IMPAACT 2021

Randomized Phase I/II Study of the Safety and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Children 6 to 24 Months of Age

A Study of the International Maternal Pediatric Adolescent AIDS Clinical Trials Network

Sponsored by:
National Institute of Allergy and Infectious Diseases
Eunice Kennedy Shriver
National Institute of Child Health and Human Development
National Institute of Mental Health

DAIDS Study ID # 38530
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DAIDS Study ID # 38530

Version 3.0

PROTOCOL SIGNATURE PAGE

I will conduct this study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Council for Harmonisation Guideline for Good Clinical Practice (E6); Institutional Review Board determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

_____ _____

Signature of Investigator of Record Date

_____

Name of Investigator of Record
(printed)

ABBREVIATIONS AND ACRONYMS

ACIP	Advisory Committee on Immunization Practices (CDC)
AE	adverse event
AGM	African green monkey
AIDS	acquired immunodeficiency syndrome
ANOVA	analysis of variance
ARI	acute respiratory illness
BSC	biological safety cabinet
cDNA	complementary deoxyribonucleic acid
cGMP	current good manufacturing practice
CFR	Code of Federal Regulations
CI	confidence interval
CIR	Center for Immunization Research
COVID-19	coronavirus disease 2019
cp	cold-passaged
CRADA	Cooperative Research and Development Agreement
CRL	Charles River Laboratories
CRPMC	Clinical Research Products Management Center
CSO	Clinical Safety Office
CTM	clinical trial material
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS
DAIDS PRO	Division of AIDS Protocol Registration Office
DC	discontinuation
DCR	Division of Clinical Research
DHHS	Department of Health and Human Services
DIR	NIAID Division of Intramural Research
DMC	Data Management Center
DMEM	Dulbecco's Modified Eagle Medium
DMID	Division of Microbiology and Infectious Diseases
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
EAE	Expedited Adverse Event
eCRF	electronic case report form
EENT	ears, eyes, nose, throat
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
F protein	fusion protein (of RSV)
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FSTRF	Frontier Science & Technology Research Foundation, Inc.
GCP	good clinical practice
GMP	good manufacturing practice
GMT	geometric mean peak titer
HEENT	head, ears, eyes, nose, throat
HIPAA	Health Insurance Portability and Accountability Act

HIV	Human Immunodeficiency Virus
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
HVTN	HIV Vaccine Trials Network
IBC	Institutional Biosafety Committee
ICF	informed consent form
ICH	International Conference on Harmonisation
ICU	intensive care unit
IgA, IgG, IgE	immunoglobulin A, G, E
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Network
IND	investigational new drug
IoR	Investigator of Record
IRB	Institutional Review Board
JHSPH	Johns Hopkins Bloomberg School of Public Health
JHU	Johns Hopkins University
LDMS	Laboratory Data Management System
LID	Laboratory of Infectious Diseases
LPC	Laboratory Processing Chart
LRI	lower respiratory illness
LRT	lower respiratory tract
MAARI	medically attended acute respiratory illness
MAALRI	medically attended acute lower respiratory illness
mAb	monoclonal antibody
MOG	IMPAACT Management Oversight Group
MOP	Manual of Procedures
MPT	median peak titers
mRNA	messenger Ribonucleic Acid
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIAID CRMS	NIAID Clinical Research Management System
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NP	nasopharyngeal
NS	nasal swab
nt	nucleotide
NW	nasal wash
OHRP	Office for Human Research Protections
ORF	open reading frame
OTC	over-the-counter
PCR	polymerase chain reaction
PE	physical exam
PFU	plaque-forming unit
PHACS	Pediatric HIV/AIDS Cohort Study
PID	participant identification number
PMTCT	prevention of mother-to-child HIV transmission
PRNT	plaque reduction neutralization titer
PSRT	Protocol Safety Review Team
r	recombinant
RE	regulatory entity
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
RSC	Regulatory Support Center
RSV	respiratory syncytial virus

RSV-MAARI	RSV-associated medically attended acute respiratory illness
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SDAC	Statistical & Data Analysis Center, Harvard School of Public Health
SDMC	Statistical and Data Management Center
SES	Study Enrollment System
SFM	serum-free medium
SID	Study Identification Number
sIRB	single Institutional Review Board
SMARTT	Surveillance Monitoring for ART Toxicities
SPDSMP	Study Progress, Data, and Safety Monitoring Plan
SOP	standard operating procedure
SPG	sucrose-phosphate-glutamate buffer
SUSAR	Serious and Unexpected Suspected Adverse Reaction
TL	tracheal lavage
URI	upper respiratory illness
URT	upper respiratory tract
US	United States
USP	United States Pharmacopeia
VAR	vaccine administration record
wt	wild-type

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SCHEMA

Purpose: To assess whether the live-attenuated RSV vaccine candidates RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 will each be safe and immunogenic in RSV-seronegative recipients.

Design: A double-blind, randomized, placebo-controlled study to evaluate the safety and immunogenicity of the vaccines in RSV-seronegative infants and children 6 to 24 months of age (referred to throughout the protocol as children). As with protocol V2.0, participants in protocol V3.0 will be randomized equally to receive RSV ΔNS2/Δ1313/I1314L vaccine, RSV 6120/ΔNS2/1030s vaccine, or placebo (a third vaccine, RSV 276 included under protocol V1.0 is not included under protocol V2.0 or V3.0). Participants will be enrolled in the study outside the time during which wild-type (wt) RSV circulates in the community at each study site. All participants will remain on study until they complete the Post-RSV Season Visit between April 1st and April 30th in the calendar year following study product administration.

Study Population: Healthy RSV-seronegative* children ≥6 months (180 days) at the time of screening to <25 months (750 days) of age at the time of enrollment

* Throughout the protocol and informed consent documents, seronegativity refers to RSV antibody status, which is defined as a serum RSV-neutralizing antibody titer <1:40.

Sample Size: Approximately 130, including approximately 40 each in the placebo and two candidate vaccine arms open under protocol V2.0 and V3.0, as well as all participants previously randomized to receive RSV 276 under protocol V1.0 prior to closure of this arm

Study Product: As with protocol V2.0, under protocol V3.0, eligible RSV-seronegative children will receive a single dose of RSV ΔNS2/Δ1313/I1314L vaccine, RSV 6120/ΔNS2/1030s vaccine, or placebo intranasally at enrollment.

~N	Product	Dose
40	RSV ΔNS2/Δ1313/I1314L Vaccine	10^6 PFU**
40	RSV 6120/ΔNS2/1030s vaccine	10^5 PFU**
10	RSV 276 Vaccine (enrollment closed under protocol V2.0 and V3.0)	10^5 PFU**
40	Placebo	0

**plaque-forming units (PFU)

Study Duration: Up to approximately 58 months total, as described below. All participants will be followed through April of the year following their enrollment. Therefore, expected length of follow-up for a given participant is between 4 and 15 months depending on time of enrollment. Given the study did not fully accrue in the first enrollment season (2019), enrollment was paused during the second enrollment season (2020) due to COVID-19, and no sites were able to resume enrollment during the third enrollment season (2021), enrollment will be extended into a fourth enrollment season (e.g., 2022), resulting in a total enrollment period of approximately 42 months and total study duration of approximately 46 months.

If a fifth enrollment season (e.g., 2023) is required and approved, the total enrollment period will be approximately 54 months and the total study duration approximately 58 months.

Primary Objectives

1. Safety: To estimate and compare (each vaccine group to placebo) the frequency and severity of adverse events (AEs) following study product administration (Day 0) through Day 56
2. Immunogenicity: To estimate and compare (between the vaccine groups and to the benchmark of 70%) the percentage of vaccinees having a ≥4-fold rise in serum RSV-neutralizing antibodies at the Day 56 Visit

Secondary Objectives

1. Immunogenicity: To estimate and compare (between the vaccine groups) the percentage of vaccinees with a ≥4-fold rise in serum IgG antibody to RSV F protein (RSV F IgG) at the Day 56 Visit
2. Immunogenicity: To estimate and compare (between the vaccine groups) the titers of serum RSV F IgG and serum RSV-neutralizing antibodies at the Day 56 Visit
3. Safety: To describe and compare the frequency and severity of RSV-associated, medically attended, acute respiratory illness (RSV-MAARI) and RSV-associated, medically attended, acute lower respiratory illness (RSV-MAALRI) in the placebo and vaccine arms during RSV season

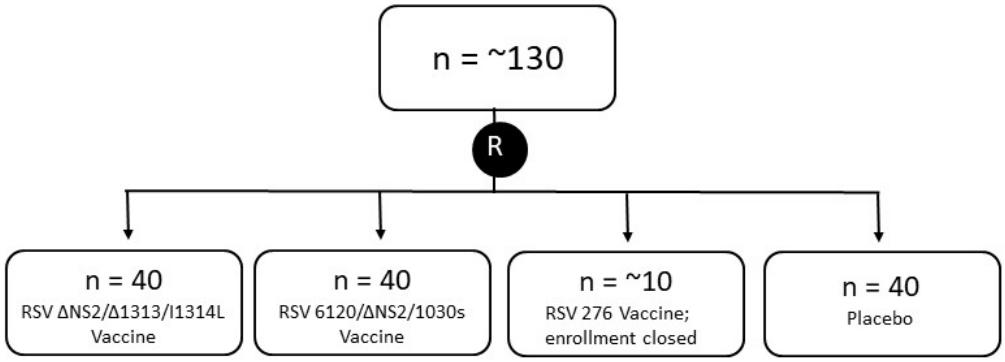
Exploratory Objectives

1. 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. 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 each vaccine group
3. Immunogenicity – magnitude of anamnestic response: To estimate and compare (each vaccine group to placebo and between the vaccine groups) the titers of serum RSV F IgG and serum RSV-neutralizing antibodies in participants infected with wt RSV during the RSV season
4. Immunogenicity – durability of vaccine-induced RSV antibodies: to estimate and compare (between the vaccine groups), 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

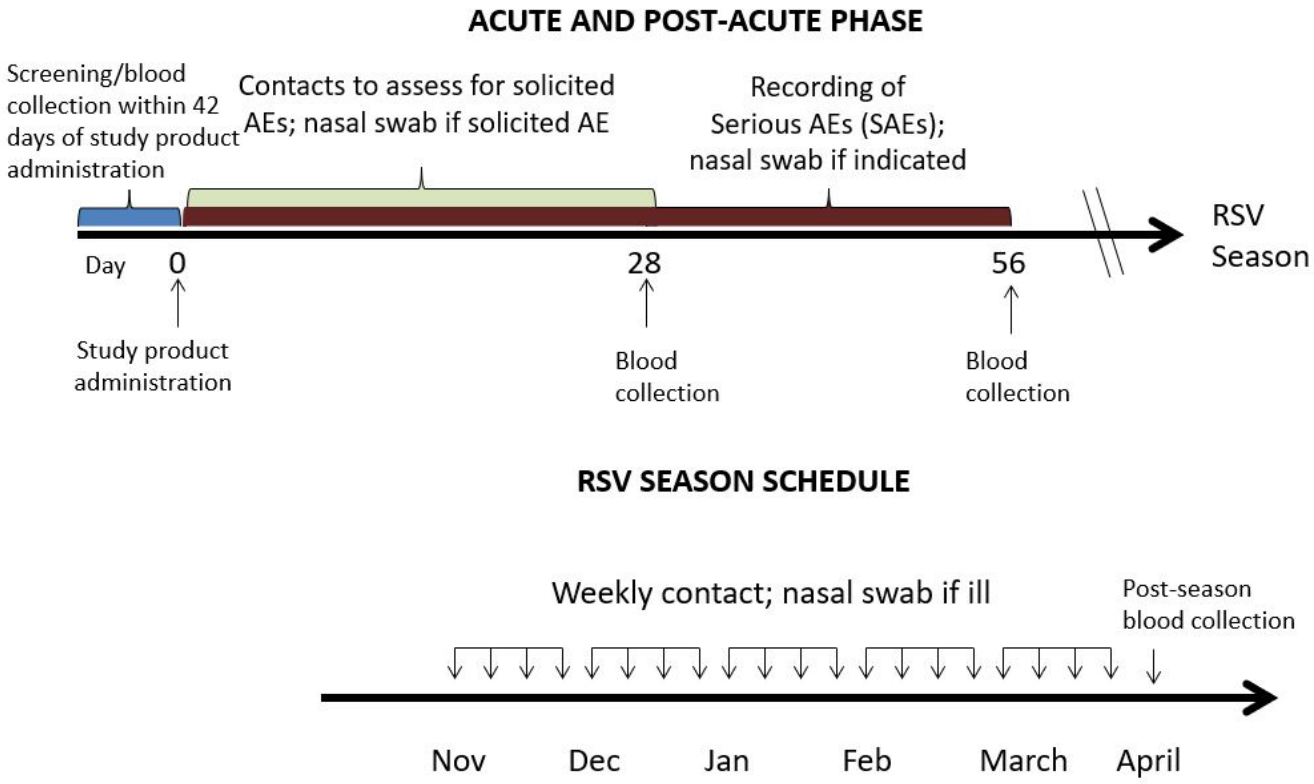
IMPAACT 2021
Randomized Phase I/II Study of the Safety and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Children 6 to 24 Months of Age

Figure 1: Study Overview

Panel A: Randomization Scheme



Panel B: Study Procedures (All Study Product Arms)



1 INTRODUCTION

1.1 Overview

Human respiratory syncytial virus (RSV) is the most common viral cause of serious acute lower respiratory illness (LRI) in infants and children under 5 years of age worldwide (1). There is broad consensus that a vaccine is needed. Attenuated live virus vaccines are a promising strategy for RSV since they have not been associated with vaccine enhanced disease (2) and they have the potential of inducing a spectrum of immune responses similar to responses induced by wild-type infection (3). This protocol is part of a multi-year development plan aimed at identifying a candidate RSV vaccine that is sufficiently attenuated but still immunogenic.

One attenuation strategy that has shown great promise is the deletion of the viral interferon antagonist NS2, resulting in an NS2 deletion mutant. Each of the two most promising NS2 deletion candidates, RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s, contains a second attenuating element in addition to the NS2 deletion. As the second element, RSV ΔNS2/Δ1313/I1314L contains a deletion Δ1313 of a single codon of the L polymerase open reading frame (ORF), stabilized by an adjacent mutation I1314L. RSV 6120/ΔNS2/1030s is identical to RSV ΔNS2/Δ1313/I1314L, but was made to be slightly less attenuated by replacing the Δ1313/I1314 mutations by the 1030s mutation, consisting of 1321K(AAA) and 1313S(TCA) in the L gene. Both ΔNS2 vaccine candidates are attenuated and moderately temperature sensitive. In a Phase I trial (NCT01893554), a single intranasal dose of 10^6 PFU of RSV ΔNS2/Δ1313/I1314L was well tolerated and immunogenic in RSV-seronegative children 6-24 months of age (20 vaccine recipients, 10 placebo recipients). 80% and 90% of recipients shed virus detected by culture and RT-qPCR, respectively (geometric mean peak titers (GMT) 1.8 $\log_{10}$ PFU/mL; 3.5 $\log_{10}$ copies/mL). Moreover, 85% and 80% had ≥4-fold increases in RSV F IgG and RSV-neutralizing serum antibody titers, respectively. The serum antibody response was durable over the RSV surveillance season, and the vaccine primed for a strong anamnestic response to wild-type RSV. There was no evidence of excess respiratory illness associated with receipt of a 10^6 dose of RSV ΔNS2/Δ1313/I1314L. [(4), manuscript in preparation].

RSV 6120/ΔNS2/1030s was also well-tolerated and highly restricted in replication in 12- to 59-month-old RSV seropositive children (10 vaccinees and 5 placebo recipients) and is currently being evaluated at a 10^5 dose in RSV-seronegative children 6-24 months of age. To date, 23 RSV-seronegative children have been enrolled in this study; information about study allocation, vaccine virus shedding, and tolerability is pending.

Another very promising strategy is based on the deletion of a large section of the open reading frame encoding the RSV RNA regulatory protein M2-2 from the RSV genome. Deletion of M2-2 increases viral mRNA production, and reduces viral RNA replication (5). The increase in mRNA production results in increased synthesis of viral antigen, with the potential for increased immunogenicity, and the decreased RNA replication results in delayed assembly of new virus particles, and in attenuation. The first candidate RSV vaccine using this strategy, MEDI/ΔM2-2, was studied sequentially in adults, seropositive children, and then seronegative infants and children (age 6 to 24 months) (6). This study found that the vaccine had excellent immunogenicity despite very low viral replication in seronegative infants and children: at a dose of 10^5 PFU, only 60% of RSV-seronegative vaccinees shed vaccine virus (mean peak titer by culture from NW samples, 1.5 $\log_{10}$ PFU/mL), yet 95% of vaccinees had ≥4-fold increases in RSV-neutralizing serum antibody titers, and substantial serum RSV-neutralizing antibody responses were achieved (GMT=1:97). RSV MEDI/ΔM2-2 thus had a very promising phenotype, but this vaccine was evaluated as part of a Collaborative Research and Development Agreement

(CRADA) with Medimmune, LLC, that has been terminated, and this material was not available for further study. Therefore, a similar virus, RSV 276, was generated to replace MEDI/ΔM2-2, as described in greater detail below.

Based on infectivity, safety, and immunogenicity data to date, these three candidate vaccines—RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276—appear to be the strongest candidates to move into expanded Phase I/II trial (IMPAACT 2021) for a further evaluation of vaccine safety and immunogenicity. Each candidate contains a large deletion mutation, which effectively reduces the potential for de-attenuation, an important concern for a live-attenuated vaccine. This expanded Phase I/II study will evaluate safety, and immunogenicity of these three lead candidates. As a secondary objective, we will assess the rates of RSV-medically attended acute respiratory illness (MAARI) and RSV-medically attended acute lower respiratory illness (MAALRI) in vaccine recipients and placebo recipients during the RSV season following receipt of study product (secondary safety outcome measure).

Table 1 includes an overview of recent RSV studies involving these and related candidates. The candidates used in IMPAACT 2021 are further described in Table 2.

Table 1: Overview of recent RSV vaccine candidates with M2-2 or NS2 deletion[a]

IMPAACT /CIR Protocol	Vaccine name	Attenuating mutations, key features	Expected effect	Study status
CIR 275	MEDI/ ΔM2-2	• Deletion of the RSV RNA regulatory M2-2 protein	• Attenuation • Increased immunogenicity	• Achieved target accrual (n=30) • Highly attenuated • Highly immunogenic (6)
2000/291	LID ΔM2-2	• Deletion of the RSV RNA regulatory M2-2 protein • Differences between LID/ΔM2-2 and MEDI/ΔM2-2: 1. Deletion of 112 nt of the 3' SH noncoding region 2. Slightly different design of M2-2 deletion 3. 19 silent nucleotide changes and two non-synonymous changes (in NS2, N) 4. Except for a single amino acid difference in NS2 and N, same amino acid sequence as MEDI/ΔM2-2	• Attenuation • Increased immunogenicity	• Closed to accrual (n=29) • Peak viral titers in NW higher than expected, exceeded MEDI/ΔM2-2 (7)
2011/311	LID ΔM2-2 1030s	• Deletion of the RSV RNA regulatory M2-2 protein • Differences to MEDI/ΔM2-2 as described for LID/ΔM2-2 above, plus: • genetically stable "1030s" attenuating point mutation [Y1321K(AAA) and S1313(TCA) mutation in L]	• Attenuation • Temperature sensitivity • Increased immunogenicity	• Achieved target accrual (n=33) • Highly immunogenic, attenuated, no safety signal

IMPAACT /CIR Protocol	Vaccine name	Attenuating mutations, key features	Expected effect	Study status
2012/312	LID cp ΔM2-2	• Deletion of the RSV RNA regulatory M2-2 protein • Differences to MEDI/ΔM2-2 as described for LID/ΔM2-2 above, plus: • five point mutations (derived from cold-passaged RSV) in the RSV nucleoprotein, fusion protein, and polymerase protein	• Attenuation (increased by addition of cp mutations) • Increased immunogenicity	• Closed to accrual (n = 17) • Over-attenuated
2013/313	D46/NS 2/N/ ΔM2-2-HindIII	• Deletion of the RSV RNA regulatory M2-2 protein; design based on MEDI/ΔM2-2. • Contains complete 3' SH noncoding region that is deleted in "LID" versions; • Differences between D46/NS2/N/ ΔM2-2-HindIII and MEDI/ΔM2-2: 19 silent nucleotide changes. • Same amino acid sequence as MEDI/ΔM2-2	• Attenuation • Increased immunogenicity	• Achieved target accrual (n=32) • Analysis finalized in February 2019
2018/321	RSV 276	• Deletion of the RSV RNA regulatory M2-2 protein; design based on MEDI/ΔM2-2. • Contains the SH noncoding region that is deleted in "LID" versions; • RSV 276 replaces MEDI/ΔM2-2, differing by two nucleotides in the 5' noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ΔM2-2; CG in RSV 276), and by two noncoding changes (A1937G; T13900C)	• Attenuation • Increased immunogenicity	• Closed to accrual (n=65) • RSV 276: associated with higher rate of solicited AEs • RSV ΔNS2/Δ1313/ I1314L: favorable safety profile
	RSV ΔNS2/ Δ1313/I1 314L	• Vaccine candidate with deletion of the RSV interferon antagonist NS2 (ΔNS2) included for comparison • Deletion of codon 1313 of the polymerase gene (Δ1313), codon 1314 was genetically stabilized (I1314L)	• Attenuation • Temperature sensitivity • Increased immunogenicity	

[a]Genomic position numbering is in reference to biological wt RSV strain A2 (Genbank accession number M74568), which is 15,222 nt in length. Thus, deletions or insertions in the recombinant viruses do not change the sequence numbering of the remaining nucleotides.

Table 2: Overview of RSV vaccine candidates with NS2 or M2-2 deletion to be studied in IMPAACT 2021

Vaccine name	Attenuating mutations, key features	Expected effect
RSV 276	• Deletion of the RSV RNA regulatory M2-2 protein (ΔM2-2); design based on MEDI/ΔM2-2. • Contains the SH noncoding region that is deleted in "LID" versions; • RSV 276 is similar to MEDI/ΔM2-2, differing by two nucleotides in the 5' noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ΔM2-2; CG in RSV 276), and by two noncoding changes in N and L (A1937G; T13900C)	•Attenuation •Increased immunogenicity
RSV ΔNS2/ Δ1313/I1314L	• Deletion of the RSV interferon antagonist NS2 (ΔNS2) • Deletion of codon 1313 of the polymerase gene (Δ1313), Codon 1314 was genetically stabilized (I1314L)	•Attenuation •Temperature sensitivity •Increased immunogenicity
RSV 6120/ΔNS2/ 1030s	• Deletion of the RSV interferon antagonist NS2 (ΔNS2) • genetically stable "1030s" attenuating point mutation [Y1321K(AAA) and S1313(TCA) mutation in L]	•Slightly less attenuated than RSV ΔNS2/Δ1313/I1314L •Temperature sensitivity •Increased immunogenicity

In IMPAACT 2021, the lead candidates from each attenuation strategy, RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276, will be evaluated side-by-side. A placebo group equal in size to each vaccine group is included because of the high background of respiratory/febrile illness among RSV-seronegative children in this age group.

It is hoped that all candidates will:

- be safe, and
- result in at least 70% of the population of vaccine recipients having a ≥4-fold rise in serum RSV-neutralizing antibody titers from pre-study product administration to the Day 56 Visit after study product administration

However, one or more of these vaccine candidates may have safety signals that become apparent only when compared to placebo recipients in this larger number of participants. The vaccine candidates may differ in the magnitude of antibody responses and in responses observed following naturally occurring RSV infection.

RSVΔNS2/Δ1313/I1314L was previously evaluated at both 10^5 PFU and 10^6 PFU; both doses were well tolerated, but only the 10^6 PFU dose was sufficiently infectious and immunogenic. For this reason, RSVΔNS2/Δ1313/I1314L will only be tested at the 10^6 dose level. RSV 6120/ΔNS2/1030s will be evaluated and RSV 276 was evaluated (under protocol V1.0) at a dose of 10^5 PFU, the dose used in previous studies of RSV 6120/ΔNS2/1030s, MEDI ΔM2-2, and RSV 276.

1.2 Background

Epidemiology, Disease Burden, and the Need for a Vaccine

In the United States alone, RSV is responsible for 75,000 to 125,000 hospitalizations of infants yearly (8), and worldwide, RSV is responsible for at least 34 million cases of lower respiratory tract disease in children under 5 years, resulting in an estimated 3.4 million hospitalizations and 66,000 to 199,000 RSV-attributable deaths each year (1). 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 airways disease during childhood (9, 10).

RSV is an enveloped RNA virus that is a member of the newly organized *Pneumoviridae* family, genus *Orthopneumovirus* (11). RSV has a single negative-sense strand RNA genome of 15.2 kilobases encoding 10 mRNAs. Each mRNA encodes a single protein, with the exception of the M2 mRNA, which contains 2 overlapping 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 RSV-neutralizing monoclonal 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 wt RSV (12). 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 (13, 14).

Following the failure of the formalin-inactivated RSV vaccine, attempts at developing RSV vaccines at National Institute of Allergy and Infectious Diseases (NIAID) have focused largely on live-attenuated approaches (3). Importantly, over a period of over 20 years, a number of live-attenuated investigational RSV vaccines have been evaluated in RSV-seronegative or RSV-naïve infants and children, and enhanced disease following wt RSV infection of vaccinees has not been observed (2). Apart from the absence of enhanced disease, live-attenuated RSV vaccines have a number of known advantages over non-replicating RSV vaccines. They can be administered intranasally, induce protective mucosal immunity in the respiratory tract (as well as systemic immunity), infect in the presence of maternally-derived RSV serum antibody, and have been well tolerated and immunogenic when administered to infants as young as four weeks of age (15).

