Phase Ib Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-attenuated Respiratory Syncytial Virus Vaccine, LID/ΔM2-2/1030s, Lot RSV#010A, Delivered as Nose Drops to RSV-Seronegative Infants and Children 6 to 24 Months of Age

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Phase Ib Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-attenuated Respiratory Syncytial Virus Vaccine, LID/ΔM2-2/1030s, Lot RSV#010A, Delivered as Nose Drops to RSV-Seronegative Infants and Children 6 to 24 Months of Age

SHORT TITLE	**LID/ΔM2-2/1030s**
PURPOSE	Assessment of safety, infectivity and immunogenicity of the respiratory syncytial virus (RSV) vaccine candidate LID/ΔM2-2/1030s in RSV-seronegative infants and children ≥ 6 months to < 25 months of age
DESIGN	A double blind, randomized, placebo-controlled study design will be used to evaluate the safety and immunogenicity of the study product in RSV-seronegative participants.
STUDY POPULATION	Groups 1 and 2: Healthy RSV-seronegative* infants and children ≥ 6 months to < 25 months of age *RSV-seronegative: RSV serum neutralizing antibody titer < 1:40; determined ≤ 42 days prior to inoculation
SAMPLE SIZE	Group 1: Approximately 21 RSV-seronegative infants and children Group 2: Approximately 60 RSV-seronegative infants and children It is expected that up to 150 children will need to be screened to accrue 81 RSV-seronegative participants.
STUDY PRODUCT	Single dose of intranasal RSV LID/ΔM2-2/1030s vaccine or placebo

Table 1: Inoculation Schedule

Group #	N	Study Product	Dose
1**	14	RSV LID/ΔM2-2/1030s	$10^{5.0}$ PFU*
	7	Placebo	0
2**	40	RSV LID/ΔM2-2/1030s	$10^{5.0}$ PFU*
	20	Placebo	0

* plaque-forming units (PFU)
** to be accrued in parallel.

Group 1 (intensive) and Group 2 (less intensive) will differ only in the frequency of study visits and nasal swab collections. Group 1 will have a study visit with nasal swabs obtained on study days 5,7,10, and 12 +/- 1 day and whenever solicited adverse events (febrile or respiratory illnesses) are reported between days 0 and 28. Group 2 will only have nasal swabs collected in the event of febrile or respiratory illnesses on study days 0 – 28. Both groups will have serum specimens obtained on days 0, 28, and 56, and post-surveillance.

STUDY DURATION

Participants will receive study product between April 1 and October 15, outside of the RSV season, and will remain on the study until they complete the post–RSV season visit. Screening may begin in March.

1 BACKGROUND

1.1 Epidemiology, Disease Burden, and the Need for a Vaccine

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

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

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 (7). Studies in experimental animals established that disease enhancement was specific to non-replicating RSV vaccines and not seen with infectious RSV or replicating vaccine vectors (8, 9).

Following the failure of the formalin-inactivated RSV vaccine, RSV vaccine development at the Laboratory of Infectious Diseases (LID) of the National Institute of Allergy and Infectious Diseases (NIAID) has focused largely on live-attenuated approaches (10). Throughout a period of over 30 years, a number of live-attenuated investigational RSV vaccines have been evaluated in RSV-naïve infants and children, and enhanced disease following wt RSV infection of vaccinees has not been observed (11). Apart from the absence of enhanced disease, live-attenuated RSV vaccines have a number of known advantages over non-replicating RSV vaccines. They can be administered intranasally (i.n.), induce protective mucosal immunity in the respiratory tract (as well as systemic immunity), infect in the presence of maternally-derived RSV serum antibody, and have been well tolerated and immunogenic when administered to infants as young as 4 weeks of age (12).

Human RSV has a single serotype with 2 antigenic subgroups, A and B. The 2 subgroups exhibit a 3- to 4-fold reciprocal difference in neutralization by polyclonal convalescent serum. Analysis of glycoprotein-specific responses in infants by enzyme-linked immunosorbent assay (ELISA) with

purified F and G glycoproteins showed that the fusion proteins (F proteins) were 50% related antigenically, and the G proteins were 7% related (13). Consistent with this level of antigenic relatedness, F protein expressed by a recombinant vaccinia virus was equally protective in cotton rats against challenge with either subgroup A or B, whereas the G protein was 13-fold less effective against the heterologous subgroup (14). Thus, the F protein is responsible for most of the observed cross-subgroup neutralization and protection, and a subgroup A vaccine virus is likely to induce a broad immune response against wt RSV of either subgroup. Antibodies to the F protein are one of the endpoints evaluated in this study.

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

This vaccine is a derivative of strain A2, subgroup A, attenuated by deletion of the M2-2 ORF, and by the "1030s" amino acid substitution in the L protein which induces temperature sensitivity. The RSV M2-2 protein is a small protein (90 amino acids in RSV strain A2) encoded by the second, downstream ORF in the M2 mRNA, which slightly overlaps the 5'-proximal, upstream M2-1 ORF (16). cDNA-derived RSV mutants in which the M2-2 ORF has been silenced or deleted grow more slowly in vitro than wt RSV (17, 18). Deletion of M2-2 results in increased accumulation of intracellular viral mRNA and decreased accumulation of genome and antigenome. This finding suggests that, during infection by wt RSV, M2-2 plays a role in shifting the balance of RNA synthesis from transcription to RNA replication (17). The increase in mRNA accumulation in cells infected with an M2-2 deleted RSV (ΔM2-2) was accompanied by an increase in the expression of RSV proteins, including expression of the F and G glycoproteins, suggesting that M2-2 deletion mutants might be more immunogenic than wt RSV. M2-2 deletion mutants are highly attenuated in non-human primates but do replicate to detectable titers (19). In previous pediatric Phase 1 studies, RSV M2-2 deletion mutants were attenuated, but highly immunogenic in infants and children, and refractory to genetic or phenotypic reversion (20-22).

Vaccine Description

RSV LID/ΔM2-2/1030s is a recombinant RSV that contains a deletion of the M2-2 ORF, combined with the "1030s" mutation in the L protein. Deletion of M2-2 is attenuating and thought to increase immunogenicity; “1030s” confers a temperature sensitivity phenotype and is further attenuating (21, 23).

Genetic stability of live-attenuated RNA viruses is a concern in clinical vaccine development. The deletion of an entire ORF, here M2-2, is refractory to genetic or phenotypic reversion. The "1030s" mutation [1313C (TCA)/1321K (AAA); (23)] of the L protein is a genetically stabilized version of the original "1030" mutation (12, 24, 25). This stabilized version remained stable under temperature stress conditions (23), and stability was substantially increased compared to previous non-stabilized versions in clinical studies (21, 26).

To generate an RSV antigenomic cDNA with an M2-2 deletion, a cDNA fragment of 241 nt length was removed from a wt RSV antigenomic cDNA, and 3 potential translation initiation codons were

silenced by site directed mutagenesis. In addition, the LID/ΔM2-2/1030s full length plasmid contains a deletion of 112 nucleotides from the downstream nontranslated region of the SH gene, and 5 translationally silent nucleotide changes in the downstream end of the SH ORF. These changes in the SH noncoding region stabilize RSV full-length cDNA plasmids during propagation in bacteria, and were deemed to be phenotypically inconsequential based on in-vitro and in-vivo studies. The "1030s" mutation was added by site directed mutagenesis of the full-length plasmid. The resulting plasmid was used to recover this recombinant RSV vaccine candidate from cDNA.

Seed virus was transferred to Charles River Laboratories (CRL; Malvern, PA) for manufacture of the Final Drug Product under cGMP (current Good Manufacturing Practice). The seed virus passed pre-production final container testing (Sterility, *Mycoplasma*, Bacteriostasis/Fungistasis**,** and testing for porcine circovirus types 1 and 2) and was accepted for manufacturing. For the production of the Final Drug Product at CRL, the 143$_{rd}$ passage of Vero cells (MF 11702; Lot #426068-2) was grown in OptiPRO™ serum-free medium. On day 3 post-infection, OptiPRO™ medium was removed, and replaced by Dulbecco's modified Eagle medium (DMEM). Antibiotics were not used in any stage of cell passage, virus growth, or vaccine development. The virus-containing supernatant was harvested on Day 6 post-infection and clarified by centrifugation. Intact cells were removed by filtration.

Clarified supernatant in 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; L-Glutamic Acid, 0.0054 M) was dispensed in 0.6 mL aliquots into labeled 2.0 mL cryogenic vials. Vials are snap-frozen and stored at -80ºC ± 15ºC. The Final Drug Product is a concentrated suspension of live recombinant LID/ΔM2-2/1030s Vero Grown Virus Vaccine (Lot RSV 010A) in DMEM without phenol red with 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; L-Glutamic Acid, 0.0054 M). The Final Drug Product has a potency of 6.0 $\log_{10}$ plaque-forming units (PFU)/mL and is diluted to dose on site.

