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/ΔNS2/1030s, Lot RSV#012A, 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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Provided by:
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LIST OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices (CDC)
AE	adverse event
AGM	African green monkey
AIDS	acquired immunodeficiency syndrome
BSC	biologic safety cabinet
CACI	compounding aseptic containment isolator
CDC	Centers for Disease Control and Prevention
cDNA	complementary deoxyribonucleic acid
CFR	Code of Federal Regulations
CGMP	Current Good Manufacturing Practice
CI	confidence interval
CIR	Center for Immunization Research
CL	clinical lot
cp	cold-passaged
CRADA	Cooperative Research and Development Agreement
CRF	case report form
CRL	Charles River Laboratories
CSO	Clinical Safety Office
CTM	clinical trial material
DAERS	DAIDS Adverse Event Reporting System
DAIDS	Division of AIDS
DAIDS PRO	Division of AIDS Protocol Registration Office
DC	discontinuation
DCR	Division of Clinical Research
DHHS	Department of Health and Human Services
DMC	Data Management Center
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
EAE	expedited adverse event
EENT	ears, eyes, nose, throat
ELISA	enzyme-linked immunosorbent assay
F protein	fusion protein
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FSTRF	Frontier Science & Technology Research Foundation, Inc.
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GMT	geometric mean titer
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
IB	investigator's brochure
ICD	informed consent document
ICH	International Conference on Harmonisation
IgA, IgG, IgE	immunoglobulin A, G, E
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Network
i.n.	intranasally
IND	investigational new drug

IoR	Investigator of Record
IRB	institutional review board
i.t.	intratracheally
JHSPH	Johns Hopkins Bloomberg School of Public Health
JHU	Johns Hopkins University
LDMS	Laboratory Data Management System
LID	Laboratory of Infectious Diseases
LPC	laboratory processing chart
LRI	lower respiratory illness
LRT	lower respiratory tract
MA-LRI	medically attended lower respiratory illness
MOP	manual of procedures
mRNA	messenger ribonucleic acid
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIAID CRMS	NIAID Clinical Research Management System
NICHD	National Institute of Child Health and Development
NIH	National Institutes of Health
nt	nucleotide
OCRPRO	Office of Clinical Research Policy & Regulatory Operations
OHRP	Office for Human Research Protections
ORF	open reading frame
OTC	over-the-counter
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PE	physical examination
PFU	plaque-forming units
PI	principal investigator
PID	participant identification number
PRNT	plaque reduction neutralization
PSRT	Protocol Safety Review Team
r	recombinant
RE	regulatory entity
RNA	ribonucleic acid
rRT-PCR	reverse transcription polymerase chain reaction
RSC	Regulatory Support Center
RSV	respiratory syncytial virus
RT-qPCR	quantitative reverse transcription polymerase chain reaction
SAE	serious adverse event
SAM	synthetic absorptive matrix
SDAC	Statistical & Data Analysis Center, Harvard School of Public Health
SDMC	Statistical and Data Management Center
SERF	Safety Expedited Report Form
SES	subject enrollment system
SID	study identification number
SMM	sponsor medical monitor
SOP	standard operating procedure
SPG	sucrose-phosphate-glutamate buffer
SRCP	Safety Review and Communication Plan
SUSAR	serious, unexpected, suspected adverse reaction
TL	tracheal lavage
ts	temperature sensitivity
T_{SH}	shut-off temperature
T_{SP}	small plaque temperature

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
ΔM2-2	deleted M2-2

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/ΔNS2/1030s, Lot RSV#012A, 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/ΔNS2/1030s

PURPOSE To determine whether study vaccine is attenuated 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.

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

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

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

SAMPLE SIZE Group 1: Approximately 15 RSV-seropositive children
Group 2: Approximately 21-30 RSV-seronegative infants and children

STUDY PRODUCT Single dose of intranasal RSV 6120/ΔNS2/1030s vaccine or placebo

Table 1: Inoculation Schedule

Group #	N	Study Product	Dose
1	10	RSV 6120/ΔNS2/1030s	$10^{5.7}$ PFU*
	5	Placebo	0
2	14-20	RSV 6120/ΔNS2/1030s	$10^{5.0}$ PFU*
	7-10	Placebo	0

*plaque-forming units (PFU)

The study will start with group 1 and proceed to group 2 if there are not more than 2 vaccine-associated solicited adverse events (AEs) (Days 0 through 10) and no vaccine-associated lower respiratory illnesses (LRIs) (Days 0 through 28) in RSV-seropositive children. If 3 or more RSV-seropositive children (group 1) have respiratory or febrile illness that coincides with shedding of > $10^{2.5}$ PFU of vaccine virus 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.

STUDY DURATION

Participants will be enrolled in the protocol between April 1 and October 31, outside of the RSV season. RSV-seropositive participants will be followed for 28 days after inoculation, and RSV-seronegative participants will remain on the study until they complete the post–RSV season visit.

After the first season of RSV surveillance is completed, research staff may invite the families of participants in group 2 to participate in an optional second season of RSV surveillance during November-March of the second year following enrollment.

Figure 1: Group 1 – Study Overview

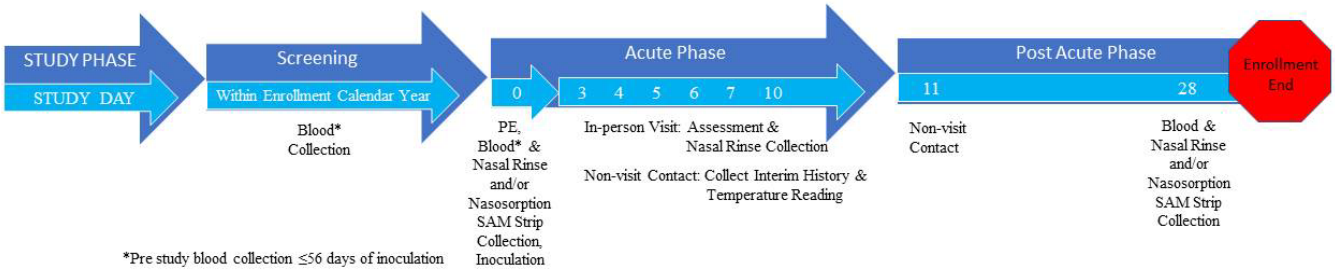
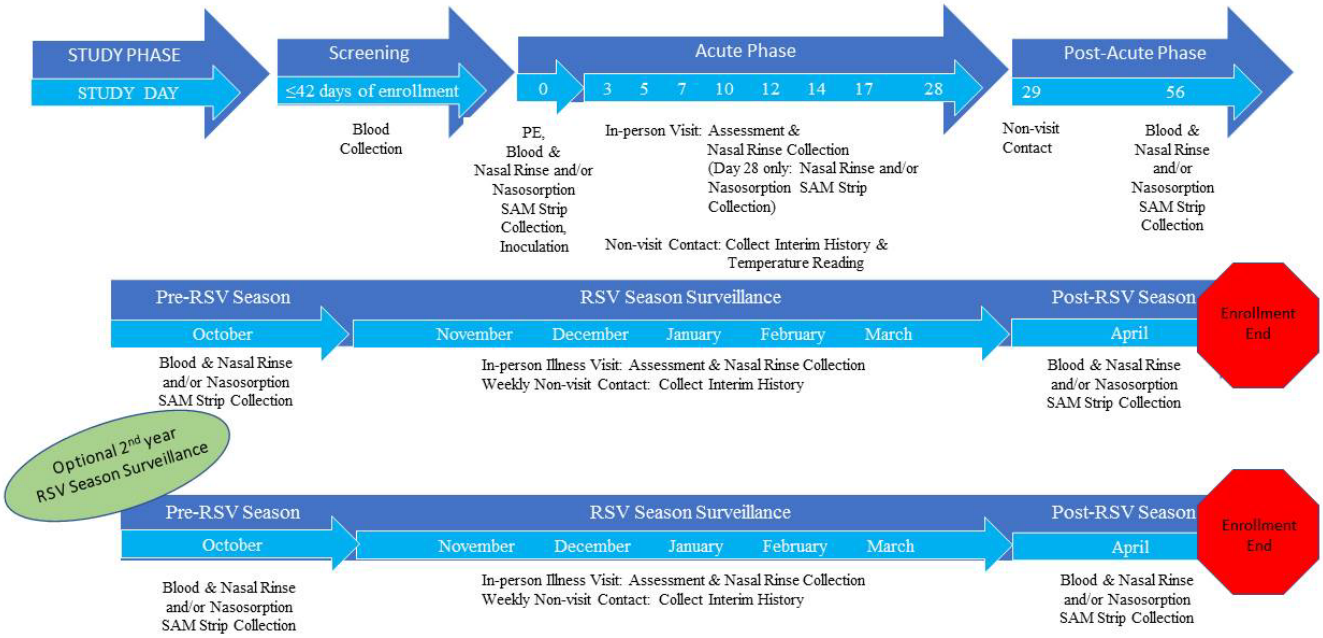


Figure 2: Group 2 – Study Overview



*Post-RSV Season 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 alone, RSV is responsible for 75,000 to 125,000 hospitalizations of infants yearly (1), and worldwide, RSV infects at least 34 million children under 5 years old, resulting in an estimated 3.4 million RSV LRI hospitalizations and 66,000 to 199,000 RSV-attributable deaths each year (2). 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 (3, 4).