Human RSV has a single serotype with two antigenic subgroups, A and B. The two subgroups exhibit a 3- to 4-fold reciprocal difference in neutralization by polyclonal convalescent serum. Analysis of glycoprotein-specific responses in infants by enzyme-linked immunosorbent assay (ELISA) with purified F and G glycoproteins showed that the fusion proteins (F proteins) were 50% related antigenically, and the G proteins were 7% related (16). 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 (17). Thus, the F protein is responsible for most of the observed cross-subgroup neutralization and protection, and a subgroup A vaccine virus is likely to induce a broad immune response against wt RSV of either subgroup. Antibodies to the F protein are one of the endpoints evaluated in this study.

The RSV vaccines to be evaluated in this study were derived using a recombinant deoxyribonucleic acid (DNA)-based technique called reverse genetics (18). 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) does not contain any recombinant DNA. The RSV vaccine candidates to be tested under this protocol are derivatives of strain A2, subgroup A.

Vaccine Description

In previous Phase I studies in RSV-seronegative infants and children 6 to 24 months of age, the live-attenuated RSV strains RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276, a virus that is similar to MEDI ΔM2-2, have emerged as the most promising candidates.

RSV ΔNS2/Δ1313/I1314L contains two independent attenuating elements: (i) a 523 nt deletion of the viral interferon/apoptosis antagonist NS2 gene, and (ii) an amino acid deletion in the L protein (Δ1313; deletion of S1313). In addition, RSV ΔNS2/Δ1313/I1314L contains the genetically stabilizing mutation I1314L (19). This stabilizing mutation was included to prevent the de-attenuating second site mutation I1314T (20). RSV 6120/ΔNS2/1030s is identical to RSV ΔNS2/Δ1313/I1314L but was made to be slightly less attenuated by replacing the Δ1313/I1314 mutations by the 1030s mutation, consisting of Y1321K(AAA) and 1313S(TCA) in the L gene. RSV with NS2 deletion is attenuated in animal models, including in chimpanzees (21). As shown in the bovine RSV/calf model (22), the deletion of an RSV interferon/apoptosis antagonist may increase immunogenicity. To study the attenuating effect of Δ1313 alone, a recombinant RSV with deletion of codon 1313 of the L gene was evaluated (RSV Δ1313). On their own, the deletion of codon 1313 or the 1030s mutation of the polymerase gene had a substantial attenuating effect on RSV. Δ1313 rendered RSV temperature sensitive (*ts)*, with a shutoff temperature (T_{SH}) of 37°C, and was strongly attenuating in mice. 1030s also induced temperature sensitivity, but at a T_{SH} that was one degree higher (38°C) than that of RSV Δ1313. The 1030s mutation also was less attenuating than Δ1313 in the mouse model (19).

MEDI/ΔM2-2 was attenuated by deletion of the RNA regulatory protein M2-2 (5). MEDI/ΔM2-2 was very highly restricted, well tolerated, safe, and immunogenic in a previous study in RSV-seronegative children and infants (6), but is not available for further study. RSV 276 was designed to be similar to MEDI/ΔM2-2. Like MEDI/ΔM2-2, RSV 276 contains a deletion of the RSV RNA-regulatory M2-2 protein, resulting in increased mRNA production and reduced RNA replication (5). Recombinant RSV with M2-2 deletion grows more slowly in vitro than wt RSV (5, 23). Deletion of M2-2 results in increased accumulation of intracellular viral mRNA and decreased accumulation of genome and antigenome (5). 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. RSV with M2-2 deletion is highly attenuated in pre-clinical studies (6, 23, 24). RSV 276 differs from MEDI/ΔM2-2 only by four nucleotides: specifically, two nucleotides in the 5' noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ΔM2-2; CG in RSV 276), and two noncoding changes (A1937G in the N ORF; T13900C in the L ORF). It is anticipated that these four nucleotide differences between

MEDI/ΔM2-2 and RSV 276 will not affect replication, attenuation, safety, and immunogenicity in humans because two of these differences are in a non-coding region adjacent to the deletion in M2-2 and the other two occur in open reading frames without changing amino acid coding.

RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 are cDNA derived live-attenuated RSV vaccine candidates. The seed viruses were generated at the Laboratory of Infectious Diseases (LID), NIAID (non-GMP), and transferred to Charles River Laboratories [CRL; Malvern, PA; operated under cGMP (current Good Manufacturing Practice)]. The seed viruses passed pre-production testing (Sterility, *Mycoplasma*, Bacteriostasis/Fungistasis and testing for porcine circovirus types 1 and 2; testing performed at CRL under cGMP) and were accepted for manufacturing of the Drug Product under cGMP in Vero cells. The Drug Products are contained in sterile 2.0 mL micro tubes, each containing 0.6 mL volumes.

The RSV ΔNS2/Δ1313/I1314L Drug Product is a concentrate of live recombinant RSV ΔNS2/Δ1313/I1314L Vero Grown Virus Vaccine (Lot RSV#006A) in OptiPro SFM 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 Drug Product has a potency of approximately 7.3 $\log_{10}$ PFU/mL and is diluted to dose on site.

The RSV 276 (Lot RSV#014A) and the RSV 6120/ΔNS2/1030s (Lot RSV#012A) Drug Products are concentrates of live recombinant Vero Grown Virus Vaccine 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 RSV 276 (Lot RSV#014A) Drug Product has a potency of approximately 6.2 $\log_{10}$ PFU/mL, and the RSV 6120/ΔNS2/1030s (Lot RSV#012A) Drug Product has a potency of approximately 6.0 $\log_{10}$ PFU/mL. Both are diluted to dose on site.

The Final Drug Products, RSV ΔNS2/Δ1313/I1314L, Lot RSV#006A, RSV 6120/ΔNS2/1030s, Lot RSV#012A, and RSV 276, Lot RSV#014A, 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, Determination of the Sucrose Level, pH Determination, Intact Cell Assay, Potency/Infectivity, Identity, Purity, Toxicology and Pharmacology testing). Sequence analysis confirmed that the genomic sequences of the Drug Products, Lots RSV#006A, RSV#012A, and RSV#014A, were identical to the cDNA that each was derived from. RSV ΔNS2/Δ1313/I1314L, Lot RSV#006A, RSV 6120/ΔNS2/1030s, Lot RSV#012A, and RSV 276, Lot RSV#014A, were released by CRL for use as investigational vaccines.

1.3 Prior Research

1.3.1 Experimental Vaccines against Respiratory Syncytial Virus

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 (2).

Several live-attenuated RSV vaccines have been evaluated in clinical trials in adult and pediatric populations as part of NIAID's ongoing RSV vaccine development program (6, 15, 25-27). RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 represent the lead vaccine candidates from these attenuation strategies. Prior to studies of RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s, three RSV vaccine viruses with NS2 deletion (rA2cpΔNS2, rA2cp248/404ΔNS2, and rA2cp530/1009ΔNS2) had been evaluated in clinical studies (27). Prior to evaluation of RSV 276 under the present protocol, several RSV candidates with M2-2 deletion have been evaluated. A short summary of preclinical studies involving these vaccine candidates is included below, and a description of previous human experience with the prior candidates that are most closely related to the candidates of the present protocol can be found in Section 1.3.3.

1.3.2 Preclinical Studies

1.3.2.1 Genetic Stability of RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s

RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s are temperature sensitive, with shut-off temperatures for virus replication of 38ºC-39°C (RSV ΔNS2/Δ1313/I1314L) and 40°C (RSV 6120/ΔNS2/1030s) and showing a small-plaque phenotype at 37°C and above (RSV ΔNS2/Δ1313/I1314L) and 38°C and above (RSV 6120/ΔNS2/1030s). In case of RSV ΔNS2/Δ1313/I1314L, this phenotype is associated with the deletion of a single codon of the polymerase ORF, codon 1313 (19, 20). To prevent a de-attenuating second-site mutation that had been observed in an in-vitro genetic stability stress test, the neighboring codon I1314 was genetically stabilized by changing it to I1314L. In further in-vitro stability stress tests, this I1314L change indeed stabilized the 1313 site of the RSV polymerase ORF. The genetic stability of RSV ΔNS2/Δ1313/I1314L was confirmed by sequence analysis of RSV ΔNS2/Δ1313/I1314L isolates from a previous Phase I study. This recent pediatric vaccine study showed that RSV ΔNS2/Δ1313/I1314L is a genetically stable RSV vaccine candidate (Karron et al., manuscript in preparation).

The moderate temperature sensitivity of RSV 6120/ΔNS2/1030s is associated with the "1030s" mutation. This mutation is also genetically stabilized (19). This "1030s" mutation was included in the previous vaccine candidate RSV cps2. Of note, no evidence for genetic instability of the "1030s" site was detected in vaccine isolates obtained during a clinical study of this candidate in infants and children (4).

1.3.2.2 Replication of RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s in an in-vitro model of mucociliary normal human tracheal/bronchial epithelial cells

Multicycle replication of experimental lots of RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s was compared to that of wt RSV in a 3D mucociliary human tissue model of normal pseudostratified differentiated primary human tracheal/bronchial airway epithelial cells (EpiAirway, Mattek, Inc.), cultured in an air-liquid interface at two different temperatures (32°C and 37°C) to model the temperatures of the upper and lower airways RSV. Differentiated human airway cells represent the best available in-vitro model to predict vaccine replication. Wt RSV A2 was included for comparison. Triplicate wells were infected at an MOI of 0.1 in 100 µl of medium per well. After 2 hours of adsorption, virus inoculum was gently removed, and the apical surfaces of the cultures were washed 3 times with 300 µL PBS. Apical washes were collected daily on days 1-7 post-infection to assay viral replication, snap frozen, and virus titers were determined by RSV immunoplaque assay on Vero cells.

Recombinant wt RSV of strain A2 replicated efficiently in these differentiated primary human tracheal/bronchial epithelial cells at 32°C and at 37°C. At 32°C, peak titers of about 5.6 $\log_{10}$ PFU/mL of wt RSV were detected on day 6, whereas at 37°C, peak titers of about 6.1 $\log_{10}$ were reached by day 5 post-infection. At 32°C, peak titers of RSV ΔNS2/Δ1313/I1314L were detected on day 5, and were about 40-fold lower than those of wildtype RSV; RSV 6120/ΔNS2/1030s also peaked on day 5, and replicated to about 4-fold higher titers than RSV ΔNS2/Δ1313/I1314L, showing that indeed, RSV ΔNS2 with the 1030s mutation is slightly less restricted for replication than RSV ΔNS2/Δ1313/I1314L. At 37°C, RSV ΔNS2/Δ1313/I1314L was too restricted for a meaningful comparison, and RSV 6120/ΔNS2/1030s replicated to about 50-fold lower peak titers than wt RSV. This difference in peak titers of RSV 6120/ΔNS2/1030s and wt RSV was statistically significant and shows that RSV 6120/ΔNS2/1030s is restricted in this most predictive pre-clinical model.

1.3.2.3 Evaluation of the Attenuation Phenotype of RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 in Experimental Animals

Recombinant RSV with deletion of ΔNS2 and codon 1313 of the polymerase ORF was evaluated for its ability to replicate in the upper and lower respiratory tract (URT and LRT, respectively) of mice. While wt RSV rA2 replicated to about 4.0 $\log_{10}$ PFU and 4.5 $\log_{10}$ PFU per gram of tissue in the upper and lower respiratory tracts of mice, replication of the deletion ΔNS2/Δ1313 mutant was reduced to below the level of detection, demonstrating attenuation.

Replication and immunogenicity of RSV ΔNS2/Δ1313/I1314L were evaluated in nonhuman primates (African green monkeys, AGMs). AGMs are semi-permissive for RSV. AGMs which were seronegative for RSV were inoculated intranasally and intratracheally with RSV ΔNS2/Δ1313/I1314L; a dose of 1 x 10^6 PFU of vaccine in a 1 mL volume was administered per site to sedated juvenile male and female AGMs (total dose per animal: 2 x 10^6 PFU). Nasopharyngeal (NP) swabs were collected daily on Days 0 through 10 and Day 14, tracheal lavage (TL) samples were collected every other day from Day 2 through Day 10 and on Day 14, and virus shedding was analyzed by plaque assay. Serum RSV-neutralizing antibody titers were determined by a complement-enhanced 60% RSV plaque reduction neutralization assay. Results from studies following the same protocol, performed in animals from the same group and origin, inoculated with recombinant wt RSV A2 at the same dose, were included for comparison. Studies were approved by the Animal Care and Use Committee of NIAID, NIH. Virus shedding was analyzed by immunoplaque assay. Substantial shedding from the upper and lower respiratory tract over several days of the wt RSV A2 control virus was detected by plaque assay, with mean peak titers of 3.5 $\log_{10}$ PFU per mL in the URT and in the LRT. Compared to wt RSV A2, mean peak titers of RSV ΔNS2/Δ1313/I1314L were slightly reduced in the URT (3.2 $\log_{10}$ PFU per mL), and greatly reduced in the LRT (1.4 $\log_{10}$ PFU per mL), with detectable shedding in TL samples of only three of four animals. The sum of daily titers (area under the curve) of shedding of RSV ΔNS2/Δ1313/I1314L from the upper and lower respiratory tract was substantially lower than that of wt RSV A2. Despite the lower level of shedding, RSV ΔNS2/Δ1313/I1314L was immunogenic in AGMs.

RSV 276 and RSV 6120/ΔNS2/1030s also were evaluated for their ability to replicate in AGMs in four independent non-GLP studies. The first two NHP studies were done to evaluate Experimental Lots of these viruses, and the subsequent two studies were done to test the clinical safety of the clinical trial materials (CTM) of RSV 276 and RSV 6120/ΔNS2/1030s. As described above, AGMs, seronegative for RSV, were inoculated intranasally and intratracheally with 1 x 10^6 PFU of RSV 276 in a 1 mL volume per site (total dose per animal: 2 x 10^6 PFU). Results from studies following the same protocol, performed in animals from the same group and origin,

inoculated with MEDI/ΔM2-2 and recombinant wt RSV A2 at the same dose, were included for comparison. Substantial shedding from the upper and lower respiratory tract over several days of the wt RSV A2 control virus was detected by plaque assay, with mean peak titers of 4.2 $\log_{10}$ PFU per mL in the URT, and 4.1 $\log_{10}$ PFU per mL in the LRT. RSV 276 was infectious for AGMs, and except for a single RSV 276 inoculated animal with undetectable shedding from the URT, all animals shed the candidate vaccine viruses over several days from the upper and lower respiratory tract. However, compared to wt RSV A2, shedding of RSV 276 was reduced in the upper and lower respiratory tract. Mean peak titers of the experimental lot and of the CTM of RSV 276 were not significantly different from those of MEDI/ΔM2-2 in a previous study. Both lots of RSV 276 induced serum neutralizing antibody titers that were higher than those to MEDI/ΔM2-2, and comparable to those to the wt RSV A2 control virus. These results show that at a total dose of 2 x 10^6 PFU, administered intranasally and intratracheally, RSV 276 is attenuated to a similar degree as MEDI/ΔM2-2, and very immunogenic in AGMs.

Compared to wt RSV A2, shedding of RSV 6120/ΔNS2/1030s was slightly reduced in the URT (mean peak titers of 3.8 log10 PFU), but higher than that of the previously tested RSV ΔNS2/Δ1313/I1314L virus (3.2 $\log_{10}$ PFU per mL). In the LRT, the mean peak titers were 4.6 $\log_{10}$ PFU per mL for the Experimental Lot and 2.9 PFU per mL for the CTM of RSV 6120/ΔNS2/1030s. Compared to the RSV ΔNS2/Δ1313/I1314L vaccine candidate (mean peak LRT titers: 1.4 $\log_{10}$ PFU per ml), RSV 6120/ΔNS2/1030s was less restricted in AGMs, and similarly immunogenic.

These study results indicate that RSV ΔNS2/Δ1313/I1314L and RSV 276 are restricted for replication in AGMs, the most permissive RSV nonhuman primate model that is currently available. At the dose of 1 x 10^6 PFU per site, shedding of these vaccine candidates was detectable from the URT and LRT, but was substantially reduced compared to recombinant wt RSV A2. Despite the low level of shedding, all of these candidates were very immunogenic in AGMs. Overall, replication and immunogenicity of RSV 276 were comparable to MEDI/ΔM2-2. RSV 6120/ΔNS2/1030s was less restricted than RSV ΔNS2/Δ1313/I1314L in AGMs. In summary, it is anticipated that these investigational RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 live vaccine candidates will be attenuated, immunogenic, and safe upon intranasal application in RSV-seronegative children.

1.3.3 Previous Clinical Experience

RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 have recently been evaluated in first-in-human Phase I studies. The RSV 276 vaccine is similar to the live-attenuated recombinant RSV MEDI ΔM2-2 vaccine virus, which has been studied in RSV-seropositive cohorts, and in RSV-seronegative children.

1.3.3.1 RSV ΔNS2/Δ1313/I1314L

Prior to RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s, three RSV vaccine viruses with NS2 deletion (rA2cpΔNS2, rA2cp248/404ΔNS2, and rA2cp530/1009ΔNS2) had been evaluated in clinical studies (27), confirming that the deletion of the NS2 gene attenuates RSV. rA2cpΔNS2 was over-attenuated for adults but under-attenuated for use in young children, whereas both rA2cp248/404ΔNS2 and rA2cp530/1009ΔNS2 were over-attenuated and insufficiently immunogenic for seronegative children (27).

Based on these results, RSVΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s were developed. In a recent Phase I trial (NCT01893554), RSVΔNS2/Δ1313/I1314L was evaluated in RSV-seropositive children 12-59 months of age at a dose of 10^6 PFU (10V, 5P), and no shedding or immune response was detected, indicative of attenuation. Next, it was evaluated in seronegative children 6-24 months of age at two sequential dose levels. At the lower dose of 10^5 PFU (15V, 7P), the vaccine was poorly infectious (7% and 73% of recipients shed virus detected by culture and RT-qPCR, respectively) and immunogenicity was low. At the higher dose of 10^6 PFU (20V, 10P), 80% and 90% of recipients shed virus detected by culture and RT-qPCR, respectively [geometric mean peak titers (GMT) 1.8 $\log_{10}$ PFU/mL; 3.5 $\log_{10}$ copies/mL]. Moreover, 85% and 80% had ≥4-fold increases in RSV F IgG and RSV-neutralizing serum antibody titers, respectively. The serum antibody response was durable over the RSV surveillance season, and the vaccine primed for a strong anamnestic response to wild-type RSV. In the 10^6 dose cohort, during the first 28 days after receipt of study product, 11 of the 20 (55%) vaccine recipients had a total of 15 episodes of mild respiratory or febrile illnesses (Grade 1, except for a single Grade 2 fever). Vaccine virus was detected by culture during one of these 15 episodes (together with parainfluenza virus type 3), and by PCR during six of these episodes. Rhinovirus was detected during five of the 15 episodes in vaccine recipients. Of the 10 placebo recipients, eight (80%) had a total of 11 episodes of respiratory or febrile illnesses during the first 28 days. The respiratory illnesses included four Grade 1 illnesses (rhinorrhea and/or cough), and two Grade 2 illnesses (croup and stridor; both protocol-specified adverse events). An episode of otitis media (Grade 2) was also observed in this group. Additionally, there were three episodes of Grade 3 fever, and one episode of Grade 2 fever among placebo recipients. Enterovirus, rhinovirus, adenovirus, and bocavirus were detected in five of these 11 illness episodes. Based on these data, there was no evidence of excess respiratory illness associated with receipt of a 10^6 dose of RSV ΔNS2/Δ1313/I1314L. ΔNS2/Δ1313/I1314L is presently being evaluated in 4- to 6-month-old infants (unscreened for RSV antibodies). In this younger cohort, seven participants have completed the post-vaccination acute and post-acute-phase follow-up, without any safety signals.

1.3.3.2 RSV MEDI/ΔM2-2 and RSV 276

Several versions of RSV vaccine candidates with M2-2 deletion have recently been evaluated [Table 1; (6, 28-31)]. The first vaccine candidate, designated RSV MEDI/ΔM2-2, was sequentially evaluated in adults, RSV-seropositive children, and RSV-seronegative infants and children (6). Fifteen healthy adults received a 10^6 PFU dose of this vaccine in an open-label study. The vaccine was generally well tolerated, and vaccine virus was not detected in nasal washes collected from any of the vaccine recipients. Serum antibody responses were not detected in any of these adult vaccinees. Thus, there was no evidence of replication of RSV MEDI/ΔM2-2 in adult vaccinees. A 10^6 PFU dose of RSV MEDI/ΔM2-2 was subsequently evaluated in RSV-seropositive children ages 12-59 months (double-blind, placebo-controlled). Ten children in this RSV-seropositive cohort received a 10^6 PFU dose of vaccine, and five received placebo. Among the vaccinees, five children had rhinorrhea or nasal congestion, which was associated in all cases with shedding of rhinovirus and with shedding of adenovirus (1 child) or enterovirus (1 child). All illnesses were mild in severity. None of the vaccinees shed vaccine virus, indicating that there was also no evidence of replication of RSV MEDI/ΔM2-2, evaluated at a dose of 10^6 PFU in RSV-seropositive children.

RSV MEDI/ΔM2-2 was subsequently evaluated at a 10^5 PFU dose in RSV-seronegative children. RSV MEDI/ΔM2-2 replicated at low titers yet induced substantial RSV-neutralizing antibody responses in RSV-seronegative children. Vaccine virus was detected by culture in only 60% (12 of 20) RSV-seronegative vaccinees, at a low mean peak titer of 1.5 $\log_{10}$ PFU/ml. However, 17 of 20 had vaccine virus detected by qRT-PCR. Four-fold or greater increases in RSV-neutralizing

antibodies occurred in 19 of 20 children, with mean log_2 titers of 2.7 ± 0.9 before study product administration and 6.6 ± 1.1 following study product administration. Thus, while the levels of infectivity and replication of RSV MEDI/ΔM2-2 were similar to what might be seen with an over-attenuated vaccine virus, the levels of RSV-specific serum antibodies indicated that this virus was surprisingly immunogenic. Respiratory illnesses were observed in 85% of vaccinees and 70% of placebo recipients, including fever (20% vs. 30%), rhinorrhea (85% vs. 50%), cough (35% vs. 30%), and otitis media (5% vs. 0%). Lower respiratory tract illness (LRI) was not detected in any participant. Transmission of vaccine virus occurred in a set of 13-month-old twin study participants; both were minimally symptomatic and vaccine virus shed retained the M2-2 deletion and remained very highly restricted in the secondary infection. When data on vaccine virus infectivity and immunogenicity in RSV-seronegative children were compared to those achieved with rA2 cp248/404/1030/ΔSH, a live-attenuated RSV vaccine candidate that was well tolerated and immunogenic in previous pediatric Phase I studies (15), it was found that the MEDI/ΔM2-2 shedding was significantly more restricted. However, the post-study product administration RSV-neutralizing serum antibody achieved (GMT = 1:97) was significantly greater with RSV MEDI/ΔM2-2 than with rA2 cp248/404/1030/ΔSH. Surveillance during the subsequent RSV season showed that several RSV-seronegative RSV MEDI/ΔM2-2 recipients had substantial antibody rises without reported medically attended illness, suggesting that the vaccine primed for strong anamnestic responses to RSV. The M2-2 deletion was stable in all shed vaccine virus samples that were tested (6).

Thus, the MEDI/ΔM2-2 phenotype appears to be very promising for an RSV vaccine; MEDI/ΔM2-2 remained the lead candidate after evaluation of several other ΔM2-2 candidates with differences affecting replication and temperature sensitivity [Table 1; (6, 28-31)]. MEDI/ΔM2-2 was not available for further clinical analysis, and RSV 276 was designed to be similar to MEDI/ΔM2-2. RSV 276 differs from MEDI/ΔM2-2 by four nucleotides in noncoding sequence. It is reasonable to expect that these two viruses will have similar levels of replication and attenuation in pre-clinical testing and in clinical testing humans, and likely will be phenotypically indistinguishable. In pre-clinical nonhuman primate studies in AGMs, the most permissive RSV animal model currently available, the level of replication of RSV 276 was similar to MEDI/ΔM2-2.

RSV 276 was evaluated under the protocol IMPAACT 2018/CIR 321 (NCT03422237, NCT03227029). Under this protocol, children 6 to 25 months of age received the RSV ΔNS2/Δ1313/I1314L or RSV 276 vaccines or placebo (randomization 2:2:1). The study enrolled 52 children in the two vaccine arms (26 each) and 13 children in the placebo arm. Three children had no data after entry and are not included in the analysis. During the 28 days post-inoculation, mild upper respiratory and/or febrile events occurred often in both vaccine and placebo recipients, with 64% [90% CI: (46%, 80%)] of RSV ΔNS2/Δ1313/I1314L recipients, 84% [90% CI: (67%, 94%)] of RSV 276 recipients, and 58% [90% CI: (32%, 82%)] of placebo recipients having one or more illness episodes. Most of the respiratory symptoms were Grade 1. The only Grade 3 events were fever, which occurred in two RSV ΔNS2/Δ1313/I1314L vaccinees and one RSV 276 vaccine recipient. Grade 2 fever occurred in one RSV ΔNS2/Δ1313/I1314L vaccinee, two RSV 276 vaccinees, and one placebo recipient. Grade 2 otitis media occurred in one RSV ΔNS2/Δ1313/I1314L vaccinee and two RSV 276 vaccine recipients. Grade 2 cough occurred in one RSV 276 vaccine recipient and one placebo recipient. There were two Grade 2 URIs reported, both in RSV ΔNS2/Δ1313/I1314L vaccinees. All other events were Grade 1. There were no LRIs or SAEs.

