IMPAACT 2013

Phase I Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-Attenuated Respiratory Syncytial Virus Vaccine, D46/NS2/N/ΔM2-2-HindIII, Lot RSV#011B, Delivered as Nose Drops to RSV-Seronegative Infants 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 ES # 30074
IND # [TBD] Held by DAIDS

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ABBREVIATIONS AND ACRONYMS

ACIP	Advisory Committee on Immunization Practices (CDC)
AE	adverse event
AGM	African green monkey
AIDS	Acquired Immunodeficiency Syndrome
cDNA	complementary deoxyribonucleic acid
cGMP	current good manufacturing practice
CFR	Code of Federal Regulations
CI	Confidence interval
CIR	Center for Immunization Research
cp	Cold Passaged
CRADA	Cooperative Research and Development Agreement
CRF	case report form
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
DMC	Data Management Center
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
EAE	Expedited Adverse Event
ELISA	enzyme-linked immunosorbent assay
EENT	ears, eyes, nose, throat
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 practices
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
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
L-15	Leibovitz-15 medium
LDMS	Laboratory Data Management System
LID	Laboratory of Infectious Diseases
LPC	laboratory processing chart
LRI	lower respiratory illness
LRT	lower respiratory tract
MA-LRI	medically attended lower respiratory illness
MOP	manual of procedures
mRNA	messenger Ribonucleic Acid
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIAID CRMS	NIAID Clinical Research Management System
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
nt	nucleotide
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
ORF	open reading frame
OTC	over-the-counter
PBMC	Peripheral blood mononuclear cell
PCR	polymerase chain reaction
PE	physical exam
PFU	plaque-forming unit
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
rRT-PCR	Reverse transcription polymerase chain reaction
RSC	Regulatory Support Center
RSV	respiratory syncytial virus
SAE	Serious Adverse Event
SDAC	Statistical & Data Analysis Center, Harvard School of Public Health
SDMC	Statistical and Data Management Center
SES	Subject Enrollment System
SID	Study Identification Number
SOP	standard operating procedure
SPG	sucrose-phosphate-glutamate buffer
SUSAR	Serious and unexpected suspected adverse reactions
TL	Tracheal lavage
URI	upper respiratory illness
URT	upper respiratory tract
US	United States
VAR	vaccine administration record
wt	wild-type

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SCHEMA

Purpose: To determine whether D46/NS2/N/ΔM2-2-HindIII vaccine is attenuated and immunogenic in this age group.

Design: A double-blind, randomized, placebo-controlled study design will be used to evaluate the safety and immunogenicity of the vaccine in RSV-seronegative infants and children. This protocol, IMPAACT 2013, is a companion protocol to the Johns Hopkins University, Center for Immunization Research (CIR) protocol CIR 313.

Study Population: Healthy RSV-seronegative* infants ≥6 months (180 days) to <25 months (750 days) of age

* 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 33 (IMPAACT 2013 and CIR 313 combined)

Study Product: Eligible RSV-seronegative infants will receive a single dose of D46/NS2/N/ΔM2-2-HindIII vaccine or placebo intranasally at entry.

N	Product	Dose
22	Vaccine	10^5 PFU**
11	Placebo	0

**plaque-forming units (PFU)

Study Duration: Approximately 13 months total. Accrual is expected to be completed in approximately 6 to 8 weeks from first enrollment, and participants will be followed through April of the year following enrollment. Therefore, expected duration of follow up for a given participant is between 6 and 13 months depending on time of enrollment.

Participants will be enrolled in the study between April 1st and October 14th (outside of RSV season) and will remain on study until they complete the post-RSV season visit between April 1st and April 30th in the calendar year following enrollment. For example, a participant enrolled on August 1st, 2017 will remain on study approximately 8-9 months (completing a final visit in April 2018) while a participant enrolled on October 14th, 2017 will remain on study approximately 6 months (also completing a final visit in April 2018).

Primary Objectives

1. Safety: To assess the frequency and severity of study product-related solicited and unsolicited adverse events (AEs), from Day 0 through midnight of the 28th day following inoculation, in vaccinated participants
2. Safety: To assess study product-related serious adverse events (SAEs) from Day 0 through midnight on the 56th day following inoculation for vaccinated participants
3. Infectivity: To determine the peak titer of vaccine virus shed and duration of virus shedding by each participant
4. Infectivity: To assess the proportion of vaccinated infants infected with study vaccine
5. Immunogenicity: To characterize antibody responses (Day 56) to the study vaccine

Secondary Objectives

1. To characterize clinical outcomes (frequency and severity of symptomatic, medically attended respiratory and febrile illness) in the vaccine and placebo recipients who experience natural infection with wild-type (wt) RSV during the subsequent RSV season
2. To characterize antibody responses in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season
3. To characterize the B cell response to vaccine and the quality and epitope specificity of RSV F specific antibody, and to characterize these responses in the vaccine and placebo recipients who experience natural infection to wt RSV during the subsequent RSV season
4. To characterize the mucosal antibody response to vaccine

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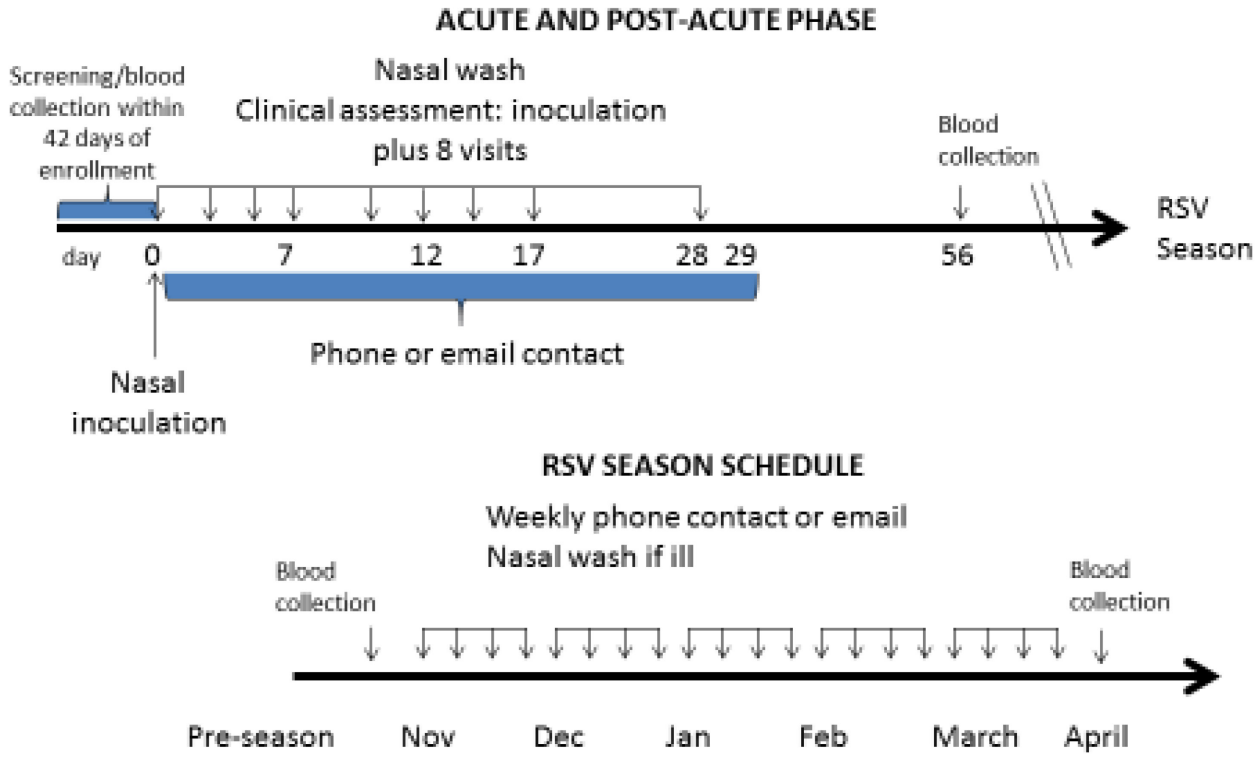
Figure 1: Study Overview



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 diseases (2) and they have the potential of inducing a spectrum of immune responses that would be similar to immunity 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 deletion of a large section of the M2-2 gene, resulting in an M2-2 deletion mutant. This mutation is associated with increased mRNA production linked with reduced RNA replication (4). 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, resulting in attenuation. A large deletion mutation effectively reduces the potential for reversion to wild type, an important concern for a live attenuated vaccine. The first candidate RSV vaccine using this strategy, MEDI ΔM2-2, was studied sequentially in adults, seropositive children, and then seronegative older infants and children (age 6 to 24 months) (5). This study found the vaccine had excellent immunogenicity associated with low viral replication. Determination of safety and tolerability of this candidate was confounded by a high incidence of adventitious respiratory infections during the study period. The MEDI ΔM2-2 phenotype may be suitable for an RSV vaccine, pending confirmation of safety. However, because of the overall low titer of shedding, and the lack of shedding in a number of vaccinees, it is possible that a virus that replicates somewhat more efficiently might be more immunogenic, provided it is suitably attenuated. This would be important since immune responses to RSV infection in infants and children are not robust. In addition, the MEDI ΔM2-2 virus lacks the temperature sensitive phenotype, a phenotype that may increase safety. Therefore, we also are evaluating several other ΔM2-2 candidates with differences affecting replication and temperature sensitivity. A slightly different vaccine, RSV LID ΔM2-2, advanced to clinical trials in RSV-seronegative children in IMPAACT 2000. This study demonstrated excellent immunogenicity and increased infectivity. However, the peak viral titers were higher than had been anticipated based on pre-clinical studies, and were deemed higher than desired. Therefore, in the interests of caution, IMPAACT 2000 was closed to accrual prior to full enrollment of the planned 50 participants [McFarland et al., manuscript in preparation], and a decision was made to further attenuate the LID ΔM2-2 vaccine as well as to consider several other candidates for evaluation.

In the current protocol and in 2 associated protocols (IMPAACT 2011 and IMPAACT 2012) that opened in sequence, 3 further attenuated versions of RSV with M2-2 deletion are being studied, 1 per protocol. Each of these versions of RSV with ΔM2-2 deletion has a different combination of mutations that are known to confer an attenuated phenotype. The 3 vectors are described in Table 1, and a short description of each candidate follows.

Table 1: Overview of RSV vaccine candidates with M2-2 deletion to be studied in IMPAACT 2011, 2012, and 2013

Protocol	Vaccine name	Mutations in addition to M2-2 deletion	Expected effect
2011	LID ΔM2-2 1030s	genetically stable "1030" attenuating point mutation	Attenuation Temperature sensitivity
2012	LID cp ΔM2-2	five point mutations in the RSV nucleoprotein, fusion protein, and polymerase protein	Attenuation
2013	D46/NS2/N/ΔM2-2-HindIII	Contains the SH noncoding region that is deleted in the other two candidates and in the previous LID ΔM2-2, one point mutation each in the NS2 and N proteins, and a modified version of the M2-2 deletion, based on RSV MEDI ΔM2-2.	Lower level of replication than LID ΔM2-2 due to the presence of the SH noncoding region

The IMPAACT 2011 virus (LID ΔM2-2 1030s) is LID ΔM2-2 with the addition of a temperature-sensitivity mutation (1030s) in the L polymerase protein. The 1030s mutation is a genetically stabilized version of a previous mutation called 1030. The 1030 and 1030s mutations (which confer the same level of temperature sensitivity and attenuation) have been characterized in a number of experimental animals including chimpanzees, and have been combined with other mutations in vaccine candidates including ones evaluated in infants and children. The LID ΔM2-2 1030s virus was highly attenuated in African green monkeys.