RSV is an enveloped RNA virus that is a member of the newly organized *Pneumoviridae* family, genus *Orthopneumovirus* (5). RSV has a single negative-sense strand RNA genome of 15.2 kilobases encoding 10 mRNAs. Each mRNA encodes a single protein, with the exception of the M2 mRNA, which contains 2 overlapping 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.

Currently, no licensed vaccine against RSV is available, although there is broad consensus that such a vaccine is urgently needed and should be a global health priority. Although 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. 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 (6). 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 (7, 8).

Following the failure of the formalin-inactivated RSV vaccine, attempts at developing RSV vaccines at NIAID have focused largely on live-attenuated approaches (9). 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 (10). 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, 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 (11).

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 (12). Consistent with this level of antigenic relatedness, F protein expressed by a recombinant 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 (13). 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 (DNA)–based technique called reverse genetics (14). 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 recombinant DNA. The RSV vaccine candidate to be tested under this protocol is a derivative of strain A2, subgroup A.

The drug substance RSV 6120/ΔNS2/1030s is a recombinant live-attenuated RSV vaccine candidate that contains a deletion of the NS2 gene (ΔNS2) and a genetically stabilized version of the attenuating temperature sensitivity mutation at codon 1321 of the L gene termed "1030s" (S1313[TCA]/Y1321K[AAA]). The RSV NS2 protein functions as an interferon antagonist, and deletion of NS2 is attenuating (15-21). The 1030s mutation is also attenuating and confers a moderate temperature-sensitive phenotype to RSV (22). RSV 6120/ΔNS2/1030s has a shutoff temperature for replication of 40°C, and it produces only small plaques at 38°C or above. Compared to recombinant wt RSV, the RSV 6120/ΔNS2/1030s candidate vaccine was significantly restricted in replication in a primary human airway epithelial cell model, and moderately attenuated and immunogenic in African green monkeys (AGM).

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 (10).

Several live-attenuated RSV vaccines have been evaluated in clinical trials in adult and pediatric populations as part of NIAID's ongoing RSV vaccine development program (11, 23-26). Four vaccine viruses with the NS2 deletion have been evaluated in clinical studies: rA2cpΔNS2, rA2cp248/404ΔNS2, rA2cp530/1009ΔNS2 (27), and RSV ΔNS2/Δ1313/I1314L. A brief summary of clinical studies involving these vaccine candidates is provided below.

rA2cpΔNS2 contains the NS2 deletion as well as a set of 5 amino acid substitutions in the N, F, and L proteins that were identified in a cold-passaged (cp) RSV (V267I in N; E218A and T523I in F; and C319Y and H1690Y in L) (28, 29). rA2cp248/404ΔNS2 contains all of the mutations in rA2cpΔNS2 as well as two additional point mutations: "248," an amino acid substitution in the L protein (Q831L) (28, 30, 31), and "404," a nucleotide substitution in the M2 gene start signal (30, 32). rA2cp530/1009ΔNS2 also contains all of the mutations in rA2cpΔNS2 as well as two additional point mutations in L: "530" (F521L) and "1009" (M1169V) (33, 34).

rA2cpΔNS2, which had the fewest attenuating mutations, was evaluated in a dose-escalating study ($10^{5.0}$ PFU, $10^{6.0}$ PFU, or $10^{7.0}$ PFU) administered to a total of 45 adults, and was also evaluated at a dose of $10^{5.0}$ PFU in eight 15- to 59-month-old RSV-seropositive children. Shedding of rA2cpΔNS2 was not detected in any of the adults, and only 2 of 16 adults who received $10^{7.0}$ PFU had an antibody response. In contrast, at the dose of $10^{5.0}$ PFU, rA2cpΔNS2 was shed by 3 of 8 vaccinated seropositive children with mean peak titers of $10^{3.4}$ PFU/mL of nasal wash. Two additional children had detectable virus by polymerase chain reaction (PCR). Based on these results, rA2cpΔNS2 was deemed under-attenuated, and was not further evaluated in seronegative children.

rA2cp248/404ΔNS2 was evaluated in adults (n = 16 vaccinees), 15- to 59-month-old RSV-seropositive children (n = 16 vaccinees), and 6- to 24-month-old RSV-seronegative children (n = 10 vaccinees). rA2cp248/404ΔNS2 was not shed by adults. Only one seropositive child shed vaccine virus (peak titer of $10^{0.4}$ PFU/mL of nasal wash), and four seronegative children shed vaccine virus (mean peak titer of $10^{2.3}$ PFU/mL of nasal wash).

rA2cp530/1009ΔNS2 was also evaluated in adults (n = 19 vaccinees), 15- to 59-month-old RSV-seropositive children (n = 14 vaccinees), and 6- to 24-month-old RSV-seronegative children (n = 15 vaccinees). rA2cp530/1009ΔNS2 was shed by one adult (peak titer of $10^{0.4}$ PFU/mL of nasal wash), none of the seropositive children, and three seronegative children (mean peak titer of $10^{1.3}$ PFU/mL of nasal wash). In summary, deletion of the NS2 gene attenuates RSV. rA2cpΔNS2 was over-attenuated for adults but under-attenuated for young children, whereas both rA2cp248/404ΔNS2 and rA2cp530/1009ΔNS2 were over-attenuated and insufficiently immunogenic for seronegative children (27).

Based on these results, RSVΔNS2/Δ1313/I1314L was developed. In a recent phase I trial (NCT01893554), this 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 and immunogenic: 7% of recipients shed virus detected by culture, and 53% developed a RSV neutralizing antibody responses. At the higher dose of 10^6 PFU (20V, 10P), 80% of recipients shed virus detected by culture, and 80% developed a RSV neutralizing antibody response.

To generate a vaccine candidate with a slightly increased level of replication compared to RSV ΔNS2/Δ1313/I1314L, the current candidate vaccine RSV 6120/ΔNS2/1030s was designed. RSV 6120/ΔNS2/1030s is expected to be slightly less restricted in replication than RSVΔNS2/Δ1313/I1314L, and more immunogenic in RSV-seronegative infants and children.

1.2.2 Preclinical Studies

1.2.2.1 Temperature sensitivity of RSV 6120/ΔNS2/1030s

The shutoff temperature (T_{SH}) was evaluated by efficiency of plaque formation at temperatures indicated in Table 2. RSV 6120/ΔNS2/1030s is moderately temperature sensitive, with a T_{SH} of 40°C. The T_{SH} is defined as the lowest restrictive temperature at which the reduction in plaque number compared to that at the permissive temperature of 32ºC is 100-fold or greater than that observed for wt RSV at the 2 temperatures. Small plaques were observed at 38°C, and plaques formed at 39°C and above were characterized as micro-plaques. Temperature sensitivity results of the vaccine candidate RSV ΔNS2/Δ1313/I1314L were included for comparison (Table 2). As expected, the temperature sensitivity of RSV ΔNS2/Δ1313/I1314L was slightly greater than that of RSV 6120/ΔNS2/1030s (T_{SH} of 39°C, small plaque temperature [T_{SP}] of 37°C for RSV ΔNS2/Δ1313/I1314L; T_{SH} of 40°C, T_{SP} of 38°C for RSV 6120/ΔNS2/1030s).

Table 2: Temperature Sensitivity of Experimental Lots of RSV 6120/ΔNS2/1030s and RSV ΔNS2/Δ1313/I1314L

Virus	Virus titer (log_{10} PFU per mL) at indicated temperature (°C) [a]									
	Repl [b]	32	35	36	37	38	39	40	T_{SH} [c]	T_{SP} [d]
wt RSV	1	8.0	8.0	7.9	7.9	7.8	7.6	7.5	> 40	> 40
wt RSV	2	6.7	6.9	6.8	6.8	6.9	6.8	6.6	> 40	> 40
RSV ΔNS2 Δ1313 I1314L	1	7.5	7.3	7.0	6.7	5.7	**1.7**	< 1	39	37
RSV 6120/ΔNS2/1030s	2	7.8	7.9	7.9	7.7	7.8	7.5[e]	**5.5**[e]	40	38

[a] *The temperature sensitivity (ts) phenotype for each virus was evaluated by plaque assay on Vero cells at the indicated temperatures. For viruses with ts phenotype, titers at shutoff temperatures are marked (bold, underlined). Vero cells were used for the ΔNS2 viruses because loss of expression of NS2 renders RSV more sensitive to type I interferon, which is not made by Vero cells. See footnote c for the definition of shutoff temperature.*

[b] *wt RSV rows 1 and 2 represent duplicate dilution series of the same virus that were examined in parallel in the same experiment. Differences between these replicas illustrate the variability that is inherent in these biological experiments.*

[c] *Shutoff temperature (T_{SH}, bold, underlined) is defined as the lowest restrictive temperature at which the reduction compared to 32ºC is 100-fold or greater than that observed for wt RSV at the two temperatures. The ts phenotype is defined as having a shutoff temperature of 40ºC or less.*

[d] *T_{SP}, small plaque temperature, is defined as the lowest restrictive temperature at which the small-plaque phenotype is observed.*