1.4 Rationale

In previous Phase IA studies in RSV-seronegative infants and children 6 to 24 months of age, RSV vaccines based on two different attenuation strategies have been evaluated. The first approach is based on deletion of the gene encoding the RSV interferon/apoptosis antagonist NS2 protein (ΔNS2). Deletion of NS2 attenuates the virus and may enhance immunogenicity. The best ΔNS2 candidates, RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s, additionally contain a genetically stabilized attenuating and temperature sensitivity mutation in the L protein (codon deletion Δ1313, as well as a missense mutation I1314L that prevents a de-attenuating mutation that otherwise can occur at position 1314 in RSV ΔNS2/Δ1313/I1314L; genetically stabilized mutation 1030s in RSV 6120/ΔNS2/1030s). The second approach is based on deletion of the RSV M2-2 open reading frame (ΔM2-2), which results in up-regulation of viral antigen synthesis, increasing immunogenicity per infectious unit. The initial ΔM2-2 vaccine candidate, MEDI/ΔM2-2, is not available for further study. A similar virus, called RSV 276, has now been prepared. Based on infectivity, safety, and immunogenicity data, these three candidate vaccines were identified as the strongest candidates to move into expanded Phase I/II trials. The overall goal of IMPAACT 2021 is to assess the safety and immunogenicity of the three vaccine candidates in a larger study and choose one or two to move forward into future large-scale studies.

In IMPAACT 2021, RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 will be evaluated side-by-side. A placebo group equal in size to each vaccine group is needed to detect safety signals, including excess solicited adverse events (respiratory or febrile illness) that might be associated with one or more of the study products, above the high background of respiratory/febrile illness in this age group. Enrollment will take place outside the time during which wt RSV circulates in the community at each study site. RSV circulation will be determined by the Protocol Team through local and national surveillance and communication with the study sites, with additional guidance provided in the Manual of Procedures (MOP). Surveillance of study participants will take place during the RSV season that starts in the calendar year of study product administration. The goal was to complete enrollment within a single enrollment season, followed by planning of subsequent, larger safety and efficacy studies. However, the study was not fully accrued in the first season. The DSMB reviewed results during the winter RSV season following the first enrollment season (2019) and recommended that the study should continue as planned when feasible in the context of circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated coronavirus disease (COVID-19) pandemic. Due to COVID-19, the study was not able to reopen during the second enrollment season (2020), and no sites were able to resume enrollment during the third enrollment season (2021); thus, enrollment is anticipated to resume for a fourth enrollment season (e.g., 2022). If a fifth enrollment season (e.g., 2023) is considered, the DSMB will review results from the fourth enrollment season (e.g., 2022) to confirm if all candidate vaccines should remain in the study.

The study population, the inclusion and exclusion criteria, most primary objectives, and other aspects of the proposed study adhere closely to previous IMPAACT RSV studies 2000, 2011, 2012, 2013, and 2018. The schedule of evaluations is simplified compared to that of the previous studies; the number of in-person visits and scheduled clinical assessments are greatly reduced from seven to a single one over the first 28 days after study product administration, which should facilitate enrollment and minimize the labor required for the study. As with previous studies, participants with illness may have additional visits to assess the severity of the illness (see Section 6.8 for Illness Visits). Nasal swabs will be collected at Illness Visits (note that nasal washes were collected under protocol Version 1.0; see Section 3 for additional detail).

Rationale for evaluation of immunogenicity on Day 28 and Day 56

To date, all candidate vaccine studies with live attenuated RSV vaccines have used Day 56 as the time point to assess serum antibody response. This time point was used for two reasons: 1) it was believed that since this is a primary response (rather than an anamnestic [memory] response), it may not have reached maximal levels by Day 28 and 2) maximal replication of these highly attenuated vaccine viruses does not typically occur until Day 7, which may also delay induction of the immune response. However, some children may have become infected with wildtype RSV by the time their Day 56 specimens are collected, resulting in a >4-fold antibody response even if there was no response to vaccine. If this window could be shortened to 28 days, a time when antibody responses are generally present after vaccine, the frequency of antibody responses due to community-acquired wild-type RSV could be greatly reduced. This will be very important for future, large-scale studies. However, the change to Day 28 measures cannot be made without first confirming that responses are detectable on Day 28 and are comparable to those measured on Day 56. It is anticipated that all future studies will measure response at a single timepoint (hopefully Day 28).

Evaluation of study products

There are four time points at which data have been/will be reviewed to allow a determination of which study arms will remain open. The first was prior to the time the study is open when interim results of IMPAACT 2018/CIR 321 and CIR 322 became available. These studies provided additional safety and immunogenicity data for each of the three candidate vaccines included in IMPAACT 2021. Each of the three products was evaluated by a subgroup of the Protocol Team using the go/no-go criteria in Table 3 below; all three products passed the go criteria. If the subgroup of Protocol Team members had determined that any product did not pass the go criteria, they would have shared this information with the DSMB members to make a final decision about whether that candidate arm may be dropped from IMPAACT 2021 prior to the opening of the study. Note that each candidate vaccine has been studied in a similar number of participants so far, so similar criteria were applied across the three vaccine candidates. The 70% cutoff specified below is considered to be a reasonable threshold to ensure that development of a potentially viable candidate vaccine is not stopped prematurely.

Table 3: Criteria for Including a Vaccine in IMPAACT 2021

	Yes	No
1. Is the upper limit of the 95% confidence interval for the percentage of participants exhibiting a vaccine take (defined as the detection of vaccine virus shedding by culture or RT-PCR, and/or ≥4-fold rise in RSV-specific serum antibodies at the Day 56 Visit) less than 70%?		
2. Have more than two participants receiving the specific product experienced LRI of ≥ Grade 2 in the 28 days following study product administration for which vaccine virus was isolated by culture at the time of illness without other potential explanation?		
3. Were there two or more participants who experienced vaccine-associated Grade 4 events of similar type (as listed below) after study product administration with presence of vaccine virus without other potential explanation? Otitis media within 28 days of study product administration Pharyngitis within 28 days of study product administration		

For any specific candidate vaccine, a response of "yes" to any of these questions could have

resulted in that candidate vaccine being removed from IMPAACT 2021 prior to the study opening.

The second time point at which data were reviewed was during the winter RSV season following the first enrollment season (2019), given that the study was not yet fully accrued. Using the criteria in Table 4 as guidance, the DSMB recommended that the study should continue as planned when feasible in the context of the COVID-19 pandemic. Due to COVID-19, the study could not open in the second enrollment season (2020). During the winter of 2021, newly available unblinded data from IMPAACT 2018/CIR 321 and CIR 322 were reviewed, and it was determined that the RSV 276 vaccine arm of IMPAACT 2021 would be closed to further enrollment under protocol V2.0 (this was the third time point at which data were reviewed); this arm remains closed under protocol V3.0. No sites were able to resume enrollment during the third enrollment season (2021); thus, enrollment is anticipated to resume for a fourth enrollment season (e.g., 2022). If a fifth enrollment season (e.g., 2023) is considered, the DSMB will review results from the fourth enrollment season (e.g., 2022) to confirm if all candidate vaccines should remain in the study (this is the fourth time point at which data will be reviewed). The DSMB will use the criteria in Table 4 as guidance in making that decision.

Table 4: Post-Accrual Season Study Review Criteria

	Yes	No
1. Is there a statistically significant ($p<0.05$) difference in the percentage with immune response (≥4-fold rise in serum RSV-neutralizing antibodies) to the candidate vaccines such that one or two products can be identified to be immunologically superior to the remaining product or products, with the absence of a safety signal among the higher performing product(s)?		
If yes, the DSMB may recommend that the study close to accrual		
If no, the DSMB will consider the following questions for each specific product:		
2. Is the upper limit of the 95% confidence interval for the percentage of participants exhibiting a ≥4-fold rise in serum RSV-neutralizing antibodies at the Day 56 Visit less than 70%?		
3. Have more than two participants receiving the specific product experienced LRI of ≥ Grade 2 in the 28 days following study product administration for which vaccine virus was isolated without other potential explanation?		
4. Were there two or more participants who experienced vaccine-associated Grade 4 events of similar type (as listed below) after study product administration with presence of vaccine virus without other potential explanation? Otitis media within 28 days of study product administration Pharyngitis within 28 days of study product administration		

For any specific candidate vaccine, a response of "yes" to any of these questions could result in that candidate vaccine arm being closed. As noted above, the RSV 276 vaccine arm was closed to further enrollment under protocol V2.0 and remains closed under protocol V3.0.

1.5 Hypotheses

The live-attenuated RSV vaccine candidates RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 will each be safe and immunogenic in RSV-seronegative recipients. However, these vaccine candidates may have safety signals that become apparent only when analyzed in a larger number of participants, and the candidates may differ in the magnitude and longevity of antibody responses, and in responses observed following naturally occurring RSV infection. To move forward, the vaccines should:

- be safe, and
- result in at least 70% of the population of vaccine recipients having a ≥4-fold rise in serum RSV-neutralizing antibody titers from pre-study product administration to the Day 56 Visit after study product administration

2 OBJECTIVES

2.1 Primary Objectives

The primary objectives of this study are the following:

2.1.1 Safety: To estimate and compare (each vaccine group to placebo) the frequency and severity of adverse events (AEs) following study product administration (Day 0) through Day 56

2.1.2 Immunogenicity: To estimate and compare (between the vaccine groups and to the benchmark of 70%) the percentage of vaccinees having a ≥4-fold rise in serum RSV-neutralizing antibodies at the Day 56 Visit

2.2 Secondary Objectives

The secondary objectives of this study are to:

2.2.1 Immunogenicity: To estimate and compare (between the vaccine groups) the percentage of vaccinees with a ≥4-fold rise in serum IgG antibody to RSV F protein (RSV F IgG) at the Day 56 Visit

2.2.2 Immunogenicity: To estimate and compare (between the vaccine groups) the titers of serum RSV F IgG and serum RSV-neutralizing antibodies at the Day 56 Visit

2.2.3 Safety: To describe and compare the frequency and severity of RSV-associated, medically attended, acute respiratory illness (RSV-MAARI) and RSV-associated, medically attended, acute lower respiratory illness (RSV-MAALRI) in the placebo and vaccine arms during RSV season

2.3 Exploratory Objectives

2.3.1 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

2.3.2 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 each vaccine group

2.3.3 Immunogenicity – magnitude of anamnestic response: To estimate and compare (each vaccine group to placebo and between the vaccine groups) the titers of serum RSV F IgG and serum RSV-neutralizing antibodies in participants infected with wt RSV during the RSV season

2.3.4 Immunogenicity – durability of vaccine-induced RSV antibodies: to estimate and compare (between the vaccine groups), 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

3 STUDY DESIGN

The study will be conducted in children at selected sites in the United States. The vaccines will be evaluated in RSV-seronegative children ≥6 months (180 days) at the time of screening to <25 months (750 days) of age at the time of enrollment. 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 (15, 26, 32). In these previous studies, live-attenuated RSV vaccines were highly restricted in replication and poorly immunogenic in children with titers ≥1:40 but were far less restricted in replication and highly immunogenic in children with titers <1:40. These data suggest that in this age group, this neutralizing antibody cutoff can distinguish effectively between children who have previously been infected with RSV and those who have not.

The study will be double-blind, randomized, and placebo-controlled. As with protocol V2.0, under protocol V3.0, RSV-seronegative participants will be randomized equally (1:1:1) to one of the two active vaccine arms or the placebo arm. The total expected sample size is approximately 130, including 40 each in the placebo and two candidate vaccine arms open under protocol V2.0 and V3.0, as well as all participants previously randomized to receive RSV 276 vaccine under protocol V1.0 prior to closure of this arm. Adjustments to the candidate vaccines included in the study may be made as outlined in Section 1.4 (e.g., the RSV 276 arm was closed to further enrollment under protocol V2.0 and remains closed under protocol V3.0). Placebo recipients are needed in pediatric studies to distinguish the background respiratory and febrile illnesses that occur in children from those attributable to study vaccine. These numbers were chosen based upon experience with Phase I evaluation of other live-attenuated respiratory virus candidate vaccines (25-27), statistical considerations (see Section 9), and feasibility for reaching the enrollment goal within one season.

Enrollment will occur outside the time during which wt RSV circulates in the community at each study site. RSV circulation will be determined by the Protocol Team through local and national surveillance and communication with the study sites, with additional guidance provided in the Manual of Procedures (MOP). The goal was to complete enrollment within a single enrollment season. Accrual was not completed in the first enrollment season (2019), and the DSMB recommended that the study continue as planned when feasible in the context of the COVID-19 pandemic. Due to the COVID-19 pandemic, enrollment was paused during the second enrollment

season (2020), and no sites were able to resume enrollment during the third enrollment season (2021). Enrollment will therefore be extended into a fourth enrollment season (e.g., 2022) and may be extended into a fifth enrollment season (e.g., 2023). This will increase the accrual time. Specific study phases are described in the paragraphs that follow.

Throughout study follow-up, reported AEs will be clinically assessed via in-person or telehealth (telephone or video) Illness Visits as described in Section 6.8. Illness Visits may be conducted by study staff or, alternatively, by other medical providers if medical records from the visit can be made available to study staff and a parent/guardian can collect the nasal swab described below.

A nasal swab to evaluate for RSV and other respiratory pathogens (adventitious agents) will be collected for each Illness Visit (see Section 6.8, Appendix I, and Appendix II). If a parent/guardian is comfortable collecting a nasal swab sample, the sample may be collected by the parent/guardian during or immediately after a telehealth visit. If parent/guardian is not comfortable collecting the sample, the nasal swab should be collected by study staff.

Note: Under protocol Version 1.0, nasal wash samples were collected from participants. As with protocol Version 2.0, under protocol V3.0, the collection has been changed to nasal swab samples, to decrease potential exposure of study staff to SARS-CoV-2 and allow parents/guardians to collect samples, if possible and feasible for parents/guardians

Duration of participation in the initial part of the study is 56 days, which consists of an Acute and a Post-Acute Phase. The schedule of events during the Acute Phase and Post-Acute Phase is shown in Appendix I. During the Acute Phase (which lasts from the day of study product administration (Study Day 0) to midnight on the 28th day following Day 0), participants will be contacted daily. These contacts will be conducted by phone, text, or email to assess interim medical history. Participants with episodes of fever Grade 2 or higher, URI (excluding rhinorrhea without cough), or otitis media will require an in-person Illness Visit; the timing of the visit depends on the grade of the event per Section 6.8. Cases of fever <Grade 2 and rhinorrhea without cough may be clinically assessed via telehealth (telephone or video) Illness Visit. Participants who have a possible LRI will have an in-person Illness Visit within one day of the reported onset of the LRI, and an in-person or telehealth (telephone or video) follow-up assessment is required one to three days after the initial assessment. See Section 6.8 for more information.

During the Post-Acute Phase (Study Day 29 to midnight on the 56th day following Day 0), study participants will have a scheduled visit on Day 56. Episodes of Serious Adverse Events from Day 29 through 56 will have an in-person or telehealth (telephone or video) Illness Visit (see Section 6.8, Appendix I).

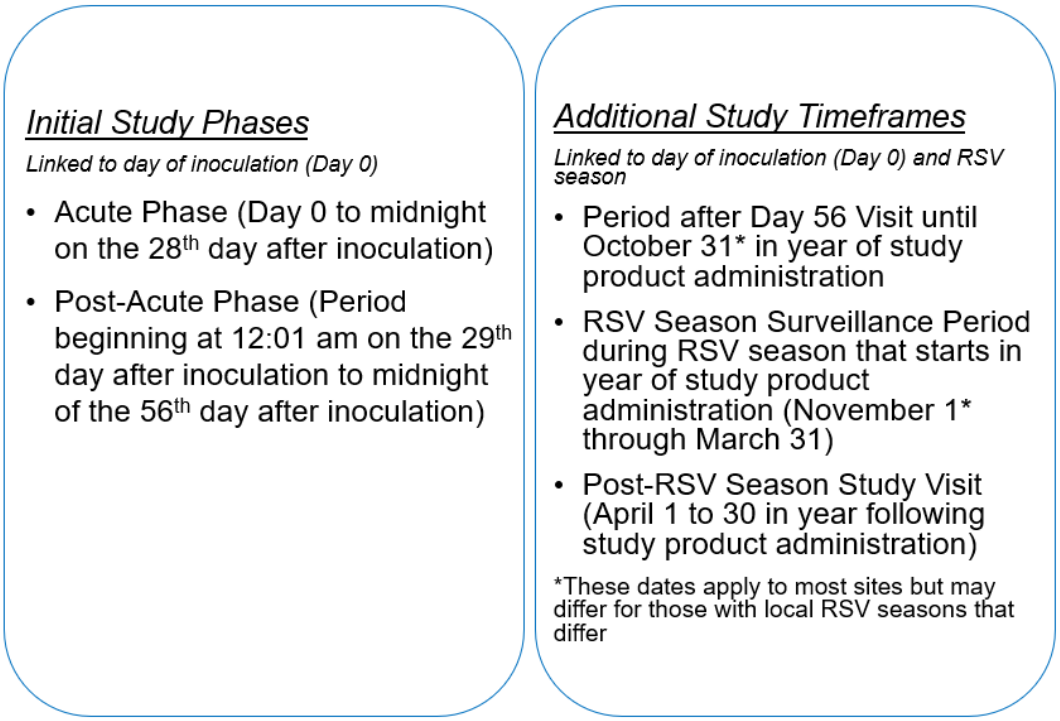
In addition to the Acute and Post-Acute Phases, the study has a third timeframe during which the incidence and severity of illness suggestive of RSV occurring during the RSV season that starts in the calendar year of the participant's study product administration are assessed. During the RSV Season Surveillance Period, encompassing November 1st to March 31st for most sites or—for sites with local RSV seasons that differ—as specified on a site-by-site basis in the MOP, site study staff will make weekly contact with the participants to identify medically attended episodes of fever, URI or LRI, or otitis media. Medically attended events during the RSV Season Surveillance Period are defined in Section 6.6. Such an episode may require an Illness Visit (see Section 6.8, Appendix II).

All participants will have a Post-RSV Season Visit (April 1st to April 30th) in the calendar year

following study product administration to collect blood for measurement of RSV immune response to further assess the durability of the vaccine response and to assess the immune response to naturally occurring wt RSV infection. Thus, the maximum duration of participation will be up to 15 months, depending upon the time of enrollment relative to the RSV season.

Figure 2 summarizes the initial study phases and additional study timeframes. There may be overlap in these various timeframes.

Figure 2: Initial Study Phases and Additional Study Timeframes



4 STUDY POPULATION

The vaccines will be evaluated in RSV-seronegative children ≥6 months (180 days) at the time of screening to <25 months (750 days) of age at the time of enrollment. As with protocol V2.0, under protocol V3.0, participants will be randomized equally (1:1:1) to receive one of the two candidate vaccines or placebo (40 per arm) from US sites only. Children will be selected for participation according to the criteria in Sections 4.1 and 4.2. The sections that follow describe study-specific co-enrollment considerations; the recruitment, screening, and enrollment process; and participant retention and withdrawal or termination. Sites are expected to obtain the potential participant's medical records from the potential participant's primary care provider to review for eligibility. The criteria related to the health status (including CD4 counts) and age of household members, day care attendance, and scheduled vaccine administration in relation to study product administration (except as noted in the medical record) may rely on parent/guardian report.

Any questions regarding interpretation of the inclusion/exclusion criteria or other considerations described in this section should be forwarded to the Protocol Team.

4.1 Inclusion Criteria

Potential participants must meet all of the following criteria in order to be included in this study:

4.1.1 ≥6 months (defined as ≥180 days) of age at the time of screening and <25 months (defined as < 750 days) of age at the time of enrollment.

4.1.2 In good health based on review of the medical record, history, and physical examination, without evidence of chronic disease.

4.1.3 Parent/guardian is willing and able to provide written informed consent as described in protocol Section 12.3.

Note: All sites must follow the policies and procedures of all applicable IRBs; this includes single IRB (sIRB) policies and procedures. Refer to Section 12.1 for more information on sIRB oversight of US sites.

4.1.4 Seronegative for RSV antibody, defined as a serum RSV-neutralizing antibody titer <1:40 at screening from a sample collected no more than 42 days prior to study product administration.

Note: results from specimens collected during screening for any study of an RSV vaccine developed by the LID (NIAID, NIH) are acceptable. If study product will not be administered the same day as randomization (see Section 6.2), it must be administered no more than 42 days after the screening sample is collected.

4.1.5 Growing normally for age in the opinion of the site clinician in the six months prior to enrollment AND has a current height and weight above the 3rd percentile for age and sex per CDC (WHO) growth standards.

4.1.6 Has received routine immunizations appropriate for age (as per national Center for Disease Control Advisory Committee on Immunization Practices [ACIP]). Note: COVID-19 vaccination will not be required unless fully licensed for this age group and ACIP-recommended. See MOP for further guidance.

4.1.7 Is expected to be available for the duration of the study.

4.1.8 If born to an HIV-infected woman, potential participant must have documentation of 2 negative HIV nucleic acid (RNA or DNA) test results from samples collected on different dates with both collected when ≥4 weeks of age and at least one collected when ≥16 weeks of age, and no positive HIV nucleic acid (RNA or DNA) test; or 2 negative HIV antibody tests, both from samples collected at ≥24 weeks of age. If potential participant was breastfed by an HIV-infected woman, each of the sampling times noted above must be measured in weeks after the last exposure to breast milk, rather than weeks of age.

4.2 Exclusion Criteria

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

4.2.1 Prior laboratory-confirmed RSV infection.

4.2.2 Known or suspected HIV infection or impairment of immunological functions.

4.2.3 Receipt of immunosuppressive therapy, including any systemic, nasal, or inhaled corticosteroids within 28 days of enrollment. Note: Cutaneous (topical) steroid treatment is not an exclusion.

4.2.4 Any receipt of bone marrow/solid organ transplant.

4.2.5 Major congenital malformations (such as congenital cleft palate) or cytogenetic abnormalities.

4.2.6 Previous enrollment in this trial, previous maternal or pediatric receipt of a licensed or investigational RSV vaccine, or previous maternal or pediatric receipt of or planned administration of any anti-RSV product (such as ribavirin or RSV IG or RSV mAb).

4.2.7 Any previous anaphylactic reaction.

4.2.8 Any known hypersensitivity to any study product component.

4.2.9 Heart disease. Note: Potential participants with cardiac abnormalities documented to be clinically insignificant and requiring no treatment may be enrolled.

4.2.10 Lung disease, including any history of reactive airway disease or medically diagnosed wheezing.

4.2.11 Member of a household that contains a person with chronic lung disease, including but not limited to chronic obstructive pulmonary disease (COPD), emphysema, or home oxygen use, reactive airway disease or asthma.

Note: Asthma or reactive airway disease in a household member is not exclusionary unless the household member has taken oral steroids for asthma management in the past month and/or has been hospitalized for asthma in the past month.

4.2.12 Member of a household that contains, or will contain, an infant who is less than 4 months of age at the enrollment date through Day 14.

4.2.13 Member of a household that contains another child/other children who is/are enrolled or is/are scheduled to be enrolled in IMPAACT 2021 on a different date and has/have not completed the Day 56 Visit (i.e., all eligible children from the same household must be enrolled/receive study product on the same date, or additional children in the household may be screened, enrolled, and randomized independently after other children in the household complete the Day 56 Visit).

4.2.14 Member of a household that contains another child who is, or is scheduled to be, enrolled in another study evaluating an intranasal live-attenuated RSV vaccine, AND there has been or will be an overlap in residency during Day 0 to 14 of that other child's participation in the study.

4.2.15 Member of a household that contains an immunocompromised individual, including, but not limited to:

- a person who has been diagnosed with cancer and who has received chemotherapy within the 12 months prior to enrollment; or
- a person living with a solid organ, cord blood, or bone marrow transplant.

4.2.16 Shares a daycare room with infants less than 4 months of age, and parent/guardian is unable or unwilling to suspend daycare for 14 days following study product administration.

4.2.17 Any of the following events at the time of enrollment:

- fever (rectal temperature of ≥100.4°F (38°C)), or
- upper respiratory signs or symptoms (including but not limited to rhinorrhea, cough, or pharyngitis) or
- nasal congestion significant enough to interfere with successful study product administration, or
- otitis media.

Note: if participant is randomized and subsequently noted to have any of the above, study product administration must be deferred per protocol Section 6.2.

4.2.18 Receipt of the following prior to enrollment (start counting backwards with '1' as the day of planned study product administration):

- any inactivated vaccine or live-attenuated rotavirus vaccine within the 14 days prior, or
- any live vaccine, other than rotavirus vaccine, within the 28 days prior, or
- another investigational vaccine or investigational drug within 28 days prior Note: if COVID-19 vaccine has EUA approval and ACIP recommendation for this age group, it is not considered investigational.

4.2.19 Scheduled administration of the following after planned study product administration (start counting with '1' as the day of planned study product administration):

- 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 Note: if COVID-19 vaccine has EUA approval and ACIP recommendation for this age group, it is not considered investigational.

4.2.20 Receipt of immunoglobulin, any antibody products, or any blood products within the past 6 months prior to enrollment

4.2.21 Receipt of any of the following medications within 3 days prior to study enrollment:

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

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

4.2.22 Born at less than 34 weeks gestation.

4.2.23 Born between 34 weeks gestation and 36 weeks and 6 days gestation and less than 1 year of age at the time of enrollment.