The IMPAACT 2012 virus (LID cp ΔM2-2) is LID ΔM2-2 with the addition of 5 amino acid substitutions in 3 proteins that in aggregate are called the “cp” mutations, and were originally identified in a cold-passaged RSV vaccine candidate cpRSV. The cp mutations have been shown to confer a relatively small amount of attenuation in adults and chimpanzees. They also have been combined with other mutations in vaccine candidates, including those evaluated in infants and children (6-9). We expect the cp mutations to confer a smaller increment of attenuation than 1030s. This virus also was highly attenuated in African green monkeys.

The IMPAACT 2013 virus (RSV D46/NS2/N/ΔM2-2-HindIII) is designed to incorporate the most significant differences between MEDI ΔM2-2 and LID ΔM2-2. In particular, it uses the LID backbone, but without a deletion and nucleotide substitutions in the SH gene. The D46/NS2/N/ΔM2-2-HindIII virus also has the MEDI assignments at the only two amino acid positions (in the NS2 and N ORFs) that differ between MEDI ΔM2-2 and LID ΔM2-2. In addition, the deletion of M2-2 in D46/NS2/N/ΔM2-2-HindIII is comparable to that of MEDI ΔM2-2. The level of attenuation of this virus in African Green Monkeys (AGMs) was slightly lower than that of LID cp ΔM2-2.

Between these three studies, we hope to identify a candidate with the following characteristics:

- >90% of vaccinees should shed vaccine virus detected by infectivity assay.
- The mean peak titer of shed virus in nasal washes should be approximately 2.5 $\log_{10}$ PFU.
- RSV-neutralizing serum antibody titers (measured 56 days post inoculation) should be similar to or better than MEDI ΔM2-2 (geometric mean titer of >1:97).
- Post-vaccination surveillance during the RSV season (Nov 1 to Mar 31) following vaccination should reveal substantial rises in RSV-neutralizing serum antibodies in a subset

of vaccine recipients in the absence of reported RSV disease, comparable to that observed with MEDI and LID ΔM2-2.

If a promising candidate can be identified in these studies, this candidate will be selected for further evaluation in expanded Phase I studies or Phase II studies.

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 (10), and worldwide, RSV infects at least 34 million children under 5 years, with an estimated 3.4 million RSV LRI 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 (11, 12).

RSV is an enveloped RNA virus that is a member of the *Paramyxoviridae* family, genus *Pneumovirus* (13). 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 nucleocapsid protein N, the phosphoprotein P, the large polymerase protein L, the attachment glycoprotein G, the fusion glycoprotein F, the small hydrophobic surface glycoprotein SH, the internal matrix protein M, the 2 nonstructural proteins NS1 and NS2, and the M2-1 and M2-2 proteins encoded by the M2 mRNA. The gene order is: 3'-NS1-NS2-N-P-M-SH-G-F-M2-L-5'. RSV transcription and genome replication take place exclusively in the cytoplasm, and virions form by budding from the apical plasma membrane of respiratory epithelial cells.

Currently, no licensed vaccine against RSV is available, although there is broad consensus that such a vaccine is urgently needed and should be a global health priority. Although passive immunoprophylaxis with the monoclonal RSV-neutralizing antibody palivizumab (Synagis®; MedImmune) is available for high-risk infants, this approach is not feasible for general use. A formalin-inactivated vaccine against RSV was evaluated clinically in the 1960s and did not confer protection; instead, disease enhancement following infection of vaccinees with wt RSV was observed (14). 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 (15, 16).

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-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 4 weeks of age (8).

Human RSV has a single serotype with 2 antigenic subgroups, A and B. The 2 subgroups exhibit a 3- to 4-fold reciprocal difference in neutralization by polyclonal convalescent serum. Analysis of glycoprotein-specific responses in infants by enzyme-linked immunosorbent assay (ELISA) with purified F and G glycoproteins showed that the fusion proteins (F proteins) were 50% related antigenically, and the G proteins were 7% related (17). 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 (18). Thus, the F protein is responsible for most of the observed cross-subgroup neutralization and protection, and a subgroup A vaccine virus is likely to induce a broad immune response against wt RSV of either subgroup. Antibodies to the F protein are one of the endpoints evaluated in this study.

The RSV vaccine to be evaluated in this study was derived using a recombinant deoxyribonucleic acid (DNA)-based technique called reverse genetics (19). The technique of reverse genetics has been used to produce a number of licensed vaccines; among them is FluMist® (MedImmune), the live-attenuated influenza vaccine currently licensed for adults and children. 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. Specific attenuating mutations were characterized in preclinical studies and combined to achieve the desired level of attenuation of this investigational vaccine. 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 trials material) does not contain any recombinant DNA.

This vaccine is a derivative of strain A2, subgroup A, with a deletion of the M2-2 ORF. The RSV M2-2 protein is a small protein (90 amino acids in RSV strain A2) encoded by the second, downstream ORF in the M2 mRNA, which slightly overlaps the 5'-proximal, upstream M2-1 ORF (20). M2-2 is expressed intracellularly at a low level (21), and it is not known whether it is packaged into the virion. cDNA-derived RSV mutants, in which the M2-2 ORF has been silenced or deleted, grow more slowly in vitro than wt RSV (4, 22). Deletion of M2-2 results in increased accumulation of intracellular viral mRNA and decreased accumulation of genome and antigenome. This finding suggests that, during infection by wt RSV, M2-2 plays a role in shifting the balance of RNA synthesis from transcription to RNA replication (4). The increase in mRNA accumulation in cells infected with an M2-2 deleted RSV (ΔM2-2) was accompanied by an increase in the expression of RSV proteins, including expression of the F and G glycoproteins, suggesting that M2-2 deletion mutants might be more immunogenic than wt RSV. M2-2 deletion mutants are highly attenuated in non-human primates but do replicate to detectable titers (22, 23). In addition, 2 previous clinical studies have shown that M2-2 deletion mutants of RSV seem to be refractory to genetic or phenotypic reversion to wt RSV. In order to further attenuate the vaccine virus, additional mutations have been added as described below.

Vaccine Description

As described in Section 1.1, two versions of RSV vaccine candidates with M2-2 deletions, MEDI ΔM2-2 (5) and LID ΔM2-2 were safe and highly immunogenic in RSV-seronegative infants and children, but the level of replication of LID ΔM2-2 (evaluated in IMPAACT 2000/CIR 291, ClinicalTrials.gov identifiers NCT02237209, NCT02040831) was greater than expected, which might be a marker for under-attenuation. To generate a vaccine candidate with a lower level of replication than LID ΔM2-2, D46/NS2/N/ΔM2-2-HindIII was designed. This candidate incorporates the most significant differences between these two RSV vaccine candidates. It uses the LID backbone, but without a deletion and nucleotide substitutions in the 3' noncoding region of the SH gene. The D46/NS2/N/ΔM2-2-HindIII virus also has the MEDI ΔM2-2 assignments at the only two amino acid positions (in the NS2 and N ORFs) that differ between MEDI ΔM2-2 and LID ΔM2-2 (24). The deletion of M2-2 in D46/NS2/N/ΔM2-2-HindIII is comparable to that of MEDI ΔM2-2.

The seed virus was 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 virus passed pre-production testing (Sterility, *Mycoplasma*, Bacteriostasis/Fungistasis**,** and testing for porcine circovirus types 1 and 2; testing performed at CRL under cGMP), and was accepted for manufacturing of the Drug Product under cGMP. For the production of the Drug Product at CRL, Vero cells (MF 11702) were grown in OptiPRO™ serum-free medium. On day 3 post-infection, OptiPro™ medium was removed, and replaced by Dulbecco's modified Eagle medium (DMEM). Antibiotics were not used in any stage of cell passage, virus growth, or vaccine development. The virus-containing supernatant was harvested on Day 6 post-infection and clarified by centrifugation. Intact cells were removed by filtration.

Clarified supernatant in 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; L-Glutamic Acid, 0.0054 M) was dispensed in 0.6 mL aliquots into labeled 2.0 mL cryogenic vials. Vials are snap-frozen and stored at -80ºC ± 15ºC. The Drug Product is a concentrated suspension of live recombinant D46/NS2/N/ΔM2-2-HindIII Vero Grown Virus Vaccine (Lot RSV#011B) 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 Drug Product has a potency of about 6.5 $\log_{10}$ PFU/mL and is diluted to dose on site.

The Final Drug Product, D46/NS2/N/ΔM2-2-HindIII, Lot RSV # 011B, passed all in-vitro and in-vivo testing required for viral vaccines (Detection of Inapparent Viruses in a Viral Vaccine Product, *in vitro* Tuberculosis Testing, PCR-based Reverse Transcriptase Testing, Porcine Circovirus Testing, Sterility, *Mycoplasma*, Bacteriostasis/Fungistasis, Residual DNA testing, DNA Sizing, Endotoxin, General Safety, Determination of the Sucrose Level, pH Determination, Intact Cell Assay, Potency/Infectivity, Identity, Purity, Toxicology and Pharmacology testing). Sequence analysis confirmed that the seed virus and Drug Product, RSV Lot #011B, were of identical sequence. D46/NS2/N/ΔM2-2-HindIII, Lot RSV #011B, was released by CRL for use as an investigational vaccine.

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 (5-9).

Two versions of RSV vaccine candidates with M2-2 deletion have recently been evaluated. The first vaccine candidate, designated RSV MEDI ΔM2-2, was sequentially evaluated in adults, RSV-seropositive children, and RSV-seronegative infants and children (5). RSV MEDI ΔM2-2 was highly restricted in replication; no shedding was observed in RSV-seropositive children, and minimal shedding was detected in RSV-seronegative children, in whom the mean peak titer of virus shed was $10^{1.5}$ PFU/mL. Rhinorrhea occurred more frequently in recipients of RSV MEDI ΔM2-2 (85%) than placebo (44%), although this difference was not significant. Despite its restriction in replication, RSV MEDI ΔM2-2 was highly immunogenic. When we compared vaccine virus replication and antibody responses in RSV-seronegative children who received RSV MEDI ΔM2-2 to those achieved with rA2cp248/404/1030ΔSH, a live-attenuated RSV vaccine candidate that was well tolerated and immunogenic in pediatric Phase I studies (8), we found that RSV MEDI ΔM2-2 vaccine virus shedding was significantly more restricted, yet the post-vaccination RSV-neutralizing serum antibody titers achieved (geometric mean titer [GMT] = 1:97) were significantly greater.

Surveillance during the subsequent RSV season showed that several RSV seronegative RSV MEDI ΔM2-2 recipients had substantial antibody rises without reported illness, suggesting that the vaccine was protective yet primed for anamnestic responses to RSV. The M2-2 deletion was stable in all shed vaccine virus samples that were tested (5). The MEDI ΔM2-2 phenotype may be suitable for an RSV vaccine, pending confirmation of safety. However, because of the overall low titer of shedding, and the lack of shedding in a number of vaccinees, it is possible that a virus that replicates somewhat more efficiently might be more immunogenic, provided it is suitably attenuated. Therefore, we also are evaluating several other ΔM2-2 candidates with differences affecting replication and temperature sensitivity.

The closely related RSV LID ΔM2-2 candidate vaccine virus was evaluated in RSV-seronegative children (IMPAACT 2000/CIR 291, ClinicalTrials.gov identifiers NCT02237209, NCT02040831), 20 of whom received vaccine and 9 of whom received placebo. RSV LID ΔM2-2 was found to be highly infectious; 95% of vaccinees shed vaccine virus, with a mean peak titer of $10^{3.4}$ PFU/mL by viral culture and a mean peak titer of $10^{5.9}$ $\log_{10}$ copies/mL by quantitative real-time polymerase chain reaction (qRT-PCR). The level of replication of RSV LID ΔM2-2 in seronegative children was greater than expected based on previous study of RSV MEDI ΔM2-2. Respiratory or febrile illnesses occurred frequently in both recipients of RSV LID ΔM2-2 (95%) and placebo (78%). One vaccinee experienced a mild LRI (rhonchi) accompanied by shedding of

vaccine virus and rhinovirus. It was not possible to determine whether the vaccine virus played a causal role in this participant's LRI, since an additional respiratory pathogen was also present. However, based upon the overall high level of vaccine virus replication and the concern that this might be a marker for under-attenuation, a decision was made to stop accrual to the study at 29 rather the targeted 51 participants.