[e] *At 39 and 40°C, RSV 6120/ΔNS2/1030s formed micro-plaques.*

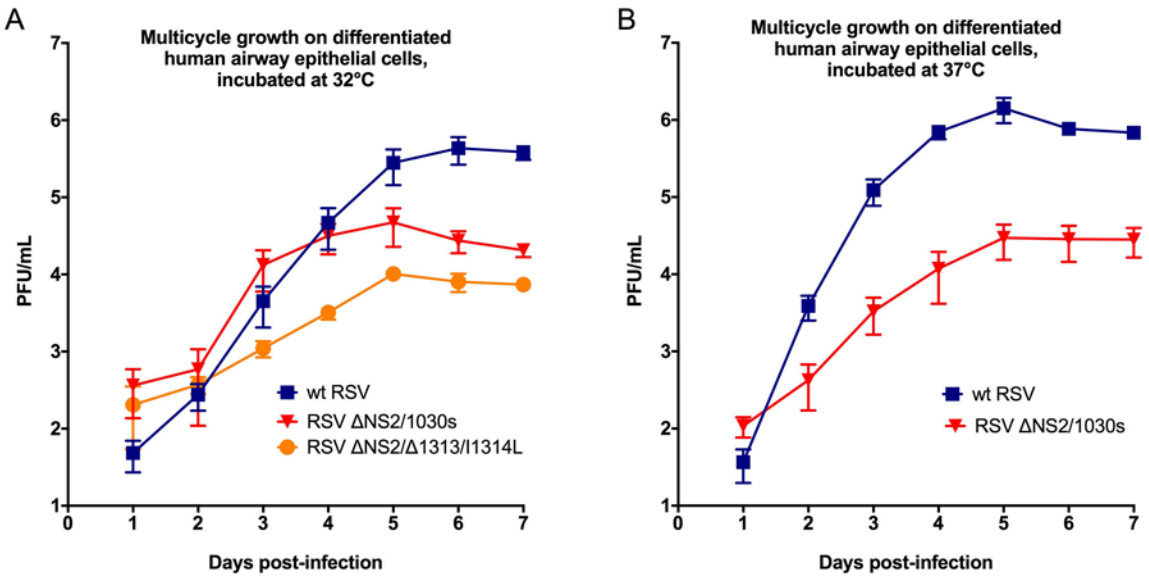
1.2.2.2 Replication of an experimental lot of RSV 6120/ΔNS2/1030s in an in-vitro model of mucociliary normal human tracheal/bronchial epithelial cells

Multicycle growth of an experimental lot of RSV 6120/ΔNS2/1030s was evaluated in a 3D mucociliary human tissue model consisting of normal differentiated primary human tracheal/bronchial airway epithelial cells (EpiAirway, Mattek, Inc.). Virus replication was evaluated at two different temperatures (32°C and 37°C) to model the temperatures of the upper and lower airways. Pseudostratified differentiated airway cells were cultured in an air-liquid interface in 12-mm transwell inserts with 0.4 μM pore sizes in Dulbecco's Modified Eagle 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 a multiplicity of infection of 0.1 with 100 µl per well of RSV 6120/ΔNS2/1030s. To evaluate replication at 32°C, recombinant wt RSV A2 and RSV ΔNS2/Δ1313/I1314L were included for comparison. At 37°C, only recombinant wt RSV was included for comparison. 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, then snap-frozen, and virus titers were determined by immunoplaque assay on Vero cells.

Recombinant wt RSV of strain A2 replicated efficiently in differentiated primary human tracheal/bronchial epithelial cells at 32°C and at 37°C (Figure 3). At 32°C, peak titers of wt RSV were detectable on day 6, whereas at 37°C, peak titers were reached by day 5 post-infection. The temperature-sensitive virus RSV ΔNS2/Δ1313/I1314L, which differs from RSV 6120/ΔNS2/1030s only by the attenuating mutation in the L ORF, was included for comparison at the lower temperature, 32°C. Peak titers of this virus were about 200-fold lower than those of wt RSV at 32°C. RSV 6120/ΔNS2/1030s replicated to slightly higher titers than RSV ΔNS2/Δ1313/I1314L, showing that indeed, RSV ΔNS2 with the 1030s mutation is slightly less restricted for replication than RSV ΔNS2 with the genetically stabilized deletion of codon 1313 of the L ORF. At 37°C, the difference in peak titers between RSV 6120/ΔNS2/1030s and wt RSV was statistically significant.

Figure 3: Multicycle Replication of Experimental Lots in an in-vitro Model of Normal Human Bronchial/Tracheal Epithelial Cells



Cultures were infected at a multiplicity of infection of 0.1, and supernatants were harvested daily for 7 days, snap-frozen, and titered later on Vero cells. A: replication at 32°C. B: replication at 37°C.

1.2.2.3 Replication of experimental lot and CTM of RSV 6120/ΔNS2/1030s in African green monkeys (AGMs)

Replication and immunogenicity of RSV 6120/ΔNS2/1030s were evaluated in nonhuman primates (NHPs), specifically African green monkeys (AGM). AGMs are semi-permissive for RSV. The first NHP study was done in AGMs to evaluate an experimental lot of RSV 6120/ΔNS2/1030s, and a second study was done to evaluate the CTM (Lot RSV#012A). AGMs that were seronegative for RSV were inoculated intranasally (i.n.) and intratracheally (i.t.) with RSV 6120/ΔNS2/1030s; a dose of 1 x 10^6 PFU of vaccine in a 1-mL volume per site was administered to sedated juvenile male and female AGMs (total dose per animal: 2 x 10^6 PFU). Nasopharyngeal swabs were collected daily on days 0 through 10 and on days 12 and 14; tracheal lavage (TL) samples were collected every other day from days 2 through 14; and virus shedding was analyzed by immunoplaque assay. Serum RSV neutralizing antibody titers were determined by a complement-enhanced 60% plaque reduction neutralization assay. Results from studies following the same protocol, performed in animals from the same group and origin, inoculated with recombinant wtRSV A2 at the same dose, were included for comparison. Studies were approved by the Animal Care and Use Committee of NIAID, NIH.

In both studies, shedding of the RSV 6120/ΔNS2/1030s virus from the upper respiratory tract (URT) and lower respiratory tract (LRT) was detectable over several days. In both studies, the mean peak titers of RSV 6120/ΔNS2/1030s in the nasopharyngeal samples was 3.8 $\log_{10}$ PFU per mL. These mean peak titers were slightly lower than that of wt RSV A2 (4.2 $\log_{10}$ PFU per mL), but higher than those of the previously tested RSV ΔNS2/Δ1313/I1314L virus (3.2 $\log_{10}$ PFU per mL). The mean peak titers in the LRT, detectable in TL samples, were 4.6 $\log_{10}$ PFU per mL for the Experimental Lot of RSV 6120/ΔNS2/1030s, and 2.9 PFU per mL for the CTM of RSV 6120/ΔNS2/1030s. Shedding was observed in all AGMs in both URT and LRT. Compared to the RSV ΔNS2/Δ1313/I1314L virus, which showed much lower mean peak titers (1.4 $\log_{10}$ PFU per mL) with only a 75% infection rate (virus replication detected in 3 out of 4 animals), the study virus RSV 6120/ΔNS2/1030s was less attenuated in the AGMs (Appendix I, Table 27, Table 28).

In both studies, the RSV 6120/ΔNS2/1030s virus induced serum neutralizing antibodies. In the first study of the experimental lot, the neutralizing antibody titers were comparable to or slightly higher than wt RSV A2 and the previously tested RSV vaccine virus (RSV ΔNS2/Δ1313/I1314L) (Appendix I, Table 29). The CTM induced titers that were slighly lower than those of wt RSV, and comparable to those of RSV ΔNS2/Δ1313/I1314L. These results show that at a total dose of 2 x 10^6 PFU, administered i.n. and i.t., RSV 6120/ΔNS2/1030s is immunogenic in AGMs.

1.2.3 Previous Clinical Experience

The live-attenuated recombinant RSV 6120/ΔNS2/1030s vaccine virus is being evaluated for the first time in humans. Based on preclinical data, RSV 6120/ΔNS2/1030s is expected to be more attenuated than the previously tested vaccine candidate rA2cpΔNS2, but less attenuated than the vaccine candidates rA2cp248/404ΔNS2 and rA2cp530/1009ΔNS2, which were deemed over-attenuated in seronegative infants and children, and slightly less attenuated and more immunogenic than RSVΔNS2/Δ1313/I1314L. As noted above, this vaccine is predicted to be attenuated and well tolerated in seropositive and seronegative children.

1.3 RATIONALE

1.4 CLINICAL DEVELOPMENT PLAN

The investigational RSV vaccine RSV 6120/ΔNS2/1030s 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 RSV 6120/ΔNS2/1030s is well tolerated and restricted in replication in RSV-seropositive children, and well tolerated, infectious, immunogenic, and genetically stable in RSV-seronegative infants and children. If the study product is found to be well tolerated, infectious, and immunogenic in RSV-seronegative infants and children, then a safety and immunogenicity study in infants < 6 months of age will be initiated. Plans will be made in consultation with industry collaborators as a lead RSV vaccine candidate is identified.

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 (13). These assays will be performed at the Johns Hopkins University (JHU) CIR laboratory.

1.5 HYPOTHESES

RSV 6120/ΔNS2/1030s will be well-tolerated in RSV-seropositive and RSV-naïve participants. In RSV-seronegative participants, RSV 6120/ΔNS2/1030s is expected to be safe and result in infection, limited vaccine replication, and the induction of a neutralizing antibody response to RSV.

2 OBJECTIVES

2.1. PRIMARY OBJECTIVES

Safety

1. To assess 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. To assess 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. To assess study product–related serious adverse events (SAEs) in RSV-seropositive participants (group 1) from day 0 through the 28th day following inoculation
4. To assess 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 (If < 70% of RSV-seronegative vaccinees are infected with vaccine virus, the study product will be considered over-attenuated.)