4.2.24 Current suspected or documented developmental disorder, delay, or other developmental problem.

4.2.25 Any previous receipt of supplemental oxygen therapy in a home setting.

4.2.26 Known or suspected SARS-CoV-2 exposure within the 14 days prior to enrollment. Note: known or suspected SARS-CoV-2 includes a known asymptomatic household member under quarantine for SARS-CoV-2 exposure but without a positive SARS-CoV-2 test.

4.3 Co-Enrollment Considerations

Co-enrollment to any biomedical interventional study is not allowed during the Acute Phase or Post-Acute Phase. After the Post-Acute Phase, co-enrollment may be considered if both Protocol Teams agree.

Note: co-enrollment into IMPAACT 2021 is allowable for participants already enrolled in the PHACS (Pediatric HIV/AIDS Cohort Study) SMARTT (Surveillance Monitoring for ART Toxicities) study.

Co-enrollment into IMPAACT 2021 is allowable for participants already enrolled in IMPAACT P1112, provided all eligibility criteria above are met, and the participant must not have received P1112 study product in the past 12 months. The P1112 and IMPAACT 2021 teams should be queried in each case to confirm (impaact.teamp1112@fstrf.org; impaact.team2021@fstrf.org).

4.4 Recruitment, Screening, and Enrollment Process

Recruitment will take place at US sites selected on the ability to recruit and enroll children in RSV vaccine studies; given IMPAACT sites' history and familiarity with select populations, this will include HIV-exposed, uninfected and/or HIV-unexposed children. Each site will identify the specific clinics (e.g., hospital, community, and private pediatric clinics) where recruitment will occur as part of the site selection process, which will be reviewed and approved by the Protocol Team. All recruitment materials must be reviewed and approved by the applicable IRB.

Study product administration (Day 0) must occur within 42 days of collection of the screening sample and ideally on the same day as randomization or, if needed, up to 5 days after randomization (see Section 6.2).

The IMPAACT Data Management Center (DMC) Study Enrollment System (SES) will be used to assist with tracking screening and enrollment. When informed consent is obtained, a participant identification number (PID) will be assigned to the child by the study staff, and a

study-specific screening number will be obtained through the SES. For children found to be eligible, randomization and enrollment will occur upon successful entry of required eligibility data into the SES. Successful entry into the SES will generate a study identification number (SID) assignment and blinded prescribing information for the study product arm to which the child has been randomly assigned; this represents the effective point of enrollment. For potential participants who are found to be ineligible for the study, or who do not enroll in the study for any reason, an electronic case report form (eCRF) will be entered to record the screening outcome. Note that any eligible children living in the same household can be enrolled and will be randomized per Section 9.3.1. Refer to Section 9.4 for more information on monitoring participant accrual in this study.

4.5 Participant Retention

Once a child has received study product, study staff will make every effort to retain him or her in follow-up for the protocol-specified duration of follow-up, i.e., through the Post-RSV Season Study Visit, thereby minimizing potential biases associated with loss to follow-up.

4.6 Participant Withdrawal or Termination from the Study

Regardless of the participant retention procedures referenced above, parents/guardians of children participating in this study may voluntarily withdraw their children from the study at any time. Any participant who has received study product will be encouraged to remain in study follow-up for the duration of the study even if sample collection is refused.

A participant may withdraw or be terminated from the study early for any of the following reasons that occur after randomization:

- Withdrawal of consent – applies to a parent/guardian who verbally or in writing withdraws consent to participate in the study for any reason.
- Exceedance of the 42-day window from collection of the screening sample to study product administration – applies to an illness or other participant-specific reason (see Section 6.2 for guidance related to exceedances due to a pause in study accrual).
- 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.
- Participant re-locates away from the study site (and it is not possible to continue participation at another IMPAACT 2021 site near the participant's new location) or is otherwise determined to be lost-to-follow-up.
- Investigator or designee determines that continued participation in the study would be unsafe or otherwise not in the best interest of the participant, after consultation with the Protocol Team.
- Other – requires an explanation.

The study may be stopped or canceled by the study investigators, sponsors, IMPAACT, government or regulatory authorities, or IRBs/IBCs for any reason.

For any participant who received study product and withdraws or is terminated from the study prior to scheduled completion of follow-up, study staff will document the reason for the withdrawal or termination in detail and will make every effort to complete final evaluations as

described in Section 6.9. In the event that the circumstances that led to a participant's withdrawal or termination change (e.g., he or she returns to the study site area after having re-located previously), the site investigator or designee should contact the Protocol Team (impaact.team2021@fstrf.org) to discuss options for resumption of follow-up.

5 STUDY PRODUCT CONSIDERATIONS

Site pharmacists should consult the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for standard pharmacy operations. Refer to Figure 1 for an overview of the study design and to the Investigator's Brochures (Ibs) for further information about the study products.

5.1 Study Products

The products that will be administered in this study are:

- Live Recombinant Respiratory Syncytial Virus RSV ΔNS2/Δ1313/I1314L 10^6 PFU per 0.5ml vaccine
- Live Recombinant Respiratory Syncytial Virus RSV 6120/ΔNS2/1030s 10^5 PFU per 0.5ml vaccine
- Live Recombinant Respiratory Syncytial Virus RSV 276 10^5 PFU per 0.5ml vaccine – this vaccine arm is closed to further enrollment under protocol V2.0 and V3.0; refer to protocol V1.0 for details related to this vaccine
- Placebo for each RSV vaccine will be Lactated Ringer's Solution for Injection, USP (0.5ml)

5.2 Study Product Regimens

Enrolled study participants will receive a single dose of the indicated vaccine or placebo, administered as nose drops within 5 days of randomization. Ideally, the date of randomization will be the same as the date of study product administration (see Section 6.2).

5.3 Study Product Formulation

5.3.1 Vaccines

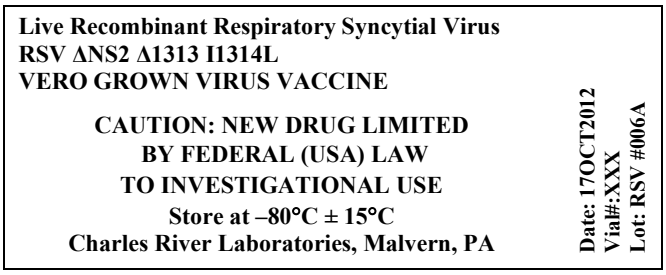
The RSV ΔNS2/Δ1313/I1314L vaccine is provided in a sterile 2.0 mL micro tube, each containing 0.6 mL of Vaccine (Lot RSV #006A), with a titer of approximately $10^{7.3}$ PFU/mL. The vaccine virus concentrate is diluted to an appropriate dose by designated licensed pharmacy personnel to prepare a dose of 10^6 PFU in a 0.5 mL volume. The vaccine vial is labeled as shown below in Figure 3.

The RSV 6120/ΔNS2/1030s vaccine is provided in a sterile 2.0 mL micro tube, each containing 0.6 mL of Vaccine (Lot RSV #012A), with a titer of approximately $10^{6.0}$ PFU/mL. The vaccine virus concentrate is diluted to an appropriate dose by designated licensed pharmacy personnel to prepare a dose of 10^5 PFU in a 0.5 mL volume. The vaccine vial is labeled as shown below in Figure 3.

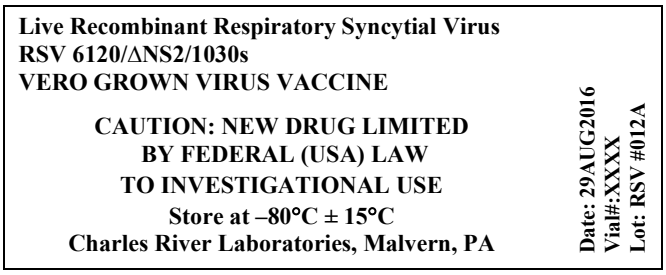
The RSV 276 vaccine will not be provided under protocol V2.0 or V3.0. Refer to protocol V1.0 for details related to this vaccine.

Figure 3: Investigational Product Label (Enlarged Sample)

RSV ΔNS2/Δ1313/I1314L Vaccine:



RSV 6120/ΔNS2/1030s Vaccine:



5.3.2 Diluent for RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s

As with protocol V2.0, under protocol V3.0, the diluent for RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s is Lactated Ringer's Solution for Injection, USP.

5.3.3 Placebo for RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s

As with protocol V2.0, under protocol V3.0, the placebo for RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s is Lactated Ringer's Solution for Injection, USP.

5.4 Study Product Storage

Vaccine will be stored in a secure freezer under pharmacy control at -80°C ±15°C. It must remain frozen until 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 in accordance with the manufacturer's recommendation and transferred to a secure 2°C to 8°C refrigerator at least 24 hours before use. Vaccine diluent/placebo will be prepared from new, unopened containers for each use.

Procedures for managing the vaccine and diluent/placebo shipment are in the Manual of Procedures (MOP).

5.5 Study Product Preparation

The diluent for the vaccines, the placebo for the vaccines, and the RSV vaccines must be prepared by following the detailed instruction in the MOP.

Prior to study product administration, an authorized prescriber will supply a prescription to the pharmacy. The prescription must include the information outlined in the MOP. Designated licensed pharmacy personnel will prepare the correct dose of vaccine or placebo for each participant in a USP <797> compliant environment appropriate for the Biosafety Level of the study products, as determined by local policies and procedures. To preserve blinding, yellow overlays will be applied to all prepared syringes.

5.5.1 Preparation of Placebo for RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s

As with protocol V2.0, under protocol V3.0, placebo for RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s will be Lactated Ringer's Solution for Injection, USP. The diluent/placebo must be kept on ice during preparation of vaccine products.

Placebo will be drawn up to a volume of 0.5 mL in a sterile 1 mL oral syringe and labeled per instructions in MOP. An auxiliary label stating "FOR INTRANASAL ADMINISTRATION ONLY" will be affixed to the syringe or outside bag. The labeled, filled syringe(s) will be transported in a cooler with a closed lid, monitored and maintained at 2ºC to 8ºC with ice or cold packs, to the clinical site for administration. Placebo must be administered within 4 hours of the time Lactated Ringer's Solution for Injection, USP is removed from the refrigerator.

Please follow the MOP for detailed instructions on preparation of placebo.

5.5.2 Preparation of Live Recombinant Respiratory Syncytial Virus RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s

If the -80°C freezer where the RSV vaccines are stored is not in close proximity to where the preparation is being done, the vaccine vials should be transported on dry ice from the freezer to the aseptic preparation area. Do not thaw this product on the bench top or allow the vial to thaw completely before putting onto wet ice. RSV is extremely sensitive to temperature fluctuations. Please follow the MOP for proper handling of the study product.

RSV ΔNS2/Δ1313/I1314L requires two vials of undiluted vaccine to prepare the vaccine dose. RSV 6120/ΔNS2/1030s requires three vials of undiluted vaccine. Refer to the MOP for additional guidance. When manipulating the undiluted vaccine, use as small a gauge needle as possible to avoid loss of vaccine in the needle and syringe hub. As with protocol V2.0, under protocol V3.0, the frozen vaccine will be thawed and diluted with Lactated Ringer's Solution for Injection, USP to the indicated dose of either 10^6 (RSV ΔNS2/Δ1313/I1314L) or 10^5 PFU (RSV 6120/ΔNS2/1030s) in 0.5 mL prior to administration.

The diluted vaccine will be drawn up to a volume of 0.5 mL in a sterile 1 mL oral syringe and labeled per instructions in MOP. An auxiliary label stating "FOR INTRANASAL ADMINISTRATION ONLY" will be affixed to the syringe or outside bag. The labeled, filled syringe(s) will be transported in a cooler with a closed lid, monitored and maintained at 2ºC to 8ºC with ice or cold packs, to the clinical site for administration. Vaccine must be prepared and administered within 4 hours of being removed from the freezer and thawing. However,

the expiration time is assigned based on the time the Lactated Ringer's Solution for Injection, USP is removed from the refrigerator in order to maintain the blind.

After the designated licensed pharmacy personnel dilutes the vaccine and draws up the vaccine into a syringe for administration, he/she will remove the label from all vaccine vials and place it in the accountability log. Please see the IMPAACT 2021 MOP for the study preparation and accountability documents. In this manner, monitoring personnel will be able to verify the accountability of all vaccine vials used for the study.

Samples of undiluted (if available) and diluted vaccine will be aliquoted from the vaccine remaining after vaccine has been prepared and delivered to the clinical staff. The samples will be snap frozen as per the MOP and stored at -80°C ±15°C in the investigational pharmacy but separate from the study product until they are shipped to the central laboratory for titration. Titration of vaccine is done to confirm the titer of the vaccine administered to the participants. To "snap freeze" diluted and undiluted vaccine aliquots, follow CoolBox procedures described in the MOP.

Please follow the MOP for detailed instructions on vaccine storage, handling, preparation, labelling and transport to the clinic.

5.6 Study Product Administration 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 1 mL oral syringe. Participant will remain supine for approximately 60 seconds following study product administration.

5.7 Study Product Acquisition

The clinical lots of RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s (and previously RSV 276 under protocol V1.0) were generated by Charles River Laboratories using the seed virus provided by the National Institutes of Health (NIH).

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

Upon successful completion of protocol registration procedures, the clinical research site (CRS) pharmacists can order the vaccine, diluent/placebo, sterile oral 1-mL syringes (commercially available, individually packaged), sterile syringe caps and yellow overlays from the CRPMC by following the instructions in Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

Please refer to the MOP for details on shipment of the study products.

5.8 Study Product Accountability

The site pharmacist is responsible for maintaining an accurate inventory and accountability record of vaccine and diluent/placebo supplies for this study. The vaccines will be prepared as instructed with the site pharmacist serving as the unblinded dispenser. A copy of the randomization code will be retained by the site pharmacist. The site pharmacist will be responsible for maintaining the blind, and pharmacy records will be maintained in the pharmacy only.

5.9 Disposition of Used Study Product

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 pharmacy personnel as per the MOP. Partially used vials of vaccine and diluent/placebo may not be saved and reused at a later time.

5.10 Final Disposition of Study Products

All unused study products must be returned to the CRPMC after the study is completed or terminated, unless otherwise instructed by the Protocol Team. Procedures and relevant forms are provided in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*.

5.11 Concomitant Medications

The term concomitant medications is used in this study to refer to medications other than the study products listed in Section 5.1. Additional guidance regarding concomitant medications is provided below and in Table 5.

5.11.1 Prohibited Concomitant Medications

The use of the following is not permitted:

- Prophylactic antipyretics, decongestants, or antihistamines during the Acute Phase (28 days following study product administration) – note that use of these medications for treatment of symptoms is allowed
- An investigational drug or investigational vaccine other than the study product within 56 days after receiving study product

5.11.2 Precautionary Concomitant Medications

Due to their potentially confounding effect on immunogenicity results, the following treatments should be avoided after study product administration unless clinically indicated:

- 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
- Immunoglobulins and/or any blood products

The following should be avoided after study product administration unless indicated in an outbreak setting:

- Licensed inactivated vaccine or live-attenuated rotavirus vaccine within 14 days after receiving study product

- Licensed live virus vaccine, other than rotavirus vaccine, within 28 days after receiving study product

Please notify the PSRT via email at impaact.psrt2021@fstrf.org if any of the concomitant medications listed above are administered.

6 STUDY VISITS AND PROCEDURES

Study visits, except study product administration, may be conducted at one of the clinical sites or as home visits. Some Illness Visits may be performed at the clinical site, in-person or via telehealth (telephone or video), or in the community by a licensed medical provider. Study product administration must be conducted at one of the clinical sites.

An overview of the study visits, evaluation schedule, and specimen collection and volumes are provided in Appendix I and II. Presented in this section is additional information on visit-specific study procedures. Study timeframes are summarized in Figure 2 of Section 3.

If necessary, blood draws may occur on a different day than other visit procedures within the allowable window for that visit. All visits and procedures must be documented in accordance with NIAID Division of AIDS (DAIDS) requirements for source documentation; refer to Section 10 for more information on documentation requirements and completion of eCRFs. Refer to Section 7 for information on expedited adverse event (EAE) reporting, which is required at specified times during follow-up.

In addition to the protocol-specified procedures listed in this section, study staff may complete other tasks consistent with site SOPs, including but not limited to collecting, reviewing, and updating demographic and locator information; reviewing elements of informed consent; scheduling telephone contacts and visits; providing instructions for contacting study staff between visits; providing visit reminders; and following up on missed visits. All such tasks should be documented consistent with site SOPs. Clinical evaluations must be performed by a medical professional. Study staff should inform participants' parents/guardians of clinically meaningful physical exam findings and laboratory test results when available.

For sites experiencing disruptions or limitations of usual operations due to COVID-19, operational guidance is provided in Appendix VAppendix VAppendix V. All sites should follow applicable government, health authority, and institutional policies with respect to conduct of study visits and procedures, with utmost importance placed on the health and well-being of study participants and study staff.

6.1 Screening Visit

Screening may begin 14 days prior to the start of the enrollment period. As sites consider scheduling for the screening visit, it is important to also consider potential dates of randomization, given that study product administration cannot occur 42 days or more after the screening sample is collected. Sites should schedule randomization and study product administration to be as soon as feasible after screening, allowing for the turnaround time for the RSV serology testing.

The parent/guardian must complete the informed consent process and sign the informed consent form before any study-related procedures are performed. Parents/guardians will also be offered a signed copy of the informed consent form (see Section 12.3).

As in previous Phase I trials of other live-attenuated RSV vaccines (15, 25-27), screening laboratory tests other than the RSV antibody will not be performed on potential participants. 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 (33).

Screening Visit Procedures*		
Administrative and Regulatory		• Obtain written informed consent per IRB-approved procedures (a separate screening ICF may be used at some sites) • Confirm parent/guardian's informed consent comprehension using the template quiz provided in the MOP or other, site-specific method. • Assign participant identification number (PID) • Assess eligibility
Clinical		• Obtain available medical records, including immunization record. If necessary, obtain release of medical records from parent/guardian to review the potential participant's medical record as required per Health Insurance Portability and Accountability Act (HIPAA). • Obtain medical history, which should include demographics, prior diagnoses, current medications, signs and symptoms, and developmental status. • Perform focused clinical examination including temperature, heart rate, respiratory rate, EENT, lung, heart, and lymph nodes. Note: if the potential participant has had a physical examination in the past 2 weeks as part of routine care, some or all of the clinical examination data may be abstracted from the medical record (i.e., the procedures would not need to be repeated at the screening visit).
Laboratory	**Blood**	*Collect blood for*: • Screening and pre-study product administration blood draw for RSV serum antibody testing. Note: if the potential participant had been screened but not enrolled into any study of an RSV vaccine developed by the LID (NIAID, NIH), the results from the sample collected for that study may be used if the sample was collected within the 42-day enrollment window for IMPAACT 2021; it is not necessary to collect another sample.
Study Product		Not applicable
Schedule Enrollment Visit		Note that study product administration must be no more than 42 days after collection of the screening sample and within 5 days of randomization.

*No more than 42 days prior to study product administration.

6.2 Enrollment Visit/Study Product Administration (Day 0)

Study enrollment and study product administration (Day 0) must occur outside the time during which wt RSV circulates in the community at each study site, and after:

- the screening sample confirms that the child is RSV-seronegative, and
- he/she meets all other inclusion/exclusion criteria.

Day 0 will correspond to the day of study product administration. Whenever possible, study product administration (Day 0) is to occur on the same day as randomization. Although sites are allowed up to 5 days after randomization to complete the Enrollment Visit and administer study product, sites should not proceed to randomization until a) final eligibility determination has been confirmed and b) it has been confirmed that study product can be administered within this window. If these 2 conditions cannot be met, randomization should be postponed.

If participant is randomized and subsequently noted to have any of the following, study product administration must be deferred:

- fever (rectal temperature of ≥100.4°F (38°C)), or
- upper or lower respiratory symptoms or signs (including but not limited to rhinorrhea, cough, or pharyngitis), or
- nasal congestion significant enough to interfere with successful study product administration, or
- otitis media

All eligible children from the same household who are enrolled on the same date must receive study product on the same date. Therefore, if one child experiences any of the illnesses above, study product administration must be deferred for all children in the household who were enrolled on the same date.

Note: if the study product administration (Day 0) is deferred for one of the reasons above but then cannot be completed within 5 days of randomization, the participant(s) must be terminated from the study. These participants will not be replaced.

If the 42-day window from collection of the screening sample to study product administration is exceeded due to an illness or other participant-specific reason and a child has already been randomized, the child must be taken off study. If the 42-day window from collection of the screening sample to study product administration is exceeded due to a pause in accrual, and a child whose parents wish to continue study participation has already been randomized, the child will be re-screened to confirm eligibility and remain in the originally assigned study arm. Email the PSRT at impaact.psrt2021@fstrf.org if the participant's parent/guardian and the site still wish for the child to participate in the study.

Enrollment Visit/Day 0 Procedures ***Must be completed no more than 42 days from collection of the screening sample and within 5 days of randomization***		
Administrative and Regulatory		• Complete eligibility determination and confirmation* • Complete paper-based eligibility checklist*, enter checklist data into SES to enroll/randomize the child, print and file a copy of the confirmation file
Clinical		• Obtain interim history, including any changes in medications and immunizations • Clinical examination: o Perform complete physical examination including temperature, heart rate, respiratory rate, weight, length and assessment of HEENT [head, ears, eyes, nose, throat], lungs, heart, abdomen, musculoskeletal, age-appropriate neurological and skin exam. o Record temperature, pulse, and respirations Note: Confirm eligibility including clinical examination prior to administering study product.
Laboratory	**Blood**	*If insufficient volume collected at screening, collect blood*: • To ensure that back-up aliquot is available for comparing pre- and post- sera.
Study Product		• Prescribe and prepare/dispense study product • Administer study product and maintain participant in a supine position for 1 minute. • Observe for at least 30 minutes after study product administration to evaluate for immediate hypersensitivity reactions.
Prepare for follow-up		• Provide the following: temperature card/memory aid with explanation, temporal and rectal thermometers with instructions for use, illness criteria explanation, and study personnel contact information. Schedule non-visit contacts for Days 1-27; schedule in-person Day 28 visit. • Provide instructions on how to collect, store, and return a nasal swab sample and dispense collection supplies.

*Perform prior to enrollment/randomization

Following the Enrollment Visit, the parent/guardian will record the child's temperatures and signs of illness on the temperature card/memory aid and provide these to study personnel during an in-person visit or non-visit day phone, email, or text contact. New rectal thermometers will be given, and temporal artery thermometers will be provided to parents/guardians for use during the study. For temperature measurements, parents/guardians 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 (34). The parent/guardian will measure temporal artery temperatures following the manufacturer's directions. If any temporal artery temperature is ≥100.0°F, parents/guardians will be asked to measure a rectal temperature within 20 minutes (34). For study-specific management of solicited AEs and grading of temperatures, see Section 7.2 and Table 7, respectively.

6.3 Acute Phase Contacts and Visit

Refer to Figure 1 for a timeline of study procedures. The Acute Phase begins with study product administration (Day 0) and ends at midnight on the 28th day after study product administration. During the Acute Phase of the study, a study physician, physician assistant, nurse practitioner, or study nurse will be available by telephone 24 hours a day for consultation with parents/guardians regarding any illnesses that may occur during this period.

Study personnel will have daily contact with participants' parents/guardians for the first 28 days after study product administration. This 28-day period is consistent with the duration of shedding of live-attenuated respiratory virus vaccines in RSV-seronegative children (34-37).

Study staff will contact the parent/guardian daily on Days 1-27 and will record parent/guardian-provided temperatures and signs of illness; in-person visit will be conducted on Day 28 as outlined below. Participants with illness may have Illness Visits to assess the severity of the illness (see Section 6.8 for Illness Visits).

6.3.1 Acute Phase Contacts: Daily on Study Days 1-27 (±1 day)

The non-visit contacts during the Acute Phase will be conducted via phone, email, or text contact.

If the parent reports symptoms suspicious for an LRI, otitis media, fever or URI (per Appendix III), then an Illness Visit should be scheduled (see Section 6.8).

Days 1-27 Daily Contact Procedures ***(each contact from day of study product administration ±1 day)***	
Interim History	• Obtain and document from parent/guardian the participant's interim history since last contact, including any changes in medications and immunizations. • Document highest temperature (temporal or rectal)
Prepare for follow-up	• Address any concerns and schedule appointment if necessary.

6.3.2 Acute Phase Visit: Study Day 28 (-1/+4 days)

An in-person study visit during the Acute Phase is scheduled to be conducted on Day 28 (-1/+4 days). If an in-person visit occurs on a date other than the target date within the allowable window, then non-visit contact is conducted in place of the original target visit date.

Events that took place through midnight of Day 28 are considered to have occurred during the Acute Phase and will be reported on the non-visit contact conducted on Day 29. Note that it is not necessary to also have a "Day 29" contact (Section 6.4.1) if the Day 28 Visit is conducted on or after Day 29.

Day 28 (-1/+4 days) Visit Procedures *(visit from day of study product administration)*		
Clinical		• Obtain interim history, including any changes in medications and immunizations • Perform focused clinical examination including temperature, heart rate, respiratory rate, EENT, lung, heart, and lymph nodes. • Record temperature, pulse, and respirations.
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV
Prepare for follow-up		• Schedule non-visit day-contact and follow-up in-person visits. • Review serious AE criteria with participants and how to contact study personnel during Post-Acute Phase (Study Day 29 through Day 56).