Because of the unique properties of the M2-2 deletion mutation, which increases antigen production and seems to increase the inherent immunogenicity per infectious unit of virus**,** a decision was made to introduce additional attenuating mutations into the LID ΔM2-2 backbone to create vaccine candidates that would be more restricted in replication than LID ΔM2-2 and similar to MEDI ΔM2-2. The vaccine for the current protocol, D46/NS2/N/ΔM2-2-HindIII, is closely related to LID ΔM2-2. It was generated by reverse genetics and uses the LID backbone but without a deletion and nucleotide substitutions in the 3' noncoding region of the SH gene. The D46/NS2/N/ΔM2-2-HindIII virus is modeled after MEDI ΔM2-2 assignments at the only two amino acid positions (in the NS2 and N ORFs) that differ between MEDI ΔM2-2 and LID ΔM2-2 (24). The deletion of M2-2 in D46/NS2/N/ΔM2-2-HindIII is comparable to that of MEDI ΔM2-2.

1.3.2 Preclinical Studies

RSV with M2-2 deletion has been extensively studied in vitro and in vivo (4, 21-23). The results indicate that RSV ΔM2-2 is attenuated in 2 small animal models and in non-human primates. RSV ΔM2-2 was also found to be immunogenic in all 3 animal models. Attenuation of an Experimental Lot and of the clinical trial material (CTM) of D46/NS2/N/ΔM2-2-HindIII was confirmed in a nonhuman primate model in African green monkeys (AGMs). Additional information about the preclinical evaluation of D46/NS2/N/ΔM2-2-HindIII can be found in the Investigator's Brochure (IB).

Evaluation of the Attenuation Phenotype of D46/NS2/NΔM2-2-HindIII in Nonhuman Primates

D46/NS2/N/ΔM2-2-HindIII was evaluated for its ability to replicate in the upper and lower respiratory tract (URT and LRT, respectively) of nonhuman primates (NHPs) [AGMs] in 2 independent non-GLP studies. AGMs are semi-permissive for RSV. The first NHP study was done to evaluate an Experimental Lot of D46/NS2/N/ΔM2-2-HindIII. Data from studies of related RSV vaccine candidates with M2-2 deletion (LID ΔM2-2 and LID ΔM2-2 1030s), performed in AGMs from the same group and origin, is included for comparison (Appendix I, Table 10, Table 11, and Table 12). The total doses administered were 2 x 10^6 PFU per animal. The second study (Appendix I, Table 13, Table 14, and Table 15) was done to test the non-clinical safety and immunogenicity of the CTM D46/NS2/N/ΔM2-2-HindIII in AGMs.

Replication and Immunogenicity of an Experimental Lot of D46/NS2/N/ΔM2-2-HindIII in AGMs

Four AGMs, seronegative for RSV, were inoculated intranasal (i.n.) and intratracheal (i.t.) with D46/NS2/N/ΔM2-2-HindIII. Results from studies performed in animals from the same group and origin, inoculated with LID ΔM2-2 and LID ΔM2-2 1030s at the same dose (n=4 per group), were included for comparison. A dose of 1 x 10^6 PFU per site was administered to sedated juvenile male and female AGMs in a 1 mL volume per site (total dose: 2 x10^6 PFU/AGM). Nasopharyngeal (NP) swabs were collected daily on Days 0 through 10 and Day 12, and tracheal lavage (TL) samples were collected every other day from Day 2 through Day 12 from all animals included in the study, and virus shedding was analyzed by plaque assay. Serum RSV neutralizing antibody titers were determined by a complement-enhanced 60% plaque reduction assay with GFP-expressing RSV A2 on Vero cell monolayer cultures incubated at 37°C. Studies were approved by the Animal Care and Use Committee of NIAID, NIH.

Substantial shedding of the RSV LID ΔM2-2 control virus was detectable by plaque assay from the upper and lower respiratory tract over several days, with mean peak titers of 2.9 $\log_{10}$ PFU per mL in the URT, and 4.2 $\log_{10}$ PFU per mL in the LRT (Appendix I, Table 10 and Table 11). In the upper respiratory tract, shedding of D46/NS2/N/ΔM2-2-HindIII was only detectable in two animals on a single day at very low titer. In the lower respiratory tract, shedding of D46/NS2/N/ΔM2-2-HindIII was detectable in all animals, but peak titers were slightly lower than those of LID ΔM2-2. Despite the lower level of virus replication, D46/NS2/N/ΔM2-2-HindIII induced serum neutralizing antibody titers comparable to LID ΔM2-2 in AGMs (Appendix I, Table 12). These results show that at a total dose of 2 x 10^6 PFU, administered i.n. and i.t., D46/NS2/NΔM2-2-HindIII is highly attenuated, yet highly immunogenic in AGMs.

Replication and Immunogenicity of D46/NS2/N/ΔM2-2-HindIII (CTM) in AGMs

In a second study, the CTM D46/NS2/N/ΔM2-2-HindIII was evaluated at a total dose of 1 x 10^6 PFU per animal for its ability to replicate and induce an immune response in AGMs inoculated intranasally and intratracheally, following the same protocol described above (total dose: 2 x 10^6 PFU/AGM). At this dose, D46/NS2/N/ΔM2-2-HindIII was restricted for replication in AGMs (Table 13, Table 14). Shedding of vaccine virus was detectable in nasopharyngeal swabs of 3 out of 4 AGMs; the peak titers detected in these animals were low. In the lower respiratory tract, a low level of virus replication was detectable in all animals over 8 days. Despite the low level of shedding, a robust serum neutralizing antibody response was induced by the CTM D46/NS2/N/ΔM2-2-HindIII (Table 15), confirming that the CTM D46/NS2/N/ΔM2-2-HindIII is highly attenuated yet immunogenic for AGMs.

In summary, compared to the previously characterized RSV vaccine candidate LID ΔM2-2, replication of D46/NS2/N/ΔM2-2-HindIII was slightly reduced in AGMs. Despite the low level of replication, intranasal and intratracheal inoculation with D46/NS2/N/ΔM2-2-HindIII induced a strong neutralizing serum antibody response.

The Drug Product Live Recombinant D46/NS2/N/ΔM2-2-HindIII Vero Grown Virus Vaccine is anticipated to be slightly more attenuated than the previous RSV vaccine candidate LID ΔM2-2. Intranasal administration of D46/NS2/N/ΔM2-2-HindIII to RSV seronegative infants and children is anticipated to result in infection, limited vaccine replication, and the induction of a robust neutralizing antibody response to RSV.

1.3.3 Previous Clinical Experience

The live attenuated recombinant D46/NS2/N/ΔM2-2-HindIII vaccine virus is being evaluated for the first time in humans. This vaccine is a derivative of the live attenuated recombinant RSV LID ΔM2-2 vaccine virus, which is genetically similar to RSV MEDI ΔM2-2, which each have been studied in RSV-seronegative children.

RSV MEDI ΔM2-2

RSV MEDI ΔM2-2, Lot RSV #002A, was evaluated in adults, RSV-seropositive children, and RSV-seronegative children. 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 vaccines. 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. Ten children in this RSV-seropositive cohort received a 10^6 PFU dose of vaccine, and 5 received placebo. Among the vaccinees, 5 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, nor did they have antibody responses to RSV, indicating that there was also no evidence of replication of RSV MEDI ΔM2-2 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 12 of 20 RSV-seronegative vaccinees with a mean peak titer of $10^{1.5}$ PFU/mL; 17 of 20 had vaccine virus detected by qRT-PCR. Four-fold or greater increases in RSV neutralizing antibody occurred in 19 of 20 children, with mean $\log_2$ titers of 2.7 ± 0.9 before vaccination and 6.6 ± 1.1 following vaccination. 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%). 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. When we compared data on vaccine virus infectivity and immunogenicity in RSV-seronegative children to those achieved with rA2 cp248/404/1030/ΔSH, a previous live-attenuated RSV vaccine candidate that was well tolerated and immunogenic in pediatric Phase I studies (8), we found that vaccine virus shedding was significantly more restricted. However, the post-vaccination 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 illness, suggesting that the vaccine was protective yet primed for anamnestic responses to RSV (5). However, conclusions about the safety and tolerability of this candidate were confounded by a high incidence of adventitious respiratory infections among participants during the trial.

RSV LID ΔM2-2

Based upon these data, with the intent to gain additional safety information about the vaccine candidates with ΔM2-2 mutation, we evaluated the closely genetically related vaccine RSV LID ΔM2-2 in RSV-seronegative children at a dose of 10^5 PFU in 0.5 mL. In pre-clinical studies, RSV LID ΔM2-2 behaved similarly to MEDI ΔM2-2. As noted in Section 1.1, clinical data show that LID ΔM2-2 replicated to higher viral titers than RSV MEDI ΔM2-2, as measured in nasal washes from RSV-seronegative vaccinees. MEDI ΔM2-2 and LID ΔM2-2 differ by 2 amino acids, which may confer a small amount of additional attenuation to MEDI ΔM2-2. The modifications in the SH gene in the LID backbone, especially the 112 nt deletion of the 3' noncoding region in the LID ΔM2-2 SH gene, may confer a small increase in replication in vivo. The available pre-clinical assays and experimental animals have been too insensitive to reliably detect these effects.

1.4 Rationale

The IMPAACT Network is testing three further-attenuated versions of RSV ΔM2-2, each bearing additional attenuating elements for increased safety, in RSV-seronegative infants 6-24 months of age in three studies with a design similar to IMPAACT 2000.

The candidates for each protocol described in Table 1, Section 1.1 include:

- IMPAACT Protocol 2011: LID ΔM2-2 1030s
- IMPAACT Protocol 2012: LID cp ΔM2-2
- IMPAACT Protocol 2013: D46/NS2/N/ΔM2-2-HindIII

Each candidate is more attenuated in non-human primates than the candidate studied in IMPAACT 2000: LID ΔM2-2.

Each of these protocols monitors safety, infectivity, and immunogenicity, with particular attention to vaccine virus infectivity and replication (i.e., peak vaccine virus titer in nasal washes, as well as duration of shedding), which are the most quantifiable metrics for the level of attenuation. Each trial stands alone with the purpose to assess safety and immunogenicity in RSV-seronegative infants 6-24 months of age. However, since the studies will have the same study design with centralized laboratory testing, comparison across the studies will be possible to evaluate which candidate vaccine(s) is most promising. It is anticipated that the most promising one or two of the candidates will move forward to an expanded study enrolling additional participants to further evaluate safety and immunogenicity.

In previous studies (summarized in Section 1.3.3), 2 RSV vaccine candidates with M2-2 deletions have been shown to be safe and immunogenic in seronegative infants and children at a dose of 10^5 PFU. Specifically, RSV MEDI ΔM2-2 was the first RSV vaccine candidate with an M2-2 deletion that was tested clinically, and was therefore sequentially evaluated (at the higher dose of 10^6 PFU) in adults and RSV-seropositive infants and children, followed by evaluation of a dose of 10^5 PFU in RSV-seronegative infants and children. As mentioned in Section 1.3.3, in RSV-seropositive cohorts, virus replication and RSV-specific antibody responses were undetectable after intranasal administration at a dose of 10^6 PFU. These results showed that RSV MEDI ΔM2-2 is highly attenuated and does not infect RSV-experienced individuals. Each of the vaccine candidates in IMPAACT 2011, 2012, and 2013 contains the M2-2 deletion, together with an additional attenuating element, and pre-clinical testing showed that this yielded further attenuation in nonhuman primates. Since it was previously shown that RSV MEDI ΔM2-2 did not

infect RSV-seropositive individuals, we would not expect to gain any relevant safety information by testing these further attenuated candidates in RSV-seropositive individuals.