Immunogenicity

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

2. To characterize serum antibody responses to the study product 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(s)
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(s)
3. To characterize mucosal antibody responses to vaccine in RSV-seropositive (group 1) and RSV-seronegative (group 2) participants

2.3. EXPLORATORY OBJECTIVE

Study samples may be used in comparative assays with samples from other RSV vaccine studies initiated by the LID, NIAID, NIH.

3 STUDY DESIGN

The study will be double-blind, randomized, and placebo-controlled. Participants in groups 1 and 2 will be block-randomized in groups of 3 at a ratio of 2:1 to receive vaccine or placebo, respectively. 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 (11, 24, 35). 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.

The study will proceed to group 2 if there are not more than 2 vaccine-associated solicited AEs and no vaccine-associated LRIs in RSV-seropositive children. If 3 or more RSV-seropositive children have respiratory or febrile illness that coincides with shedding of > $10^{2.5}$ PFU of vaccine virus as detected by culture, then we will enroll an additional 15 RSV-seropositive children to continue evaluating the safety, infectivity, and immunogenicity of the vaccine virus.

Enrollment occurs at the time of inoculation with study product, Day 0, and will occur between April 1 and October 31 to avoid the time during which wt RSV typically circulates in the community (Appendix V, Figure 6). Accrual will stop prior to November 1.

For group 1, RSV-seropositive participants, the duration of participation in the initial phase of the study is 28 days. The initial phase consists of an Acute Phase from inoculation through the 10th day after inoculation, and a Post-Acute Phase from the 11th day through the 28th day after inoculation. For group 2, RSV-seronegative participants, the duration of participation in the initial phase of the study is 56 days. The initial phase consists of an Acute Phase from inoculation through the 28th day after inoculation, and a Post-Acute Phase from the 29th day through the 56th day after inoculation.

During the Acute Phase, 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 an interim medical history conducted by a mutually agreed upon communication method. During the Acute Phase, the participants will be evaluated for AEs or SAEs. Participants who have a febrile or respiratory illness or otitis media will have a study visit to perform a nasal rinse. The nasal rinse will be tested for RSV and for other respiratory pathogens that are considered adventitious agents. During the Post-Acute Phase, study participants' parents/guardians will be instructed to contact the study staff if any SAEs occur. The RSV-seropositive participants will have a scheduled follow-up visit on the 28th day after inoculation, and the RSV-seronegative participants 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 30.

For the RSV-seronegative participants (group 2), the study has a third phase that assesses the incidence and severity of illness suggestive of RSV occurring during the RSV season following inoculation. During the RSV Season Surveillance Period, encompassing November 1 to 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 nasal rinse to evaluate for RSV and other respiratory pathogens that are considered adventitious agents (Appendix III).

The RSV-seronegative participants will also have a study visit during the pre-RSV season between October 1 and 31 to collect a blood sample and nasal rinse sample and/or nasosorption synthetic absorptive matrix (SAM) strip for immunological assays. The samples will be used to assess the durability of the vaccine response. Participants will have a post-RSV season visit to collect blood and nasal rinse and/or nasosorption SAM strip specimens to further assess the durability of the vaccine response and to assess the immune response to naturally occurring wt RSV infection.. 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

Approximately 15 RSV-seropositive children ≥ 12 months to < 60 months of age will be enrolled; 10 will receive a single dose of $10^{5.7}$ PFU of vaccine and 5 will receive a single dose of placebo. Subsequently, 21 to 30 RSV-seronegative infants and children ≥ 6 months to < 25 months of age will be enrolled in the study and will receive a single dose of either $10^{5.0}$ PFU of vaccine or placebo in a 2:1 ratio. 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 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 (11,23,25) and statistical considerations (Section 9).

Infants and children will be selected for participation according to the described study-specific co-enrollment considerations; the recruitment, screening, and enrollment process; and participant retention and withdrawal or termination.

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 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 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 10th day after inoculation

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

- a person who is HIV-infected
- a person who has received chemotherapy within the 12 months prior to enrollment
- a person receiving immunosuppressant agents
- 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 10th 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 in the 28 days after, or
- another investigational vaccine or investigational drug in 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 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.

4.2.27. 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 physical examination 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 innoculation 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 antibody product including ribavirin, RSV Ig, or RSV mAb

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 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 in the 28 days after, or
- another investigational vaccine or investigational drug in 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 medications 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

4.5 INCLUSION CRITERIA FOR RSV-SERONEGATIVE CHILDREN PARTICIPATING IN A SECOND SEASON OF RSV SURVEILLANCE

4.5.1. RSV-seronegative participants who have completed the initial RSV season surveillance portion of the study and whose parent/guardian is willing and able to provide written informed consent

4.5.2. Expected to be available during the second year of RSV surveillance

4.6 EXCLUSION CRITERIA FOR RSV-SERONEGATIVE CHILDREN PARTICIPATING IN A SECOND SEASON OF RSV SURVEILLANCE

4.6.1. Currently enrolled in another RSV study

4.7 CO-ENROLLMENT CONSIDERATIONS

Co-enrollment to an investigational vaccine or investigational drug study is not allowed during the Acute Phase or Post-Acute Phase of this study. After the Post-Acute Phase, co-enrollment may be considered at the discretion of the principal investigator (PI).

4.8 RE-ENROLLMENT CONSIDERATIONS

Participants who receive placebo may re-enroll in the initial study following completion of the initial RSV surveillance period if they continue to meet all eligibility requirements.

4.9 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 approach parents/guardians, present them with IRB-approved informational brochures, and obtain contact information. Study staff will contact interested parents/guardians to elaborate upon the details and requirements of the study.

CIR research staff may also recruit participants through mailing IRB-approved documents 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 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.

After the first year of RSV surveillance is completed, the parents/guardians of children enrolled in group 2 will be invited to have their children participate in a second year of RSV surveillance. Participation is optional, and separate consent will be obtained for participation in this portion of the study.

4.10 PARTICIPANT RETENTION

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

4.11 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 PI to discuss options for resumption of follow-up. Withdrawn subjects will not be replaced.

Participants enrolled in the optional second year of RSV season surveillance may voluntarily withdraw before the conclusion of the second year RSV surveillance season.

5 STUDY PRODUCT

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

- Group 1: Live Recombinant Respiratory Syncytial Virus RSV 6120/ΔNS2/1030s, approximately $10^{5.7}$ PFU per 1.0 mL vaccine
- Group 2: Live Recombinant Respiratory Syncytial Virus RSV 6120/ΔNS2/1030s, 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 RSV 6120/ΔNS2/1030s vaccine or placebo, administered as nose drops.

5.2 STUDY PRODUCT FORMULATION

5.2.1 RSV 6120/ΔNS2/1030s

The RSV 6120/ΔNS2/1030s vaccine is provided in a sterile 2.0-mL cryovial, each containing 0.6 mL of vaccine (Lot RSV#012A). The vaccine virus concentrate is diluted by trained research personnel to a dose of approximately $10^{5.7}$ 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 4.

Figure 4: Investigational Product Label Sample

Live recombinant Respiratory Syncytial Virus
RSV 6120/ΔNS2/1030s
VERO GROWN VIRUS VACCINE

CAUTION:NEW DRUG LIMITED
BY FEDERAL (USA) LAW
TO INVESTIGATIONAL USE
Store at –80°C ± 15°C
Charles River Laboratories, Malvern, PA

Date: 29AUG2016
Vial#:XXXX
Lot: RSV#012A

(Enlarged Sample)

5.2.2 Diluent for RSV 6120/ΔNS2/1030s

The diluent for RSV 6120/ΔNS2/1030s is Lactated Ringer's Solution for Injection, USP.

5.2.3 Placebo for RSV 6120/ΔNS2/1030s

The placebo for RSV 6120/ΔNS2/1030s 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 MOP.

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 MOP.

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/ΔNS2/1030s

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

Approximately three vials per dose of undiluted vaccine will be used to prepare the administration dose. 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. Concentration of the undiluted study product is approximately 10^6 PFU per mL. The frozen study product will be thawed and diluted with Lactated Ringer's Solution for Injection, USP to a dose of approximately $10^{5.7}$ PFU in 1.0 mL prior to administration for group 1. For subjects in group 2, the frozen study product will be thawed and diluted with Lactated Ringer's Solution for Injection, USP to a dose of approximately $10^{5.0}$ PFU in 0.5mL prior to administration.

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 in a cooler at 2ºC to 8ºC with ice or cold packs to the clinical site for administration. 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 MOP provides detailed instructions on vaccine storage, handling, preparation, labeling and transport to the clinic.

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 lot of RSV 6120/ΔNS2/1030s was 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 at the JHSPH for confirmation of the vaccine titer. However, only after receipt of IRB approval and FDA safe to proceed determination, will vials of vaccine be used for administration to study subjects. Such an initial shipment may contain the full number of vials needed for implementation of the protocol, but no vaccine will be administered to participants until all necessary approvals have been received. 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
- 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 clinical sites. Unless otherwise specified, visits may be split, with required procedures performed on more than one day within the allowable visit window. All clinical tasks will be performed by a medical professional. The physical examination 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, neurological exams. A focused clinical examination 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 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 back 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 informed consent document (ICD) before screening or study procedures are performed. The consenting process for the study may take place during the screening visit or may be conducted at a separate visit. 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.