The Final Drug Product, LID/ΔM2-2/1030s, Lot RSV # 010A, passed all in-vitro and in-vivo testing required for viral vaccines (Detection of Inapparent Viruses in a Viral Vaccine Product, *in-vitro* Tuberculosis Testing, PCR-based Reverse Transcriptase Testing, Porcine Circovirus Testing, Sterility, *Mycoplasma*, Bacteriostasis/Fungistasis, Residual DNA testing, DNA Sizing, Endotoxin, General Safety, Determination of the Sucrose Level, pH Determination, Intact Cell Assay, Potency/Infectivity, Identity, Purity, Toxicology and Pharmacology testing). Sequence analysis confirmed that the working seed virus and Final Drug Product, RSV Lot #010A, were of identical sequence. LID/ΔM2-2/1030s, Lot RSV #010A, was released by CRL for use as an investigational vaccine.

1.2 Prior Research

1.2.1 Experimental Vaccines Against RSV: M2-2 Deletion Mutants

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

A number of RSV vaccine candidates with M2-2 deletion have recently been evaluated. The first vaccine candidate evaluated in humans, designated RSV MEDI ΔM2-2, was sequentially evaluated in adults, RSV-seropositive children, and RSV-seronegative infants and children (20). RSV MEDI ΔM2-2 was highly restricted in replication; no shedding was observed in RSV-seropositive children, and minimal shedding was detected in RSV-seronegative children, in whom the mean peak titer of virus shed was $10^{1.5}$ PFU/mL. Rhinorrhea occurred more frequently in recipients of RSV MEDI ΔM2-2 (85%) than placebo (44%), although this difference was not significant. Despite its restriction in replication, RSV MEDI ΔM2-2 was highly immunogenic. When we compared vaccine virus replication and antibody responses in RSV-seronegative children who received RSV MEDI ΔM2-2 to those achieved with rA2cp248/404/1030ΔSH, a live-attenuated RSV vaccine candidate that was well tolerated and immunogenic in pediatric Phase I studies, we found that RSV MEDI ΔM2-2 vaccine virus shedding was significantly more restricted, yet the post-vaccination RSV-neutralizing serum antibody titers achieved (geometric mean titer [GMT] = 1:97) were significantly greater. Surveillance during the subsequent RSV season showed that several RSV seronegative RSV MEDI ΔM2-2 recipients had substantial antibody rises without reported illness, suggesting that the vaccine was protective yet primed for anamnestic responses to RSV. The M2-2 deletion was stable in all shed vaccine virus samples that were tested (20).

The closely related RSV LID ΔM2-2 candidate vaccine was evaluated in RSV-seronegative children [IMPAACT 2000/CIR 291, ClinicalTrials.gov identifier NCT02237209, NCT02040831; (21, 22)], 20 of whom received vaccine and 9 of whom received placebo. RSV LID ΔM2-2 was found to be highly infectious; 95% of vaccinees shed vaccine virus, with a mean peak titer of $10^{3.4}$ PFU/mL by immunoplaque assay and a mean peak titer of $10^{5.9}$ $\log_{10}$ copies/mL by quantitative real-time polymerase chain reaction (qRT-PCR). The level of replication of RSV LID ΔM2-2 in seronegative children was greater than expected based on previous study of RSV MEDI ΔM2-2. Respiratory or febrile illnesses occurred frequently in both recipients of RSV LID ΔM2-2 (95%) and placebo (78%). One vaccinee experienced a mild LRI (rhonchi) accompanied by shedding of vaccine virus and rhinovirus. It was not possible to determine whether the vaccine virus played a causal role in this participant's LRI, since an additional respiratory pathogen was also present. However, based upon the overall high level of vaccine virus replication, a decision was made to stop accrual to the study at 29 rather the targeted 51 participants.

Because of the unique properties of the M2-2 deletion mutation, which increases antigen production and seems to increase the inherent immunogenicity per infectious unit of virus**,** a decision was made to introduce additional attenuating mutations into the LID ΔM2-2 backbone to create vaccine candidates that would be more restricted in replication than LID ΔM2-2. The vaccine for the current protocol, LID/ΔM2-2/1030s, is closely related to LID ΔM2-2. It contains 2 attenuating elements, namely the M2-2 deletion, and the further attenuating "1030s" mutation, a genetically stabilized version of a temperature sensitivity mutation [Y1321K (AAA) in conjunction with the stabilizing mutation S1313 (TCA) in the L protein (21)]. As described below, LID/ΔM2-2/1030s appears to be a promising vaccine candidate based on evaluation in a small number of RSV-seronegative children (21). The purpose of this study is to further evaluate the safety and immunogenicity of LID/ΔM2-2/1030s in RSV-seronegative children.

1.2.2 Preclinical Studies

RSV with M2-2 deletion has been extensively studied in vitro and in vivo (17-19, 27). The results indicate that RSV ΔM2-2 is attenuated in small animal models and in non-human primates. RSV ΔM2-2 was also found to be immunogenic in these animal models. In mice and in 2 nonhuman primate models, namely in rhesus macaques and African green monkeys (AGMs), LID/ΔM2-2/1030s was confirmed to be further attenuated than RSV LID/ΔM2-2. Additional information about the preclinical evaluation of LID/ΔM2-2/1030s can be found in the Investigator's Brochure (IB).

Evaluation of Temperature Sensitivity of LID/ΔM2-2/1030s

In an in-vitro temperature sensitivity assay in Vero cells, LID/ΔM2-2/1030s exhibited a shut off temperature for plaque formation of 40°C, while LID ΔM2-2 and wt RSV have a shutoff temperature above 40°C. The shut-off temperature 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 (28). Starting at 38°C, LID/ΔM2-2/1030s exhibited a small-plaque phenotype. RSV ΔNS2 Δ1313 I1314L, a virus with a known shutoff temperature in Vero cells of 39°C, was included in these assays as a control (Table 2). This moderate level of temperature sensitivity is likely to somewhat restrict replication of LID/ΔM2-2/1030s in the lower respiratory tract, contributing to safety.

Table 2: Temperature Sensitivity of LID/ΔM2-2/1030s (Experimental Lot), RSV ΔNS2 Δ1313 I1314L, and Additional Controls

	Virus titer (log_{10} PFU per mL) at indicated temperature (°C) [a]								
Virus	32	35	36	37	38	39	40	T_{SH} [b]	T_{SP} [c]
LID/ΔM2-2/1030s	7.1	7.1	7.1	7.0	6.5*	5.4	< 1	40	38
RSV cp ΔSH ΔM2-2	6.9	6.8	6.9	6.8	6.7	6.5	6.3*	> 40	40
RSV ΔSH ΔM2-2	6.8*	6.8	6.9	6.8	6.7	6.7	6.4	> 40	32
RSV ΔNS2 Δ1313 I1314L	7.3	7.2	7.3	7.1*	6.3	3.3	< 1	39	37
LID ΔM2-2	7.3*	7.4	7.4	7.4	7.4	7.3	6.9	> 40	32
wt RSV	7.7	7.7	7.7	7.7	7.6	7.6	7.3	> 40	> 40

[a] *The ts phenotype for each virus was evaluated by plaque assay on Vero cells at the indicated temperatures. For viruses with ts phenotype, titers at shut-off temperatures are marked (bold, underlined). See footnote b for the definition of shut-off temperature.*

[b] *Shut off temperature (T_{SH}, bold, underlined) 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 (28). The ts phenotype is defined as having a shut off temperature of 40° C or less.*

[c] *T_{SP}, Small plaque temperature is defined as the lowest restrictive temperature at which the small-plaque phenotype is observed. Titers at lowest restrictive temperature are marked with an asterisk.*

1.2.3 Previous Clinical Experience

The live-attenuated recombinant LID/ΔM2-2/1030s vaccine virus was previously evaluated in 32 RSV-seronegative children (21 vaccinees and 11 placebo recipients; NCT02952339 and NCT02794870). The vaccine was highly infectious in RSV-seronegative children: Eighteen of the 20 vaccinees (90%) were infected with vaccine virus (i.e. shed vaccine virus and/or had a ≥4-fold rise in serum RSV antibodies). Vaccine virus was detected by immunoplaque assay and/or RT-qPCR for 17 of the 20 vaccinees (85%) [90% CI: (78%, 99.7%)] and in none of the placebo recipients. The mean peak titer of vaccine virus detected by immunoplaque assay was 2.8 log_{10} PFU/mL [compared to 3.4 log_{10} PFU/mL for LID/ΔM2-2 (22)]; 85% of vaccinees had RSV neutralizing antibody responses detectable on day 56 after immunization (29).Vaccine virus was detected for a median duration of 10 days (IQR 9-12) by immunoplaque assay and 12 days (IQR 10-14) by RT-qPCR. Daily viral shedding was highest on study days 7 to 10.