If the child is diagnosed as having an LRI, otitis media, fever, or URI (per Appendix III) during the Acute Phase, evaluations required for the Illness Visit need to be performed. See Section 6.8 for expectations regarding timing and requirements for in-person versus telehealth Illness Visits. If illness criteria are met or suspected (Appendix III), an Adventitious Agent Assay will be performed on the Illness Visit nasal swab (see MOP and LPC).

6.4 Post-Acute Phase (Days 29 to 56)

The Post-Acute Phase begins at 12:01 am on the 29th day after study product administration and ends at midnight on the 56th day after study product administration. During the Post-Acute Phase, parents/guardians will be instructed to monitor for and contact the study site if their child has symptoms that are suggestive of a Serious Adverse Event (serious AE). If the parent/guardian reports a serious AE that may meet the study pause or stop criteria (Section 9.4.2), then an Illness Visit should be scheduled (see Section 6.8).

6.4.1 Day 29 Contact (+1 day)

There will be a non-visit contact on Day 29 to obtain interim history through midnight on the 28th day following study product administration. If the Day 28 Visit is conducted on or after Day 29, it is not necessary to have an additional non-visit contact with the family.

Day 29 Contact Procedures *(+1 day)*	
Interim History	• Obtain interim history through midnight of the 28th day following study product administration, including any changes in medications and immunizations • Document highest temperature (temporal or rectal)
Prepare for follow-up	• Address any concerns. • Review serious AE criteria with participants and how to contact study personnel during Post-Acute Phase (Day 29 through Day 56).

6.4.2 Day 56 Visit (+7 Days)

The Day 56 Visit should be conducted between 56 and 63 days following study product administration. Because the Post-Acute Phase ends as of midnight on the 56th day following study product administration, only events through that time should be evaluated as having occurred during the Post-Acute Phase. If the Day 56 Visit is conducted on the 56th day after study product administration, sites should arrange a non-visit contact the next day to confirm that there were no events between the time of the study visit and midnight of the 56th day after study product administration. The evaluations expected at this visit are outlined below.

Day 56 Visit *(from day of study product administration +7 days)*		
Clinical		• Obtain interim history since last contact, including any changes in medications and immunizations • Record temperature, pulse, and respirations.
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV
Prepare for follow-up		• Review plans for weekly contact during the RSV Season Surveillance Period (see Section 6.6).

6.5 Period after Day 56 Visit until October 31st or as specified in the MOP

During this period, contact with the participant is not required. No clinical data will be entered into eCRFs or reported under this protocol except for data as outlined in Table 5 in Section 7.2.

6.6 RSV Season Surveillance (November 1st through March 31st following study product administration or as specified in the MOP)

Based on previous data regarding the seasonality of RSV in the Baltimore, MD area (Appendix IV), surveillance for RSV-associated disease will be conducted during the RSV season that starts in the calendar year of each participant's study product administration. RSV season is between November 1st and March 31st for most sites or—for sites with local RSV seasons that differ—as specified on a site-by-site basis in the MOP. Note, RSV Season Surveillance may overlap with the Study Acute and Post-Acute Phase. In this case, evaluations required for each of the relevant phases of the study will be conducted.

During the RSV season that starts in the calendar year of study product administration, participants enrolled in this study will be monitored for symptomatic, medically attended, RSV-like illnesses listed below. Medically attended events during the RSV Season Surveillance Period are those for which care is sought in person or through formal telehealth (telephone or video) visits conducted by study staff or other medical provider; a telephone call to a nurse line is not considered medically attended. Information about these illnesses will be obtained during the RSV Season Surveillance Period by weekly communication between study personnel and the participant's parent/guardian. These contacts may be by telephone, email, or text contact or by in-person visit. For this period, determine if any of the following medically attended events occurred. Please note that the symptoms below do not need to meet the Appendix III criteria.

- Medically attended fever
- Medically attended upper respiratory illness
- Medically attended otitis media

- Medically attended lower respiratory illness

Study staff should conduct an Illness Visit within 3 days of a site's study staff notification of any of these events; note that the Illness Visit can be conducted by a licensed provider in the community only if medical records for the medically attended event are available to the site (see Section 6.8).

RSV Season Surveillance contacts (November 1st* through March 31st following study product administration)	
Clinical	• Obtain interim history, including any changes in medications and immunizations
Prepare for follow-up	• Continue with weekly contacts through March 31st • Schedule an Illness Visit if warranted (see Section 6.8) • Schedule Post-RSV Season Study Visit to take place between April 1st and 30th in the calendar year following study product administration

*This date applies to most sites but may differ for those with local RSV seasons that differ.

6.7 Post-RSV Season Study Visit (April 1st to 30th in the calendar year following study product administration)

There will be a single in-person visit between April 1st and 30th in the calendar year following study product administration. This window applies to all sites. For sites experiencing disruptions or limitations of usual operations due to COVID-19, operational guidance related to this visit (including broadened visit window specifications) is provided in Appendix V.

Post-RSV Season Study Visit (April 1st to 30th in the calendar year following study product administration)		
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV

6.8 Illness Visit

The timeframe after site notification in which the Illness Visit must occur depends on grading of the fever and respiratory symptoms per Section 7.3.3 and Appendix III and the phase of the study. Following an Illness Visit, sites should continue to follow participants until resolution of symptoms. Refer to the MOP for guidance regarding additional Illness Visits for the same illness.

Illness Visits may be conducted by study staff or, alternatively, by other medical providers if medical records from the visit can be made available to study staff and a parent/guardian can collect the nasal swab sample.

All Illness Visits require nasal swab collection, which can be performed by study staff or by parents/guardians, if they are comfortable collecting. If a participant experiences an illness that may meet protocol-specified pausing/stopping rules (see Section 9.4.2), and parent/guardian collected the nasal swab, study staff should rapidly retrieve swab. See MOP and LPC for additional guidance on collection, storage, and return of the nasal swab samples.

Illness Visits may occur during any of the following phases of the study:

- Acute Phase:
  - For Grade 1 otitis media or URI (except rhinorrhea without cough), an in-person Illness Visit must be conducted within 3 days after illness onset.
  - For rhinorrhea without cough or for fever <Grade 2, the Illness Visit may be performed via telehealth (telephone or video) rather than in-person, if a parent/guardian can collect nasal swab sample at home.
  - For Grade 2 or higher fever, otitis media, or URI (except rhinorrhea without cough), an in-person Illness Visit must be conducted within 2 days after illness onset.
  - For a possible LRI, with any grade, the initial in-person assessment will occur within 1 day of the reported onset of the LRI. A follow-up assessment (in person or via telehealth (telephone or video)), including nasal swab, is required 1 to 3 days after the initial assessment.
- Post-Acute Phase: For a serious AE that may meet the study pause or stop criteria (Section 9.4.2), an in-person Illness Visit must be conducted within 3 days of site notification. As noted above, the in-person visit can be performed by study staff or, alternatively, by another medical provider if medical records from the visit can be made available to study staff and a parent/guardian can collect the nasal swab sample.
- RSV Season Surveillance Period (between November 1st and March 31st for most sites or—for sites with local RSV seasons that differ—as specified on a site-by-site basis in the MOP): A nasal swab should be collected as outlined below (by parent/guardian or by study staff) within 3 days of site notification of a medically attended illness (as defined in Section 6.6) of the following types: fever, URI, LRI or otitis media.
  - If study staff can obtain the medical records for the medically attended visit, the medical records can be used in lieu of an Illness Visit, with parent/guardian collection of a nasal swab within 3 days of notification of the event. However, if this phase overlaps with the Acute or Post-Acute Phases, the time frames specified for the relevant Acute or Post-Acute phase should be used.
  - If obtaining medical records is not possible, study staff should conduct an in-person or telehealth (telephone or video) Illness Visit within 3 days of notification of the event. However, if this phase overlaps with the Acute or Post-Acute Phases, the time frames specified for the relevant Acute or Post-Acute phase should be used.

Illness Visit Procedures		
Clinical		• Obtain interim history, including any changes in medications and immunizations, and request release of medical records, if available, from non-study visit(s) related to the event prompting the Illness Visit. • If an in-person visit is conducted per Section 6.8, perform focused clinical examination including temperature, heart rate, respiratory rate, EENT, lung, heart, and lymph nodes. • If an in-person visit is conducted per Section 6.8, or if using medical records, record temperature, pulse, and respirations.
Laboratory	**Nasal Swab**	*Collect nasal swab for:* • Viral detection and quantification • If a participant experiences an illness that may meet protocol-specified pausing/stopping rules (see Section 9.4.2) and parent/guardian collected nasal swab, study staff should make plans to rapidly retrieve swab.
Prepare for follow-up		• Schedule follow-up as appropriate

6.9 Early Discontinuation Study Visit

In the event that a participant is unable to continue participation in the study, every effort should be made to schedule a final Early Discontinuation Visit. Participants who discontinue prior to study product administration are not required to complete an Early Discontinuation Visit.

Early Discontinuation		
Clinical		• Obtain interim history, including any changes in medications and immunizations
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV

6.10 Additional Considerations for Laboratory Procedures

Each study site and laboratory involved in this study will comply with the DAIDS policy on Requirements for DAIDS Funded and/or Sponsored Laboratories in Clinical Trials Policy, which is available at:
https://www.niaid.nih.gov/research/daids-clinical-research-laboratory-specimens-management

6.10.1 Specimen Collection

Specimens will be collected for this study as indicated in the Schedule of Evaluations and per detailed guidance provided in the Laboratory Processing Chart (LPC), which will be available on the IMPAACT web site: www.impaactnetwork.org. Further information on collection of blood and nasal swab specimens will also be provided in the MOP.

In accordance with US National Institutes of Health (NIH) recommendations, pediatric blood collection will not exceed 5 mL/kg in a single day or 9.5 mL/kg in any eight-week period. See Appendix I and II for specific blood volumes for IMPAACT 2021. Blood volumes of 5 mL are required for serology assays. This is the minimal required volume to determine the antibody response to the vaccines by the "gold standard" RSV plaque reduction neutralization assay, and by RSV glycoprotein ELISAs.

Virus Detection

Nasal swab specimens for detection of RSV and adventitious respiratory viruses by rRT-PCR (and culture when possible) will be collected from participants who meet illness criteria during the initial phase (Day 0 through Day 56) and RSV Season Surveillance Period (November 1st – March 31st for most sites or—for sites with local RSV seasons that differ—as specified on a site-by-site basis in the MOP). Laboratory testing will be performed by personnel that are not involved with clinical assessment to maintain the blinding of the study.

6.10.2 Specimen Preparation, Testing, Storage, and Shipping

All specimens collected for this study will be labeled, transported, processed, tested, stored and/or shipped in accordance with the DAIDS policy referenced in Section 6.10, site and local laboratory SOPs, and the LPC. The frequency of specimen collection and testing will be directed by the Schedule of Evaluations (Appendix I and II). The Laboratory Data Management System

(LDMS) will be used to document specimen collection, testing, storage, and shipping as specified in LPC.

Virologic and Immunologic Assays

Serum specimens will be collected up to 42 days prior to study product administration for the screening prior to study product administration, and at the Day 28 and Day 56 Visits for measurement of serum antibodies to RSV. In addition, post-RSV season serum specimens will be collected. These later samples will be used to determine whether a 2.5-fold or greater rise in RSV-specific serum antibody titer has occurred during the RSV season, which would signify infection with wt RSV. This will allow comparison of the rate and severity of significant RSV illness following infection with wt virus, as well as comparison of the antibody responses in vaccine and placebo recipients. Screening serum samples will be rapidly shipped to JHU for measurement of RSV-neutralizing antibody. All other sera will be shipped to JHU as specified in the MOP.

Nasal swab specimens collected during Illness Visits will be used for quantitation of the amount of vaccine virus shed by quantitative reverse-transcription PCR. These assays, as well as the assessment of nasal swabs for the presence of adventitious viral agents, will be performed at the Johns Hopkins University Center for Immunization Research (JHU CIR). Cytokine/chemokine assays may also be performed on nasal washes from participants (enrolled under protocol V1.0) infected with vaccine virus if sufficient material is available. Selected specimens may be sent to LID, NIAID for confirmatory testing.

If a participant experiences an illness meeting protocol-specified pausing/stopping rules, nasal swab specimens from the Illness Visit will be rapidly shipped to JHU for testing for adventitious agents by PCR; in this scenario, if parent/guardian collected a nasal swab, study staff should rapidly retrieve the specimen.

Plan for Future 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 JHU CIR's approved biospecimen repository for vaccine research or at the LID, NIAID. These samples may be used for future screening for respiratory virus vaccine studies and to learn more about RSV infection and other diseases. These samples will not be sold or used to make commercial products. Samples will be stored only with the parent/guardian's permission.

All samples stored in the repository will be labeled with the participant identification (PID) numbers 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' PID numbers. A master log linking the study participants' PID numbers is maintained at the individual enrolling site and will not be shared with the Protocol Team or the laboratory at the JHU CIR. Study participants, or their parents/guardians, may withdraw consent for future testing of their specimens at any time.

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. In that case, IMPAACT and LID approval must be sought prior to any sharing of samples and/or data/clinical information.

6.10.3 Biohazard Containment

Sites will use local standards to guide the use of personal protective equipment when collecting nasal swabs. 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 in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health. Additional specimen collection and processing precautions are included in the MOP. All infectious specimens will be transported using packaging mandated in Title 42 of the Code of Federal Regulations, Part 72 (42 CFR 72) and in accordance with individual carrier guidelines (e.g., Federal Express, Airborne Express).

7 SAFETY ASSESSMENT, MONITORING, AND REPORTING

Participant safety will be carefully assessed, monitored, and reported at multiple levels throughout this study. Sections 7.1-7.3 describe safety-related roles, responsibilities, and procedures for site investigators. The safety monitoring roles of the Protocol Safety Review Team (PSRT) and the Data and Safety and Monitoring Board (DSMB) are briefly referenced in Section 7.1 and described in detail in Section 9.4. For this study, the DSMB will include two to three subject matter experts (i.e., *ad hoc* DSMB Expert Members) with expertise in pediatric respiratory virus vaccine development.

7.1 Safety-Related Roles and Responsibilities

7.1.1 Site Investigators

Site investigators are responsible for continuous monitoring of all study participants and for alerting the Protocol Team if unexpected concerns arise. Site investigators will enter safety-related data into eCRFs as indicated in Section 7.2 and complete expedited adverse event (EAE) reporting as indicated in Section 7.3.

Site investigators are also responsible for prompt reporting of any unanticipated problems involving risks to participants or others to all applicable IRBs and other applicable review bodies, per the procedures of each applicable review body.

7.1.2 Protocol Safety Review Team (PSRT)

A Protocol Safety Review Team (PSRT) will routinely review clinical and laboratory safety data reports prepared by the SDMC. Designees for PSRT members (e.g., a colleague who serves in a similar role as the PSRT member) will be allowed in the event of their non-availability for a review. To meet minimum quorum for a safety data review, the PSRT must include (but is not limited to):

- A Protocol Chair or Vice Chair
- A Data Manager
- DAIDS or NICHD Medical Officer
- A Protocol Statistician

The content, format, and frequency of safety monitoring will be agreed upon in advance by the PSRT. The PSRT will be blinded to group assignment.

The PSRT will convene via teleconference or other means routinely throughout the study to review data relevant to safety monitoring and discuss any safety concerns – approximately twice per month during the Acute Phase and once a month thereafter – as well as on an *ad hoc* (as needed) basis outside of regularly scheduled calls. The PSRT will also provide rapid consultation to site clinicians regarding toxicity management as needed.

On behalf of the full Protocol Team, the PSRT will monitor participant safety through routine review of study data reports as described in Section 9.4.2.

7.1.3 Data and Safety Monitoring Board (DSMB)

An independent DSMB will monitor participant safety through routine and as needed reviews of study data. Refer to Section 9.4.3 for more information on the composition and role of the DSMB in monitoring of this study. Two to three *ad hoc* subject matter experts (i.e., *ad hoc* DSMB Expert Members) with expertise in pediatric respiratory virus vaccine development who are not otherwise affiliated with the Protocol Team will be added to the DSMB for review of this protocol. These *ad hoc* DSMB Expert Members will also perform *ad hoc* reviews of LRIs occurring during the Acute Phase and other safety concerns identified by the PSRT in real time as outlined in Section 9.4.

7.2 Safety-Related Data Collection

Note: This section describes eCRF data collection for pre-existing conditions, adverse events, and laboratory test results. As part of this description, reference is made to severity grading and criteria for EAE reporting; refer to Sections 7.3.3 and 7.3.2, respectively, for detailed information on these topics.

The definition of the term adverse event provided in Version 2.0 of the Manual for Expedited Reporting of Adverse Events to DAIDS (DAIDS EAE Manual) will be used in this study. This definition will be applied to all participants, beginning at the time of randomization, regardless of subsequent administration of or exposure to study product. Solicited AEs are predefined AEs that can occur after randomization, may be expected to occur if the study product is insufficiently attenuated, and have protocol-specific criteria for reporting. Any untoward medical conditions (including abnormal laboratory test results, signs, symptoms, or diagnoses) identified prior to enrollment will be considered pre-existing conditions. Refer to Section 4.4 for more information on defining the effective point of enrollment in the study.

Pre-existing conditions, adverse events, and laboratory test results will be entered into eCRFs as specified below.

Pre-Existing Conditions

All pre-existing conditions identified as present during the screening period (prior to randomization) will be entered into medical history log eCRFs.

Adverse Events

Adverse events — inclusive of abnormal laboratory test results and clinical signs, symptoms, and diagnoses — will be entered into adverse events log eCRFs as outlined in Table 5. In addition, for any event assessed as serious as defined in Version 2.0 of the DAIDS EAE Manual due to the event resulting in hospitalization or prolongation of hospitalization, data regarding the hospitalization will be entered into eCRFs.

For IMPAACT 2021, solicited AEs, whether identified by a parent/guardian or clinician, are only entered into eCRFs 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 entered into the eCRFs. From randomization through Day 28, solicited AEs meeting the criteria for reporting will be entered into eCRFs, assigned a severity grade (see Section 7.3.3, Table 6, and Table 7), and assessed for relationship to study product (see Section 8.1). For this study, the solicited AEs are defined in Appendix III and include the following:

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

Solicited Adverse Events Elicited by History Unconfirmed by Clinical Assessment

With the exception of fever, solicited AEs reported by parents/guardians are NOT entered into eCRFs 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 entered into eCRFs 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:

- Entered into the eCRFs as AEs if the parent/guardian description meets the "definition" column criteria in Appendix III.
- Recorded only on the source document, and NOT entered into the eCRF, if the parent/guardian description fails to meet the "definition" column criteria in Appendix III. For example, both rhinorrhea and cough must each occur on 2 consecutive days to meet the definition required for reporting per Appendix III.

Laboratory Test Results

In addition to the recording specified above, laboratory test results will be entered into the relevant laboratory eCRFs, regardless of whether the test was protocol-specified or ordered by the site investigator for clinical purposes.

This study has several periods of AE surveillance that have different AE eCRF entry requirements. In addition, there may be a period when no AEs are entered into eCRFs if Day 56 is before the start of the RSV Season Surveillance Period (November 1st for most sites or—for sites with local RSV seasons that differ—as specified on a site-by-site basis in the MOP). The adverse events (solicited and unsolicited; and serious AEs) to be entered into eCRFs and the study timeframe and the calendar dates during which they are to be reported are defined in Table 5.

Adverse events identified in this study will be entered into adverse event log eCRFs as described in Table 5. Expectations regarding the recording of concomitant medications are also detailed in this table.

Table 5: AE eCRF Entry Requirements

Study Phase at the time of event onset	Calendar Date	AEs to enter into eCRFs	Concomitant Medications to enter into eCRFs
From randomization through midnight of 28th day following study product administration (Acute Phase)	ANY	• All serious AEs • All solicited AEs that meet Appendix III criteria • All unsolicited AEs (Grades 1 to 4), with the exception of the following conditions if not treated with prescription medication or OTC medications with antipyretic properties: diaper rashes, teething pain, and spitting up. See MOP for additional guidance. Note: serious AEs and LRIs must be reported via DAIDS Adverse Experience Reporting System (DAERS; see Section 7.3.4).	Enter these medications into eCRFs regardless of whether the related event is entered into eCRFs: • All cough and cold remedies including decongestants, cough suppressants, expectorants • All nasal sprays (except saline spray) • All antihistamines • All antipyretics • All prescription medications For serious AEs and LRIs: • All concomitant medications administered to treat the entered event
From 12:01 am on 29th day after study product administration to midnight of the 56th day following study product administration (Post-Acute Phase)	ANY	• All serious AEs Note: serious AEs must be reported via DAERS (see Section 7.3.4).	• All concomitant medications administered to treat the entered event
After Day 56 and until RSV Season Surveillance Period	Up to October 31st* in year of study product administration	• SUSARs	• All concomitant medications administered to treat the entered event
RSV Season Surveillance Period	November 1st* to March 31st following study product administration	• Fever, LRI, URI, and/or otitis media that are medically attended • All serious AEs Note: these events do not need to meet the Appendix III criteria. Serious AEs and Grade ≥3 LRIs must be reported via DAERS (see Section 7.3.4).	For serious AEs and LRIs (all grades): • All concomitant medications administered to treat the entered event Medications administered to treat entered medically attended illness should be documented in source notes.
Post-RSV Season	April 1st-April 30th in the year after the study product administration	• Grade ≥3 AEs or serious AEs that is deemed related to Post-RSV Season Study Visit procedures. • SUSARs	• All concomitant medications administered to treat the entered event

Study Phase at the time of event onset	Calendar Date	AEs to enter into eCRFs	Concomitant Medications to enter into eCRFs
Throughout study	ANY	• Updated data on unresolved AEs or serious AEs with onset date during the timeframe from study product administration (Day 0) to midnight on the 28th day after Day 0 • Updated data on unresolved serious AEs with onset date prior to midnight on the 56th day following Day 0 • Updated data on unresolved serious AEs with onset date during RSV Surveillance Period or related to the Post-RSV Season Study Visit Note: unresolved events should be followed to resolution or until no further change is expected.	• All concomitant medications administered to treat the entered event

*These dates apply to most sites but may differ for those with local RSV seasons that differ (see MOP).

7.3 Expedited Adverse Event (EAE) Reporting

7.3.1 EAE Reporting to DAIDS

Requirements, definitions, and methods for expedited reporting of adverse events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. This form is available on the DAIDS RSC website at: https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting.

For questions about DAERS, please contact NIAID CRMS at CRMSsupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com.

7.3.2 EAE Reporting Requirements for this Study

- Both the Serious Adverse Event and SUSAR Reporting Categories, as defined in Version 2.0 of the DAIDS EAE Manual, will be used by this study. Reporting periods for both categories are defined in Table 8.
- The study agents for which expedited reporting is required are recombinant live-attenuated respiratory syncytial virus vaccines RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 (protocol V1.0 only) and placebo.

- In addition to the Serious Adverse Event Reporting Category identified above, other AEs that must be reported in an expedited manner are LRIs occurring during the periods specified in Table 8.

7.3.3 Grading Severity of Events (applies to EAEs and all other adverse events)

All solicited AEs and fever will be graded following the protocol-defined grading system outlined in Table 6 and Table 7. Other AEs (i.e., excluding solicited AEs and fever) will be assessed for severity by the site investigator using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table): Corrected Version 2.1, dated July 2017**.** In the event that this table is updated following protocol implementation, events will continue to be evaluated per this version of the DAIDS AE Grading Table. The DAIDS AE Grading Table is available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

Solicited AE Grading

Table 6: Grading Table for Solicited AEs

Severity	Defined
Grade (1) Mild	No medical intervention required; may include use of over-the-counter medications managed by the caregiver for treatment of symptoms; does not interfere with usual activities (e.g., eating, sleeping, playing)
Grade (2) Moderate	Moderate symptoms, i.e., symptoms interfering to some degree with usual activities. In most cases, symptoms severe enough to necessitate a medical care visit would likely meet this criterion; however, if medical care is sought and symptoms are assessed as only mild, the event may remain Grade 1. If prescription medication is used or recommended for symptoms, the event automatically moves to at least Grade 2.
Grade (3) Severe	Prolonged medical intervention and/or hospitalization required
Grade (4) Life threatening	Illness requiring hospitalization with intensive care
Grade (5) Death	Event resulting in fatal outcome to the participant

Fever Grading: Temperature Measurement

Table 7: Fever Grading*

Severity	Defined
Grade (0)**	≥100.0ºF but <100.4ºF (≥37.8ºC but <38ºC)
Grade (1)	≥100.4ºF but <101.5ºF (≥38ºC but <38.6ºC)
Grade (2)	≥101.5ºF but <102.5ºF (≥38.6ºC but <39.2ºC)
Grade (3)	≥102.5ºF but <104.9ºF (≥39.2ºC but <40.5ºC)
Grade (4)	≥104.9ºF (≥40.5ºC)

*Applies to any modality of temperature measurement
**Grade 0 is to be used only when temporal temperature is ≥100.0 but <100.4 and no rectal temperature is measured.

The expedited AE reporting period for this study is defined in Section 7.3.4.

7.3.4 EAE Reporting Period

Table 8 details the events that must be reported in an expedited fashion via DAERS during specific periods of the study.

Table 8: EAE Reporting

Study Phase at the time of event onset	Calendar Date	Events to Report via DAERS
From randomization through midnight of 28th day following study product administration (Acute Phase)	ANY	• Serious AEs • LRIs
From 12:01 am on 29th day after study product administration to midnight of the 56th day following study product administration (Post-Acute Phase)	ANY	• Serious AEs
After Day 56 and until RSV Season Surveillance Period	Up to October 31st* in year of study product administration	• Follow-up related to Serious AEs/LRIs already reported • SUSARs
RSV Season Surveillance Period	November 1st* to March 31st following study product administration	• Serious AEs • Grade ≥3 LRIs
Post-RSV Season	April 1st to April 30th in the year after the study product administration	• Serious AEs related to Post-RSV Season Study Visit procedures • SUSARs

*These dates apply to most sites but may differ for those with local RSV seasons that differ (see MOP).