For group 2, enrollment into the second RSV surveillance period will be initiated under a separate consent from the ICD used for the primary study.

During the consenting process, the parent will be given the opportunity to provide permission for use of their child's picture in advertisement 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 physical examination. 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 SOP. As in previous phase I trials of other live-attenuated RSV vaccines (11, 23-25), 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 (36).

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 3: Screening Visit Procedures

Screening Visit Procedures		
Administrative Tasks		• Conduct consenting process • Confirm parent/guardian's informed consent comprehension • Obtain release of medical records as required per HIPAA • Obtain available medical records • Assess eligibility •
Clinical Tasks		• Obtain, review, and document medical history • Perform complete physical examination, or physical examination may be deferred until study day 0 • Address any concerns • Document findings • Obtain and 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		• Schedule 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

6.2.1 Participants Enrolled in Surveillance for a Second RSV Season

No screening is needed prior to the second RSV surveillance period.

6.3 RANDOMIZATION

Randomization numbers will be assigned by the unblinded laboratory vaccine dispenser and will be forwarded to the Data Safety Monitoring Board (DSMB) Executive Secretary. Unblinded laboratory personnel will label the vaccine syringes with the study virus vaccine name, PI initials, study number, subject's randomization number, preparer and vaccine verifier's initials and expiration date and time. The vaccine administrator and verifier will initial the label and place the label in the subject'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 VAR will be sent to the laboratory.

A copy of the randomization code will be retained by the unblinded dispenser in the laboratory where the vaccine is prepared. 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 subject within the randomization group. Pediatric subjects will be block-randomized in groups of 3 (2 vaccinees and 1 placebo recipient per block); this allows for maintenance of the blind during each subject'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 laboratory study binder.

6.4 ENROLLMENT

6.4.1 Enrollment - Inoculation (Day 0)

Enrollment will occur between April 1 and October 31 to avoid the RSV season. 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, an authorized prescriber will provide a 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. Study staff will use incorrect answers from the comprehension assessment to identify those 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 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, or pharyngitis), or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media.

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 4: 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 physical exam: o Complete physical exam if deferred at screening, OR o Focused physical exam 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
	Nasosorption SAM Strip and/or Nasal Wash	*Collect nasosorption strip for:* • RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash.
		The nasal wash must be obtained prior to administering the study product. *Collect nasal wash for:* • RSV antibody assays • RSV viral detection and quantification
Study Product Administration		• Administer study product and maintain participant in a supine position for 1 minute • Observe for approximately 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

Following the inoculation day visit, the parent/guardian will record the infant/child's temperatures and signs of illness on the temperature card and provide these to study personnel during an in-person 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 (37). 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 (37). For study-specific management and grading of temperatures, see Section 8, Table 22.

6.4.2 Enrollment in the Second Season of RSV Surveillance (Group 2 Only)

Participation in RSV surveillance for a second season will require completion of an additional consent document by the parent or guardian. The consent document for this optional portion of the study may be done at the pre–RSV second seasonal surveillance visit or at a separate visit prior to the pre–RSV second seasonal surveillance visit.

6.5 STUDY PHASES

Refer to Figures 1 and 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 (28, 37-39).

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 6.8, 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 5: 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		• Obtain and document from parent/guardian the participant's previous days' interim history including: ▪ 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 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 6: Acute Phase Non-Visit Contact Procedures – RSV-Seropositive Children

Days 1, 2, 8, and 9 Contact Procedures *(each visit ± 1 day)*	
Clinical Tasks	• Obtain and document from parent/guardian the participant's interim history from previous days, including: ▪ 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 7: Day 11 Non-Visit Procedures – RSV-Seropositive Children

Day 11 Non-Visit Procedures	
Clinical Tasks	• Obtain and document from parent/guardian the participant's previous days' interim history including: ▪ 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

6.5.1.4 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. If the parent reports an SAE that may meet the study pause or stop criteria (Section 8.3), then an Illness Visit will be scheduled (Section 6.8).

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 8: Day 28 Visit Procedures – RSV-Seropositive Children

Day 28 Visit *(+7 Days)*		
Administrative Tasks		• Provide study compensation
Clinical Tasks		• Obtain and document from parent/guardian the participant's interim history 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 criteria • Address any concerns • Review safety data • Document findings
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to RSV
	Nasosorption SAM Strip and/or Nasal Wash	*Collect nasosorption strip for:* • RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash.
		The nasal wash must be obtained prior to administering the study product. *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.

Table 9: 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		• Obtain and document from parent/guardian the participant's previous days' interim history including: ▪ 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 and/or Nasosorption SAM Strip**	*Collect nasal wash for:* • RSV viral detection and quantification ***Day 28 only**: Collect nasal wash for:* • RSV antibody assays • RSV viral detection and quantification
		***Day 28 only**: Collect nasosorption strip for:* • RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash.
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

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 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 10: 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	• Obtain and document from parent/guardian the participant's previous days' interim history including: ▪ 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 11: Day 29 Non-Visit Procedures – RSV-Seronegative Children

Day 29 Non-Visit Procedures	
Clinical Tasks	• Obtain and document from parent/guardian the participant's previous days' interim history including: ▪ 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 6.8).

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.

Table 12: Day 56 Visit Procedures – RSV-Seronegative Children

Day 56 Visit *(+7 Days)*		
Administrative Tasks		• Provide first study compensation payment
Clinical Tasks		• Obtain and document from parent/guardian the participant's interim history 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
	Nasosorption SAM Strip and/or Nasal Wash	*Collect nasosorption strip for:* • RSV antibody assays • Note: The nasosorption must be performed prior to the nasal wash.
		The nasal wash must be obtained prior to administering the study product. *Collect nasal wash for:* • RSV antibody assays
Follow-up Preparation		Follow-up depends on when, during the calendar year, the Day 56 Visit is conducted. 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 October 31st

During this period, contact with the participant is not required except for the Pre-RSV Season Study Visit described in Section 6.6.1. No clinical data will be recorded on CRFs or reported under this protocol except for data as outlined in Section 7.2.

6.6 RSV SURVEILLANCE: FIRST SEASON

6.6.1 Pre-RSV Season Surveillance Study Visit

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. Otherwise, an in-person visit is expected of participants during the Pre-RSV Season for collection of a blood sample, nasal wash sample and/or nasosorption strip for RSV antibody assays.

Table 13: Pre-RSV Season Surveillance Study Visit Procedures

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:* • Serum antibodies to RSV
	Nasosorption SAM Strip and/or Nasal Wash	*Collect nasosorption strip for:* • RSV antibody assays • Note: The nasosorption must be performed prior to the nasal wash.
		The nasal wash must be obtained prior to administering the study product. *Collect nasal wash for:* RSV antibody assays
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), surveillance for RSV-associated disease will be conducted between November 1 and March 31 during the first RSV season following receipt of study product. For some RSV-seronegative participants, surveillance during the first RSV season may overlap with the Acute and/or Post-Acute study phases. In this case, all evaluations required for each of the relevant phases of the study will be conducted.

During the first 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 visit. (Rhinorrhea and cough need not meet the Appendix IV criteria if they are documented by the health care provider):

- 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 6.8).

Table 14: RSV Season Surveillance Procedures

RSV Season Surveillance (November 1 through March 31 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 1st - April 30th with allowable window through September 30th)

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 1st and April 30th through September 30th.

Table 15: Post-RSV Seasonal Surveillance Study Visit Procedures

Post-RSV Season Study Visit		
Administrative Tasks		• Provide second study compensation
Clinical Tasks		• Document findings related to study procedures
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to RSV
	Nasosorption SAM Strip	*Collect nasosorption strip for:* • RSV antibody assays

6.7 RSV SURVEILLANCE: SECOND SEASON

The second RSV seasonal surveillance is November 1 through March 31 in the second season following inoculation and will be optional for RSV-seronegative children (group 2) who have participated in the first surveillance season. An ICD for the second season of RSV surveillance must be signed before any procedures are performed. If a participant's parent/guardian agrees to have their child participate in a second season of RSV surveillance, the procedures for the second season will be identical to those outlined for the first season in Section 6.6, except that a Pre-Season 2 Visit with blood and nasal sample will always be required, and the Post-Season 2 Visit will occur in the second calendar year following enrollment in the study.

6.8 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 If the Illness Visit occurs on a day concurrent with an in-person study visit, a single nasal wash 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.

If the acute or post-acute phases overlap the surveillance period (between November 1 and March 31), then the timelines for the acute and post-acute phases will be used.

Table 16: 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	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

Table 17: Illness Visit Procedures

Illness Visit Procedures		
Administrative Tasks		• Complete Adventitious Agent Assay Request for rRT-PCR on nasal wash for adventitious agents
Clinical Tasks		• Obtain and document from parent/guardian the participant's interim history including: ▪ 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:* • Viral detection and quantification
Follow-up Preparation		• Schedule follow-up as appropriate

6.9 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 18: Early Discontinuation Procedures

Early Discontinuation		
Administrative Tasks		• Record data on CRF
Clinical Tasks		• Obtain 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 findings
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to RSV
	Nasal Wash and/or Nasosorption SAM Strip	*If Early Discontinuation Visit is within 28 days of inoculation for RSV-seropositive participants or 56 days for RSV-seronegative participants (Appendix II), collect nasal wash for:* • Viral detection and quantification • RSV antibody assays
		Collect nasosorption strip for: • RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash.

