	
Total blood volume	5mL	--	--	--	--	--	--	--	--	--	--	--	--	--	5mL	--	5mL
*If a family reports an SAE that may meet the study pause or stop criteria, complete the indicated tasks																	

[a] Blood for RSV immunological assay is obtained not more than 56 days prior to inoculation. Screening RSV antibody can be obtained greater than 56 days prior to inoculation so long as it is obtained within the same calendar year.

A physical exam can be completed at screening or enrollment.

Table 30: Schedule of Events: Group 2: RSV-Seronegative Participants

		ACUTE PHASE																					POST-ACUTE PHASE			
	Screening	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18-27 (contact each day)	Day 28	Day 29	Day 30-55	Day 56	Illness Visit	Early DC
Study window		± 1 day																						+7 days		
In person visit[c]	X	X			X		X		X			X		X		X			X		X		*	X	X	X
Non-visit contact			X	X		X		X		X	X		X		X		X	X		X		X	*			
Informed consent	X	(X)																								
History	X																									
Interim history		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	*	X	X	X
Physical exam (full)	(X)	X																								
Clinical assessment (focused PE)[c]		(X)			X		X		X			X		X		X			X		X		*		X	
Administer study product		X																								
Blood for: immunologic assays	5mL[a]																							5mL		5mL
Nasal wash and/or nasosorption SAM strip for: RSV antibody[b]		X																			X			X		X
Nasal wash or swab for: viral detection & quantification[c]		X			X		X		X			X		X		X			X		X		*		X	X
Request adventitious agent assay																							*		X	
Total blood volume	5mL	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	5mL	--	5mL
*If a family reports an SAE that may meet the study pause or stop criteria, complete the indicated tasks																										

[a]Blood for screening is obtained not more than 42 days prior to inoculation. A physical exam can be completed at screening or enrollment.
[b]For the 13 subjects enrolled prior to March 2020
[c] If a Stay at Home Order is initiated or in the case of respiratory illness, the parent may be asked to obtain the nasal swab, and the clinical assessment may be completed via a remote or in-person research visit. During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate.

Appendix III: Schedule of Events: RSV Seasonal Surveillance

Table 31: Schedule of Events: RSV Seasonal Surveillance

	Pre-RSV season[a]	Weekly contact	Post-RSV season	Illness Visit	Early DC
Visit period	Oct 1st to Oct 31st	Date of seasonal enrollment pause to Mar 31st	Apr 1st to Apr 30th Allowable through September 30th in the event of delays due to a Stay at Home Order		
Clinical assessment (focused PE)[b]				X	
Interim history		X		X	X
LABORATORY EVALUATIONS					
Blood for: immunologic assays	5 mL		5 mL		5 mL
Nasal wash and/or nasosorption SAM strip for immunologic assay[a]	X		X		
Nasal wash or swab for viral detection & quantification[b]				X	
Request adventitious agent assay				X	
TOTAL BLOOD VOLUME	5 mL	--	5 mL	--	5 mL

[a] For the 13 subjects enrolled prior to March 2020

[b] If a Stay at Home Order is initiated, the parent may be asked to obtain the nasal swab, and the clinical assessment may be completed via a remote or in-person research visit.

Appendix IV: Definitions of Solicited Adverse Events

Table 32: Definitions of Solicited Adverse Events

Event	Defined
Fever	Temporal or rectal temperatures ≥100.4°F
Acute Otitis Media[1]	Loss of tympanic membrane landmarks, accompanied by erythema and loss of mobility. May or may not be associated with fever or other respiratory symptoms. Confirmed with tympanometry if possible.
Upper Respiratory Tract Illness (URI)	
Rhinorrhea	Two or more consecutive days of clear or purulent discharge from the nares. Note: Not associated with crying, change of room temperature, or eating and drinking.
Pharyngitis[1]	Pharyngeal erythema accompanied by exudate or pharyngeal erythema with enlarged, tender lymph nodes. Note: May be associated with sore throat, or painful or difficult swallowing.
Cough Without LRI	Two or more consecutive days of 3 or more episodes of cough during a 15-minute timed observation period, or cough awakens child from sleep. Note: Not associated with eating, drinking or choking.
Hoarseness	An unnaturally deep or rough quality of voice.
Lower Respiratory Tract Illness (LRI)	
Wheezing[2,3]	Sustained, high pitched, musical breath sounds, especially during the expiratory phase, which do not clear with cough.
Pneumonia[1,2,3]	Rales and crackles, originating in the lower respiratory tract, usually accompanied by tachypnea, which do not clear with cough. May be confirmed by x-ray showing areas of consolidation.
Laryngotracheobronchitis (Croup)[1,2,3]	Barking cough, hoarseness, and inspiratory stridor.
Rhonchi[2,3]	Coarse breath sounds which are not transmitted noises from the upper airway and do not clear with cough.
Rales[2,3]	Abnormal lung sound heard through a stethoscope. Rales may be sibilant (whistling), dry (crackling) or wet (sloshy) depending on the amount and density of fluid refluxing back and forth in the air passages.

[1] Diagnosis must be made by a medical professional
[2] Must be sustained over 20 minutes.
[3] Clinical assessments must be made by a medical professional and confirmed by a second medical professional, if possible.
NOTE: Solicited AEs will only be recorded on CRFs according to criteria defined in Section 8.1.1.

Figure 6.6-1: RSV Seasonality in Baltimore