As is commonly observed in our vaccine trials in young RSV-seronegative children, respiratory and febrile illnesses occurred frequently in both vaccinees and placebo recipients. During the 28 days post-inoculation, respiratory and/or febrile illnesses occurred in 60% [90% CI: (39%, 78%)] of vaccinees and 27% [90% CI: (8%, 56%)] of placebo recipients. These differences in rates of illness were not statistically significant. Among the 12 vaccinees with illness, multiplex reverse transcription polymerase chain reaction (RT-PCR) testing (FastTrack Diagnostics, Luxembourg) of

NW detected vaccine alone in 3, vaccine plus ≥1 other adventitious agent in 4, and no vaccine but ≥1 other agent in 5. Among the 3 placebo recipients with illness, rhinovirus was detected in one and no agent in 2. Grade 3 fever occurred in 2 participants, one vaccinee and one placebo recipient. The vaccinee with grade 3 fever (104°F, Day 3) and respiratory symptoms (Days 5-22), did not have detectable vaccine virus shedding or a serum RSV antibody response (thus no evidence of infection by vaccine virus) but did have 4 other viruses detected in NW collected between Days 5-22. All other events in both groups were grade 2 or lower in severity. One vaccinee had a grade 2 LRI on day 21 after inoculation, characterized by wheezing, cough, and URI symptoms. This vaccinee had vaccine virus detectable in NW only on day 9. At the time of his LRI, vaccine virus was no longer detected in the NW, but rhinovirus was present. No SAEs were observed during the postvaccination follow-up period (21).

Based on the results from this small Phase 1 study, RSV LID/ΔM2-2/1030s was selected for further evaluation.

1.3 Clinical Development Plan

In the small Phase 1 study in RSV seronegative infants and children described above (21), the investigational RSV vaccine RSV LID/ΔM2-2/1030s showed promising levels of infectivity, immunogenicity, and safety. Under the present protocol, RSV LID/ΔM2-2/1030s will be further evaluated in additional cohorts of RSV-seronegative infants and children.

The main purposes of this study are (i) to collect additional data on vaccine infectivity, including magnitude and kinetics of vaccine virus shedding (Group 1), and (ii) to further evaluate in a larger Phase 1 study whether this vaccine is well tolerated and immunogenic in RSV-seronegative infants and children (Groups 1 and 2). The vaccine virus shedding results from Group 1 will provide additional insights on magnitude and kinetics of vaccine replication. Both Groups 1 and 2 will include follow-up for medically-attended RSV during the winter RSV surveillance season. Based on the results from this safety and immunogenicity study, evaluation of RSV LID/ΔM2-2/1030s may proceed to larger studies with efficacy endpoints.

In preparation for these larger studies, Groups 1 and 2 will include a blood draw on days 28 and 56 after study product administration to evaluate the timing of the maximum serum antibody response following vaccination. The nasal swab sampling performed in Group 1 will allow further exploration of the replication characteristics of RSV LID/ΔM2-2/1030s, and will add to the data collected in the previous Phase 1 study described in section 1.2.3 (21). Information generated from this study will provide the basis for larger studies focusing exclusively on safety and efficacy endpoints.

The primary immunogenicity endpoint to be evaluated is the proportion of vaccines with a ≥4-fold rise in RSV neutralizing antibody titers on Day 56 (the time point used in previous studies to evaluate the immune response to live-attenuated vaccines). The induction of neutralizing antibodies 56 days after immunization is a well-established and important surrogate marker of effective immunity to RSV disease. Antibodies to the F protein are also associated with cross-subgroup neutralization and protection (14). These assays will be performed at the Johns Hopkins University (JHU) CIR laboratory. Antibody detection assays may also be performed at the LID.

2 OBJECTIVES

2.1 Primary Objectives

Safety: Groups 1 and 2 (combined):

1. To assess the frequency and severity of study product–related solicited and unsolicited AEs from day 0 through the 28th day following inoculation
2. To assess the frequency and severity of study product–related SAEs from day 0 through the 56th day following inoculation

Immunogenicity: Groups 1 and 2 (combined):

1. To evaluate the percentage of vaccinees with a ≥4-fold rise in RSV-neutralizing serum antibodies at day 56 after inoculation

Infectivity: Group 1

1. To determine the peak titer of vaccine virus shed by each participant in Group 1
2. To determine the proportion of vaccinees infected with vaccine virus in Group 1 [defined as shedding vaccine virus, detected by RT-qPCR, and/or ≥4-fold rise in RSV-specific serum antibodies, detected by ELISA against the RSV F protein and/or an RSV plaque reduction neutralization test (RSV-PRNT)]

2.2 Secondary Objectives

1. *Groups 1 and 2 (combined): Safety* - To characterize the frequency and severity of symptomatic medically attended respiratory and febrile illness in vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season(s) in Groups 1 and 2

2. *Groups 1 and 2 (combined): Immunogenicity* - To evaluate the percentage of vaccines with a ≥4-fold rise in serum RSV F immunoglobulin G (IgG) antibodies at day 56 after inoculation

2.3 Exploratory Objectives

1. *Groups 1 and 2 (combined):* Safety - To evaluate the association of vaccine virus shedding with adverse events: To assess the incidence and magnitude of vaccine virus shedding in samples collected at Illness Visits associated with solicited AEs on Study Days 0 through 28 and with serious AEs from Study Day 0 through Day 56
2. *Groups 1 and 2 (combined):* Immunogenicity – timing of maximum response: To estimate and compare serum RSV F IgG and serum RSV-neutralizing antibody responses at the Day 28 and 56 Visits in vaccine recipients
3. *Groups 1 and 2 (combined):* Immunogenicity - magnitude of anamnestic response: To estimate and compare in vaccine recipients to placebo recipients the titers of serum RSV F IgG and serum RSV-neutralizing antibodies in participants infected with wt RSV during the RSV season
4. *Groups 1 and 2 (combined):* Immunogenicity – durability of vaccine-induced RSV antibodies: to estimate and compare the magnitude of RSV serum antibody titers in samples collected at the Day 56 and Post-RSV Season Visits among vaccine recipients who do not have evidence of RSV infection during RSV season
5. *Group 1:* The genetic stability of vaccine isolates will be evaluated by sequence analysis.

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 performed in RSV-seronegative children, and will be double-blind, randomized, and placebo-controlled. Groups 1 and 2 will be accrued in parallel. Group 1 (Intensive) and Group 2 (Less Intensive) will differ only in the frequency of study visits and nasal swab collections during the acute phase (days 0-28 after study product administration). Participants in Groups 1 and 2 will be block-randomized in groups of 3 at a ratio of 2:1 to receive a single intranasal dose of 10^5 PFU of vaccine or placebo, respectively. Evaluations in Group 1 will include nasal swabs during the predicted time of peak vaccine shedding. Infectivity endpoints will be evaluated only for Group 1. For evaluation of safety and immunogenicity endpoints, results from Groups 1 and 2 will be combined. All sites will follow relevant CDC and institutional guidance regarding in-person visits and use of personal protective equipment to ensure protection of subjects, families, and staff in the context of the SARS-CoV-2 pandemic.

For the purpose of this study, 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, 12, 20-22). In these and other 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.

At Vanderbilt University Medical Center (VUMC), participants will be screened and enrolled under this protocol. If eligible, participants will be randomized and will receive study product. At the University of Rochester Medical Center and the CIR, a separate screening protocol may be used to determine eligibility. Enrollment will occur at the time of inoculation with study product, Day 0. At all sites, inoculation with study product will occur between April 1 and October 15 to avoid the time during which wt RSV typically circulates in the community (Appendix IV, Figure 2). Participants will remain on the study until they complete the post–RSV season visit.

The first 28 days after inoculation is considered the acute phase of the study and 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 swab, or an interim medical history conducted by a mutually agreed upon communication method. Children will not be enrolled in this study while a Stay at Home Order is in place. However, in the event that a Stay at Home Order is initiated within the first 28 days following inoculation, or if a child develops a respiratory illness, parents may be asked to obtain the nasal swabs, and clinical assessments may be completed via remote or in-person research visits. During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate. Parents will be informed of these possibilities prior to enrollment, and will be trained in the methods used to collect an anterior nasal swab specimen. During the Acute Phase, the participants will be evaluated for adverse events (AEs) or serious adverse events (SAEs).

Group 1 will have intensive follow-up with nasal swabs obtained on study days 5, 7, 10, and 12 and whenever febrile or respiratory illnesses or otitis media (solicited AEs) are reported. Group 2 will have a less intensive follow-up with nasal swabs only in the event of solicited AEs during the acute phase of the study. Participants who have a solicited AE will have a nasal swab obtained and a remote or in person clinical assessment. The nasal swab will be tested for RSV, and for other respiratory pathogens that are considered adventitious agents. During the Post-Acute Phase, study days 29 – 56, participants' parents/guardians will be instructed to contact the study staff if any SAEs occur. Groups 1 and 2 will have a scheduled follow-up visit on the 28th and 56th day after

inoculation. The schedules of evaluations during the Acute Phase and Post-Acute Phase are shown in Appendix I, Table 21 and Table 22.

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 October 16 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 swab to evaluate for RSV and other respiratory pathogens that are considered adventitious agents (Appendix II). As noted above, if a Stay at Home Order is initiated during the RSV Surveillance Period, parents may be asked to obtain nasal swabs and clinical assessments may be completed via remote or in-person research visits.

Participants in both groups will have a post-RSV season visit to collect blood to assess the durability of the vaccine response and to assess the immune response to naturally occurring wt RSV infection. Some 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 21 RSV-seronegative infants and children ≥ 6 months to < 25 months of age will be accrued in Group 1; 14 will receive a single dose of $10^{5.0}$ PFU of vaccine and 7 will receive a single dose of placebo. 45-60 RSV-seronegative infants and children ≥ 6 months to < 25 months of age will be accrued in Group 2 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, 12, 20-22) and statistical considerations (Section 9.1).