6.10 LABORATORY PROCEDURES

6.10.1 Specimen Collection

Specimens will be collected for this study as indicated in the Schedule of Events. Further information on collection of blood and nasal wash specimens will also be provided in the MOP.

6.10.2 Virus Detection

Specimens for viral culture and quantification of vaccine virus shedding will be obtained by nasal wash with approximately 20 mL of Ringer's lactate solution 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. These specimens may also be tested for adventitious respiratory viruses if needed. Additional nasal wash specimens for detection of RSV and adventitious respiratory viruses by culture and 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 First Seasonal Surveillance Period (November 1 – March 31) for all RSV-seronegative participants and the RSV Second Season Surveillance for RSV-seronegative participants whose parents/guardians elect to participate. If a participant becomes ill during the Acute or Post-Acute Phase, then up to 10 additional nasal washes over a 28-day period for group 1 participants and over a 56-day period for group 2 participants 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.

6.10.3 Immunologic Assays

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) postinoculation; and in RSV-seronegative infants and children not more than 42 days prior to inoculation and on Day 56 (+7) postinoculation. In addition, pre-first RSV season and post-first 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 first 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.

For subjects in group 2 enrolled in surveillance during the second RSV season, RSV neutralizing antibodies will also be measured in serum specimens collected before and after the second RSV season: between October 1 and October 31 (pre-season 2), and with a target window of April 1 to April 30 which can be extended through September 30 (post-season 2). This will allow assessment of the duration of antibody responses to vaccination, and comparison of the rate and severity of significant RSV illness following infection with wt virus in vaccine and placebo recipients during the second season.

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 subjects for study purposes. A maximum of 20 mL of blood will be taken from RSV-seronegative subjects for study purposes from screening through season 1 surveillance. A maximum of an additional 10 mL of blood will be taken from RSV-seronegative subjects who participate in season 2 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 RSV-seronegative participants, 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 1 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. If the parent/guardian opts to participate in the second year of RSV surveillance, a nasal wash specimen and/or nasosorption strip will also be obtained at the second year pre- and post-RSV surveillance visits to measure secretory immunity.

6.10.4 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 SOPs. The frequency of specimen collection and testing will be directed by the Schedule of Evaluations (Appendix II and Appendix III).

6.10.5 Research Laboratories

Quantitation of the amount of vaccine virus shed, assays to measure immune responses before and after inoculation, and assessment of nasal washes 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 from participants infected with vaccine virus if sufficient material is available. Selected specimens may be sent to LID, NIAID for confirmatory testing.

6.10.6 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 fluid, 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 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.

6.10.7 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 7.1.2 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 protocol team 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) was 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. SUSARs will be reported to the FDA and all participating investigators as IND Safety Reports. The Regulatory 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 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, physical examination, 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 (November 1). 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 19 and Table 20.**

Concomitant medications and AEs identified in this study will be recorded on CRFs. AEs will be recorded as signs, symptoms, laboratory test results, and diagnoses, as shown in Table 19 and Table 20.

Table 19: AE CRF Recording Requirements for RSV-Seropositive Subjects

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, criteria • All unsolicited AEs (Grades 1 to 4), with the exception of the following conditions if not treated with prescription medication or non-prescription medications with antipyretic properties: diaper rashes, teething pain, and spitting up	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 LRIs and SAEs	• All medications related to the recorded event
Throughout study	ANY	• Unresolved AEs or SAEs with onset from Day 0 to midnight on the 10th day after inoculation • Unresolved LRIs and SAEs with onset prior to the 28th day following inoculation •	• All medications related to the recorded event

Table 20: AE CRF Recording Requirements for RSV-Seronegative Subjects

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 IVcriteria • All unsolicited AEs (Grades 1 to 4), with the exception of the following conditions if not treated with prescription medication or non-prescription medications with antipyretic properties: diaper rashes, teething pain, and spitting up	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 October 31 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 First and Second Season Surveillance Periods	November 1 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 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
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

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 subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF and the SERF. 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 10 in groups 1 and study days 0 to 28 in 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 WIRB (contact information below) based on its reporting requirements:

- Western Institutional Review Board (WIRB), 1019 39th Ave SE, Puyallup, WA 98374, Phone: 1-800-562-4789

7.4 REPORTING OF UNANTICIPATED PROBLEMS TO OCRPRO

An 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. Possibly, probably, or definitely 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 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 study clinician 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 322: 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 Individual symptoms listed in the "events" column that fail to meet the criteria in the "definition" column in Appendix IV 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/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 patient 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 Investigator's Brochure 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.

8.2.1 AE Grading

Table 21: 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	Outpatient medical intervention by a health care provider required; may include use of over-the-counter and/or prescription medications
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 22: 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 at all sites (Table 23).

Table 23: Pausing and Stopping Rules

Specified Phase	Event	Reporting
Acute and Post-Acute Days 0 through 28 following inoculation for RSV-seropositive subjects and days 0 through 56 following inoculation for RSV-seronegative subjects	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.
Acute Only Days 0 through 10 following inoculation for RSV-seropositive subjects and days 0 through 28 following inoculation for RSV-seronegative subjects	An LRI per Appendix IV, OR A fever of Grade 4 OR Any Grade 3 or above solicited AE (other than fever)	

The regulatory sponsor (OCRPRO) will determine if the FDA needs to be notified. The DSMB and regulatory 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 in Appendix II (Table 30).

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

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 protocol team considers a significant safety issue for participants.

The DSMB will notify the PI (via the study sponsor) of their recommendations as to whether enrollment can resume, or if the study needs to be stopped. The regulatory sponsor (OCRPRO) will be informed about this decision. 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, blinded, randomized, placebo-controlled vaccine trial is to assess the safety, infectivity, and immunogenicity of the RSV 6120/ΔNS2/1030s vaccine candidate in RSV-seropositive and RSV-seronegative pediatric participants. Fifteen RSV-seropositive participants will be randomized in a 2:1 ratio to receive either the candidate vaccine at a dose of $10^{5.7}$ PFU or placebo. Twenty-one to thirty RSV-seronegative participants will be randomized in a 2:1 ratio to receive either the candidate vaccine at a dose of $10^{5.0}$ PFU or placebo.

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.

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 from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative (a binary outcome based on nasal washes done throughout the study period; Day 0 nasal wash will be counted as baseline); or
    2) ≥4-fold rise in RSV neutralizing antibody titer from Study Days 0-28 for RSV-seropositive and Study Days 0-56 for RSV-seronegative subjects
- Peak titer of vaccine virus shed from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative subjects
- Duration of virus shedding in nasal washes as determined by a) culture and b) RT-PCR from Study Days 0-10 for RSV-seropositive and Study Days 0-28 for RSV-seronegative subjects

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 subjects
- ≥4-fold rise in IgG antibody responses to RSV F glycoprotein (ELISA) from study entry to Study Day 28 for RSV-seropositive and from study entry and Study Day 56 for RSV-seronegative subjects

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 first RSV season and through the second RSV season for group 2 subjects who participate in a second RSV season surveillance.
- The antibody responses in the RSV-seronegative vaccine and placebo recipients who experience natural infection with wt RSV during the first RSV season and during a second RSV season for those who choose to participate.
- The quality and epitope specificity of RSF F-specific antibody

9.3 SAMPLE SIZE AND ACCRUAL

9.3.1 Sample Size and Randomization

In group 1, approximately 15 RSV-seropositive children will be enrolled in the study and will receive either $10^{5.7}$ PFU of vaccine or placebo, at a ratio of 2:1. In group 2, approximately 21-30 RSV-seronegative infants and children will be enrolled in the study and will receive either $10^{5.0}$ PFU of vaccine or placebo at a ratio of 2:1. If 30 seronegative participants are enrolled, we anticipate that 20 seronegative vaccinees and 10 seronegative 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 (24-26). The 2:1 randomization ratio will be used to maximize the information obtained regarding the response of children to the RSV 6120/ΔNS2/1030s vaccine. In the event that a participant is discontinued early from the study and the team decides that additional data would be needed to answer the study objectives, an additional participant may be enrolled in the same treatment arm as the discontinued participant.

Eligible subjects whose parents/guardians decline participation in the second RSV season surveillance will not be replaced.