After the protocol-defined EAE reporting period, unless otherwise noted, only serious and unexpected suspected adverse reactions (SUSARs) as defined in Version 2.0 of the DAIDS EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

8 PARTICIPANT MANAGEMENT

8.1 Management of Adverse Events

All adverse events identified in this study will be source documented in participant research records, consistent with the requirements referenced in Section 10. Among other details, source documentation will include the severity of each event (graded as described in Section 7.3.3) and its relationship to study product, assessed by the site clinician (see below).

Relationship categories for Adverse Events are as follows:

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.

Further standardized guidance on determining whether there is a reasonable possibility of a relationship is available in the DAIDS EAE Manual, referenced in Section 7.3.1.

Study staff who administer study product and assess AEs will be blinded. If the need arises to unblind a specific participant's assignment to guide management of a serious illness or medical emergency or if knowing the participant's treatment assignment would otherwise affect decisions regarding the participant's immediate medical management as determined by the site Investigator of Record (IoR) or treating clinician, the procedures for emergency unblinding specified in the IMPAACT Network Manual of Procedures (MOP) must be followed. In the event that unblinding is required, only that specific participant's assignment will be unblinded.

9 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The goal of this Phase I/II blinded, randomized, placebo-controlled study is to assess the safety and immunogenicity of three RSV vaccine candidates (RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s and RSV 276) in RSV-seronegative children, and to determine whether any of the vaccines are good candidates to move forward into larger safety and efficacy studies. Note that adjustments to the candidate vaccines included in the study may be made as outlined in Section 1.4.

Since clinical testing of RSV vaccines is only possible outside the time during which wt RSV circulates in the community, and since vaccine studies have long preparation and follow-up periods, it is essential that vaccine development planning be as efficient and timely as possible. To move forward, the vaccines should:

1. be safe, and
2. result in at least 70% of vaccine recipients having a ≥4-fold rise in serum RSV-neutralizing antibody titers from pre-study product administration to the Day 56 Visit after study product administration.

At this stage in the development of a vaccine for RSV, it is difficult to predict the behavior of each candidate vaccine with respect to replication, tolerability, and vaccine-induced immunity, and difficult to set precise benchmarks for each of these individual characteristics to determine the 'best' candidate vaccine. The goal of this study is to obtain more precise estimates of vaccine-induced immune responses (≥4-fold rise in serum RSV-neutralizing antibody titers from pre-study product administration to the Day 56 Visit) and gain additional knowledge about safety and replication, to help inform whether any of the candidate vaccines should go forward into larger studies. The proposed sample size is not sufficient to definitively assess safety or superiority in immunogenicity of one vaccine candidate over the others unless one has a very poor safety profile or immunogenicity.

As with protocol V2.0, under protocol V3.0, participants will be randomized equally (1:1:1) to receive one of the two candidate vaccines or placebo (40 per arm). Eligible children living in the same household (including twins, siblings, or other non-related children living in the same household) will be allowed to enroll and will be randomized per Section 9.3.1. Participants will be enrolled outside the time during which wt RSV circulates in the community and followed through the RSV season that starts in the calendar year in which they receive study product with a final study visit in April of the following calendar year.

The study will be reviewed by the NIAID Intramural Data and Safety Monitoring Board (DSMB), which will include *ad hoc* DSMB Expert members who are experts in pediatric respiratory vaccine development. The DSMB will review safety twice per year, Day 56 immune response rates at the conclusion of Day 56 follow-up during each calendar year, and if there are safety concerns between scheduled reviews. If targeted enrollment is achieved in one season, data collection for the primary objectives and two secondary objectives will be complete once all participants have completed their Day 56 Visit. Once immunologic samples have been assayed, safety and immunogenicity data will be analyzed and an unblinded primary analysis report distributed to the DSMB and a subset of the Protocol Team not involved in ongoing safety monitoring. This report will contain key information needed to plan vaccine studies for the subsequent year, and release of this information to selected members of the Protocol Team will not compromise safety monitoring through the remainder of the surveillance season. The study was not fully accrued in the first enrollment season (2019), and the DSMB recommended that the study continue when feasible in the context of the COVID-19 pandemic. Due to COVID-19, the study did not reopen during the second enrollment season (2020). During the winter of 2021, newly available unblinded data from IMPAACT 2018/CIR 321 and CIR 322 were reviewed, and it was determined that the RSV 276 vaccine arm of IMPAACT 2021 would be closed to further enrollment under protocol V2.0, and this arm remains closed under protocol V3.0.

No sites were able to resume enrollment during the third enrollment season (2021); thus, enrollment is anticipated to resume for a fourth enrollment season (e.g., 2022). If a fifth enrollment season (e.g., 2023) is considered, the DSMB will review results from the fourth enrollment season (e.g., 2022) to confirm if all candidate vaccines should remain in the study. If enrollment is closed to an arm, the remaining arms would continue to enroll to the 40-per-arm goal.

9.2 Objectives and Outcome Measures

The primary and secondary outcome measures listed below will be addressed in the study's primary Statistical Analysis Plan, which will define the content of the Primary Analysis Report. This report will form the basis for the primary study manuscript and results reporting to ClinicalTrials.gov.

9.2.1 Primary Outcome Measures

Objective 1: Safety: To estimate and compare (each vaccine group to placebo) the frequency and severity of adverse events (AEs) following study product administration (Day 0) through Day 56

Outcome measures:

1. Grade 1 or higher solicited AEs as defined in Appendix III from Day 0 through Day 28
2. Grade 2 or higher LRIs as defined in Appendix III from Day 0 through Day 28
3. Serious AEs from Day 0 through Day 56

Objective 2: Immunogenicity: To estimate and compare (between the vaccine groups and to the benchmark of 70%) the percentage of vaccinees having a ≥4-fold rise in serum RSV-neutralizing antibodies at the Day 56 Visit
Outcome measure:

4. ≥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.

9.2.2 Secondary Outcome Measures

Objective 1: Immunogenicity: To estimate and compare (between the vaccine groups) the percentage of vaccinees with a ≥4-fold rise in serum IgG antibody to RSV F protein (RSV F IgG) at the Day 56 Visit

Outcome measure:

1. ≥4-fold rise in serum RSV F IgG from pre-study product administration (screening) to the Day 56 Visit

Objective 2: Immunogenicity: To estimate and compare (between the vaccine groups) the titers of serum RSV F IgG and serum RSV-neutralizing antibodies at the Day 56 Visit

Outcome measures:

2. Titer of serum RSV F IgG at the Day 56 Visit
3. Titer of serum RSV-neutralizing antibodies at the Day 56 Visit

Objective 3: Safety: To describe and compare the frequency and severity of RSV-associated medically attended acute respiratory illness (RSV-MAARI) and RSV-associated medically attended acute lower respiratory illness (RSV-MAALRI) in the placebo and vaccine arms during RSV season

Outcome measures:

4. RSV-MAARI and maximum grade (if more than one illness within a participant)
5. RSV-MAALRI and maximum grade (if more than one illness within a participant)

9.2.3 Exploratory Outcome Measures

Objective 1: 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
2. culture (when possible)

For serious AEs between Days 0 and 56 (more than one per participant possible). Detection and magnitude of vaccine virus shedding detected in Illness Visit specimens as determined by:

3. RT-PCR
4. culture (when possible)

Objective 2: 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 each vaccine group

Outcome measures:

5. Titer of serum RSV F IgG at the Day 28 and 56 Visits
6. Titer of serum RSV-neutralizing antibodies at the Day 28 and 56 Visits

Objective 3: Immunogenicity – magnitude of anamnestic response: To estimate and compare (each vaccine group to placebo and between the vaccine groups) 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):

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

Objective 4: Immunogenicity – durability of vaccine-induced RSV antibodies: to estimate and compare (between the vaccine groups), 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):

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

9.3 Randomization and Sample Size

9.3.1 Randomization

As with protocol V2.0, under protocol V3.0, RSV-seronegative children will be randomized equally (1:1:1) to receive one of two candidate vaccines (RSV ΔNS2/Δ1313/I1314L (10^6 PFU) or RSV 6120/ΔNS2/1030s (10^5 PFU)) or placebo. Under protocol V1.0, prior to the closure of the RSV 276 (10^5 PFU) arm, participants were previously randomized equally to one of three candidate vaccines (RSV ΔNS2/Δ1313/I1314L (10^6 PFU), RSV 6120/ΔNS2/1030s (10^5 PFU), or RSV 276 (10^5 PFU)) or placebo.

Randomization will use permuted blocks. Eligible children from the same household are allowed to enroll: they must either be enrolled on the same date and to the same study arm to reduce potential cross-contamination that could occur if children living together were to receive different study products, or additional children in the household can be screened, enrolled, and randomized independently after other children in the household complete the Day 56 Visit.

9.3.2 Sample Size

A target sample size of approximately 40 per arm was chosen to gather more data on safety and to yield sufficiently precise confidence intervals around Day 56 immunogenicity to help inform which candidate vaccines should go forward into larger studies. The total expected sample size is approximately 130, including approximately 40 each in the placebo and two candidate vaccine arms open under protocol V2.0 and V3.0 (RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s), as well as all participants previously randomized to receive RSV 276 (10^5 PFU) under protocol V1.0 prior to closure of this arm.

To fully assess safety, much larger sample sizes will be required, and additional studies will need to be conducted on the candidate vaccines identified as promising in this trial. Declaring superiority in immunogenicity of one vaccine candidate over another will only be possible if one candidate has very poor immunogenicity.

9.3.2.1 Immunogenicity

If multiple children living in the same household are enrolled on the same date, one will be randomly chosen for the primary immunogenicity analysis. Assuming enrollment of 40 participants per arm and an exclusion rate of 5% (losses-to-follow-up, non-evaluability, or non-selected siblings), approximately 38 participants per arm will be evaluable for the primary initial response to vaccine immunogenicity outcome measure. The goal of the study is to estimate the response rates in each candidate vaccine arm with sufficient precision to make a decision about whether that vaccine should move forward into larger studies. Table 9 shows exact 95% confidence intervals (CIs) around various observed response rates with a sample size of 38. For example, if the response rate is estimated to be 79%, the 95% CI would be (63%, 91%), and the study can be reasonably sure that the actual response rate is greater than 63%.

Table 9: Exact 95% confidence intervals for various observed vaccine response rates with a sample size of 38

Observed ≥4-fold rise in RSV antibody N (%)	95% CI
20 (53%)	(36%, 69%)
24 (63%)	(46%, 78%)
28 (74%)	(57%, 87%)
30 (79%)	(63%, 91%)
32 (84%)	(69%, 94%)
34 (90%)	(75%, 97%)

For a product to be considered a good candidate, a response rate of at least 70% is desirable. With 38 participants, the study will have greater than 80% power to reject the null hypothesis if the true response rate is at least 90% (two-sided hypothesis test with Type I error of 5%).

Response rates among the vaccine arms will be compared with two-sided tests of the null hypothesis that the response rates are the same. With 38 participants per arm, there would be at least 80% power to detect differences of more than 33% if the true response rate in the inferior arm is less than 50% (Type I error of 5%).

9.3.2.2 Safety

Safety summaries will include all enrolled participants, including all children from within the same household who enrolled on the same date and each counted separately. Safety outcome measures include types of events that will be frequent but less serious (e.g., Grade 1 solicited AEs) and some that will be rare but serious (Grade 3 or higher AEs, serious AEs and LRIs), which if they occurred in the vaccine arms, would be of concern.

Safety of each vaccine must be interpreted relative to the rates of comparable events over a comparable time period in the placebo arm. Rates will be compared using two-sided 95% CIs around the differences in rates of AEs between the vaccine arms (P_V) and the placebo arm (P_C). Table 10 shows exact CIs for differences in various observed rates of AEs between the vaccine and placebo groups with 40 participants per arm. If the observed AE rate in the placebo arm is 50%, differences in rates of more than 20% between arms would result in CIs not covering the value 0.

Table 10: Differences (exact 95% CIs) in observed rates of AEs between vaccine and placebo arms

Observed N (%) of AEs		Observed difference ($p_v - p_c$)	95% CI for ($p_v - p_c$)
Placebo (p_c)	Vaccine (p_v)		N=40
20 (50%)	24 (60%)	10%	(-12%, 32%)
	28 (70%)	20%	(-1%, 41%)
	32 (80%)	30%	(10%, 50%)
14 (35%)	18 (45%)	10%	(-11%, 31%)
	22 (55%)	20%	(-1%, 41%)
	26 (65%)	30%	(9%, 51%)
10 (25%)	14 (35%)	10%	(-10%, 30%)
	18 (45%)	20%	(0%, 40%)
	22 (55%)	30%	(10%, 50%)
4 (10%)	8 (20%)	10%	(-5%, 25%)
	12 (30%)	20%	(3%, 37%)
	16 (40%)	30%	(12%, 48%)
2 (5%)	6 (15%)	10%	(-3%, 23%)
	10 (25%)	20%	(5%, 35%)

Potentially serious LRIs will be monitored closely and are likely to occur. Previous studies indicate that about 2.8% of participants will develop an LRI. With this underlying rate in 160 children, there would be an 83% chance of seeing >2 LRIs. With 40 participants, if the underlying LRI probability is 0.01, the chance of seeing >2 LRIs is <1%. If the underlying probability of an LRI is 5 times higher (0.05), the chance of seeing >2 LRIs is 32%. If the underlying probability of an LRI in the placebo arm is 0.01, and in each vaccine arm it is 0.05, the average probability across arms would be 0.04, and with 160 participants, the chance of seeing >2 LRIs would be about 96%.

Table 11 shows two-sided exact 95% CIs for a range of observed numbers of AEs. For example, if no AE of a given type is observed among 40 participants, the team is 95% confident that the underlying probability of that AE in that study arm is between 0% and 9%.

Table 11: Exact 95% confidence intervals for range of numbers of observed events among 40 participants

Observed N (%) of AEs	95% CI
0 (0%)	(0%, 9%)
2 (5%)	(1%, 17%)
3 (8%)	(2%, 20%)
5 (13%)	(4%, 27%)
7 (18%)	(7%, 33%)
10 (25%)	(13%, 41%)
15 (38%)	(23%, 54%)

9.4 Monitoring

Full details of monitoring reports will be described in a separate Study Progress Data and Safety Monitoring Plan (SPDSMP). The study will be monitored by the groups described in Table 12.

Table 12: Groups responsible for monitoring

Group	Membership	Responsibility	Approximate frequency	Blinding status
IMPAACT Leadership	IMPAACT Management Oversight Group (MOG)	Accrual according to standard operating procedures	Monthly	Blinded
Protocol Team		Accrual and retention	Monthly	Blinded
Protocol Safety Review Team (PSRT)*	Protocol Chair(s), Vice Chair(s), Medical Officer(s), LID investigator(s), Protocol Statistician(s), Protocol and Lab Data Manager(s), CRM(s)	Safety	Twice monthly while participants are within 28 days post-study product administration and at least monthly thereafter	Blinded
Data and Safety Monitoring Board (DSMB)	Standing NIAID Intramural DSMB with 2-3 *ad hoc* DSMB Expert Members who are experts in pediatric respiratory virus vaccine development	Safety; data monitoring, recommendations on continuation, study modifications, suspension, or termination 2-3 *ad hoc* DSMB Expert Members who are experts in pediatric respiratory virus vaccine development will adjudicate LRIs during Days 0 – 28 and AEs/serious AEs at request of PSRT in real time.	Twice a year (September/February); Expert Member *ad hoc* review of LRIs during Days 0 – 28 and AEs/serious AEs at request of PSRT Full DSMB *ad hoc* review if requested by *ad hoc* DSMB Expert Members	Masked for twice yearly reviews with option to unblind if necessary Unblinded for *ad hoc* reviews
DAIDS Medical Officer	DAIDS	Pharmacovigilance, medical review of EAEs, safety reports to the FDA as needed	As EAEs are reported by sites through DAERS	Blinded

*See Section 7.1.2 for a listing of PSRT members required to meet minimum quorum for a safety data review.

9.4.1 Monitoring of Accrual and Retention by the Protocol Team

The Protocol Team is responsible for continuous monitoring of study progress, including timely achievement of key milestones, and quality of study conduct. The team will closely monitor participant accrual and retention based on reports generated at least monthly by the Statistical and Data Management Center (SDMC). The team has developed an accrual plan that includes site-specific and total enrollment projections over the course of the accrual period, and actual accrual will be monitored relative to these projections. The team will monitor the timing of site-specific study activation, which will determine when each site will begin accruing participants, and actual accrual following activation. For any site that is delayed in completing the study activation process, or that falls short of its accrual projections, the team will communicate with the site to identify the barriers the site has encountered and the operational strategies and action plans to address these. If relatively few of the eligible sites have been activated after the study has been open to accrual, the team will periodically re-assess the feasibility of the study and the reasons why sites have not been activated and may make adjustments as needed.

The Protocol Team will similarly review participant retention and other key indicators of the quality of study conduct (e.g., data quality, and data and specimen completeness) based on reports generated by the SDMC and will follow up with study sites as needed to ensure high quality study conduct throughout the period of study implementation.

9.4.2 Monitoring of Safety by the Protocol Safety Review Team (PSRT) and *ad hoc* DSMB Expert Members

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 serious adverse events 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 SDMC will generate reports of adverse events pooled across arms (with the vaccine and placebo arms presented together). Safety reports will be reviewed by the Protocol Safety Review Team (PSRT), which includes the Protocol Chair(s) and/or Vice Chair(s), Medical Officer(s), LID investigator(s), Protocol Statistician(s), Protocol and Lab Data Manager(s), and CRM(s). The PSRT will monitor participant safety via conference call or other meeting approximately twice a month during the Acute Phase (i.e., until the last participant enrolled in that season has been followed for 28 days post-study product administration) and monthly until all participants have reached Day 56. On these calls, the DAIDS Medical Officer will also review any EAEs reported to the DAIDS Safety Office not yet entered in the study database.

Ad hoc DSMB Expert Member reviews will be requested by the PSRT if:

- any LRIs, intensive care unit (ICU) admissions, or deaths occur within 28 days of study product administration or
- >1 serious AE occurs within 56 days of study product administration or
- >1 Grade 4 fever occurs within 14 days of study product administration or
- >1 Grade ≥3 solicited AE (excluding rhinorrhea and fever) occurs within 28 days of study product administration.

In the event of a participant's ICU admission or death within 28 days of study product administration that is considered related to study product administration, accrual and study product administration will stop pending review. In all other 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 (results will ultimately be included in the study database). Independent SDMC personnel other than the Protocol Statistician will send the *ad hoc* DSMB Expert Members the participant's treatment assignment. 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.

9.4.3 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 who do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. Standing DSMB membership is outlined in the NIAID Policy on the Intramural Research Program Data and Safety Monitoring Board, and for this study, the DSMB will also include *ad hoc* DSMB Expert Members who are experts in pediatric respiratory vaccine development (Section 9.4.2).

The DSMB will review the protocol and Study Progress, Data, and Safety Monitoring Plan (SPDSMP) prior to the study opening to enrollment and then at least twice a year (August/September and February) or on a schedule specified by the DSMB. All reviews will focus on accrual, data completeness, adherence to the protocol, and safety. If full enrollment is not achieved in the first season, once Day 56 immunogenicity results are available (February of the subsequent calendar year), the DSMB will review the immunogenicity data. Once enrollment and follow-up through Day 56 is complete, a primary analysis report addressing the study's primary and first two secondary objectives will be finalized. This report will be sent to the DSMB and a subset of the Protocol Team not reviewing ongoing safety during the surveillance period. This report will be used to plan vaccine studies for the subsequent calendar year. Once follow-up is complete through the end of the surveillance period, the DSMB will be sent a copy of the second analysis report.

During enrollment, two reports will be submitted to the DSMB Executive Secretary for distribution to the DSMB members: one that pools data across arms and a second broken down by masked treatment arm. Codes identifying the arms will be provided separately. The goals of the reviews are to drop any vaccine arms that have clearly inferior immunogenicity or safety

concerns. Since this is a Phase I/II study, no adjustments are made to account for the impact of multiple looks on Type I error rates.

The DSMB might consider halting enrollment to a vaccine arm:

1. for clear differences in immunogenicity: if there a statistically significant difference in the percentage with immune response (≥4-fold rise in serum RSV-neutralizing antibodies) to the candidate vaccines such that one or two products can be identified to be immunologically superior to the remaining product or products, with the absence of a safety signal among the higher performing product(s)
2. for inadequate immunogenicity: the upper limit of a 95% CI around the response rate (≥4-fold rise in RSV-neutralizing antibody titer) for a vaccine arm is less than 70%. [With a sample size of 10 (15, 20, 25, 30, 38), this would occur if the observed response rate was <30% (<40%, <45%, <48%, <50%, <53%)]
3. for safety concerns (also considering vaccine virus shedding and adventitious data):
    a. if >2 participants receiving the specific product experience LRIs of ≥Grade 2 in the 28 days following study product administration for which vaccine virus is isolated without other potential explanation
    b. if there are ≥2 participants with vaccine-associated Grade 4 events of similar type (otitis media or pharyngitis within 28 days of study product administration) with presence of vaccine virus without other potential explanation.

After each *ad hoc* or interim review, the DSMB Executive Secretary will promptly provide the Protocol Chair with the DSMB's recommendations, and the official DSMB Recommendations will be provided in a timely fashion through the office of the NIAID Clinical Director. The Protocol Team will circulate written DSMB recommendations to the sites upon receipt for submission to applicable IRBs.

9.5 Analyses

A detailed description of analyses will be described in a separate Statistical Analysis Plan (SAP). Since this is a Phase I/II study, statistical comparisons will not be adjusted for multiple comparisons or interim looks by the DSMB. Exact confidence intervals will be calculated using the method of Clopper-Pearson.

9.5.1 Primary Safety Outcome Measures

All children who receive study product will be included in safety analyses. Safety outcome measures will be included if they occur after study product administration.

The number and proportion (two-sided 95% CI) of participants who experience each primary safety outcome measure will be summarized by study arm. The difference in proportions (95% CI) between each vaccine group and the placebo group will be calculated. Supportive analyses that stratify by time of enrollment will be performed to account for potential ascertainment differences related to COVID-19. In addition, for each primary safety outcome measure, the worst grade experienced by each participant will be summarized, including all graded events. Rare events (LRIs and serious AEs) will be summarized with details including site-assessed relationship with the study product.

9.5.2 Primary Immunogenicity Outcome Measures

This will be a per-protocol analysis including children who receive the as-randomized study vaccine and have serum RSV-neutralizing antibody titer results at pre-study product administration (screening) and the Day 56 Visit. In the event that twins, siblings, or other non-related children living in the same household are enrolled on the same date, one will be randomly selected to be included in the primary analysis.

Children will be classified as a responder to the vaccine if they achieve a ≥4-fold increase in serum RSV-neutralizing antibody titers from pre-study product administration to the Day 56 Visit. Proportions (95% CIs) of responders will be summarized by arm. Pair-wise differences in response rates with 95% CIs between the vaccine arms (and differences between vaccine and placebo arms) will be summarized. Sensitivity analyses to ensure that results support the same overall conclusion will be performed in which the selected child is replaced by other siblings or other children from the same household who enrolled on the same date. Secondary analyses will include (i) classifying participants missing their Day 56 antibody titer as failures, (ii) in the intent-to-treat population including all participants as-randomized regardless of vaccine receipt, and (iii) using an outcome measure defined as a ≥4-fold increase in serum RSV-neutralizing antibody titers from pre-study product administration to the Day 56 Visit and no wild-type RSV infection detected before Day 56. If enrollment takes place over more than one season, response rates will be summarized by calendar year of enrollment.

9.5.3 Secondary Outcome Measures

Children will be classified as responders to the vaccine if they achieve a ≥4-fold increase in serum RSV F IgG antibody levels from pre-study product administration to the Day 56 Visit. Participants will be included in the analysis if they receive the as-randomized study vaccine and have RSV F IgG results at screening and the Day 56 Visit. These data will be summarized using the same methods as for the primary initial response to vaccine immunogenicity outcome measure.

Titers of serum RSV F IgG and serum RSV-neutralizing antibodies will be summarized at the Day 56 Visit, as well as changes from pre-study product administration. Levels and changes will be compared between vaccines using pair-wise Wilcoxon rank sum tests. Sensitivity analyses will be performed replacing the selected child by other siblings or other children living in the same household who enrolled on the same date. If relevant, seasonal effects will be examined using analyses stratified by calendar year of enrollment.

The frequency and severity of RSV-associated MAARI and MAALRI in children who experience these conditions during the subsequent RSV season will be summarized within each arm. Event rates may need to be adjusted for the varying length of the RSV surveillance season across sites. Supportive analyses that stratify by time of enrollment will also be performed to account for potential ascertainment differences related to COVID-19.

9.5.4 Exploratory Outcome Measures

Participants who experience solicited AEs (Days 0-28) and serious AEs (Days 0-56) are required to have a nasal swab specimen collected. Viral shedding will initially be summarized at the participant level, with additional analyses done depending on observed patterns.

Other continuous exploratory outcome measures (infectivity, titers, etc.) will use methods similar to the secondary continuous immunogenicity outcome measures.