4.1 Recruitment Process

Research staff will recruit participants from pediatric practices and clinics in the surrounding area. At the CIR and the University of Rochester, an IRB-approved screening protocol may be used for this study. At VUMC, children will be screened under this protocol. Upon referral by the participants' primary care provider or the provider's staff, staff will contact parents/guardians in person or remotely as appropriate, via telephone, email, or text message, send them IRB-approved informational brochures, and obtain contact information. Study staff will follow up with interested parents/guardians to elaborate upon the details and requirements of the screening and study process.

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

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

4.2 Study Visits

An overview of the study visits, evaluation schedule, and specimen collection is provided in Appendix I and Appendix II. 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. As noted above, sites will follow relevant CDC and institutional guidance regarding in-person visits and use of personal protective equipment. 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 assessment will include temperature, heart rate, respiratory rate, EENT, lung, heart and lymph nodes assessment. As previously stated, for visits following the inoculation visit, a parent may be asked to obtain the nasal swabs and clinical assessment may be done via a remote or in-person research visit if a Stay at Home order is initiated or if a child is ill.

All specimens will be obtained, processed, stored and transported per the Manual of Procedures (MOP).

In addition to the protocol-specified procedures listed in this section, study staff may complete other tasks consistent with standard operating procedures (SOPs), including but not limited to collecting, reviewing, and updating demographic and locator information; reviewing elements of informed consent; scheduling telephone (or text/email) 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.

4.3 Consenting Process

At the CIR and the University of Rochester, the screening process may be completed under a separate screening protocol and consent. At VUMC, screening will be performed under this protocol. 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 provided with a copy of the ICD.

During the consenting process, the parent will be given the opportunity to provide permission for use of their child's picture in 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.

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. The portions of the medical record that are pertinent to the study will be maintained in the study chart.

4.4 Screening Visit

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

The screening visit will include obtaining a serum sample to test for the presence of RSV antibodies. Approximately 3-5 mL of blood is sufficient quantity for the screening and pre-inoculation assays. The procedure will be performed and documented per the MOP. As in previous phase I trials of other live-attenuated RSV vaccines (11, 12, 20-22), hematologic and metabolic 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 (29).

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 physical examination or defer 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[1] sample for RSV serum antibody testing
Follow-up Preparation		□ Schedule enrollment visit if eligible

[1.] Obtained no more than 42 days prior to inoculation

4.5 Randomization

Randomization numbers will be assigned by Research Electronic Data Capture (REDCap) and will be forwarded to the Data and Safety Monitoring Board (DSMB) Executive Secretary. A copy of the randomization code will be retained by the unblinded dispenser.

Randomization will be implemented using the Randomization Module within Vanderbilt's REDCap database. The investigational pharmacy at each of the 3 institutions will distribute the assigned study vaccine. 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, 12, 20-22). Detailed procedures for randomization and unblinding will be placed in the MOP.

Unblinded study personnel will label the vaccine syringes and complete the appropriate parts of the vaccine administration record (VAR). After the participant has been inoculated, the vaccine administrator and verifier will initial the label and place the label in the subject's study chart after inoculation. The inoculation time will be documented on the VAR and a copy will be sent to the pharmacy. All syringes will be appropriately disposed of by the study staff.

4.6 Inoculation (Day 0)

Inoculation with study product will occur between April 1 and October 15 to avoid the RSV season. Study product administration occurs on study day 0, and must be completed at a clinic or medical office site or pediatric practice where emergency supplies are available in case of an adverse event (e.g. allergic reaction). 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.

Informed consent 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. At the VUMC, study-specific informed consent will be obtained prior to screening, whereas at Johns Hopkins CIR and the University of Rochester, screening may be performed under a separate protocol and study-specific consent will be obtained prior to inoculation. 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 the study visit 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, or

If the 42-day window from screening to inoculation is exceeded, then the infant or child must be rescreened, and an additional blood draw is needed.

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 □ Complete physical exam if deferred at screening, OR 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
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		□ Confirm contact method □ Complete teaching □ Schedule next visit

*No more than 42 days from screening

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 a remote or in-person research visit or non-visit day contact. New rectal thermometers will be given, and temporal artery thermometers will be provided to parent/guardian for use during the study. For temperature measurements, parent/guardian will be instructed to use the study-provided temporal artery thermometer to screen for elevated temporal artery temperatures. This device is used to minimize the number of rectal temperature measurements and has been shown to be an effective screening tool for rectal fever (30). 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 (30). For study-specific management and grading of temperatures, see Section 8.2.2, Table 17.

4.7 Study Phases

The Acute Phase begins with inoculation and ends at midnight on the 28th day after inoculation. 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 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 (11, 12, 20-22).

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 remote or in-person research visits to assess the illness (Section 4.8, Illness Visit).

Timing and amounts of study compensation will vary according to site and are included in the site-specific consent forms.

4.7.1 Acute Phase: In-person Study Visit Days

4.7.1.1 Group 1: Study Days 1-27

An in-person study visit and clinical assessment performed by a healthcare professional will be completed during visits on days 5, 7, 10, and 12 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. All sites will follow relevant CDC and institutional guidance regarding in-person visits and use of personal protective equipment. If a Stay at Home Order is initiated following inoculation, or if the child has a respiratory or febrile illness, the parent may be asked to obtain the nasal swab and the clinical assessment may be done via a remote or in-person research visit. Transport of specimens from the home to the research laboratory will be arranged as needed.

Table 5: Group 1: Acute Phase In-Person Visit Procedures

Days 5, 7, 10 and 12 In-person Visit Procedures *(each visit ± 1 days) (Group 1)*		
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 assessment □ Address any concerns □ Review safety data □ Document findings
Laboratory Tasks	**Nasal Swab**	*Collect nasal swab for*:* □ RSV viral detection and quantification
Follow-up Preparation		□ Schedule non-visit day contact and follow-up, in-person visits

*During the Acute Phase study visit, if the participant is diagnosed with or suspected of having URI, LRI, otitis media or fever (as defined in Appendix III), testing of the nasal swab specimen for adventitious agents will be performed as described in the MOP. Additional remote or in-person research visits with nasal swabbing will be scheduled if the participant experiences febrile or respiratory illnesses before Day 5 or after Day 12

4.7.1.2 Group 2: Study Days 1-27

A study visit is conducted if the participant is diagnosed with or has a parental report of having symptoms of URI, LRI, otitis media or fever (as defined in Appendix III) and testing of the nasal swab specimen for adventitious agents will be performed as described in the MOP. Parents will be provided with the necessary materials and will receive video instruction on collection of anterior nasal swab specimens to use if a remote visit is scheduled. Transport of specimens from the home to the research laboratory will be arranged as needed.

4.7.1.3 Groups 1 and 2: Study Day 28

Table 6: Groups 1 and 2: Day-28 In-Person Visit Procedures

Day 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 assessment □ Address any concerns □ Review safety data □ Document findings
Laboratory Tasks	**Nasal Swab***	*Collect nasal swab* for:* □ RSV viral detection and adventitious agents
	Blood	*Collect blood for*: Serum antibodies to RSV
Follow-up Preparation		□ Schedule day 56 follow-up, in-person visit

*A nasal swab specimen is only obtained if the participant has febrile or respiratory illness or otitis media. Timing of visit and collection are outlined in Table 12 and Table 13.

4.7.2 Acute Phase: Non-Visit Study Day Contacts

4.7.2.1 Group 1

In the 28 days following inoculation, contact with parents/guardians will be made on days that an in-person visit is not completed: days 1, 2, 3, 4, 6, 8, 9, 11, 13-27 (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 remote or in-person research visits to assess the severity of the illness and obtain a nasal swab for testing for adventitious agents. The families may be asked to obtain the nasal swabs for testing for adventitious agents if a remote visit is completed (Section 4.8). If a Stay at Home Order is initiated, the parent may be asked to obtain nasal swabs for routine study visits or illness visits, and clinical assessments may be completed via remote research visits. During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate.

4.7.2.2 Group 2

In the 28 days following inoculation, parental contact will be made on days that an in-person visit is not completed: days 1-27. On non-visit days, study staff will contact the parent/guardian and will record the parent/guardian-provided temperatures and signs of illness. For participants with febrile or respiratory illnesses or otitis media, a remote or in-person research illness visit will be scheduled to assess the severity of the illness and obtain a nasal swab for testing for adventitious agents. The families may be asked to obtain the nasal swabs for testing for adventitious agents if a remote visit is completed (Section 4.8). During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate.

Table 7: Groups 1 and 2: Acute Phase Non-Visit Contact Procedures

Days 1, 2, 3, 4, 6, 8, 9, 11, 13-27 (Group 1) and Days 1 – 27 (Group 2) Contact Procedures	
Clinical Tasks	☐ Obtain and document from parent/guardian the participant's interim history from previous days, including: o medications and/or immunizations o signs and symptoms of illness o highest temperature reading, indicate temperature method ☐ Address any concerns ☐ Review safety data ☐ Document findings
Follow-up Preparation	☐ Schedule an illness appointment if necessary

4.7.3 Study Day 29 Visit

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 8: Groups 1 and 2: Day 29 Non-Visit Procedures

Day 29 Non-Visit Procedures	
Clinical Tasks	☐ Obtain and document from parent/guardian the participant's previous days' interim history including: o medications and/or immunizations o signs and symptoms of illness o 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

4.7.4 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 a remote or in-person Illness Visit will be scheduled to assess the severity of the illness and obtain a nasal swab for testing for adventitious agents. The families may be asked to obtain the nasal swabs for testing for adventitious agents if a remote visit is completed (Section 4.8). During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate.