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 24 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 24: 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 25 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 25: Percent of Participants Experiencing LRI or AEs with Exact 90% CI

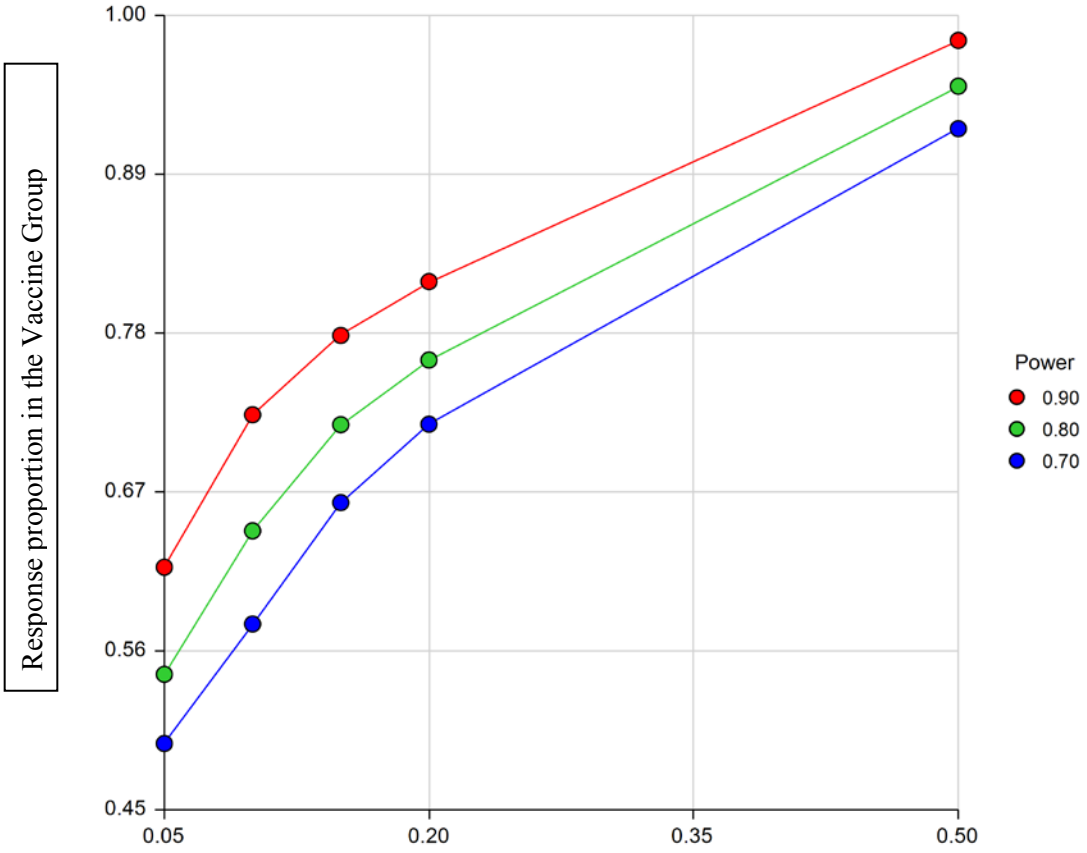
N	% LRI or AEs	90% CI
10	0%	0% -- 26%
20	0%	0% -- 14%
10	10%	1% -- 39%
20	10%	2% -- 28%
10	20%	4% -- 51%
20	20%	7% -- 40%
10	30%	9% -- 61%
20	30%	14% -- 51%

Group sample sizes of 20 in the vaccinated group and 10 in the placebo group would achieve 80% power to detect a difference between the 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 26 presents examples of true group differences which can be detected with 80% power, and Figure 5 graphically shows power curves for 90% power, 80% power, and 70% power given the sample sizes.

Table 26: 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 5: 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, the 90% CI around a sample mean peak titer of 2.5 $\log_{10}$, with a SD of 1.5 (from 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 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 subject; 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 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 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 regulatory 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 in CIR 322 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% confidence intervals, during Study Days 0 to 10 in RSV-seropositive subjects and during Study Days 0 to 28 in RSV-seronegative subjects and of vaccine-related SAE during Study Days 0 to 28 in RSV-seropositive subjects and during Study Days 0 to 56 in RSV-seronegative subjects will be summarized. In addition, line listing of individual clinical solicited AEs and unsolicited AEs, graded by severity, will be prepared for events occuring during Study Days 0 to 10 in RSV-seropositive subjects and during Study Days 0 to 28 in RSV-seronegative subjects and vaccine-related SAE during Study Days 0 to 28 in RSV-seropositive subjects and during Study Days 0 to 56 in RSV-seronegative subjects.

The proportion of participants with infection defined as recovery of vaccine virus from a nasal wash as determined by culture and RT-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 by each individual will be prepared. In addition, the geometric mean peak titer and mean duration of virus shed will be provided for each treatment 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 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.

Where appropriate, a 1-tailed Wilcoxon rank sum test will be used to test the hypothesis that the vaccinated group will exhibit greater peak viral titers and antibody titers following vaccination compared to the placebo group. A 1-tailed Fisher's exact test will be used to test the hypothesis

that the vaccinated group will exhibit a greater proportion of participants who develop fourfold or greater rises in RSV-neutralizing antibody titer following vaccination compared to the placebo group.

These will be the only formal statistical comparisons between the vaccinated and placebo groups. These tests will be carried out at a 5% significance level.

The study results will be compared with the criteria listed in Section 1.1 to determine if this vaccine is a promising candidate 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 first RSV season will be presented. A summary will also be presented of the frequency and severity of symptomatic, medically attended respiratory and febrile illness in the RSV-seronegative vaccine and placebo recipients who participate in a second RSV season surveillance and who experience natural infection with wt RSV during the second RSV season. In addition, a line listing of the individual RSV antibody titer pre- and post-First RSV Season Surveillance Period will be prepared, as well as a listing of the RSV antibody titer pre- and post-Second RSV Season Surveillance Period for those seronegative subjects who participate in the second RSV season surveillance. In addition, the geometric mean and median antibody titers will be provided, for each treatment group. The adaptive immune responses to vaccine will be summarized for each treatment group. A line listing of the mucosal antibody response detected in nasal wash specimens will be prepared.

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 SOP.

Data from source documentation for subjects enrolled in the study will be entered into the study data system. The data entry is to be completed on an ongoing basis during the study. Data entry shall be performed by authorized individuals who have a unique log-on and password. Corrections to the data system shall be tracked electronically (password protected) with time, date, individual making the correction, and what was changed.

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 subject's medical records and laboratory reports. Data will be collected directly from subjects 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/ΔNS2/1030s vaccine 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 BROCHURE

The current version of the IB comprehensively describes all the available preclinical experience with the experimental vaccine. If relevant new information becomes available during the course of the trial, the PI will receive a revised IB or an amendment to the current version.

10.4 QUALITY CONTROL AND QUALITY ASSURANCE

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

11 CLINICAL SITE MONITORING

Site monitors under contract to NIAID will inspect study facilities and review participant study records including ICD, CRFs, medical records, laboratory records, and study product records, to ensure protection of study participants, compliance with the IRB approved protocol, and accuracy and completeness of records. The monitors also will review essential document files to ensure compliance with all applicable regulatory requirements. Study staff will make study facilities and documents available for inspection by the monitors.

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 (40, 41). 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 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 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

Risks occasionally associated with nasal wash 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 the study vaccine 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

12.6.1 Study Compensation: Acute Phase and First Season Surveillance

Compensation will be in the form of either check or gift 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.

12.6.2 Study Compensation: Second Season Surveillance

Subjects' parents/guardians will receive compensation at the rate of $50 for the enrollment visit, $30 per scheduled or unscheduled study visit, $5 per weekly surveillance report and a $50 bonus for study completion.

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 subject 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 physical exam 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 322 is sponsored by the OCRPRO, Division of Clinical Research, 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.

For any future protocol amendments, upon receiving final IRB/IBC and any other applicable regulatory entity 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 SECTION

Table 27: Viral Titers of Nasopharyngeal Swab Samples from AGMs Inoculated with the Experimental Lot or CTM of RSV 6120/ΔNS2/1030s, with RSV ΔNS2/Δ1313/I1314L, or with Recombinant wt RSV rA2

Virus Test Article[a] and Study Number	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 rA2 CTM RSV#004A[d]	7209	-	-	3.1	4.4	4.4	5.2	3.6	4.1	3.4	2.0	-	5.2	31.3
	7467	-	0.7	2.8	3.8	2.1	3.9	3.4	3.2	1.9	2.0	-	3.9	24.5
	7468	-	1.7	2.6	4.0	3.9	3.7	3.6	2.7	3.6	2.6	-	4.0	29.1
	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***
RSV ΔNS2/Δ1313/ I1314L CTM RSV#006A[e]	7648	-	-	1.7	1.2	1.8	2.9	2.2	4.0	3.7	3.4	-	4.0	22.0
	7692	-	-	-	-	1.9	-	-	3.3	1.0	1.5	-	3.3	10.2
	7714	-	-	0.7	-	1.3	-	-	1.4	2.4	2.8	-	2.8	10.7
	7764	-	-	-	0.7	0.7	1.0	-	2.3	1.2	2.9	-	2.9	10.5
	Mean:												***3.2***	***13.3***
6120/ΔNS2/ 1030s Exp. Lot	8494	-	-	2.4	2.4	2.8	3.7	4.1	4.8	>3.3	3.0	-	4.8	28.0
	8542	-	-	1.4	2.0	2.0	1.8	2.9	2.9	3.3	1.4	-	3.0	18.5
	8554	0.7	-	-	1.5	2.0	2.1	4.1	1.3	3.0	3.6	-	4.1	19.3
	8562	-	-	-	2.4	3.2	2.9	2.3	1.8	3.2	3.4	-	3.4	20.7
	Mean:												***3.8***	***21.6***
6120/ΔNS2/ 1030s CTM RSV#012A[f]	8928	-	1.7	3.3	3.9	4.1	4.9	3.4	3.6	3.1	1.0	0.7	4.9	30.0
	8940	-	-	-	1.7	1.5	3.4	0.7	4.3	4.0	3.4	2.2	4.3	22.3
	8983	-	-	-	0.7	1.5	2.9	2.7	0.7	2.4	1.3	2.7	2.9	15.9
	9045	-	0.7	0.7	1.5	1.3	1.8	3.0	2.6	1.3	-	0.7	3.0	13.8
	Mean:												***3.8***	***20.5***

[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).

[b] Virus titrations were performed on Vero cells. The lower limit of detection was 0.7 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 used for samples with no detectable virus.

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

[e] Lot RSV#006A, vial numbers 0004, 1377, 2505.

[f] Lot RSV#012A, vial numbers 0019-0021, 1267-1269, 2483-2486.