The primary immunogenicity endpoints to be evaluated are RSV neutralizing antibody titer, and RSV F protein antibody (by ELISA). Neutralizing antibody is a well-established and important surrogate marker of effective immunity to RSV disease. Antibodies to the F protein are also associated with cross-subgroup neutralization and protection (18). These assays will be performed at a central laboratory at the CIR at JHU that has performed the assays for the preceding clinical trials.

As a secondary objective, we will study additional details of the B cell response to RSV. RSV neutralizing serum antibody levels represent the most reliable correlate of protection from RSV LRI; the protective role of antibody has been established infants over years of preventive use of palivizumab (25). Recent findings suggest that antibodies to the post-fusion form of the F protein may be most effective in neutralizing RSV. However, new experimental approaches to discern antibody specificities to epitopes present on the pre- and post-fusion forms of the fusion protein have become available. We are planning to study the epitope specificity and the quality (affinity and avidity) of the primary immune response to RSV vaccines. The induction of memory B cells is essential for long-term protection from severe RSV disease. RSV F protein specific B cells will be isolated, and studies on class switching, antibody maturation, and induction of B cell memory will be performed.

1.5 Hypotheses

D46/NS2/N/ΔM2-2-HindIII will be safe and immunogenic in RSV-naive infants.

2 OBJECTIVES

2.1 Primary Objective

The primary objectives of this study are the following:

2.1.1 Safety: To assess the frequency and severity of study product-related solicited and unsolicited adverse events (AEs) from Study Day 0 through midnight of the 28th day following inoculation in vaccinated participants

2.1.2 Safety: To assess study product-related SAE from Study Day 0 through midnight on the 56th day following inoculation for vaccinated participants

2.1.3 Infectivity: To determine the peak titer of vaccine virus shed and duration of virus shedding by each participant

2.1.4 Infectivity: To assess the proportion of vaccinated infants infected with study vaccine

2.1.5 Immunogenicity: To characterize antibody responses (Day 56) to the study vaccine

2.2 Secondary Objectives

The secondary objectives of this study are to:

2.2.1 To characterize clinical outcomes (frequency and severity of symptomatic, medically attended respiratory and febrile illness) in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season

2.2.2 To characterize antibody responses in the vaccine and placebo recipients who experience natural infection to wt RSV during the subsequent RSV season

2.2.3 To characterize the B cell response to vaccine and the quality and epitope specificity of RSV F specific antibody, and to characterize these responses in the vaccine and placebo recipients who experience natural infection to wt RSV during the subsequent RSV season

2.2.4 To characterize the mucosal antibody response to vaccine

2.3 Exploratory Objective

2.3.1 Study samples may be used to compare to samples from other RSV vaccine studies initiated by the Laboratory of Infectious Diseases, NIAID, NIH

3 STUDY DESIGN

IMPAACT 2013 is a companion study to the Johns Hopkins University (JHU) Center for Immunization Research (CIR) protocol 313. The CIR 313 and IMPAACT 2013 protocols have identical primary and secondary objectives, investigational agents, inoculation schedules, evaluation assays and schedules, and safety monitoring and reporting. Because the CIR site does not enroll HIV-exposed infants, the eligibility criteria pertaining to that population are not included in CIR 313.

The study will be conducted in infants at the JHU CIR and selected IMPAACT sites in the United States. The vaccine will be evaluated in RSV-seronegative (i.e., RSV-naïve) infants ≥6 months (180 days) to <25 months (750 days) of age. 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 (6, 8, 26). In these previous studies, live-attenuated RSV vaccines were highly restricted in replication and poorly immunogenic in children with titers ≥1:40 but were far less restricted in replication and highly immunogenic in children with titers <1:40. These data suggest that this neutralizing antibody cutoff can distinguish effectively between RSV-experienced and RSV-naïve children.

The study will be double-blind, randomized, and placebo-controlled. Thirty-three RSV-seronegative participants will be randomized at a ratio of 2:1 to receive vaccine or placebo, respectively. Placebo recipients are needed in pediatric studies to distinguish the background respiratory and febrile illnesses that occur in infants and children from those attributable to study vaccine. These numbers were chosen based upon experience with Phase I evaluation of other live-attenuated respiratory virus candidate vaccines (6, 7, 9) and statistical considerations (see Section 9).

Enrollment will occur between April 1st and October 14th to avoid the time during which wt RSV typically circulates in the community. Specific study phases are described in the paragraphs that follow.

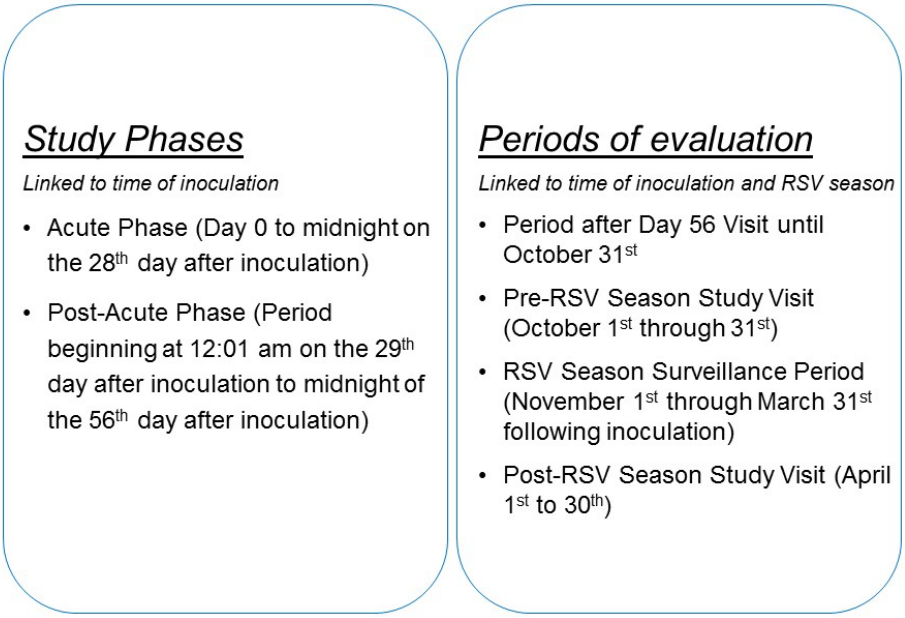
Duration of participation in the initial phase of the study is 56 days, which consists of an Acute and a Post-Acute Phase. During the Acute Phase (Study Day 0 to midnight on the 28th day following inoculation), participants will be contacted daily. These contacts will consist either of: 1) in-person evaluation of interim medical history, clinical assessment, and nasal wash or 2) interim medical history conducted by phone, text, or email. During the Post-Acute Phase (Study Day 29 to midnight on the 56th day following inoculation), study participants will have a scheduled visit on Day 56. The schedule of evaluations during the Acute Phase and Post-Acute Phase is shown in Appendix II.

The study has a third phase that assesses the incidence and severity of illness suggestive of RSV occurring during the RSV season following inoculation. During the RSV Season Surveillance Period, encompassing November 1st to March 31st, site study staff will make weekly contact with the participants to identify medically attended episodes of fever, URI or LRI, or otitis media. Participants who have such an episode will have a study visit to perform a nasal wash to evaluate for RSV and other respiratory pathogens (adventitious agents) (see Appendix III).

Participants will also have a study visit during the pre-RSV season (between October 1st and 31st) to collect a blood sample for immunological assays, which will be used to assess the durability of the vaccine response and to serve as a pre-RSV season specimen. Participants will have a post-RSV season visit (April 1st to April 30th) 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 395 days, depending upon the time of enrollment relative to the RSV season.

Figure 2 summarizes the study phases and periods of evaluation. There may be overlap in these various phases and periods. Accrual will stop effective on October 14th.

Figure 2: Study Phases and Periods of Evaluation



4 STUDY POPULATION

The vaccine will be evaluated in RSV-seronegative infants ≥6 months (180 days) to <25 months (750 days) of age. Approximately 33 evaluable participants will be enrolled (22 receiving the vaccine and 11 receiving a placebo) from US sites only. Infants will be selected for participation according to the criteria in Section 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 infant's medical records from the infant's primary care provider to review for eligibility. The criteria related to the health status and age of household members, day care attendance, scheduled vaccine administration in relation to inoculation of study product, and use of salicylates (except as noted in the medical record) may rely on parent 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 The participant is in good health based on review of the medical record, history, and physical examination, without evidence of chronic disease.

4.1.3 Parents/guardians are willing and able to provide written informed consent as described in protocol Section 12.3.

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 inoculation. Note: results from specimens collected during screening for any study of an RSV vaccine developed by the LID (NIAID, NIH) are acceptable as long as within the 42-day window.

4.1.5 Is growing at a normal velocity for age (as demonstrated on a standard growth chart) AND

- If less than 1 year of age: has a current height and weight above the 5th percentile
- If 1 year of age or older: has a current height and weight above the 3rd percentile for age.

4.1.6 Participant has received routine immunizations appropriate for age (as per national Center for Disease Control Advisory Committee on Immunization Practices [ACIP]). Note: if rotavirus immunization was delayed, "catch-up" rotavirus immunization is indicated only if the participant is age-eligible per ACIP.

4.1.7 Participant is expected to be available for the duration of the study.

4.1.8 If born to an HIV-infected woman, participant must not have been breastfed and 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.

4.2 Exclusion Criteria

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

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

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

4.2.3 Bone marrow/solid organ transplant recipient.

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

4.2.5 Previous receipt of a licensed or investigational RSV vaccine (or placebo in any IMPAACT RSV study) or previous receipt of or planned administration of any anti-RSV product (such as ribavirin or RSV IG or RSV mAb).

4.2.6 Previous anaphylactic reaction.

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

4.2.8 Known hypersensitivity to any study product component.

4.2.9 Heart disease. Note: 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, or will contain, an infant who is less than 6 months of age at the enrollment date through Day 28.

4.2.12 Member of a household that contains another child who is, or is scheduled to be, enrolled in IMPAACT 2011, 2012 or 2013 or another study evaluating an intranasal live-attenuated RSV vaccine, AND there has been or will be an overlap in residency during that other child's participation in the study's Acute Phase (Days 0 to 28).

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

- a person who is greater than or equal to 6 years of age with HIV-related immunodeficiency, defined as having a most recent CD4 T lymphocyte cell count <300 cells/mm^3. CD4 T lymphocyte count must have been measured within 6 months prior to enrollment, or
- a person age 1 year up to less than 6 years with HIV-related immunodeficiency,

defined as having a most recent CD4 T lymphocyte cell percentage <25 or CD4 T lymphocyte count <750 cells/mm^3 (if both values are available, use the lower of the two). CD4 T lymphocyte parameter must have been measured within the 6 months prior to enrollment; or

- a person age less than 1 year with HIV-related immunodeficiency, defined as having a most recent CD4 T lymphocyte cell percentage <30 or CD4 T lymphocyte count <1000 cells/mm^3 (if both values are available, use the lower of the two). CD4 T lymphocyte parameter must have been measured within the 6 months prior to enrollment; or
- a person who has received chemotherapy within the 12 months prior to enrollment; or
- a person receiving immunosuppressant agents; or
- a person living with a solid organ or bone marrow transplant.