4.7.5 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 9: Groups 1 and 2: Day 56 Visit Procedures

Day 56 Visit *(+7 Days) (Groups 1 and 2)*		
Administrative Tasks		□ Provide study compensation
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: o visits to medical provider/hospitalizations o medications and/or immunizations o 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 (if Stay-at-Home orders are in place at a given site, sera will be collected as soon as possible thereafter)
Follow-up Preparation		Review plans for weekly contact during the RSV Season Surveillance Period (Section 4.7.7.1)

4.7.6 Period After Day 56 through October 15th

During this period, contact with the participant is not required. No clinical data will be recorded on CRFs or reported under this protocol.

4.7.7 RSV Surveillance

4.7.7.1 Weekly Contact for Surveillance During the RSV Season

Based on previous data regarding the seasonality of RSV in the Baltimore, MD, Rochester, NY, and Nashville, TN areas (Appendix IV, Figure 2), surveillance for RSV-associated disease will be conducted between October 16th and March 31st 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 III 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 4.8). All sites will follow relevant CDC and institutional guidance regarding in-person visits and use of personal protective equipment to ensure protection of subjects, families, and staff in the context of the SARS-CoV2 pandemic. If a Stay at Home Order is initiated, the parent may be asked to obtain the nasal swab for the RSV Surveillance illness, and the clinical assessment may be completed via a remote or in-person research visit.

Table 10: Groups 1 and 2: RSV Season Surveillance Procedures

RSV Season Surveillance (October 15 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 to take place (targeted April 1 - April 30 with allowable window through September 30th if Stay at Home Orders require that this study visit be delayed)

4.7.7.2 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 with an allowable window through September 30th if Stay at Home Orders require that this study visit be delayed.

Table 11: Groups 1 and 2: Post-RSV Seasonal Surveillance Study Visit Procedures

Post-RSV Season Study Visit		
Administrative Tasks		□ Provide study compensation
Clinical Tasks		□ Document findings related to study procedures
Laboratory Tasks	**Blood**	*Collect blood for:* □ Serum antibodies to RSV

4.8 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. If the Illness Visit occurs on a day concurrent with an in-person study visit, a single nasal swab collection is required, and adventitious agent testing will be requested. All sites will follow relevant CDC and institutional guidance regarding in-person visits and use of personal protective equipment to ensure protection of subjects, families, and staff in the context of the SARS-CoV-2 pandemic. For illness visits, the parent may be asked to obtain the nasal swab. During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate. As otitis media and LRI cannot be detected by remote study visit, medical records from provider visits will be obtained to document these events if a remote visit is completed or an in-person visit cannot be completed in a timely fashion.

If the acute or post-acute phases overlap the surveillance period (between October 15 and March 31), then the timelines for the acute and post-acute phases will be used.

Table 12: 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; a follow-up visit, including nasal swab is required one to three days after the initial assessment.
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 13: Illness Visit Procedures

Illness Visit Procedures		
Administrative Tasks		□ Complete Adventitious Agent Assay Request for rRT-PCR on nasal swab for adventitious agents □ Complete medical record release if needed
Clinical Tasks		□ Obtain and document from parent/guardian the participant's interim history including: o medications and/or immunizations o signs and symptoms of illness o highest temperature reading, indicate temperature method □ Perform focused clinical assessment □ Address any concerns □ Review safety data □ Document findings
Laboratory Tasks	**Nasal Swab**	*Collect nasal swab for* □ Viral detection and quantification
Follow-up Preparation		□ Schedule follow-up as appropriate

4.9 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 56. Every effort should be made to schedule a final Early Discontinuation Visit.

Table 14: 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 56 □ Document findings
Laboratory Tasks	**Blood**	*Collect blood for:* □ Serum antibodies to RSV
	Nasal Swab	*If Early Discontinuation Visit is within 56 days (Appendix I), collect nasal swab for:* □ Viral detection and quantification

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. Compensation will be given only for the visits and contacts that are completed.

4.12 Specimen Collection

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

4.12.1 Virus Detection

Specimens for viral detection, quantification of vaccine virus shedding by rRT-qPCR, and to obtain vaccine isolates foranalysis of genetic stability will be obtained by nasal swab approximately 4 times after inoculation in Group 1, as shown in Appendix I. These specimens may also be tested for adventitious respiratory viruses and bacteria if needed. Additional anterior nasal swab specimens for detection of RSV and adventitious respiratory viruses by rRT-qPCR will also be obtained from participants in Group 1 who meet illness criteria during the Acute phase (Days 0 through Day 28); participants in Group 2 will only have nasal swabs obtained during the Acute Phase if they experience febrile or respiratory illnesses. During the RSV Season Surveillance Period, (October 16 – March 31) nasal swabs will be obtained from all study participants in Groups 1 and 2 in the event of medically-attended febrile or respiratory illnesses. If a participant becomes ill during the Acute or Post-Acute Phase, then additional nasal swabs may be obtained to exclude infection with an adventitious virus. Laboratory testing will be performed by personnel who are not involved with clinical assessment to maintain the blinding status.

4.12.2 Immunologic Assays

For measurement of RSV serum antibodies, serum specimens will be obtained not more than 42 days prior to inoculation and on Days 28 and 56 (+7) post-inoculation. In addition, post RSV season serum specimens will be collected from all participants.

These samples will be used to assess antibody responses to vaccine, and to determine whether a four-fold or greater rise in antibody titer has occurred following the RSV season, which would signify infection with wt RSV. This will allow comparison of the rate and severity of significant RSV illness following infection with wt virus, as well as comparison of the antibody responses, in vaccine and placebo recipients. Obtaining serum samples for antibody testing on days 28 and 56 will allow an assessment of the kinetics of the antibody response, which will be useful in determining the optimal time to obtain post-inoculation sera in future studies.

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 20 mL of blood will be obtained from participants for study purposes from screening through the surveillance period.

4.12.3 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 I and Appendix II).

4.12.4 Research Laboratories

Quantitation of the amount of vaccine virus shed, assays to measure immune responses before and after inoculation, and assessment of nasal swabs for adventitious viral agents will be performed at

the CIR. Specimens may be sent to LID, NIAID for confirmatory testing, for specialized immune assays, and for sequence analysis, including deep sequencing, of vaccine isolates.

4.12.5 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.

4.12.6 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).

5 Inclusion/Exclusion Criteria

5.1 Inclusion Criteria

Potential participants must meet all of the following inclusion criteria to be enrolled in this study and randomized to receive study product or placebo:

5.1.1. ≥6 months of age and <25 months of age at the time of inoculation

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

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

5.1.4. In good health based on review of the medical record, history, and physical examination at the time of inoculation

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

5.1.6. Growing normally for age as demonstrated on a WHO growth chart, AND

5.1.6.1. If < 1 year of age: has a current height and weight above the 5th percentile for age

5.1.6.2. If ≥ 1 year of age: has a current height and weight above the 3rd percentile for age

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

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

5.2 Exclusion Criteria

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

5.2.1. ≤ 6 months of age and > 25 months of age at the time of inoculation

5.2.2. Born at less than 34 weeks gestation

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

5.2.4. Maternal history of a positive HIV test before or during pregnancy

5.2.5. Evidence of chronic disease

5.2.6. Known or suspected infection or impairment of immunological functions

5.2.7. Bone marrow/solid organ transplant recipient

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

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

5.2.10. Cardiac abnormality requiring treatment

5.2.11. Lung disease or reactive airway disease

5.2.12. More than one episode of medically diagnosed wheezing in the first year of life

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

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

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

5.2.16. Previous receipt of an investigational RSV vaccine

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

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

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

5.2.20. Previous anaphylactic reaction

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

5.2.22. Known hypersensitivity to any study product component

5.2.23. 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

5.2.24. 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

5.2.25. 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

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

- inactivated influenza vaccine within 3 days prior, or
- any other 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

5.2.27. 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

5.2.28. 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

5.2.29. 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.

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

- fever (temporal or rectal temperature of ≥100.4°F), or
- upper respiratory signs or symptoms (rhinorrhea, cough, or pharyngitis) or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media
- contact with a person diagnosed with COVID-19 disease or active SARS-CoV-2 infection within the preceding 10 days

Eligible siblings may be enrolled in this study provided that they are enrolled simultaneously. In this event, they will be co-randomized to receive the same study product (vaccine or placebo), and randomization will be expanded to groups of 6 or 9 as needed.

5.3 Co-Enrollment Considerations

Co-enrollment in 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).

5.4 Re-Enrollment Considerations

Participants who receive placebo may re-enroll in the initial study following completion of the RSV surveillance period if the study is ongoing at that time and they continue to meet all eligibility requirements

6 STUDY PRODUCT

The unblinded dispenser should consult the study MOP. Refer to the IB for further information about the study product.