Abbreviations: AGM- African green monkey, CTM- clinical trial material, RSV- respiratory syncytial virus, NP- nasopharyngeal, PFU- plaque-forming units, i.n.- intranasally, i.t.- intratracheally.

Table 28: Viral Titer of Tracheal Lavage Samples from AGMs Inoculated with the Experimental Lot or CTM of RSV 6120/ΔNS2/1030s, with RSV ΔNS2/Δ1313/I1314L, or with Recombinant wt RSV rA2

Virus Test Article[a] and Study Number	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 rA2 CTM RSV#004A[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***
RSV ΔNS2/Δ1313/ I1314L CTM RSV#006A[e]	7648	-	-	-	1.5	-	-	1.5	5.0
	7692	-	-	-	1.0	1.7	-	1.7	5.5
	7714	-	-	-	-	-	-	-	4.2
	7764	-	-	-	1.3	-	-	1.3	4.8
	Mean:							***1.4***	***4.9***
6120/ΔNS2/ 1030s Exp. Lot	8494	2.5	3.7	3.8	3.5	3.0	-	3.8	17.2
	8542	-	5.8	4.1	4.5	3.5	-	5.8	19.3
	8554	2.0	1.5	4.3	4.0	3.3	2.1	4.3	17.2
	8562	-	2.4	2.0	4.5	3.3	1.0	4.5	14.0
	Mean:							***4.6***	***16.9***
6120/ΔNS2/ 1030s CTM RSV#012A[f]	8928	2.3	1.0	2.0	3.2	1.0	1.0	3.2	9.9
	8940	1.0	3.1	2.9	3.4	2.8	1.0	3.4	13.6
	8983	1.0	1.0	1.0	1.0	1.0	2.1	2.1	5.6
	9045	1.0	2.4	2.7	3.1	2.3	1.0	3.1	12.1
	***Mean*:**							***2.9***	***10.5***

[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).*

[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] *CTM vial numbers 0004, 1377, 2505*

[f] *Lot RSV#012A, vial numbers 0019-0021, 1267-1269, 2483-2486.*

Abbreviations: AGM- African green monkey, CTM- clinical trial material, RSV- respiratory syncytial virus, TL- tracheal lavage, PFU- plaque-forming units, i.n.- intranasally, i.t.- intratracheally.

Table 29: Serum $PRNT_{60}$ Titers in AGMs Inoculated with the Experimental Lot or with CTM of RSV 6120/ΔNS2/1030s or RSV ΔNS2/Δ1313/I1314L, or Recombinant wt RSV rA2

Virus Test Article[a] and Study Number	AGM ID	RSV Neutralization Titer (Log2 of reciprocal) on Indicated Days		
		0	21	28
RSV rA2 wt RSV CTM RSV#004A[a]	7638	-	7.0	6.6
	7744	-	6.5	7.9
	7781	-	8.0	8.6
	7799	-	7.6	7.8
	***Mean*:**		**7.3**	**7.8**
RSV ΔNS2/Δ1313/ I1314L CTM RSV#006A[b]	7648	-	6.4	6.8
	7692	-	6.6	6.4
	7714	-	4.4	6.1
	7764	-	5.5	6.0
	***Mean*:**	**-**	**5.8**	**6.3**
6120/ΔNS2/ 1030s Exp. Lot	7638	-	9.6	9.7
	7744	-	9.3	9.6
	7781	-	7.5	9.4
	7799	-	8.7	9.4
	***Mean*:**		**8.8**	**9.5**
6120/ΔNS2/ 1030s CTM RSV#012A [c]	8928	-	6.8	6.8
	8940	-	5.3	5.7
	8983	-	3.3	3.3
	9045	-	5.6	5.6
	***Mean*:**		**5.3**	**5.4**

[a] *Lot RSV#004A; vial numbers 263, 886, 1108, 1567*
[b] *Lot RSV#006A, vial numbers 0004, 1377, 2505.*
[c] *Lot RSV#012A, vial numbers 0019-0021, 1267-1269, 2483-2486. 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 "-".*

Abbreviations: AGM- African green monkey, CTM- clinical trial material, RSV- respiratory syncytial virus.

APPENDIX II: SCHEDULE OF EVENTS: SCREENING, ACUTE PHASE, AND POST-ACUTE PHASE

Table 30: Schedule of Events: Group 1: RSV-Seropositive Participants

	ACUTE PHASE												POST-ACUTE PHASE				
	Screening[a]	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																
Clinical assessment (focused PE)		X			X	X	X	X	X			X		*		X	
Administer study product		X															
Blood for: immunologic assays	5mL														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.

Table 31: Schedule of Events: Group 2: RSV-Seronegative Participants

		ACUTE PHASE																					POST-ACUTE PHASE			
	Screening[a]	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	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																									
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	X)	X																								
Clinical assessment (focused PE)		X			X		X		X			X		X		X			X		X		*		X	
Administer study product		X																								
Blood for: immunologic assays	5mL																							5mL		5mL
Nasal wash and/or nasosorption SAM strip for: RSV antibody		X																			X			X		X
Nasal wash for: viral detection & quantification		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.

APPENDIX III: SCHEDULE OF EVENTS: RSV SEASONAL SURVEILLANCE

Table 32: Schedule of Events: RSV Seasonal Surveillance

	Pre-RSV season	Weekly contact	Post-RSV season	Illness Visit	Early DC
Visit period	Oct 1st to Oct 31st	Nov 1st to Mar 31st	Ideally Apr 1st to Apr 30th; allowable through Sept 30th		
Clinical assessment (focused PE)				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	X		X		
Nasal wash for viral detection & quantification				X	
Request adventitious agent assay				X	
TOTAL BLOOD VOLUME	5 mL	--	5 mL	--	5 mL

APPENDIX IV: DEFINITIONS OF SOLICITED ADVERSE EVENTS

Table 33: Definitions of Solicited Adverse Events

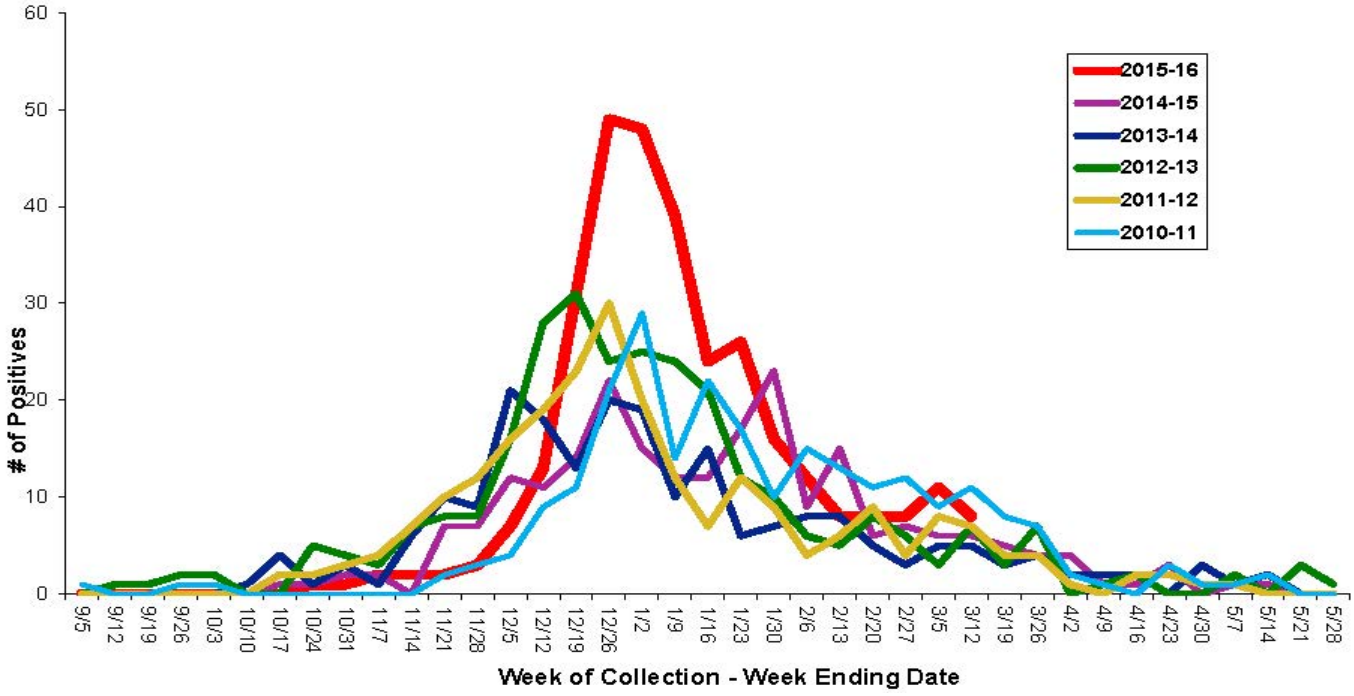
Event	Defined
Fever	Temporal or rectal temperatures ≥100.4ºF
Acute Otitis Media[1]	Loss of tympanic membrane landmarks, accompanied by erythema and loss of mobility. May or may not be associated with fever or other respiratory symptoms. Confirmed with tympanometry if possible.
Upper Respiratory Tract 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 7.2

APPENDIX V: RSV SEASONALITY IN BALTIMORE

Figure 6: RSV Seasonality in Baltimore



All specimens collected and tested at Johns Hopkins Hospital through 10 March 2016.