Verbal report of CD4 T cell lymphocyte is sufficient documentation if the parent/guardian is confident of history.

4.2.14 Attends a daycare facility and shares a room with infants less than 6 months of age, and parent/guardian is unable or unwilling to suspend daycare for 28 days following inoculation.

4.2.15 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 (rhinorrhea, cough, or pharyngitis) or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media.

4.2.16 Receipt of the following prior to enrollment:

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

4.2.17 Scheduled administration of the following after planned inoculation:

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

4.2.18 Receipt of immunoglobulin, any antibody products, or any blood products within the past 6 months

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

- systemic antibacterial, antiviral, antifungal, anti-parasitic, or antituberculous agents, whether for treatment or prophylaxis, or
- intranasal medications, or
- other prescription 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.

4.2.20 Receipt of salicylate (aspirin) or salicylate-containing products within the 28 days prior to enrollment.

4.2.21 Born at less than 34 weeks gestation.

4.2.22 Born at less than 37 weeks gestation and less than 1 year of age at the time of enrollment.

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

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

4.3 Co-Enrollment Considerations

Co-enrollment to an 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 2013 is allowable for participants already enrolled in IMPAACT P1112, provided all eligibility criteria above are met. The P1112 and IMPAACT 2013 teams should be queried in each case to confirm.

4.4 Recruitment, Screening, and Enrollment Process

Recruitment will take place at IMPAACT sites selected on the ability to recruit and enroll both HIV-exposed, uninfected and HIV-unexposed infants in RSV vaccine studies. Each site will identify the specific 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 site IRBs.

The IMPAACT Operations Center will monitor screening and enrollment through close contact with sites. These data will be provided to the team during regular team calls.

The IMPAACT Data Management Center (DMC) Subject Enrollment System (SES) will be used to track enrollment. When informed consent is obtained, participant identification numbers (PIDs) will be assigned to the infant through the SES. For infants found to be eligible, randomization will occur upon successful entry of required eligibility data into the SES. Successful entry into the SES will generate a study identification number (SID) and blinded prescribing information for the study vaccine regimen to which the infant has been randomly assigned. For infants who are found to be ineligible for the study, or who do not enroll in the study for any reason, a case report form (CRF) will be completed to record the screening outcome. Refer to Section 9.4 for more information on monitoring participant accrual in this study.

4.5 Participant Retention

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

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

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

The study may be terminated for the following reasons:

- Research terminated by sponsor or investigator – applies to the situation where the entire study is terminated by the sponsor or investigator for any reason.
- The study sponsor, IMPAACT, the site IRB, the Office for Human Research Protections (OHRP), NIAID, or the US FDA may decide to end the study.

For any participant who 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 6.11. 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 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 product.

5.1 Study Products

The products that will be administered in this study are:

- Live Recombinant Respiratory Syncytial Virus (RSV) D46/NS2/N/ΔM2-2-HindIII 10^5 PFU per 0.5ml vaccine
- Placebo for RSV vaccine will be 1X L-15 Leibovitz medium (1X L-15) 0.5ml

5.2 Study Product Regimens

Enrolled study participants will receive a single dose of D46/NS2/N/ΔM2-2-HindIII vaccine or placebo, administered as nose drops within 3 days of randomization. Ideally, the date of randomization will be the same as the date of inoculation (see Section 6.2).

5.3 Study Product Formulation

5.3.1 Vaccine

The D46/NS2/N/ΔM2-2-HindIII vaccine is provided in a sterile 2.0 mL cryovial, each containing 0.6 mL of Vaccine (Lot RSV #011B), with a titer of approximately $10^{6.5}$ 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.

Figure 3: Investigational Product Label (Enlarged Sample)

Live recombinant Respiratory Syncytial Virus
D46/NS2/N/ΔM2-2-HindIII
VERO GROWN VIRUS VACCINE

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

Date: 19JUL2016
Vial#:XXXX
Lot: RSV#011B

5.3.2 Diluent for D46/NS2/N/ΔM2-2-HindIII

The diluent for D46/NS2/N/ΔM2-2-HindIII is 1X Leibovitz L-15 medium.

Sterile Water For Injection, USP (SWFI) and 2X Leibovitz L-15 medium are required to prepare the diluent for the vaccine. 2X Leibovitz L-15 medium is a specific lot of Leibovitz L-15 medium. It is a solution of amino acids, sugar, and salt that has been safety tested as described in a Master File (MF 12959), which has been submitted to the FDA.

5.3.3 Placebo for D46/NS2/N/ΔM2-2-HindIII

The placebo for D46/NS2/N/ΔM2-2-HindIII is 1X Leibovitz L-15 medium.

Sterile Water For Injection, USP (SWFI) and 2X Leibovitz L-15 medium are required to prepare the Placebo for the vaccine.

5.4 Study Product Storage

Vaccine will be stored in a secure freezer -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.

Leibovitz L-15 medium will be stored in a secure refrigerator at 2°C to 8°C. Vaccine diluent/placebo will be prepared from new, unopened containers for each use.
Sterile Water for Injection, USP (SWFI) must also be stored in the refrigerator at 2°C to 8°C.

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

Prior to inoculation, 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 for each participant in a Biological Safety Cabinet (BSC) or Compounding Aseptic Containment Isolator (CACI) using aseptic technique. To preserve blinding, yellow overlays will be applied to all prepared syringes.

5.5.1 Diluent for D46/NS2/N/ΔM2-2-HindIII

The diluent is prepared by mixing concentrated 2X Leibovitz L-15 medium with sterile water for injection in 1:1 ratio. The prepared vaccine diluent will be 1X Leibovitz L-15 medium.

Please follow the MOP for detailed instructions on diluent preparation.

5.5.2 Placebo for D46/NS2/N/ΔM2-2-HindIII

Placebo for D46/NS2/N/ΔM2-2-HindIII will be prepared by mixing the concentrated 2X Leibovitz L-15 medium with sterile water for injection in 1:1 ratio. The prepared product will be 1X Leibovitz L-15 medium.

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 syringes will be transported in a cooler 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 concentrated 2X Leibovitz L-15 being removed from the refrigerator.

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

5.5.3 Live Recombinant Respiratory Syncytial Virus (RSV) D46/NS2/N/ΔM2-2-HindIII

Diluent must be prepared prior to removal of the RSV vaccine from the freezer.

If the -80°C freezer where the RSV vaccine is 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 biologic safety cabinet/isolator. 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.

Three vials per dose of undiluted vaccine are always used to prepare the vaccine dose to account for potential differences in titers of the concentrated vaccine. When manipulating the undiluted vaccine, use as small a gauge needle as possible to avoid loss of vaccine in the needle and syringe hub. Concentration of the undiluted vaccine is about $10^{6.5}$ PFU per mL. The frozen vaccine will be thawed and diluted with 1X L-15 to a dose of 10^5 PFU 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 syringes will be transported in a cooler 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 concentrated 2X Leibovitz L-15 is removed from the refrigerator in order to maintain the blind.

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 separate from the study product in the investigational pharmacy 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 BioCision 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/ Inoculation Procedure

All study participants will receive a single dose of study product, administered as a 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 inoculation.

5.7 Study Product Acquisition

The clinical lot preparation of D46/NS2/N/ΔM2-2-HindIII was generated by Charles River Laboratories using the seed virus provided by the National Institutes of Health (NIH).

Specific lot concentrated 2X L-15 Leibovitz Medium that will be used to prepare the Diluent and Placebo is provided by the National Institutes of Health (NIH).

Sterile Water for Injection, USP (SWFI) must be obtained by each site.
Upon successful completion of protocol registration procedures, the Clinical research site (CRS) pharmacists can order the vaccine, the 2X L-15 Leibovitz Medium (which is used to make the diluent and placebo), sterile oral 1ml 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 the vaccine and diluent/placebo shipment.

5.8 Study Product Accountability

The site pharmacist is responsible for maintaining an accurate inventory and accountability record of vaccine and Leibovitz L-15 medium supplies for this study. The vaccine 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. Without the Protocol Chair's written request to unblind, the randomization code may not be released. The site pharmacist will be responsible for maintaining the blind, and pharmacy records will be maintained in the pharmacy only. Partially used vials of vaccine and Leibovitz L-15 medium components may not be saved and reused at a later time.

5.9 Disposition of Used/Unused Study Product

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 the three vaccine vials and place it in the accountability log. Please see the IMPAACT 2013 MOP Appendix VI 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. 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.

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

5.11.1 Prohibited Concomitant Medications

The use of prophylactic antipyretics, decongestants, or antihistamines is not permitted during the Acute Phase (28 days following inoculation); however, use of these medications for treatment of symptoms is allowed.

5.11.2 Precautionary Concomitant Medications

Due to their potentially confounding effect on study immunogenicity results, the following treatments should be avoided after inoculation unless determined to be clinically indicated after consultation with the PSRT:

- Use of an investigational drug or investigational vaccine other than the study product

within 56 days after receiving study product
- 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
- Licensed inactivated vaccine or live-attenuated rotavirus vaccine within 14 days after receiving study product
- Immunoglobulins and/or any blood products
- Licensed live virus vaccine, other than rotavirus vaccine, within 28 days after receiving study product

6 STUDY VISITS AND PROCEDURES

Study visits, except inoculation, may be conducted at one of the clinical sites or as home visits. Inoculation 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 Appendices II and III. Presented in this section is additional information on visit-specific study procedures. Study phases and periods of evaluation are summarized in Figure 2 of Section 3.

Unless otherwise specified, visits may be split, with required procedures conducted on more than one day within the allowable visit window, if necessary. All visits and procedures must be documented in accordance with the NIAID Division of AIDS (DAIDS) policies for source documentation; refer to Section 10 for more information on documentation requirements and completion of CRFs. 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 participant parents/guardians of clinically meaningful physical exam findings and laboratory test results when available.

6.1 Screening Visit

Screening may begin 14 days prior to the start of the 2017 spring enrollment period (i.e., screening may begin on March 18, 2017). As sites consider scheduling for the screening visit, it is important to also consider potential dates of randomization, given that inoculation cannot occur 42 days or more after the screening evaluation. Sites should schedule randomization and inoculation 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 (6-9), screening laboratory tests other than the RSV antibody will not be performed on 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 (27).

Screening Visit Procedures (no more than 42 days prior to enrollment; not prior to March 18)		
Administrative and Regulatory		• Obtain written informed consent • 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 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 complete infant 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.
Laboratory	**Blood**	*Collect blood for*: • Screening and pre-study inoculation 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 2013; it is not necessary to collect another sample. • *Only from participants who are at sites with capacity for processing viable PBMCs:* Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored. If the potential participant had been screened but not enrolled into any study of an RSV vaccine developed by the LID (NIAID, NIH), the stored blood collected for that study at screening may be used for IMPAACT 2013, if it was collected within the 42-day enrollment window for IMPAACT 2013.
Study Product		Not applicable
Schedule Enrollment Visit		Note that Enrollment must be no more than 42 days after screening and within 3 days of randomization.

6.2 Enrollment Visit

Study enrollment and inoculation must occur between April 1st and October 14th inclusive, (outside of the RSV season) and after:

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

Day 0 will correspond to the day of inoculation with study product. Whenever possible, inoculation is to occur on the same day as randomization. Although sites are allowed up to 3 days after randomization to conduct 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, inoculation 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 inoculation, or
- otitis media

Note: if the inoculation will not occur within 3 days of randomization, the site MUST contact the Protocol Safety Review Team (PSRT) via email at impaact.psrt2013@fstrf.org to explain the circumstances and obtain permission to inoculate after the 3-day window period.

If the 42-day window from screening to inoculation is exceeded during the deferral and the infant has already been randomized, the team may grant permission for the infant to be rescreened. If found eligible, the infant will be taken off study, re-enrolled and randomized again (possibly to a different arm than the original randomization). Sites are advised to avoid this situation by scheduling the enrollment visit early in the screening window.