- Groups 1 and 2: Live Recombinant Respiratory Syncytial Virus RSV LID/ΔM2-2/1030s, approximately $10^{5.3}$ PFU per 1.0 mL vaccine
- Groups 1 and 2: Placebo for the RSV vaccine will be Lactated Ringer's Solution for Injection, USP 1.0 mL

6.1 Study Product Regimens

Enrolled study participants will receive a single dose of RSV LID/ΔM2-2/1030s vaccine or placebo, administered as nose drops.

6.2 Study Product Formulation

6.2.1 RSV LID/ΔM2-2/1030s

The LID/ΔM2-2/1030s vaccine is provided in a sterile 2.0-mL cryovial, each containing 0.6 mL of vaccine (Lot RSV#010A) with a titer of approximately $10^{5.7}$ PFU/mL. The vaccine virus concentrate is diluted by trained pharmacy personnel to a dose of approximately $10^{5.0}$ PFU in a 0.5-mL volume. The vaccine vial is labeled as shown in Figure 1.

Live Recombinant Respiratory Syncytial Virus LID ΔM2-2 1030s VERO GROWN VIRUS VACCINE	Date: 09JUN2015 Vial#:XXXX Lot: RSV #010A
CAUTION:NEW DRUG LIMITED	
BY FEDERAL (USA) LAW	
TO INVESTIGATIONAL USE	
Store at –80°C ± 15°C Charles River Laboratories, Malvern, PA	

Figure 1: Investigational Product Label Sample

6.2.2 Diluent for RSV LID/ΔM2-2/1030s

The diluent for RSV LID/ΔM2-2/1030s is Lactated Ringer's Solution for Injection, USP.

6.2.3 Placebo for RSV LID/ΔM2-2/1030s

The placebo for RSV LID/ΔM2-2/1030s is Lactated Ringer's Solution for Injection, USP.

6.3 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.

6.4 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.

6.4.1 Diluent

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

6.4.2 Placebo

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

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

6.4.3 Live RSV LID/ΔM2-2/1030s

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 3 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 $10^{5.7}$ 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 $10^{5.0}$ PFU in 0.5mL prior to administration.

The diluted study product will be drawn up to a volume of 0.5 mL 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.

6.5 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 0.5 mL of study product will be delivered as nose drops (approximately 0.25 mL per nostril) using a sterile, needle-less, syringe. Participant will remain supine for approximately 60 seconds following inoculation.

6.6 Study Product Acquisition

The clinical lot of RSV LID/ΔM2-2/1030s was generated by 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. Following institutional biosafety committee (IBC) approval, and prior to IRB approval, the PI/designee may request that vials of vaccine be transferred from the Sponsor to the research pharmacy at the clinical site for confirmation of the vaccine titer. However, only after receipt of IRB approval 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.

6.7 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.

6.8 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.

6.9 Final Disposition of Study Products

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

6.10 Concomitant Medications

Permitted concomitant medications at enrollment (prescription or non-prescription) include nutritional supplements, medications for gastroesophageal reflux, gas, 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 through the 28th day following inoculation. However, use of these medications for treatment of symptoms is allowed.

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

- Licensed inactivated vaccine or live-attenuated rotavirus vaccine within 14 days of inoculation, with the exception of inactivated influenza vaccine
- Licensed live virus vaccine, other than rotavirus vaccine, within 28 days of inoculation
- Systemic corticosteroids for more than 14 days at a dosage equivalent to prednisone at ≥ 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 56 days of inoculation

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 DSMB are briefly referenced in Section 7.1.4 and described in detail in Section 9.4.2.

7.1 Safety-Related Roles and Responsibilities

7.1.1 Principal Investigator

Each site PI is responsible for continuous monitoring of 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 and 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, 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.

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 15.

Table 15: AE CRF Recording Requirements

Study Phase at the Time of Event Onset	Calendar Date	AEs to Record on CRFs	Concomitant Medications to Record on CRFs
Day 0 through midnight of 28th day following inoculation (Acute Phase)	ANY	□ All SAEs □ All solicited AEs that meet Appendix IV. 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 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 15 in year of inoculation	□ Grade ≥ 3 AEs or SAEs that is deemed related to Pre-RSV Season Study Visit procedures	□ All medications related to the recorded event
RSV Season Surveillance Periods	October 16 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 III 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	April 1 to April 30 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

SAEs are described in Section 8.1.2. All SAEs will be reviewed by a study physician, recorded as specified by the CSO, through REDCap or 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 III if they occur during study days 0 to 28.

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 301-846-5360, 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 UP nonAE 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 2 categories of AEs specific to CIR 335: 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 III Individual symptoms listed in the "events" column that fail to meet the criteria in the "definition" column in Appendix III are recorded in source documents but are not recorded on the CRFs. During the Acute Phase of this study, days 0 through 28, 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 III 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 occurring on the day of the study visit and 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 III 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 III.
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 III.

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 IB or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

8.2 Grading the Severity of Adverse Events

The Investigator will assess all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research). All AEs and fever will be graded using the protocol-defined grading system outlined below.

8.2.1 AE Grading

Table 16: 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. Does not interfere with usual activities, including eating and/or sleeping.
Grade 2 Moderate	Symptoms interfering to some degree with usual activities, including eating and/or sleeping. In most cases, symptoms severe enough to necessitate a medical care visit would likely meet this criterion; however, if medical care is sought and symptoms are assessed as only mild, the event may remain Grade 1. If prescription medication is used or recommended for symptoms, the event automatically moves to at least Grade 2.
Grade 3 Severe	Prolonged medical intervention and/or hospitalization required
Grade 4 Life threatening	Illness requiring hospitalization with intensive care
Grade 5 Death	Event resulting in fatal outcome to the participant

8.2.2 Fever Grading

Table 17: 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

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 18).

Table 18: Pausing and Stopping Rules

Specified Phase	Event	Reporting
Acute and Post-Acute Days 0 through 56 following inoculation	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. The event will be reviewed by the DSMB prior to resuming enrollment.

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 I (Table 21 and Table 22).

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. The regulatory sponsor (OCRPRO) will determine if the FDA needs to be notified.

Ad hoc DSMB Expert Member review will also be requested by the PI if any of the events listed below occur. The CSO and Study Medical Monitor will also be notified.

- >1 Grade 4 fever occurs within 14 days of study product administration, or
- >1 Grade ≥3 solicited AE (excluding rhinorrhea or fever) occurs within 28 days of study product administration.
- Any LRI occurs during the acute 28 day follow up period

In these cases, accrual and study product administration will continue while ad hoc DSMB Expert Member review is in progress (See Section 9.4.2, Monitoring by the NIAID Intramural DSMB, for details).

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 LID/ΔM2-2/1030s vaccine candidate in RSV-seronegative pediatric participants.

Group 1 (intensive sampling): Twenty-one 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 to evaluate safety and immunogenicity.

Group 2 (less intensive sampling): Sixty 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 to evaluate safety and immunogenicity.

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

Primary Objective: Safety: Groups 1 and 2 (combined):

1. To assess the frequency and severity of study product–related solicited and unsolicited AEs from day 0 through the 28th day following inoculation

   Outcome measures:
   Grade 1 or higher solicited AEs as defined in Appendix III from Day 0 through Day 28
   Grade 2 or higher LRIs as defined in Appendix III from Day 0 through Day 28

2. To assess the frequency and severity of study product–related SAEs from day 0 through the 56th day following inoculation

   Outcome measure:
   Serious AEs from Day 0 through Day 56

Primary Objective: Immunogenicity: Groups 1 and 2 (combined):

1. To evaluate the percentage of vaccinees with a ≥4-fold rise in RSV-neutralizing serum antibodies at day 56 after inoculation

    Outcome measure:
    ≥4-fold rise in serum RSV-neutralizing antibody titer from pre-study product administration (screening) to the Day 56 Visit

    Titer is quantified even if <1:40; therefore, a ≥4-fold rise can be determined even for lower titers.

Primary Objective: Infectivity: Group 1

1. To determine the peak titer of vaccine virus shed by each participant in Group 1

    Outcome measure:
    Peak titer of vaccine virus shed from Study Day 0-28

2. To determine the proportion of vaccinees infected with vaccine virus in Group 1 [defined as shedding vaccine virus, detected by RT-qPCR, and/or ≥4-fold rise in RSV-specific serum antibodies, detected by ELISA against the RSV F protein and/or an RSV-PRNT]

    Outcome measure:
    Shedding vaccine virus, detected by RT-qPCR, and/or ≥4-fold rise in RSV-specific serum antibodies, detected by ELISA against the RSV F protein and/or an RSV-PRNT]

9.2.2 Secondary Outcome measures

Secondary Objective: Safety: Groups 1 and 2 (combined): - To characterize the frequency and severity of symptomatic medically attended respiratory and febrile illness in vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season(s) in groups 1 and 2.