Day 28 and 56 Visit antibody levels will be summarized using means/geometric means and 95% confidence intervals. It is unlikely that formal equivalence can be established with the relatively small sample size.

10 DATA HANDLING AND RECORD KEEPING

10.1 Data Management Responsibilities

As described in Section 4.4, data on screening and enrollment in this study will be collected using the DMC Study Enrollment System (SES).

Study sites must maintain adequate and accurate research records containing all information pertinent to the study for all screened and enrolled participants, including paper-based CRFs (if used), eCRFs, and supporting source data. In maintaining these records, sites must comply with the standards of source documentation specified in the DAIDS Site Clinical Operations and Research Essentials (SCORE) Manual, which is available on the website referenced in Section 10.2.

eCRFs and an eCRF completion guide will be made available to study sites by the DMC. Study site staff will enter required data into eCRFs, with system checks applied and data queries generated immediately upon saving the entered data. Data must be entered within timeframes specified by the DMC; queries must also be resolved in a timely manner. Selected laboratory data will be transferred electronically to the DMC through the LDMS.

The Protocol Team and/or study oversight bodies (e.g., DSMB) may determine that additional source data associated with procedures or evaluations performed per protocol should be entered into eCRFs so that the data can be used for analysis or to otherwise assist with interpretation of study findings. In such cases, sites will be officially instructed to enter the additional data into eCRFs from available source documentation.

Further information on eCRFs and IMPAACT data management procedures will be provided by the DMC. A User Manual for the Study Enrollment System (SES) is available on the DMC portal at https://www.frontierscience.org.

10.2 Essential and Source Documents and Access to Source Data

All DAIDS policies referenced in this section are available at:
https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

Study-related documentation will be completed as required by applicable IRBs, the sponsor, and regulatory authorities. Continuing review documentation will be submitted to applicable IRBs. An annual report will be submitted by the sponsor to the FDA based on the anniversary date that the IND for the RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, and RSV 276 vaccines went into effect. These reports will provide a brief description of the progress of the investigation as outlined in 21 CFR 312.33 and will include any revisions of the protocol not previously submitted to the FDA.

Study-related documents will be maintained by the site investigator 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 site investigator 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 Protocol Chair. 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 site investigator wish to assign the study records to another party and/or move them to another location, the site investigator 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 by the site from the sponsor prior to destruction or relocation of research records.

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, IMPAACT, the US Food and Drug Administration, the European Medicines Agency (EMA), site drug regulatory authorities, the sIRB and any applicable local IRBs, OHRP, and other US, local, and international 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.

10.3 Clinical Investigator's Brochure

Investigators will receive the current version of the Clinical Investigator's Brochures (Ibs) that comprehensively describe all the available preclinical experience with each experimental vaccine. If relevant new information becomes available during the course of the trial, the investigators will receive a revised IB or an amendment to the current version.

10.4 Quality Control and Quality Assurance

Study sites must ensure that essential documents and participant research records are subject to continuous quality control and quality assurance procedures consistent with the DAIDS SCORE Manual, which is available on the website referenced in Section 10.2.

11 CLINICAL SITE MONITORING

Under contract to DAIDS or NICHD, site monitors will inspect study site facilities and review participant study records – including informed consent forms, paper-based CRFs (if used), eCRFs, medical records, laboratory records, and pharmacy records – to ensure protection of study participants, compliance with the IRB/IBC-approved protocol, and accuracy and completeness of records. Monitors also will review essential document files to ensure compliance with all applicable regulatory requirements. Site investigators will make study facilities and documents available for inspection by the monitors.

Monitoring visits may be conducted on-site or remotely. Remote visits may include remote source document verification using methods specified for this purpose by DAIDS or NICHD. Remote monitoring visits may be performed in place of, or in addition to onsite visits to ensure the safety of study participants and data integrity (38). Site investigators will make study documents available for site monitors to review utilizing a secure platform that is 21 CFR Part 11 and HIPAA compliant. Potential platform options include: Veeva SiteVault, Medidata Rave Imaging Solution, Medidata Remote Source Review, site-controlled SharePoint or cloud-based portal, and direct access to electronic medical records. Other secure platforms that are 21 CFR Part 11 and HIPAA compliant may be utilized, as allowed by DAIDS Office of Clinical Site Oversight (OCSO) or NICHD.

12 HUMAN SUBJECTS PROTECTIONS

12.1 Institutional Review Board/Ethics Committee Review and Approval

Prior to study initiation, site investigators must obtain IRB/IBC review and approval of this protocol and site-specific ICFs in accordance with 45 CFR 46; subsequent to initial review and approval, IRBs/IBCs must review the study at least annually. Site investigators must promptly report to IRBs/IBCs any changes in the study and must comply with the requirements of 45 CFR 46.108(a)(4) and 21 CFR 56.108(b) for promptly reporting the following: unanticipated problems involving risks to participants or others; serious or continuing noncompliance with applicable regulations or the requirements or determinations of their IRBs/IBCs; and any suspension or termination of IRB approval.

US sites are overseen by an sIRB, with additional review by local IRBs if required per their agreements with the sIRB. Site investigators are responsible for awareness of and adherence to the policies and procedures of all applicable IRBs/IBCs. All such policies and procedures must be followed and complete documentation of all correspondence to and from all applicable IRBs/IBCs must be maintained in site essential document files. Sites must submit documentation of both initial review and approval and continuing review to the DAIDS Protocol Registration Office (PRO) in accordance with the DAIDS Protocol Registration Manual (see also Section 13.2).

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

During the study, applicable IRBs will be provided with all documents subject to review (i.e., protocol amendments, informed consent form updates, advertisements, and any written information that may be provided to the participant's parents/guardians). Study progress reports will be made to applicable IRBs 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 (39, 40). This study responds to that mandate and will provide clinical research data to inform RSV vaccine safety and immunogenicity in children. Nonetheless, the children who take part in this study are considered vulnerable participants per the US Code of Federal Regulations, the IRBs/IBCs must consider the potential risks and benefits to child participants as described in 45 CFR 46 Subpart D (for children).

With respect to 45 CFR 46 Subpart D, IRBs/IBCs must determine the level of risk to children in the categories specified in 45 CFR 46.404-407. Documentation of this determination is required to complete the DAIDS protocol registration process described in Section 13.2, and the risk category assigned by the IRB/IBC further determines the parental informed consent requirements for the study at each site. Per 45 CFR 46.408 (b), the IRB/IBC may find that the consent of one parent is sufficient for research to be conducted under 46.404 or 46.405. If the IRB/IBC finds that the research is covered by 46.406 or 46.407, both parents must give their consent, unless one parent is deceased, unknown, incompetent, or not reasonably available or when only one parent has legal responsibility for the care and custody of the child (as determined locally). The IRB/IBC must document their risk determination, and study sites should adapt the signature pages of their site-specific ICFs as needed to accommodate the parental consent requirements associated with the IRB/IBC determination.

Study sites must comply DAIDS requirements for enrolling minors in clinical research as specified in the DAIDS SCORE Manual, which is available on the website referenced in Section 10.2.

12.3 Informed Consent

A sample informed consent form is provided in Appendix VI. In obtaining and documenting informed consent, the site investigator must comply with the applicable regulatory requirements, ICH GCP guidelines, and ethical principles. The written informed consent form must be approved by the sIRB prior to its use.

Written informed consent for the child's 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. The process will emphasize the unproven efficacy of the study vaccine products.

As part of the informed consent process, parents/guardians will also be asked whether they agree to storage and future research testing of biological specimens 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 the child's study participation.

Parental consenting requirements at each site will depend on the IRB/IBC risk determination described in Section 12.2; all IRB/IBC requirements will be followed.

12.4 Potential Benefits

Participants may not receive direct study product-related benefit from enrollment in this study. However, based on prior studies, there is the possibility of a reduced risk of disease from RSV for vaccine recipients; placebo recipients will not receive any direct benefit from enrollment in this study. The three vaccines included in this study have been tested on small numbers of children, with Phase I data available/pending as outlined in protocol Section 1.3.3. The existing data suggest the vaccines lead to immunogenicity, but a reduced risk of disease has not been demonstrated so far; the prospect of direct benefit for participants is therefore uncertain. 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.

12.5 Potential Risks

The burden of repeat study visits and examinations may be a risk associated with study participation. Additional potential risks are summarized below.

12.5.1 Venipuncture

As outlined in Appendix I and Appendix II, each participant will have four scheduled blood draws of 5 mL each. Risks occasionally associated with venipuncture include pain and bruising at the site of venipuncture, lightheadedness, infection, and syncope (rarely).

12.5.2 Nasal Swabs

As outlined in Appendix I and Appendix II, one nasal swab (anterior nares swab) will be collected at each Illness Visit; note that, for possible LRI during the Acute Phase, a follow-up nasal swab is to be collected (see Section 6.8). Nasal swabs may be collected by participants' parents/guardians and will be retrieved by study staff or returned to the study site. The total number of nasal swabs performed for each participant during study participation will depend on how many times the participant meets illness criteria. Risks occasionally associated with nasal swab include discomfort and occasionally epistaxis. Nasal swabs are not standard care in well children and are not routinely performed on ill children.

12.5.3 Receipt of Study Product

If a vaccine (RSV ΔNS2/Δ1313/I1314L or RSV 6120/ΔNS2/1030s) is insufficiently attenuated, participants could experience rhinorrhea, cough, fever, hoarseness, otitis media, or LRI. Immediate hypersensitivity reactions—which could be life threatening—including urticaria, anaphylaxis, or other Immunoglobulin E (IgE)-mediated responses are possible, as with any vaccine. There is a theoretical possibility, as with any investigational vaccine, of risks about which there is no present knowledge. Parents/guardians will be informed of any such risks should further data become available. As noted in Section 1.4, the RSV 276 vaccine arm was closed to further enrollment under protocol V2.0 and remains closed under protocol V3.0; RSV ΔNS2/Δ1313/I1314L and RSV 6120/ΔNS2/1030s will continue to be evaluated to determine which study arms will remain open in IMPAACT 2021 as outlined in Table 3 and Table 4.

12.6 Reimbursement/Compensation

Pending sIRB approval, compensation will be provided to the participant's parent/guardian based on each site's standard. Specific compensation amounts will be determined by the sIRB in collaboration with local IRB. The amount must be reviewed and approved by the sIRB. Compensation will be specified in site-specific ICFs and/or other materials if applicable per IRB policies and procedures.

12.7 Privacy and Confidentiality

All study procedures will be conducted in private and every effort will be made to protect participant privacy and confidentiality to the extent possible. Participant information will not be released without written permission to do so except as necessary for review, monitoring, and/or auditing as described in Section 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, eCRFs, and other documents that may be transmitted off-site (e.g., EAE report forms, photographs of observed reactions) will be identified by PID only. Likewise, communications between study staff and Protocol Team members regarding individual participants will identify participants by PID only.

Study sites are encouraged but not required by DAIDS to store study records that bear participant names or other personal identifiers separately from records identified by PID. All local databases must be secured with password protected access systems. Lists, logbooks, appointment books, and any other documents that link PID numbers to personal identifying information should be stored in a separate, locked location in an area with limited access.

In addition to the above, a Certificate of Confidentiality has been deemed issued for the IMPAACT Network by the US Department of Health and Human Services. This certificate protects study staff from being compelled to disclose study-related information by any US Federal, state, or local civil, criminal, administrative, legislative, or other proceedings. It thus serves to protect the identity and privacy of study participants.

12.8 Management of Incidental Findings

Site clinicians will inform parents (or other authorized guardians if applicable) of all clinically meaningful physical exam findings and laboratory test results. When applicable, site 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

This study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), *Eunice Kennedy Shriver* National Institute of Child Health and Development (NICHD), and National Institute of Mental Health (NIMH), which are part of the United States National Institutes of Health (NIH).

The Division of AIDS (DAIDS) within the NIAID is responsible for regulatory oversight of this study and holds the Investigational New Drug (IND) application under which the study will be conducted. DAIDS will distribute safety-related information pertaining to the study products prior to and during the conduct of the study, in accordance with its sponsor obligations.

NIAID and NICHD provide funding to the clinical research sites at which this study will be conducted. Each institute contracts with an independent clinical site monitoring group to perform clinical site monitoring as described in Section 11. As part of this activity, monitors will inspect study-related documentation to ensure compliance with applicable US, local, and international regulatory requirements.

13.2 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol ICFs approved by the sIRB and, as applicable, by their local IRBs/IBCs, and any other applicable regulatory entities. Upon receiving final approval, sites

will submit all required protocol registration documents to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific ICFs will be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

For any future protocol amendments, upon receiving final sIRB and any other applicable regulatory entity approvals, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICFs will not be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual, which is available on the RSC website: https://rsc.niaid.nih.gov/clinical-research-sites/protocol-registration.

13.3 Study Implementation

This study will be conducted in accordance with the protocol, international good clinical practice guidelines, and all applicable US, local, and international regulations. Study implementation will also be guided by the IMPAACT Network MOP, study-specific MOP, LPC, and other study implementation materials, which will be available on the IMPAACT website: www.impaactnetwork.org.

Study implementation at each site will also be guided site-specific SOPs. The DAIDS SCORE Manual specifies the minimum set of SOPs that must be established at sites conducting DAIDS funded and/or sponsored clinical trials (available on the website referenced in Section 10.2). These SOPs should be updated and/or supplemented as needed to describe roles, responsibilities, and procedures for this study.

13.4 Protocol Deviation Reporting

Per the requirements for source documentation specified in the DAIDS SCORE Manual (available at the website referenced in Section 10.2), all 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.

Deviations should be reported to applicable IRBs/IBCs 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 within IMPAACT, following procedures specified in the IMPAACT Manual of Procedures.

13.5 ClinicalTrials.gov

The NIH Policy on Dissemination of NIH-funded Clinical Trial Information establishes the expectation that clinical trials funded in whole or in part by the NIH will be registered and have summary results information submitted to ClinicalTrials.gov for public posting. The Protocol Team will comply with this policy as well as the requirements of 42 CFR 11.

14 PUBLICATIONS

All presentations and publications of data collected in this study are governed by IMPAACT policies, which are available in the IMPAACT Network MOP. Any presentation, abstract, or manuscript will be made available for review by the pharmaceutical and NIAID sponsors prior to submission. Publication or presentation clearance will conform to any CRADA or other collaborative agreement in place.

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

		ACUTE PHASE			POST-ACUTE PHASE					
	Screening	Enrollment Visit/Study Product Administration (Day 0)	Day 1-27 (contact each day ±1 day)	Day 28 (-1/+4 days)	Day 29 (+1 day)	Day 29 to Day 56 Visit (monitoring throughout)	Day 56 (+7 days)	Day 57	Illness Visit	Early Discontinuation
In-person visit (all except Day 0 may be in clinic or home)	X	X		X			X		X*	X
Non-visit contact			X		X	Per Section 6.4		If needed per Section 6.4.2		
Informed consent	X									
History	X									
Interim history		X	X	X	X	Per Section 6.4	X	If needed per Section 6.4.2	X	X
Physical exam (full)		X								
Clinical assessment (focused PE)	X			X					X	
Administer study product		X								
Blood for: immunologic assays	5mL	If needed per Section 6.2		5mL			5mL			5mL
Nasal swab for viral detection & quantification									X	
Total blood volume	5mL	--	--	5mL	--	--	5mL		--	5mL

* Select Illness Visits can be conducted via telehealth (See Section 6.8)

Appendix II: Schedule of Events: RSV Seasonal Surveillance and Post-season Sampling

	Weekly contact	Post-RSV Season Visit	Illness Visit***	Early Discontinuation
Visit Period	Nov 1st* to Mar 31st	Apr 1st to Apr 30th**		
Clinical assessment (focused PE)			X	
Interim history	X		X	X
LABORATORY EVALUATIONS				
Blood for: immunologic assays		5 mL		5 mL
Nasal swab for viral detection & quantification			X	
TOTAL BLOOD VOLUME	--	5 mL	--	5 mL

*These dates apply to most sites but may differ for those with local RSV seasons that differ.
** For sites experiencing disruptions or limitations of usual operations due to COVID-19, broadened visit window specifications for this visit are provided in Appendix V.
*** Select Illness Visits can be conducted via telehealth (See Section 6.8)

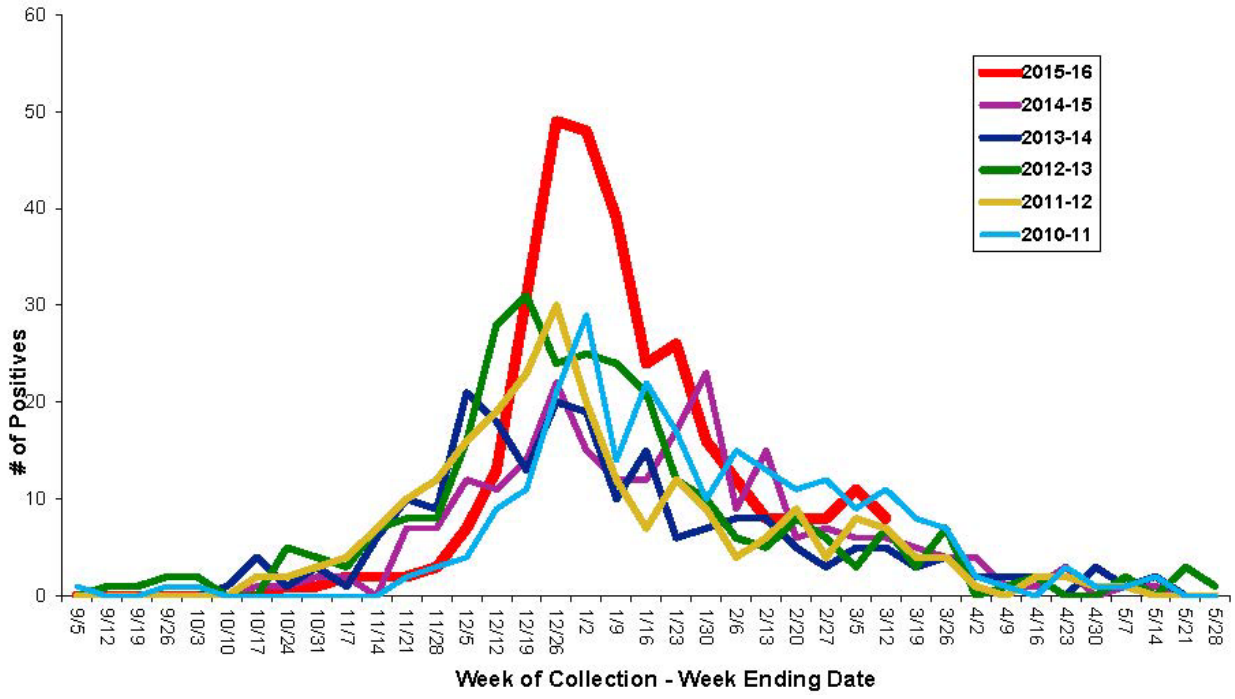
Appendix III: Definitions of Solicited Adverse Events

Event	Defined
Fever	Temporal temperatures ≥100.0ºF unconfirmed by rectal temp -or- Rectal temperature of ≥100.4ºF.
Acute Otitis Media[1]	Loss of tympanic membrane landmarks, accompanied by erythema and loss of mobility. May or may not be associated with fever or other respiratory symptoms. Confirmed with tympanometry if possible.
Upper Respiratory Tract Illness (URI)	
Rhinorrhea	Two or more consecutive days of clear or purulent discharge from the nares. Note: Not associated with crying, change of room temperature, or eating and drinking.
Pharyngitis[1]	Pharyngeal erythema accompanied by exudate or pharyngeal erythema with enlarged tender lymph nodes. Note: May be associated with sore throat, or painful or difficult swallowing.
Cough without LRI	Two or more consecutive days of 3 or more episodes of cough during a 15-minute timed observation period, or cough awakens child from sleep. Note: Not associated with eating, drinking or choking.
Hoarseness	An unnaturally deep or rough quality of voice.
Lower Respiratory Tract Illness (LRI)	
Wheezing[2,3]	Sustained, high pitched, musical breath sounds, especially during the expiratory phase, which do not clear with cough.
Pneumonia[1,2,3]	Rales and crackles, originating in the lower respiratory tract, usually accompanied by tachypnea, which do not clear with cough. May be confirmed by x-ray showing areas of consolidation.
Laryngotracheobronchitis (croup) [1,2,4]	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 (more sloshy) depending on the amount and density of fluid refluxing back and forth in the air passages.

1 Diagnosis must be made by a medical professional
2 Must be sustained over 20 minutes.
3 Clinical assessment must be made by a medical professional and confirmed by a second medical professional, if possible.
4 It is not necessary for medical professional(s) to witness inspiratory stridor as long as parent or guardian report is consistent with stridor and a medical professional judges the symptoms in total to be consistent with croup.

NOTE: Solicited AEs will only be entered into eCRFs according to guidance outlined in Section 7.2.

Appendix IV: RSV Seasonality in Baltimore



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

Appendix V: Operational Guidance for Study Implementation at Sites Experiencing Operational Disruptions Due to COVID-19

To safeguard the health and well-being of study participants and study staff in the context of circulating SARS-CoV-2 and the associated coronavirus disease 2019 (COVID-19), the guidance provided in this appendix may be implemented at sites experiencing disruptions due to COVID-19.

The extent to which site operations may be disrupted by COVID-19 may vary across sites and over time. **All sites should follow applicable government, health authority, and institutional policies with respect to conduct of study visits and procedures, with utmost importance placed on the health and well-being of study participants and study staff.** All sites must also comply with any directives received from the study sponsor, the IMPAACT Network, the IMPAACT 2021 the Protocol Team and/or Protocol Safety Review Team (PSRT) and should contact the PSRT (impaact.psrt2021@fstrf.org) with any questions or concerns regarding this guidance or management of study participants.

Should a determination be made in the future that the guidance provided in this appendix is no longer applicable, sites will be formally notified and instructed to inform their IRBs and other applicable regulatory entities.

Visit Scheduling

- Sites should continue to conduct weekly non-visit contacts per protocol with participants' families during the remainder of RSV Season Surveillance (i.e., through 31 March in a given year).
- Should sites have research visits suspended due to COVID-19, data should be collected via chart abstractions. The participants' parents/guardians may collect nasal swab specimens as directed by the site staff.
- Sites should conduct Post-RSV Season Study Visits; effective when sites experience disruption due to COVID-19 and implement this guidance, the target window for the Post-RSV Season Study Visit is broadened to include 1 April to 31 July in a given year and the allowable window for this visit is broadened to include up to 30 September in a given year.
  - Sites should utilize the broadened visit window as needed to permit continued completion of in-person Post-RSV Season Study Visits.
  - Sites that anticipate operational disruptions or closures in the near future are advised to conduct visits early in the target window. Visits conducted prior to opening of the target window would also be preferred to completely missing a visit at a later date.
  - Sites that are currently experiencing operational disruptions or closures are advised to conduct visits late in the target window. Visits conducted after closing of the target window (i.e., after 31 July in a given year) but prior to the closing of the allowable window (i.e., by 30 September in a given year) would also be preferred to completely missed visits.
  - Sites with no ability to conduct Post-RSV Season Study Visits in-person during any of the windows noted above should notify the protocol team.

Prioritization of Post-RSV Season Study Visit Procedures

- Sites with full capacity to conduct Post-RSV Season Study Visits in-person at the study clinic during the month of April in a given year should continue to do so in full compliance with the current protocol language. If it is not possible to conduct visits in April, the broadened windows noted above may be used.
- Sites may also conduct in-person Post-RSV Season Study Visits off-site, in full or in part, if permitted by applicable government, health authority, and institutional policies during any of the windows noted above. Where this option is permitted, site staff should communicate with parents/guardians to determine in advance where and when such visits will take place, with adequate protections for

safety, privacy, and confidentiality. Off-site visit procedures should be conducted by site staff who are adequately qualified and trained to conduct the procedures, as determined by the site Investigator of Record (IoR), with attention paid to occupational health, biohazard containment, and specimen and data chain of custody. These staff should also be adequately trained and qualified to immediately assess and/or manage any adverse events and/or social impacts that may occur during the visits. If adverse events requiring further evaluation or management are identified during an off-site visit, study staff conducting the visit should arrange for appropriate clinical management, in consultation with the IoR or designee, as needed.

- The only protocol-specified procedure for the Post-RSV Season Study Visit is collection of blood for serum antibodies to RSV. Sites with limited capacity to conduct Post-RSV Season Study Visits in-person with blood collection during any of the windows noted above should contact the protocol team.

Documentation

- Site-specific contingency plans, and the implementation thereof, should be documented in essential document files for IMPAACT 2021.
- Documentation should be entered in participant study charts in real-time should any of the following occur:
  - Missed visits
  - Out-of-window visits
  - Off-site visits (document the location of the visit)
  - Incomplete or partial visits (document which procedures were performed and which were not)
  - Remote contacts (e.g., by telephone) performed in lieu of in-person visits (document method used to complete the contact and which procedures were performed)
  - Any other participant contacts
  - Non-standard laboratory assays (e.g., use of alternate laboratories or alternate laboratory assays)
- In consultation with the Division of AIDS, the IMPAACT Network has developed and disseminated guidance for documenting and/or reporting protocol deviations that may occur due to limited site capacity to conduct study visits or procedures due to COVID-19. Please contact the IMPAACT Operations Center Clinical Research Managers with any questions related to documentation and reporting requirements.