Enrollment Visit Procedures ***Must be no more than 42 days from screening and within 3 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 infant, print and file a copy of the confirmation file
Clinical		• Obtain interim history • Clinical examination: o Perform focused clinical examination including temperature, heart rate, respiratory rate, EENT, lung, heart, and lymph nodes. 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.
	Nasal wash	*Collect nasal wash for:* • RSV antibody assays • RSV viral detection and quantification Note: The nasal wash must be performed prior to administering the study product.
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 inoculation to evaluate for immediate hypersensitivity reactions.
Prepare for follow-up		• Provide the following: temperature card with explanation, temporal and rectal thermometers with instructions for use, illness criteria explanation, and study personnel contact information. Schedule non-visit day-contact for Days 1 and 2 and schedule an in-person visit for Day 3.

*Perform prior to enrollment/randomization

Following the Entry Visit, the parent/guardian will record the infant's temperatures and signs of illness on the temperature card and provide these to study personnel during an in-person visit or non-visit day 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 (28). 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 (28). For study-specific management and grading of temperatures, see Section 8.1.1 and Table 4.

6.3 Acute Phase Visits and Contacts

Refer to Figure 1 for a timeline of study visits. The Acute Phase begins with inoculation and ends at midnight on the 28th day after inoculation. During the Acute Phase of the study, a study 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 participant' parents/guardians for the first 28 days after inoculation. This 28-day period is consistent with the duration of shedding of live-attenuated respiratory virus vaccines in RSV-seronegative infants and children (28-31). A clinical assessment will be completed during visits on Days 3, 5, 7, 10, 12, 14, 17, and 28 (each visit ±1 day) after inoculation. This clinical assessment must be performed by a medical professional.

On non-visit days, study staff will contact the parent/guardian and will record the parent/guardian-provided temperatures and signs of illness. Participants with illness may have additional visits to assess the severity of the illness (see Section 6.10 for Illness Visits).

There will be a final non-visit contact on Day 29 to obtain interim history through midnight on the 28th day following inoculation.

Certain events may trigger study pause or stop during the first 56 days following inoculation, review Section 8.2 for details associated with pausing and stopping rules.

6.3.1 Acute Phase Visits: Study Days 3, 5, 7, 10, 12, 14, 17, and 28 (±1 day)

Study visits during the Acute Phase are scheduled to be conducted at the frequency noted above with a visit window of ± 1 day. If an in-person visit is moved by ± 1 day, then telephone contact is conducted in place of the original interim 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) if the Day 28 Visit is conducted on this day.

Days 3, 5, 7, 10, 12, 14, 17, and 28 Visit Procedures *(each visit from day of inoculation ± 1 day)*		
Clinical		• Obtain interim history • Perform focused clinical examination including temperature, heart rate, respiratory rate, EENT, lung, heart, and lymph nodes. • Record temperature, pulse, and respirations.
Laboratory	**Nasal wash**	*Collect nasal wash for:* • RSV viral detection and quantification
		Day 28 visit only: Collect nasal wash for: • RSV antibody assays • RSV viral detection and quantification
Prepare for follow-up		• Schedule non-visit day-contact and follow-up in-person visits. • *Day 28 only:* review SAE criteria with participants and how to contact study personnel during Post-Acute Phase (Study Day 30 to the Day 56 Visit).

If the infant is diagnosed as having an LRI or otitis media, fever or URI (per Appendix IV) during the Acute Phase, evaluations required for the Illness Visit need to be performed and processed. If illness criteria are met or suspected (see Section 6.10 and Appendix IV), also request that an Adventitious Agent Assay be performed on the nasal wash (see MOP and LPC).

6.3.2 Acute Phase Contacts: Study Days 1, 2, 4, 6, 8, 9, 11, 13, 15,16, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 (±1 day)

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

If the parent reports symptoms suspicious for an LRI, otitis media, fever or URI (per Appendix IV), then an Illness Visit should be scheduled (see Section 6.10).

Days 1, 2, 4, 6, 8, 9, 11, 13, 15, 16, 18,19, 20, 21, 22, 23, 24, 25, 26 and 27 Contact Procedures ***(each visit from day of inoculation ± 1 day)***	
Interim History	• Obtain and document from parent/guardian the participant's previous days' interim history, including any changes in medications and/or immunizations. • Document highest temperature (temporal or rectal)
Prepare for follow-up	• Address any concerns and schedule appointment if necessary.

6.4 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 inoculation. If the Day 28 Visit is conducted on Day 29, it is not necessary to have an additional telephone or email contact with the family on Day 29.

Day 29 Non-Visit Procedures ***(+ 1 day)***	
Interim History	• Obtain interim history through midnight of the 28th day following inoculation • Document highest temperature (temporal or rectal)
Prepare for follow-up	• Address any concerns. • Review SAE criteria with participants and how to contact study personnel during Post-Acute Phase (Day 30 to the Day 56 Visit).

6.5 Post-Acute Phase (Days 30 to 56)

The Post-Acute Phase begins at 12:01 am on the 29th day after inoculation and ends at midnight on the 56th day after inoculation. During the Post-Acute Phase, parents/guardians will be instructed to monitor for and contact the study site if their infant has symptoms that are suggestive a Serious Adverse Event (SAE). If the parent reports an SAE that may meet the study pause or stop criteria (Section 8.2) then an Illness Visit should be scheduled (see Section 6.10).

Certain events may trigger study pause or stop during the first 56 days following inoculation; review Section 8.2 for details associated with pausing and stopping rules.

6.5.1 Day 56 Visit (+7 Days)

The Day 56 Visit should be conducted between 56 and 63 days following inoculation. Because the Post-Acute Phase ends as of midnight on the 56^{h} day following inoculation, 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 inoculation, 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 inoculation. The evaluations expected at this visit are outlined below.

Day 56 Visit *(from day of inoculation +7 Days)*		
Clinical		• Obtain interim history since last contact • Record temperature, pulse, and respirations.
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV • *Only from participants who are at sites with capacity for processing viable PBMCs:* Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasal Wash	*Collect nasal wash for:* • RSV antibody assays
Prepare for follow-up		Follow-up depends on when, during the calendar year, the Day 56 visit is conducted. If the Day 56 Visit is conducted: • Prior to October 1st, schedule for Pre-RSV Season Visit (Section 6.7) • On or after October 1st-the Day 56 Visit will also be the Pre-RSV Season Visit. Therefore, review plans for weekly contact during the RSV Season Surveillance Period (see Section 6.8).

6.6 Period after Day 56 Visit until October 31st

During this period, contact with the participant is not required except for the Pre-RSV Season Study Visit described in Section 6.7. No clinical data will be recorded on CRFs or reported under this protocol except for data as outlined in Table 2 in Section 7.2.

6.7 Pre-RSV Season Study Visit (October 1st to 31st)

The Pre-RSV Season Study Visit is not required if the Day 56 Visit is conducted on or after October 1st (i.e., the samples collected at the Day 56 Visit are sufficient for the Pre-RSV Season Study Visit). Otherwise, an in-person visit is expected of participants during the Pre-RSV Season for collection of a blood sample and nasal wash for RSV antibody assays.

Pre-RSV Season Study Visit (October 1st to 31st)		
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV • *Only from participants who are at sites with capacity for processing viable PBMCs:* Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasal Wash	*Collect nasal wash for:* • RSV antibody assays
Prepare for follow-up		• Review plans for weekly contact during the RSV Season Surveillance Period (see Section 6.8).

6.8 RSV Season Surveillance (November 1st through March 31st following inoculation)

Based on previous data regarding the seasonality of RSV in the Baltimore, MD area (Appendix V), surveillance for RSV-associated disease will be conducted between November 1st and March 31st. 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 following receipt of study product, participants enrolled in this study will be monitored for symptomatic, medically attended, RSV-like illnesses listed below. These contacts may be by weekly telephone, email, or an in-person visit. 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. 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 IV criteria.

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

An Illness Visit should be scheduled within 3 days of a site's study staff notification of any of these events (see Section 6.10).

RSV Season Surveillance (November 1st through March 31st following inoculation)	
Clinical	• Obtain interim history
Prepare for follow-up	• Continue with weekly contacts through March 31st • Schedule an Illness Visit if warranted • Schedule Post-RSV Season Study Visit to take place between April 1st and 30th

6.9 Post-RSV Season Study Visit (April 1st to 30th)

There will be a single in-person visit between April 1st and 30th.

Post-RSV Season Study Visit (April 1st to 30th)		
Administrative and Regulatory		• Inform participant's parent/guardian of study randomization, if known.
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV. • *Only from participants who are at sites with capacity for processing viable PBMCs:* Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasal Wash	*Collect nasal wash for:* • RSV antibody assays

6.10 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 IV and the phase of the study. If the Illness Visit occurs on a day concurrent with a routine Study Visit, a single nasal wash collection is required, however, an Adventitious Agent request form should be completed. Following an Illness Visit, sites should continue to follow participants until resolution of symptoms. Illness visits may occur during any of the following phases of the study.

- Acute Phase: For fever, otitis media, or URI, the Illness Visit must be conducted within 3 days if Grade 1 and within 2 days if Grade ≥ 2.
- Acute Phase: For a possible LRI, with any grade, the assessment will occur within 1 day.
- Post-Acute Phase: For an SAE that may meet the study pause or stop criteria (Section 8.2), an Illness Visit must be conducted within 3 days of site notification.
- RSV Season Surveillance Period (between November 1st and March 31st): The Illness Visit should be scheduled within 3 days of site notification of a medically-attended illness of the following types: fever, URI, LRI or otitis media. 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 • Perform focused clinical examination including temperature, heart rate, respiratory rate, EENT, lung, heart, and lymph nodes. • Record temperature, pulse, and respirations.
Laboratory	**Nasal Wash**	*Collect nasal wash for:* • Viral detection and quantification • Complete Adventitious Agent Assay Request for rRT/PCR on nasal wash for adventitious agents (see MOP and LPC).
Prepare for follow-up		• Schedule follow-up as appropriate

6.11 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.

Early Discontinuation		
Clinical		• Obtain interim history
Laboratory	**Blood**	*Collect blood for:* • Serum antibodies to RSV • *Only from participants who are at sites with capacity for processing viable PBMCs:* Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasal Wash	*If Early Discontinuation Visit is conducted within 56 Days of inoculation (Appendix II), collect nasal wash for:* • Viral detection and quantification • RSV antibody assays

6.12 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

6.12.1 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 wash 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.

Virus Detection

Specimens for viral culture and quantification of vaccine virus shedding will be collected by nasal wash with approximately 20 mL of Ringer's lactate solution once before and approximately 8 times after inoculation as shown in Appendix II. These specimens may also be tested for adventitious agents if the participant is ill. Additional nasal wash specimens for detection of RSV and adventitious respiratory viruses by culture and rRT-PCR will also be collected from participants who meet illness criteria during the initial phase (Day 0 to the Day 56 Visit) and RSV Season Surveillance Period (November 1st – March 31st). Laboratory testing will be performed by personnel that are not involved with clinical assessment to maintain the blinding of the study.

6.12.2 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 6.12, site and local laboratory SOPs, and the LPC. The frequency of specimen collection and testing will be directed by the Schedule of Evaluations (Appendices II and III). 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 inoculation for the screening and the study pre-inoculation, and at the Day 56 Visit for measurement of post-inoculation serum antibodies to RSV. In addition, pre-RSV season and post-RSV season serum specimens will be collected. These samples will be used to determine whether a fourfold or greater rise in antibody titer has occurred during the RSV season, which would signify infection with wt RSV. This will allow comparison of the rate and severity of significant RSV illness following infection with wt virus, as well as comparison of the antibody responses, in vaccine and placebo recipients.