Outcome measures:

1. RSV-medically attended acute respiratory illness (MAARI) and maximum grade (if more than one illness within a participant)
2. RSV-medically attended acute lower respiratory illness (MAALRI) and maximum grade (if more than one illness within a participant)

Groups 1 and 2 (combined): Immunogenicity - To evaluate the percentage of vaccines with a ≥4-fold rise in serum RSV F IgG antibodies at day 56 after inoculation

Outcome measures:
≥4-fold rise in serum RSV F IgG from pre-study product administration (screening) to the Day 56 Visit

9.2.3 Exploratory Outcome Measures

Groups 1 and 2 (combined): Safety - To evaluate the association of vaccine virus shedding with adverse events: To assess the incidence and magnitude of vaccine virus shedding in samples collected at Illness Visits associated with solicited AEs on Study Days 0 through 28 and with serious AEs from Study Day 0 through Day 56

Outcome measures:
For solicited AEs between Days 0 and 28 (more than one per participant possible).
Detection and magnitude of vaccine virus shedding detected in Illness Visit specimens as determined by:
1. RT-PCR

Groups 1 and 2 (combined): Immunogenicity – timing of maximum response: To estimate and compare serum RSV F IgG and serum RSV-neutralizing antibody responses at the Day 28 and 56 Visits in vaccine recipients

Outcome measures:
Titer of serum RSV F IgG at the Day 28 and 56 Visits
Titer of serum RSV-neutralizing antibodies at the Day 28 and 56 Visits

Groups 1 and 2 (combined): Immunogenicity - magnitude of anamnestic response: To estimate and compare in vaccine recipients to placebo recipients the titers of serum RSV F IgG and serum RSV-neutralizing antibodies in participants infected with wt RSV during the RSV season

Outcome measures:
In subset of children who had RSV infection documented during the RSV season (via viral testing confirming presence of RSV or a further ≥2.5-fold rise in serum RSV-neutralizing antibody titers from the Day 56 Visit to the Post-RSV Season Visit):

Titer of serum RSV F IgG at the Day 56 and Post-RSV Season Visits
Titer of serum RSV-neutralizing antibodies at the Day 56 and Post-RSV Season Visits

Groups 1 and 2 (combined): Immunogenicity – durability of vaccine-induced RSV antibodies: to estimate and compare the magnitude of RSV serum antibody titers in samples collected at the Day 56 and Post-RSV Season Visits among vaccine recipients who do not have evidence of RSV infection during RSV season

Outcome measures:
In subset of children who achieve ≥4-fold rises in serum RSV-neutralizing antibody titers between pre-study product administration and the Day 56 Visit and with no evidence of RSV infection after Day 56 (no RSV infection identified by viral testing done at the site or centrally and <2.5-fold rise in serum RSV-neutralizing antibody titers from the Day 56 Visit to the Post-RSV Season Visit):

Titer of serum RSV F IgG at Day 56 and Post-RSV Season Visits
Titer of serum RSV-neutralizing antibodies at Day 56 and Post-RSV Season Visits

Group 1: The genetic stability of vaccine isolates will be evaluated by sequence analysis. Study samples may be used in comparative assays with samples from other RSV vaccine studies initiated by the LID, NIAID, NIH.

9.3 Sample Size and Accrual

9.3.1 Sample Size and Randomization

In Group 1, 21 RSV-seronegative infants and children will be accrued and will be randomized to receive either $10^{5.0}$ PFU of vaccine or placebo at a ratio of 2:1. In Group 2, 60 RSV-seronegative infants and children will be accrued and will receive either $10^{5.0}$ PFU of vaccine or placebo at a ratio of 2:1. The results from groups 1 and 2 will be combined for evaluation of safety and immunogenicity. The 2:1 randomization ratio will be used to maximize the information obtained regarding the response of children to the LID/ΔM2-2/1030s vaccine.

It may be difficult to accrue 81 (21 in Group 1 and 60 in Group 2) RSV-seronegative infants in one season. Therefore, at the end of the first season, the accrual numbers will be evaluated and if at least 66 (21 Group 1 and 45 Group 2) RSV-seronegative infants have been accrued enrollment will stop; otherwise, enrollment will continue during a second season. In the statistics section we provide power calculations for Group 2 sizes of 60 and 45.

The following calculations focus on the assessment of the safety of the vaccine and, in particular, occurrence of LRI due to vaccine virus from vaccine inoculation through day 56, 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.

The probability of observing 3 or more LRI events with a sample of 54 (14 vaccinees from Group 1 and 40 from Group 2) seronegative vaccine recipients is 0.02 if the true LRI rate is 1% and the probability is 0.82 if the true rate is 8%. Therefore, if we observe 3 or more LRI events we will consider the vaccine safety to be concerning, since there is high probability of having increased the rate of LRI over the low background rate. If after 1 season we are only able to accrue 44 vaccine recipients (14 in Group 1 and 30 from Group 2), we would still consider 3 more LRI events to be troubling: With the smaller sample size, the probability of observing 3 or more events out of 44 is 0.01 for a true LRI rate of 1% and 0.83 for a true LRI rate of 10%. This means that the true increase in LRI rate would need to be slightly higher to have good power to detect an increase if only 44 vaccine recipients have been accrued. For all sample sizes between 44 and 54, we would also consider 3 or more LRIs to be concerning.

We are also interested in estimating AE rates and will calculate 90% confidence intervals (CIs) for AEs. Table 19 gives the probability of 90% CI half widths less than a specified amount for sample sizes of 54 and 44 vaccines.

Table 19: Probability of Observing a 90% Confidence Interval Half Width Less than a Specified Amount with Various True AE Rates

True AE rate	N=54		N=44	
	Specified half width, h	Probability half width will be less than h	Specified half width, h	Probability half width will be less than h
0.01	0.04	0.92	0.04	0.90
0.05	0.07	0.82	0.06	0.87
0.10	0.08	0.91	0.09	0.85

There will be very little power for between-group (placebo and vaccine) comparisons of AEs. Table 20 gives the detectable differences with 80% power for both samples sizes of 27 vs 54 and 22 vs 44 for various true differences. The one-sided Fishers exact test is used. The alpha level of the test that was targeted is one sided 0.05 to see if the AE rate has been increased in the vaccine group.

Table 20: Detectable Differences with 80% Power for Various True Differences

True Response Proportion in the Placebo Group	True Response proportion in vaccine group (difference)	
	N= 54 vs 27	N= 44 vs 22
0.01	0.20 (0.19)	0.23(0.22)
0.05	0.29 (0.24)	0.33 (0.27)
0.1	0.37 (0.27)	0.40 (0.30)
0.15	0.44 (0.29)	0.48 (0.33)
0.20	0.50 (0.30)	0.53 (0.33)
0.30	0.61 (0.31)	0.64 (0.34)

Table 20 also gives the detectable differences in the proportion of infants with ≥4-fold rise in serum RSV-neutralizing antibody titer between the groups with 80% power. We will also use a one sided Fishers exact test at the 0.05 level for this comparison. This is done because this is an early study and we are willing to increase the probability of considering an inactive vaccine interesting in order to increase the probability of finding an active vaccine while maintaining small sample size. Any positive findings in this study will need to be explored further.

With a sample size of 14 seronegative vaccine recipients, the 90% CI around a sample mean peak titer of 2.5 $\log_{10}$, with a standard deviation (SD) of 1.5 is (1.79, 3.21). This calculation uses a t-test. This ensures with 90% confidence that the true population mean peak titer is between 1.79 and 3.21 $\log_{10}$, and with 95% confidence that the true population mean is not lower than 1.79 $\log_{10}$.

With the same sample size of 14, if the true proportion of vaccine recipients who shed vaccine virus is 80% than there is probability 0.87 that the half width of the 90% CI will be less than 20%. If the true proportion of vaccine recipients who shed vaccine virus is 90% than there is probability 0.84 that the half width of the 90% CI will be less than 18%.

9.4 Monitoring

9.4.1 Site Monitoring Plan

As per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (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 informed consent form (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 REDCap 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, REDCap 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. Two to three additional *ad hoc* DSMB Expert Members with expertise in pediatric respiratory virus vaccine development will be added to the DSMB for review of this protocol. These *ad hoc* DSMB Expert Members will perform real-time *ad hoc* reviews of individual (unblinded) cases of LRIs and SAEs not attributed to an etiology or cause unrelated to the study product to assess whether detection of vaccine virus is associated with the illness. 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.

Ad hoc DSMB Expert Member reviews will be requested by the PI if pausing and stopping rules are triggered (Section 8.3, Table 18):

- An SAE that cannot be attributed to an etiology or a cause unrelated to the study product occurs within 56 days of study product administration.

Accrual and study product administration will stop pending review.

Ad hoc DSMB Expert Member reviews will also be requested if

- More than one Grade 4 fever occurs within 14 days of study product administration or
- More than one Grade 3 or higher solicited AE (excluding rhinorrhea or fever) occurs within 28 days of study product administration.
- Any LRI that occurs within 28 days of study product administration

In these cases, accrual and study product administration will continue while *ad hoc* DSMB Expert Member review is in progress. The review will be convened per DSMB guidelines. The site will send respiratory viral samples to the JHU CIR laboratory to determine shedding of RSV or adventitious viral agents, and the lab will send their results for *ad hoc* review directly to the *ad hoc* DSMB Expert Members. Information on treatment assignment will be provided to the *ad hoc* DSMB Expert Members by the unblinded dispenser. The *ad hoc* DSMB Expert Members will review the relevant safety information and available data, including presence of vaccine virus shedding, whether other adventitious agents are present, and whether the participant received placebo or one of the candidate vaccines. The *ad hoc* DSMB Expert Members will review whether the AE, serious AE, or LRI can be attributed to an etiology, a cause, or a diagnosis unrelated to the study vaccine, and if it is associated with shedding of vaccine virus at the time of the event (even if another pathogen is identified). The *ad hoc* DSMB Expert Members will report their assessment to the DSMB. Based on (1) the severity of the event, (2) whether the participant received a candidate vaccine and (3) whether RSV and/or other agents are present in the nasal swab, the *ad hoc* DSMB Expert Members may recommend to the DSMB that enrollment be continued or paused pending full DSMB review.