Appendix VI: Informed Consent Form for Study Participation

Version 3.0, dated 28 April 2022

Sponsor / Study Title:	**National Institutes of Health (NIH) / National Institute of Allergy and Infectious Diseases (NIAID) / National Institute of Child Health and Human Development (NICHD) / National Institute of Mental Health (NIMH) / "Randomized Phase I/II Study of the Safety and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV ΔNS2/Δ1313/I1314L, RSV 6120/ΔNS2/1030s, RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Children 6 to 24 Months of Age"**
Protocol Number:	**IMPAACT 2021**
Principal Investigator: (Study Doctor)	**«PiFullName»**
Telephone:	**«IcfPhoneNumber»**
Address:	**«PiLocations»**

KEY INFORMATION

You are being asked to allow your child to take part in this research study to test vaccines to prevent respiratory syncytial virus (RSV) illness in children. Research studies include only people who choose to take part or whose parents/legal guardians choose to allow them to take part. The study staff will explain the study to you and will answer any questions you might have. You should take your time to make your decision.

Important details you should know:

- The purpose of the study is to look at the safety (side effects) and antibody (germ fighter) response of children to a single dose of one of two investigational vaccines against RSV. Investigational means that the study vaccines have not been approved for use by the U.S. Food and Drug Administration (FDA).
- If you choose to allow your child to participate in this study, he/she will receive either 1 dose of one of the study vaccines or 1 dose of placebo (inactive substance), which has no study vaccine in it.
- After the visit where your child receives the study vaccine or placebo, he/she will complete 3 more in-person study visits, which will include answering questions about your child's health, completing a physical exam on your child and collecting a small amount of blood from your child. In between these visits, study staff will contact you by telephone, text, or email to check on your child. If your child becomes ill, he/she will have a nasal swab collected to test for the RSV vaccine virus or any other virus that may be in your child's nose. The swab may be collected by you or by study staff.

- Your child will be in this study until April of the year after he/she started the study, which is between 4 and 15 months from now, depending on which month of the year he/she starts the study.
- Risks and side effects related to receipt of a study vaccine could include mild respiratory illnesses or colds or allergic reaction (rarely). If your child receives a nasal swab as part of the study, he/she could experience brief discomfort, or rarely, a nosebleed. The study blood draws could cause bleeding, pain, bruising, infection, and/or lightheadedness/passing out (rarely).
- Your child may receive a study vaccine that may reduce RSV disease; however, your child may receive no direct benefit from participating in the study. This study will help doctors learn more about RSV, and it is hoped that this information will help in the prevention of RSV in children in the future.
- You may choose to not allow your child to take part in this study. There are currently no licensed vaccines to protect against RSV illness.

INTRODUCTION

You are being asked to allow your child to take part in this research study to test vaccines to prevent respiratory syncytial virus (RSV) illness in children. This study is sponsored by the National Institutes of Health (NIH). The study doctor in charge of this study at this study site is listed on the first page of this form. Before you decide if you want your child to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The clinical research staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to allow your child to take part in this study, you will be asked to sign and date this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

The study is being done to look at the safety (side effects) and antibody (germ fighter) response of children to a single dose of one of two study vaccines against a virus called respiratory syncytial virus, or RSV. The study will look to see if the vaccine increases protection against RSV. It will tell us how safe and strong the study vaccine was during the study. The experimental vaccines in this study have not been approved by the US Food and Drug Administration (FDA). Your child was chosen to be in this study because your child is at least 6 months (180 days) old and less than 25 months (750 days) old and is healthy.

RSV is a virus (germ) that can cause breathing problems in children. Symptoms of infection with RSV may include:

- Fever
- Runny nose
- Sore throat
- Ear infection
- Cough
- Croup (barky cough with hoarseness)

RSV can cause serious lung infections such as pneumonia and wheezing. At this time, there is no approved vaccine to prevent RSV illness.

Doctors who develop vaccines at the NIH have made live virus vaccines that may help prevent RSV illness in babies and children. A live virus vaccine contains a weakened, live virus that is made to help your body respond in a way that will protect you from getting sick from the virus. This is called an "immune response." The investigational RSV vaccines in this study contain a live, weakened form of RSV and are given as nose drops. The study vaccines have been tested in humans before, and other RSV vaccines very similar to these have also been tested in both adults and children. There were not many side effects, and there was an immune response.

We are asking you to allow your child to participate in this study. We will ask you to review, sign, and date this study consent prior to conducting any study procedures with your child. As part of reviewing the consent, we will ask you to answer questions to see how well you understand the study. If you agree that your child can participate, we will give your child either 1 dose of one of the study vaccines or 1 dose of placebo, which has no investigational vaccine in it. The placebo is made of water and salt and is gentle on the inside of the nose. It is sterile, which means it is very clean, and is safe to use in people. About 100 additional children are planned to take part in the study.

WHAT DOES MY CHILD HAVE TO DO IF HE/SHE IS IN THIS STUDY?

General Information About the Study

If you agree to allow your child to take part in this study, you will be asked some questions to be sure he/she can be in this study. Your child's blood will be tested at the first visit to see if your child has had RSV in the past. You will be told the result of this test. Your child cannot take part in this study if he/she already has antibodies against RSV, which means he/she already had the RSV illness. Your child will not be able to be in the study if your child lives with people who have weak immune systems or is not well.

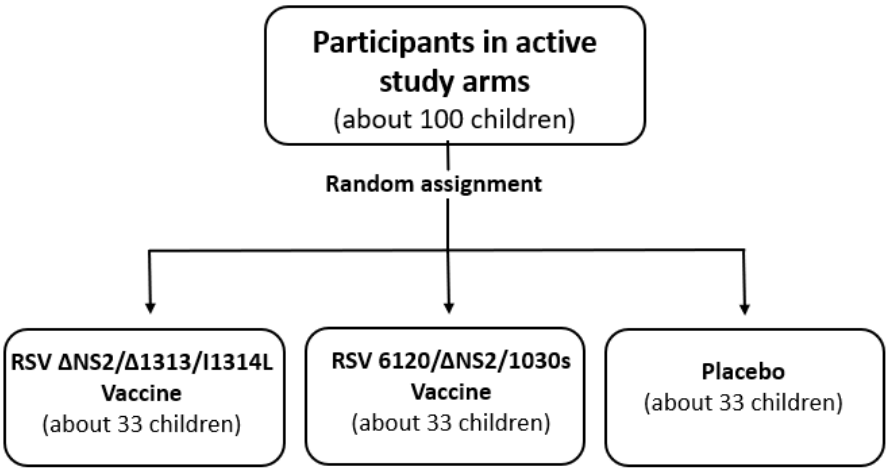
Your child cannot take part in this study if you know or think that he/she has been exposed to SARS-CoV-2, the new coronavirus that causes the COVID-19 illness, within 14 days prior to enrollment. Your child cannot take part in this study if he/she lives with or is in a daycare room with babies younger than 4 months of age, unless you are able to keep your child out of daycare for 14 days after he/she receives the study vaccine or placebo.

Your child should not get any vaccines, including rotavirus vaccine for at least 14 days and other live vaccines for at least 28 days after getting the study vaccine or placebo. We ask that you talk with the study staff before your child gets any routine vaccines for the 28 days after the study vaccine or placebo. We ask that your child does not take part in any other experimental vaccine or drug studies for 8 weeks after they receive vaccine or placebo.

The study vaccine/placebo will be given to your child as described under "Day Study Vaccine/Placebo is Given" below. If multiple children living in the same household are eligible to participate in the study, they must be enrolled on the same date, or they may enroll on different dates if additional children in the household are screened and enrolled after other children in the household complete the Day 56 Visit. All children living in the same household who are enrolled on the same date must receive the study vaccine/placebo on the same date.

About 2 out of each 3 enrolled children will get one of the two RSV study vaccines, and about 1 out of each 3 enrolled children will get nose drops without vaccine (placebo). Whether your child gets one of the study vaccines or nose drops without vaccine (placebo) will be decided randomly by computer, like flipping a coin. All children living in the same household who are enrolled on the same date will be randomly assigned to the same study group. Neither you nor the study doctors or study nurses will know

whether your child got one of the study vaccines or placebo until the study ends, but this information can be made available to the study doctor if needed. The graphic below shows the study groups.



Your child will be in this study until April of the year after he/she started the study. For the first 8 weeks after getting the study vaccine or placebo, your child will be followed closely. During this part of the study, there will be about 2 days when your child is seen by the study staff and 28 days when your child will not be seen but you will be contacted by telephone, text, or email by the study staff. Your child will also be followed during the RSV season after your child gets the vaccine or placebo. During this time, we will contact you by telephone, text, or email each week to ask about your child's health and arrange for follow-up visits if needed.

Study visits will last about 30 to 60 minutes, except on the day when your child is screened and on the day he/she is given the study vaccine or placebo; those 2 visits may take about 1 to 2 hours each.

Please note the following for the study visits:

- If your child has RSV symptoms, such as runny nose, sore throat, cough, fever, or difficulty breathing, he/she might need to be seen for an evaluation, sometimes as soon as within 24 hours.
- Study visits, except the visit where your child gets the study vaccine or placebo, may take place at your home or at one of the research sites/clinics. The visit where your child receives the study vaccine or placebo must take place at one of the research sites/clinics where emergency equipment is available.
- Other study visits may take place at your home or at one of the research sites/clinics. Study staff will provide you with information on the location of these visits.
- For temperature measurements, you will be asked to use a temporal thermometer, which is used on your child's forehead. You will measure forehead temperatures 3 times in a row, following the directions. The highest of the 3 readings will be recorded on a chart we will give to you. If your child has a forehead temperature greater than or equal to 100.0^{o} Fahrenheit, you will be asked to check your child's rectal temperature within 20 minutes. Forehead and rectal thermometers will be given to you for use during the study.

In the sections below, we will tell you more about what will happen at the different study visits.

Screening Visit
The purpose of the screening visit is to find out if your child may enter the study. It will take about 1 to 2

hours and will include:

- The study staff telling you about the study and asking you questions to be sure you understand the study.
- Going over, signing, and dating the consent form.
- Going over your child's medical history and doing a physical examination on your child. The physical examination will include checking your child's temperature, pulse (heart rate), breathing rate, and examination of your child's ears, eyes, nose, throat, lungs, heart, and lymph nodes. If the physical examination results are not normal, the study staff will tell you and refer your child for follow-up care with your child's primary medical provider. If your child has had a physical examination recently, we may be able to record the results from his/her medical record, rather than doing another examination.
- Answering questions about the health of your child and people living in your house.
- Collecting a small amount of blood (about 1 teaspoon) to test for antibodies (germ fighters) against RSV. If your child had been screened for any study of an RSV vaccine developed by the NIH doctors, we may not need to collect this sample, because we may be able to use the results and blood from the other study.
- If requested, giving written permission to review your child's medical records.
- If we think your child may be eligible for the study, your child will be asked to return for a series of study visits, beginning with the visit when we will confirm that he/she is eligible and then give your child the study vaccine or placebo.

Day Study Vaccine/Placebo is Given

This visit will take about 1 to 2 hours and will include the following:

- We will confirm that your child has not been ill recently and do a physical examination, which will include checking your child's temperature, pulse (heart rate), breathing rate, weight, length, head, ears, eyes, nose, throat, lungs, heart, abdomen, musculoskeletal system, nervous system, and skin.
- Your child will receive 1 dose of study vaccine or placebo given as nose drops using a small syringe (without a needle). Your child will be lying on his/her back while we give a small amount of nose drops and into both nostrils. Your child will remain lying down for about 1 minute afterwards. Your child can be in your lap during this time.
- After the nose drops are given, we will watch your child in the clinic for 30 minutes.
- We will provide you with the dates of the rest of the visits and telephone/email contact days.
- You will be given a forehead thermometer, a rectal thermometer, and a temperature chart to record your child's temperature daily for 29 days (including the day the study vaccine/placebo is given to your child), and at any other time you are concerned about fever.

Monitoring for 56 Days after Study Vaccine/Placebo is Given

- Your child will have a study visit on Day 28 (-1/+4 days) after the study vaccine or placebo is given. This visit will take about 30 to 60 minutes, and we will:
    - Check your child's temperature, pulse, breathing rate, ears, eyes, nose, throat, lungs, heart, and lymph nodes.
    - Do a brief clinical assessment.
    - Ask about your child's health since the last visit.
    - We will take a small amount of blood (about 1 teaspoon) from your child to measure antibodies (germ fighters) against RSV.
    - Because study visits will be less frequent after the first month, on Day 28, we will review when you should contact the study staff in the event your child becomes ill during the following month.

- The study nurse will contact you daily from Days 1 through 27, and on Day 29. The study staff will ask you to report your child's temperatures and any illness your child has had since the last visit or contact. The contact may be by telephone, text, or email, whichever you prefer.
- Your child will have a follow-up visit about 56 days after the study nose drops were given. At this visit, we will ask about your child's health since the last visit and take a small amount of blood (about 1 teaspoon) from your child to measure antibodies (germ fighters) against RSV. We will check your child's temperature, pulse, and breathing rate.
- We also ask you to call us right away to tell us about any illness that your child has from the day he/she receives the nose drops up to the follow-up visit (8 weeks).
- A study nurse or study doctor will be available by telephone to answer your questions 24 hours a day during the 28 days after your child receives the study vaccine or placebo.
- If your child becomes ill, you may be asked to complete a telehealth (telephone or video) visit, an in-person visit with a licensed medical provider in the community, and/or bring him/her to the clinic for an examination, sometimes as quickly as within 24 hours. We may request your permission to review your child's medical records, including records from visits with other medical providers related to the illness. A nasal swab will be collected at that time to look for the RSV vaccine virus or any other virus that may be in your child's nose. The swab may be collected by study staff or by you, if you are comfortable doing this. To do the nasal swab, a swab will be gently inserted into your child's nose, rotated for a few seconds, and then removed.

Monitoring Before, During, and After RSV Season

- Your child will also be followed during the RSV season after getting the study nose drops. We will be in contact with you each week to inquire about your child's health. If your child has a fever, a respiratory illness (a cold), or an ear infection that requires medical care, we will work with you to schedule an in-person visit at the clinic or with a licensed medical provider in the community, or telehealth (telephone or video) visit, to collect a nasal swab and clinical assessment. We may request your permission to review your child's medical records, including records from visits with other medical providers related to the illness.
- We will collect a small amount of blood (about 1 teaspoon) in April of the year after he/she started the study to look at the antibodies (germ fighters) against natural RSV infection.
- You will not be told whether your child received the study vaccine or placebo while on study. You will receive that information after the end of the study. You will not receive information about your child's response to the study vaccine, but you will receive a summary of the overall response to the study vaccine for everyone in the study.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

There will be approximately 100 additional children taking part in this study.

WHAT ARE THE RISKS OF THE STUDY?

Risks of the Study Vaccines

- If the study vaccines are not weakened enough, they may cause a runny nose, sore throat, cough, or other signs of a cold. Some children who previously received a different vaccine that is no longer included in this study had coughs. It is also possible that the study vaccines may cause a sinus infection, croup (infection of the upper airway), ear infection, fever, wheezing, or pneumonia (infection of the lungs). In other studies with similar study vaccines, mild respiratory illnesses or colds were observed frequently in children who received either study vaccine or placebo. Runny nose occurred more often in children who got vaccine than those who got placebo.

- Study investigators have used the same or similar placebo for studies of RSV, parainfluenza (virus causing cold, pneumonia, bronchitis, etc.), and influenza (flu) vaccines in several hundred babies and children over the past 20 years. They have not noticed side effects with this placebo.
- There is no specific medicine to treat RSV illness. If any symptoms of RSV occur, such as runny nose, sore throat, cough, or difficulty breathing, your child will receive prompt medical care, if needed.
- The study vaccines were made in a way that was designed to minimize the inclusion of other ingredients. However, as with all biological products, there is a small chance that they contain unidentified material. There is a very small chance that such material may cause illness, including possibly serious illness.
- There may be other side effects of the study vaccines that are not yet known. If new information about possible side effects of the study vaccines becomes available, we will let you know.
- It is possible that the study vaccine virus could be spread from your child to other people in the home or daycare and may make them sick. It could be spread to young children and people with weakened immune systems. This is why children who will live with/share a daycare room with young babies during the 14 days after receiving the study vaccine or placebo or who live with people who have weak immune systems are not allowed to participate in the study.
- The study vaccines could cause a severe allergic reaction. A severe reaction can cause hives, throat swelling, rapid heart rate, weakness, difficulty breathing, or death. These reactions are rare and have not been seen to date with the vaccines in this study.

Risks of Nasal Swab

Nasal swabs may cause brief discomfort and may rarely cause a nosebleed.

Risks of Having Blood Drawn

Blood drawing can cause bleeding, pain, bruising, or infection at the place where the blood is taken. Sometimes, blood drawing can cause your child to feel lightheaded or to faint. It sometimes takes more than one try to get blood from a small child. You may ask study staff about options for reducing the discomfort from drawing blood, such as numbing cream.

WHY WOULD THE STUDY DOCTOR TAKE MY CHILD OFF OF THIS STUDY EARLY?

The study doctors or the sponsor also have the right to end your child's participation in the study at any time without your consent for any of the following reasons:

- For your child's safety;
- You do not follow study procedures as directed by the study doctors;
- New information becomes available regarding the safety of the study vaccine;
- If it is in your child's best interest;
- You do not consent to continue in the study after being told of changes in the research that may affect your child;
- The study sponsor, the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT), the Advarra Institutional Review Board (IRB), the Office for Human Research Protections (OHRP), the National Institute of Allergy and Infectious Diseases (NIAID), or the United States Food and Drug Administration (FDA) decide to end the study. An IRB is a committee that watches over the safety and rights of research participants.

WHAT HAPPENS IF MY CHILD IS INJURED?

If your child suffers physical injury from this study, the study doctor will provide or will refer your child for appropriate medical treatment. The study doctor will also provide referrals to appropriate health care

facilities. The cost for this treatment will be charged to you or your insurance company. There is no program for compensation either through this institution or the National Institutes of Health (NIH). No financial compensation by the doctors that gave your child the study vaccine or placebo will be made for any discomfort suffered because of participation in this study. You will not be giving up any of your child's legal rights by signing and dating this consent form.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

- Your child may receive an active study vaccine that may reduce RSV disease; however, your child may not receive any direct benefit from being in the study. Other studies of these vaccines have shown an immune response, but they have not shown a reduced risk of disease so far. If your child receives placebo, your child will not receive protection against RSV.
- Being in the study may help find a vaccine that works well to prevent serious RSV illness. Such a vaccine may be of future benefit to babies and children in this country and in the rest of the world.

WHAT OTHER CHOICES DOES MY CHILD HAVE BESIDES THIS STUDY?

There are no licensed vaccines to protect against RSV illness at this time. There is no other similar study or approved vaccine that we can offer your child. You may choose to not allow your child to take part in this study.

WHAT ABOUT CONFIDENTIALITY?

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, the researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about you or your child's participation in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate of Confidentiality to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without your consent, information that would identify you as a participant in the research project under certain circumstances such as child abuse.

Your child's name, birth date, and Social Security number are not routinely given to anyone unless required by law. All of the information you give us during this study will be put in locked file cabinets and/or in password-protected computer files. The only people who will have access to this information will be those who are involved in the study.

There will be people involved in the study who need to see your child's health information. These people may include the researchers, study and laboratory personnel, and other clinical research staff. Others who may see your child's information are the groups of people who make sure that the study is being done as it

should be: Advarra Institutional Review Board (IRB), the Center for Immunization Research (CIR), the National Institute of Allergy and Infectious Diseases (NIAID; NIH) Intramural Data and Safety Monitoring Board and others who need to see your child's information to make sure that the study is going as planned.

Other groups of people who may be involved in the study and may need to see your child's information are:

- The government agency "Office for Human Research Protections," that makes sure that we are conducting the research as planned, and the US FDA, and the European Medicines Agency (EMA)
- The sponsor of the study and people with whom the sponsor may contract for the study, such as study monitors.
- Other US, local, and international regulatory groups

At the end of the study, whatever we learn from the research may be used in a medical journal or used for teaching. Your child's name or other details about his/her health will not be used in a manner such that anyone can personally identify your child.

WHAT ARE THE COSTS TO ME?

There are no costs to you or your child for him/her being in the study. The costs for the study vaccine/placebo, study visits, or study procedures are covered by the sponsor (NIH/NIAID). However, taking part in this study may lead to added costs to you or your child and your/your child's insurance company if medical complications arise or if your child's doctor decides extra tests are needed. In some cases, it is possible that your/your child's insurance company will not pay for these costs, because your child is taking part in a research study.

WILL MY CHILD RECEIVE ANY COMPENSATION?

«Compensation»

You will be paid up to a total of $xx.xx for your child's participation in this study at the following rate:

- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.

You will also be paid during the RSV surveillance period as follows:

- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.
- $xx.xx for Visits xxx.

If you stop your child from taking part in the study early, you will only be paid for the days of the study that your child completed. Your child may also receive age-appropriate books or small toys. If needed, bus tokens or parking passes may be given to you.

If you have any questions regarding compensation for your child's participation, please contact the study staff.

You may be required to provide your Social Security number to be paid. If your payment for study participation exceeds $600 per year, this information must be reported to the Internal Revenue Service.

WHAT ARE MY CHILD'S RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to have your child take part in this study or leave this study at any time. Your decision will not have any impact on your child's participation in other studies and will not result in any penalty or loss of benefits to which you or your child are otherwise entitled.

A study doctor, physician assistant, nurse practitioner, or study nurse will inform you of any significant abnormal physical findings and/or test results for your child and will make appropriate referrals back to your child's primary care giver, if necessary.

We will tell you about new information from this or other studies that may affect your child's health, welfare, or willingness to stay in this study. You may be asked to sign and date a revised consent form if this occurs. If you want the results of the study, let the study staff know.

At the end of the study, you will be told in writing whether your child was given one of the vaccines or the placebo.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify your child. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT ARE MY RESPONSIBILITIES?

- If you decide to withdraw your child from the study early, we ask that you notify the study nurse or study doctor.
- If your child comes off the study early, we will ask you to bring him/her into the clinic for an early discontinuation visit. At that visit, we will ask about your child's health since the last visit and do a final blood draw (about 1 teaspoon) to measure antibodies (germ fighters) against RSV.
- Any child who has received the study product will be encouraged to remain in the study so that safety information can be collected.
- It is important that you do not enroll your child in other studies where your child receives the study vaccines or medications for 8 weeks after he/she receives vaccine/placebo.
- If your child becomes ill, or this study site is closed due to COVID-19 safety concerns when a nasal swab should be collected, and you are comfortable collecting a nasal swab from him/her using supplies we give you, we may ask you to return the swab to the study team, or a study team member may come get the swab from you. You will receive instructions on how to collect, store, and return nasal swab samples and receive collection supplies during the visit when your child is enrolled and receives the study vaccine/placebo.

WHOM TO CONTACT ABOUT THIS STUDY

During the study, if your child experiences any medical problems or suffers a research-related injury or if you have questions, concerns, or complaints about the study, please contact the study doctor at the telephone number listed on the first page of this consent document. If you seek emergency care for your child, or hospitalization is required, alert the treating physician that your child is participating in this research study.

An institutional review board (IRB) is an independent committee established to help protect the rights of research participants. If you have any questions about your child's rights as a research participant and/or concerns or complaints regarding this research study, contact:

- By mail:
  Study Subject Adviser
  Advarra IRB
  6100 Merriweather Dr., Suite 600
  Columbia, MD 21044
- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: Pro000XXXXX.

STORAGE AND FUTURE USE OF UNUSED SPECIMENS

If you agree, any unused blood or nasal swab samples taken from your child will be stored indefinitely (with protectors of identity) once this study is complete. These unused blood and nasal swab samples will be stored as part of the Johns Hopkins University Center for Immunization Research's approved biospecimen repository for vaccine research or at the Laboratory of Infectious Diseases at the National Institutes of Health. The samples may be used for future laboratory studies (including studies done by different investigators) to learn more about RSV and other viruses. These studies will be conducted without obtaining additional informed consent from you. The studies will not include human genetic research; that is, the study doctors will not look at your child's genes (also known as DNA).

- Your child's unused blood or nasal swab samples, if any, will be used only for laboratory studies and will not be sold or used directly to make products that will be for sale.
- The samples will not contain your child's private information. They will be coded so that your child's name cannot be easily identified.
- Reports about studies done with your child's unused samples will not be put in your child's health or study records.
- There will be no direct benefit to your child in using the samples as noted above, but from studying the unused samples of children taking part in the studies, we may learn more about the RSV germ or other viruses that cause illness in babies and children.
- Results from future studies using your child's unused samples may be included in medical papers and meeting reports, but your child's name will not be used.

You can change your mind at any time about allowing your child's unused samples to be used for future laboratory studies. If you do change your mind, contact the study doctor or study nurse and let him/her know. Then the samples will no longer be used for laboratory studies and will be destroyed.

PERMISSION FOR STORAGE AND FUTURE USE OF UNUSED SPECIMENS
Your choice will not have any effect on your child's taking part in this study.

I will allow the use of my child's unused blood or nasal swab samples to be stored indefinitely and to be used in future laboratory studies for the purposes described above. Your child's name will not be available to the laboratory or to the scientists who may be doing any future tests. (Please check one and initial below)

Yes: Initials _____ Date _____

No: Initials _____ Date _____

If NO, your child's study samples will only be used for the testing described in this study.

SIGNATURE AND DATE

If you have read this consent form (or had it explained to you), all of your questions have been answered, and you agree your child can take part in this study, please sign and date your name below.

_____
Study Participant's Name (print)

_____ _____
Participant's Parent's/Legal Guardian's Name (print) Parent's/Legal Guardian's Signature and Date

_____ _____
Study Staff Conducting Consent Discussion's Name (print) Study Staff's Signature and Date

_____ _____
Witness' Name (print) (As appropriate) Witness' Signature and Date