Blood specimens for cryo-preservation of PBMCs will be collected up to 42 days prior to inoculation and at the Day 56 Visit to analyze the B cell response to the vaccine. In addition, pre-RSV season and post-RSV season PBMCs will be collected. These samples will be used to analyze the B cell response to vaccination, and to wt RSV infection during the surveillance season. Plasma leftover from the processing of the PBMCs will be saved. Analysis of the B cell response may include sequencing of participant genes related to their immune response to the vaccine or RSV if the parent/guardian consents to limited genetic testing.

Nasal wash specimens for measurement of secretory immunity will be collected from participants before inoculation, at the Day 28 and 56 Visits after inoculation, and at the Pre-RSV Season and Post-RSV Season Visits. These specimens may be generated from the same nasal wash used for viral culture, except that the aliquot for measurement of secretory immunity will not contain viral transport medium.

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

Plan for Future Use and Storage of Biological Sample

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. With permission, limited human genetic tests may be performed on these samples. 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 of the participants that, by themselves, cannot identify study participants but are linkable to the study databases generated by the main study. The repository database will contain only the study participants' 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 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, IRB approval must be sought prior to any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior IRB approval.

6.12.3 Biohazard Containment

As the transmission of blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel 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 Safety and Monitoring Board (DSMB) are briefly referenced in Section 7.1 and described in detail in Sections 9.4.1 and 9.4.2.

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 record safety-related data on CRFs 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 to their IRBs/IBCs and other applicable review bodies of any unanticipated problems involving risks to participants or others.

7.1.2 Protocol Safety Review Team

A Protocol Safety Review Team (PSRT) will routinely review clinical and laboratory safety data reports prepared by the SDMC. Designees for PSRT members 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):

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

The content, format and frequency of safety monitoring will be agreed upon in advance by the PSRT. Representatives of the product developer may also be included on PSRT discussions, but not in decisions.

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 – at least twice per month during the active immunization phases -- and at least 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 9.4.1.

7.1.3 Data Safety Monitoring Board

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

7.1.4 Sponsor Reporting

Serious and unexpected suspected adverse reactions (SUSAR) as defined in 21 CFR 312.32 will be determined by the IND Sponsor and reported to the FDA and all participating investigators as IND Safety Reports. The sponsor will also submit a brief report of the progress of the investigation to the FDA on an annual basis as defined in 21 CFR 312.33

7.2 Safety-Related Recording on Case Report Forms

Any event that occurs during protocol-specified AE reporting periods (see Table 2), following inoculation with study product, is to be considered an AE and should be evaluated as a potential Expedited AE (per DAIDS). The current section outlines which events should be collected on source documents and which should be recorded on CRFs for inclusion in the database.

AEs may be observed by the site investigator, elicited from the parent/guardian or participant, captured on participant's temperature cards, or volunteered by the parent/guardian or participant. Assessment of safety will include clinical observation and monitoring of hematological, chemical, and immunologic parameters as necessary. Follow-up such as history, physical examination, and laboratory testing and/or treatment may be necessary if a participant experiences an AE. Details of AEs will be properly collected on the source documents, recorded on CRFs, and provided to PSRT and the DSMB in separate semi-annual and annual reports. AEs will be provided to the IRB as defined by the individual IRB policy.

This study has several periods of AE surveillance that have different AE CRF recording requirements. In addition, there may be a period when no AEs are recorded on CRFs if the Day 56 Visit is conducted in advance of the start of the RSV Season Surveillance Period (November 1st). The adverse events (solicited and unsolicited; and SAEs) to be recorded on CRFs and the study phase and the calendar dates during which they are to be reported are defined in Table 3.

Adverse events identified in this study will be recorded on CRFs as signs, symptoms, laboratory test results, and diagnoses, as described in Table 2. Expectations regarding the recording of Concomitant Medications are also detailed in this table.

Table 2: AE CRF Recording Requirements

Study Phase at the time of event onset	Calendar Date	AEs to record on CRFs	Concomitant Medications to record on CRFs
Days 0 through midnight of 28th day following inoculation (Acute Phase)	ANY	• All SAEs • All solicited AEs that meet Appendix IV criteria • All unsolicited AEs (Grades 1 to 4), with the exception of the following conditions if not treated with prescription medication or OTC medications with antipyretic properties: diaper rashes, teething pain, and spitting up. Note: SAEs and LRIs must be reported via DAIDS Adverse Experience Reporting System (DAERS; see Section 7.3.4).	Record these medications on CRFs regardless of whether the related event is recorded on CRFs: • All cough and cold remedies including decongestants, cough suppressants, expectorants • All nasal sprays (except saline spray) • All antihistamines • All antipyretics • All prescription medications For SAE and LRIs: • All concomitant medications related to the recorded event
From 12:01 am on 29th day after inoculation to midnight of the 56th day following inoculation (Post-Acute Phase)	ANY	• All SAEs Note: SAEs must be reported via DAERS (see Section 7.3.4).	• All concomitant medications related to the recorded event
After Day 56 Visit and until RSV Season Surveillance Period	Up to October 31st in year of inoculation	• Grade ≥3 AE or SAE that is deemed related to Pre-RSV Season Study Visit procedures.	• All concomitant medications related to the recorded event
RSV Season Surveillance Period	November 1st to March 31st following inoculation	• Fever, LRI, URI, and/or otitis media that are medically attended • All SAEs Note: these events do not need to meet the Appendix IV criteria. SAEs and Grade ≥3 LRIs must be reported via DAERS (see Section 7.3.4).	For SAE and LRIs (all grades): • All concomitant medications related to the recorded event Medications related to recorded medically attended illness should be documented in source notes.
Post-RSV Season	April 1st-April 30th in the year after the inoculation	• Grade ≥3 AE or SAE that is deemed related to Post-RSV Season Study Visit procedures.	• All concomitant medications related to the recorded event
Throughout study	ANY	• Unresolved AE or SAE with onset date during Day 0 to midnight on the 28th day after inoculation • Unresolved SAE with onset date prior to midnight on the 56th day following inoculation • Unresolved SAE with onset date during RSV Surveillance Period or related to the Pre- or Post-RSV Season Study Visit	• All concomitant medications related to the recorded event

7.3 Expedited Adverse Event (EAE) Reporting

7.3.1 Adverse Event 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 RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual.

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

Where DAERS has not been implemented, sites will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids/paper-eae-reporting. For questions about expedited reporting, please contact DAIDS RSC (DAIDSRSCSafetyOffice@tech-res.com).

7.3.2 Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used by this study.
- In addition to the EAE Reporting Category identified above, other AEs that must be reported in an expedited manner are LRIs occurring during the periods specified in Table 5.
- The study agent for which expedited reporting is required is recombinant live-attenuated respiratory syncytial virus vaccine D46/NS2/N/ΔM2-2-HindIII, Lot RSV#011B/placebo.
- After the protocol-defined AE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).
- With respect to the relationship categories specified for purposes of participant and toxicity management in Section 8.1, the categories of definitely related, probably related, and possibly related will correspond to an assessment of "related" for EAE reporting; the categories of probably not related and not related will correspond to an assessment of "not related" for EAE reporting.

7.3.3 Grading Severity of Events

All solicited AEs and fever will be graded following protocol-defined grading system outlined in Table 3 and Table 4. 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): Version 2.0, dated November 2014**.** 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 http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf?sfvrsn=8 .

Solicited AE Grading

Table 3: Grading Table for Solicited AEs

Severity	Defined
Grade (0) None	Not applicable
Grade (1) Mild	No medical intervention required; may include use of over-the-counter medications managed by the caregiver for treatment of symptoms
Grade (2) Moderate	Outpatient medical intervention by a health care provider required; may include use of over-the-counter and/or prescription medications
Grade (3) Severe	Prolonged medical intervention and/or hospitalization required
Grade (4) Life threatening	Illness requiring hospitalization with intensive care
Grade (5) Death	Event resulting in fatal outcome to the participant

Fever Grading: Temperature Measurement

Table 4: Fever Grading*

Severity	Defined
Grade (0)	≥100.0ºF but <100.4ºF (≥37.8ºC but <38ºC)
Grade (1)	≥100.4ºF but ≤101.4ºF (≥38ºC but <38.6ºC)
Grade (2)	≥101.5ºF but ≤102.4ºF (≥38.6ºC but ≤39.1ºC)
Grade (3)	≥102.5ºF but ≤104.8ºF (≥39.2ºC but ≤40.4ºC)
Grade (4)	≥104.9ºF (≥40.5ºC)

*Applies to any modality of temperature measurement

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

7.3.4 Expedited AE Reporting Period

Table 5 details the events that must be reported in an expedited fashion via DAERS during specific periods of the study.

Table 5: EAE Reporting

Study Phase at the time of event onset	Calendar Date	Events to Report via DAERS
Days 0 through midnight of 28th day following inoculation (Acute Phase)	ANY	• SAEs • LRIs
From 12:01 am on 29th day after inoculation to midnight of the 56th day following inoculation (Post-Acute Phase)	ANY	• SAEs
After Day 56 Visit and until RSV Season Surveillance Period	Up to October 31st in year of inoculation	• Follow-up related to SAEs/LRIs already reported
Pre-RSV Season	October 1st-October 31st in the year of inoculation	• SAEs related to Pre-RSV Season Study Visit procedures
RSV Season Surveillance Period	November 1st to March 31st following inoculation	• SAEs • Grade ≥3 LRIs
Post-RSV Season	April 1st-April 30th in the year after the inoculation	• SAEs related to Post-RSV Season Study Visit procedures

8 PARTICIPANT MANAGEMENT

8.1 Management of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. This includes exacerbation of pre-existing conditions and intercurrent illnesses.

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

Relationship categories for Adverse Events are as follows:

Definitely related The event and administration of study drug are related in time, and a direct association can be demonstrated.

Probably related	The event and administration of study drug are reasonably related in time and the event is more likely explained by study drug than other causes.
Possibly related	The event and administration of study drug are reasonably related in time and the event can be explained equally well by causes other than study drug.
Probably not related	A potential relationship between the event and study drug could exist (i.e., the possibility cannot be excluded), but the event is most likely explained by causes other than study drug.
Not related	The event is clearly explained by another cause not related to study drug.

There are two categories of AEs specific to IMPAACT 2013: solicited and unsolicited. Solicited AEs are described in Section 8.1.1. Unsolicited AEs are all other AEs.

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

8.1.1 Solicited Adverse Events

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

Solicited AEs, whether identified by a parent/guardian or clinician, are only recorded on CRFs if they met the definitions per Appendix IV. Individual symptoms listed in the "events" column that fail to meet the criteria in the "definition" column in Appendix IV are recorded in source documents but are not recorded on the CRFs. During the Acute Phase of this study, Days 0 to 28, solicited AEs meeting the criteria for reporting will be recorded on CRFs, assigned a severity grade (Section 7.3.3 and Table 3 and Table 4), and assessed for relationship to study product (see Section 8.1). For this study, the solicited AEs are defined in Appendix IV 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 recorded on CRFs if a clinical assessment done on the day of the event(s) does/did not confirm their presence. For example, if a parent/guardian reports rhinorrhea on the day of visit, and there is/was no rhinorrhea upon exam, then the participant is considered to not have rhinorrhea that day.