If the DSMB decides the study can continue unchanged, they will communicate their findings to the Protocol Team. Alternatively, the DSMB may recommend modifications to the study design.

9.5 Analyses

9.5.1 Assessment of Primary Objectives

Safety and immunogenicity data from all participants in Groups 1 and 2 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 or for the Day 56 Visit follow-up (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 6.10 within the first 56 days after inoculation may be excluded from the immunogenicity evaluations after the time of the treatment. These participants, however, will be included in the safety evaluations for the duration of the study. These participants will not be replaced. Details of the analyses listed below will be included in the statistical analysis plan.

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

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.

For Group 1, the proportion of participants with infection defined as recovery of vaccine virus from a nasal swab as determined by RT-PCR, and/or a ≥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 swabs by each individual will be prepared. In addition, the geometric mean peak titer and mean duration of virus shed will be provided for Group 1.

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 four-fold 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.

9.5.2 Assessment of Secondary Objectives

The summary of the frequency and severity of symptomatic, medically attended respiratory and febrile illness in vaccine and placebo recipients who experience natural infection with wt RSV during the RSV season will be presented. In addition, a line listing of the individual RSV antibody titer pre- and post-RSV Season Surveillance Period will be prepared. 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.

10 DATA HANDLING AND RECORD KEEPING

10.1 Data Management and Coordination

The Data Coordinating Center for this study will be housed at VUMC. The clinical sites will maintain adequate and accurate research records containing all information pertinent to the study for all screened and enrolled participants, including CRFs and supporting source data per the MOP.

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. Data will be entered into the REDCap database developed by the Vanderbilt Coordinating Center.

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 LID/ΔM2-2/1030s 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 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 site 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 (31, 32). 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 Swab

Nasal swabs can cause discomfort and on rare occasions are associated with nose bleeds.

12.5.3 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.

12.5.4 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 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 Surveillance

Compensation will be in the form of either check or gift card. In addition, the parent/guardian will receive bus tokens, taxi fare, or parking passes as needed for study visits. Parent/guardian will be compensated only for those portions of the study that are completed. Compensation will be in accordance with IRB policies and procedures and will be subject to IRB approval.
Participants may receive age-appropriate books or small toys. The total value of the books or toys will not exceed $10 per participant.

12.7 Privacy and Confidentiality

All study procedures will be conducted in private and every effort will be made to protect participant privacy and confidentiality to the extent possible. Participant information will not be released without written permission to do so except as necessary for review, monitoring, auditing or as required by law, or 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 335 is sponsored by the OCRPRO, DCR, NIAID, NIH.

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

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

13.2 Protocol Registration

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

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.

15 ABBREVIATIONS

AE	adverse event
ALRI	acute lower respiratory infection
cDNA	complementary deoxyribonucleic acid
CI	confidence interval
CIR	Center for Immunization Research
CRADA	Cooperative Research and Development Agreement
CRF	case report form
DCR	Division of Clinical Research
DMEM	Dulbecco's modified Eagle medium
DSMB	data and safety monitoring board
EENT	eyes, ears, nose, and throat
ELISA	enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FDAAA	US Food and Drug Administration Amendments Act of 2007
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
i.n.	intranasally
IB	investigator's brochure
IBC	institutional biosafety committee
ICD	informed consent document
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IgG	immunoglobulin G
IND	Investigational New Drug application
IRB	institutional review board
JHU	Johns Hopkins University
JHSPH	Johns Hopkins Bloomberg School of Public Health
LID	Laboratory of Infectious Diseases
LRI	lower respiratory infection
MOP	Manual of Procedures
mRNA	messenger RNA
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
ORF	open reading frame
PCR	polymerase chain reaction
PFU	plaque forming unit(s)
PI	principal investigator
PRNT	plaque reduction neutralization test
rDNA	recombinant deoxyribonucleic acid
REDCap	Research Electronic Data Capture
RNA	ribonucleic acid
RSV	respiratory syncytial virus
RT-PCR	reverse transcription polymerase chain reaction
rRT-PCR	real-time reverse transcription polymerase chain reaction
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SERF	Safety Expedited Report Form

SOP	standard operating procedure
SRCP	Safety Review and Communications Plan
SUSAR	serious and unexpected suspected adverse reaction
UP	unanticipated problem
URI	upper respiratory tract illness
US	United States
VAR	vaccine administration record
VUMC	Vanderbilt University Medical Center
WIRB	Western Institutional Review Board
wt	wild-type

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Appendix I: Tables Referenced in the Background Section

Table 21: Schedule of Events: Group 1: RSV-Seronegative Participants; Intensive

		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 13-27 (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
Non-visit contact			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
Physical exam	(X)	X																			
Clinical assessment (focused PE)[b]		X					X		X			X		X				*		X	
Administer study product		X																			
Blood for: immunologic assays	5mL															5mL			5mL		5mL
Nasal swab for: viral detection & quantification[b]							X		X			X		X		X**		*		X***	X
Request adventitious agent assay																		*		X	
Total blood volume	5mL	--	--	--	--	--	--	--	--	--	--	--	--	--	--	5mL	--	--	5mL	--	5mL

* If a family reports an SAE that may meet the study pause or stop criteria, complete the indicated tasks (Table 18)
**A nasal swab specimen is only obtained if the participant has febrile or respiratory illness or otitis media. Timing of visit and collection are outlined in Table 12 and Table 13.

[a] Blood (at least 2 mL) for screening is obtained not more than 42 days prior to inoculation.

[b] If a Stay at Home Order is initiated or in the case of respiratory illness, the parent may be asked to obtain the nasal swab, and the clinical assessment may be completed via a remote or in-person research visit. During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate.

Table 22: Schedule of Events: Group 2: RSV-Seronegative Participants; Less Intensive

		ACUTE PHASE				POST-ACUTE PHASE			
	Screening[a]	Day 0	Day 1-27 (contact each day)	Day 28 (+/- 1 day)	Day 29	Day 30-55	Day 56	Illness Visit	Early DC
Study window							+7 days		
In person visit	X	X		X		*	X	X	X
Non-visit contact		X	X		X	*			
Informed consent	X								
History	X								
Interim history		X	X	X	X	*	X	X	X
Physical exam	(X)	X							
Clinical assessment (focused PE)[b]		X				*		X	
Administer study product		X							
Blood for: immunologic assays	5mL***	(X)		5 mL			5mL		5mL
Nasal swab for: viral detection & quantification[b]			(X)**	(X)**		*		X	X
Request adventitious agent assay						*		X	
Total blood volume	5mL	--	--	5 mL	--	--	5mL	--	5mL

* If a family reports an SAE that may meet the study pause or stop criteria, complete the indicated tasks (Table 18)

**A nasal swab specimen is only obtained if the participant has febrile or respiratory illness or otitis media. Timing of visit and collection are outlined in Table 12 and Table 13.

***A minimum of 2 mL is required for screening, to be obtained not more than 42 days prior to inoculation.Separate blood draws for screening and preinoculation sample may be performed if necessary.

[a] Blood (at least 2 mL) for screening is obtained not more than 42 days prior to inoculation.

[b] If a Stay at Home Order is initiated or in case of respiratory illness, the parent may be asked to obtain the nasal swab, and the clinical assessment may be completed via a remote or in-person research visit. During remote research visits parents will also be asked to measure the child's temperature, heart rate and respiratory rate.

Appendix II: Schedule of Events: RSV Seasonal Surveillance

Table 23: Schedule of Events: RSV Seasonal Surveillance

	Weekly contact	Post-RSV season	Illness Visit	Early DC
Visit period	Oct 16 th to Mar 31st	Apr 1st to Apr 30th Allowable through September 30th in the event of delays due to a Stay at Home Order		
Clinical assessment[a] (focused PE)			X	
Interim history	X		X	X
LABORATORY EVALUATIONS				
Blood for: immunologic assays		5 mL		5 mL
Nasal swab for viral detection & quantification[a]			X	
Request adventitious agent assay			X	
TOTAL BLOOD VOLUME	--	5 mL	--	5 mL

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

Appendix III: Definitions of Solicited Adverse Events

Table 24: Definitions of Solicited Adverse Events

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

[1] Diagnosis must be made by a medical profession
[2] Must be sustained over 20 minute
[3] Clinical assessments must be made by a medical professional and confirmed by a second medical professional, if possible

Appendix IV: RSV SEASONALITY IN BALTIMORE, MD, Nashville, TN, and Rochester, NY

Figure 2: RSV Seasonality in Baltimore, MD (top), Nashville, TN (middle), and Rochester, NY (bottom)