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

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

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

8.1.2 Serious Adverse Event

A Serious Adverse Event (SAE) is an AE, whether considered related to the study product or not, that:

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

8.2 Pausing and Stopping Rules

If any of the following occur in a participant during the specified period after he/she receives study product), additional inoculations and continued enrollment will be temporarily suspended at all sites:

- Through midnight of the 56th day following inoculation:
  - An SAE that cannot be attributed to an etiology or cannot be attributed to a cause unrelated to the study product, OR
- Through midnight of the 28th day following inoculation:
  - An LRI per Appendix IV, OR
  - A fever of Grade 4 OR
  - Any Grade 3 or above solicited AE (other than fever)

If any of these events occur, the site will report the event (as outlined in Sections 7.3) AND will notify the PSRT of the event (including a description of the event) via email at impaact.psrt2013@fstrf.org within 24 hours of site notification. The Protocol Team will notify all sites to suspend enrollment and inoculation. The site reporting the event will complete the event assessment including the collection of viral samples. Respiratory viral samples collected from the participant up to that point will be shipped to the Johns Hopkins University laboratory as soon as possible (see MOP). Study accrual will remain suspended while the SDMC checks study product assignment and virology studies are started. The DSMB will be informed and receive pertinent data regarding the event from the Protocol Chair. The study sponsor will also be notified. Follow-up visits for participants already inoculated will continue as outlined in Appendix II.

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

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

The DSMB will notify the Protocol Chair (via the study sponsor) of their recommendations, and the sponsor and PSRT will determine if enrollment can resume, or if the study needs to be stopped. In the event of an SAE, the study may be resumed if it can be demonstrated to the DSMB that there is no proven causal relationship with the vaccine. In all cases, once a pause occurs, the sites cannot resume enrollment or inoculation until notified to do so by the Protocol Team.

9 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

9.1.1 General Design

The goal of this Phase I, blinded, randomized, placebo-controlled vaccine trial is to assess the safety, infectivity, and immunogenicity of the D46/NS2/N/ΔM2-2-HindIII vaccine candidate in RSV-seronegative pediatric participants. Thirty-three participants will be randomized in a 2:1 ratio to receive either the candidate vaccine or placebo. Data from this study and from a companion study of this vaccine conducted at the Center for Immunization Research (CIR), Johns Hopkins Bloomberg School of Public Health, will be maintained in the same database and will be analyzed together. A subset of the study team will be unblinded to study results prior to completion of the study follow-up (see Section 9.4.1 for details).

9.1.2 Description of the Statistical Methods to be Employed

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

9.2 Outcome Measures

9.2.1 Primary Outcome Measures

- Safety: types and grades of study product-related:
  - solicited AEs as defined in Appendix IV from Study Day 0-28
  - unsolicited AEs from Study Day 0-28
  - SAE (as defined in Section 8.1.2) from Study Day 0-56
- Infectivity:
  - infection with RSV defined as 1) vaccine virus identified in a nasal wash from Study Day 0-28 (a binary outcome based on nasal washes done throughout the study period; Day 0 nasal wash will be counted as baseline) or 2) ≥4-fold rise in RSV neutralizing antibody titer from Study Day 0-56
  - peak titer of vaccine virus shed from Study Day 0-28
  - duration of virus shedding in nasal washes as determined by a) culture and b) RT-PCR from Study Day 0-28

- Immunogenicity:
  - ≥4-fold rise in RSV-neutralizing antibody titer from study entry to Study Day 56
  - antibody responses to RSV F glycoprotein as assessed by ELISA at study entry and Study Day 56

9.2.2 Secondary Outcome Measures

- The frequency and severity of symptomatic, medically attended respiratory and febrile illness in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season.
- The antibody responses in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season.
- The B cell responses to vaccine and the quality and epitope specificity of RSV F specific antibody
- The mucosal antibody responses to vaccine, determined from nasal wash samples

9.3 Sample Size and Accrual

9.3.1 Sample Size and Randomization

Approximately 33 RSV-seronegative infants will be enrolled in the study (IMPAACT 2013 and CIR 313 combined) and will receive either vaccine or placebo in a 2:1 ratio. Assuming an attrition rate of about 10%, approximately 20 vaccine and 10 placebo recipients will provide data for the primary objectives. The sample size was chosen based upon past experience with Phase I evaluation of other live-attenuated respiratory virus candidate vaccines (5-7). The 2:1 randomization ratio will be used to maximize the information obtained regarding the response of

infants to the D46/NS2/N/ΔM2-2-HindIII vaccine. Permuted block randomization will be used to ensure that the 2:1 ratio of treated to control participants will be maintained across time. In the event that a participant is discontinued early from the study and the team decides that additional data would be needed to answer the study objectives, an additional participant may be enrolled in the same treatment arm as the discontinued participant.

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

The following calculations focus on the assessment of the tolerability of the vaccine (Section 2.1) and in particular, occurrence of LRI, which occurs very infrequently in children who participate in these types of studies but would be considered a sentinel safety event if observed in participants infected with vaccine virus.

Table 6 shows the probability of observing 0 events of LRI within the sample of 20 vaccinees, as well as the probability of observing 1 or more events, or 2 or more events, under a range of assumptions concerning the true rate of such events in the participant population represented by this sample. From this table, it can be seen that if the true proportion of LRI (or other AE) is at least 10%, there is a 61% chance of observing 2 or more events in a group of size 20, and an 88% chance of observing at least a single event.

Table 6: The Probability of Observing LRI events in Vaccinees

	N=20		
True underlying probability of LRI or AEs	Pr (0 events)	Pr (1+ events)	Pr (2+ events)
.01	.82	.18	.02
.03	.54	.46	.12
.05	.36	.64	.26
.1	.12	.88	.61
.15	.04	.96	.82

Table 7 presents 90% confidence intervals (CIs) around potential rates of LRI or AEs that might be observed in the sample of 20 vaccinees. The CIs around similar rates in a sample of 10 placebo recipients are also presented. Note that if no LRI or AEs are detected among the 20 vaccinees, we are 90% confident that the true probability of AEs in the population from which the sample is drawn is between 0 and 14%.

Table 7: Percent of Participants Experiencing LRI or AEs with Exact 90% Confidence Intervals

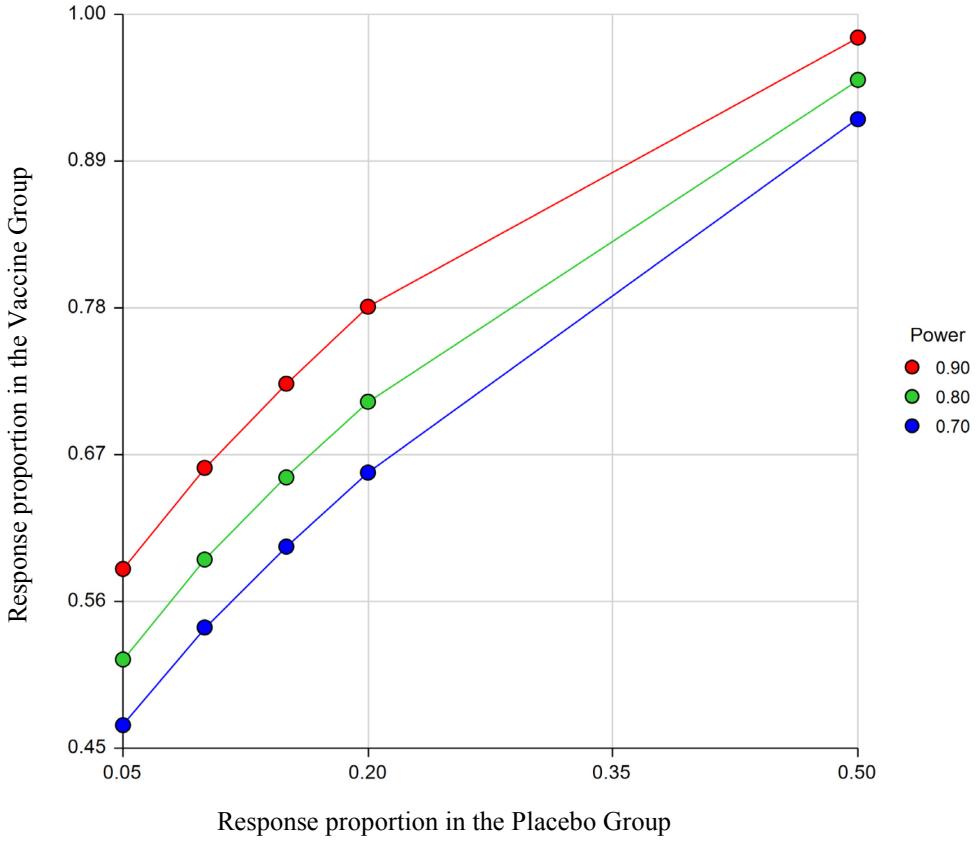
N	% LRI or AEs	90% CI
10	0%	0% -- 26%
20	0%	0% -- 14%
10	10%	1% -- 39%
20	10%	2% -- 28%
10	20%	4% -- 51%
20	20%	7% -- 40%
10	30%	9% -- 61%
20	30%	14% -- 51%

Group sample sizes of 20 in the vaccinated group and 10 in the placebo group would achieve 80% power to detect a difference between the group proportions of about 0.45. The test statistic used is the one-sided Fisher's Exact test. The alpha level of the test was targeted at 0.05. Table 8 presents examples of true group differences that can be detected with 80% power, and Figure 4 displays graphically power curves for 90% power, 80% power, and 70% power given the sample sizes.

Table 8: Magnitude of Difference in Responses Detectable with 80% Power

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

Figure 4: Power curves for comparisons between vaccine and placebo responses



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

With a sample size of 20 vaccinees, the 90% CI around a sample mean peak titer of 2.5 $\log_{10}$, with a SD of 1.5 (from IMPAACT 2000) is (1.92, 3.08). This ensures with 90% confidence that the true population mean peak titer is between 1.92 and 3.08 $\log_{10}$, and with 95% confidence that the true population mean is not lower than 1.92 log10.

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

9.4 Monitoring

Accrual to this study will be monitored by the IMPAACT leadership in accordance with standard operating procedures. The team will monitor feasibility monthly, first based on site activation and then on accrual. Initially, the team will monitor site activation weekly to ensure that an adequate number of sites have been activated to participate in the study. If relatively few of the eligible sites have been activated after the study has been approved for 1 month, the team will re-assess

the feasibility of the study and the reasons why sites have not been activated, and may amend the protocol accordingly.

9.4.1 Monitoring by the Protocol Team

Study Progress and Quality of Study Conduct

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 that will be generated at least monthly by the Statistical and Data Management Center (SDMC). The team has developed a study 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. Accrual performance will be reported by the DMC, by site and across sites, and the team will review and discuss study progress at least monthly. 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.

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 take action with study sites as needed to ensure high quality study conduct throughout the period of study implementation.

Participant Safety

The Protocol Team and the PSRT will closely monitor participant safety through routine review of safety data reports generated by the SDMC. These reports will provide tabulations of adverse events (defined in Section 8.1) identified in enrolled infants, including abnormal laboratory test results, signs, symptoms, and diagnoses, pooled across arms (with the vaccine and placebo arms presented together). The PSRT will review these reports via conference call or other meeting at least twice a month during the first 56 days post inoculation and at least monthly thereafter. At the time of each call, the DAIDS Medical Officer will also review any EAEs (defined in Section 7.3) reported to the DAIDS Safety Office that are not yet reflected in the data reports. The PSRT will continually evaluate the pattern and frequency of reported events and assess for any individual occurrences or trends of concern.

If there is a report of any event that meets the pause/stop criteria, procedures as per 8.2 will be followed.

Blinding/Unblinding

At the end of the RSV Season Surveillance Period, each site will receive a list of the assignments so that each participant's parent/guardian may be informed of their infant's assignment at the final visit (between April 1st and 30th following inoculation).

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

A subset of protocol team members limited to the Protocol Statistician, Protocol Vice Chair, and the two Scientific Investigators of the Laboratory of Infectious Diseases will be unblinded to all data at the completion of the Post-Acute Phase of follow-up (Day 56) for the last participant enrolled in each calendar year to enable more efficient and timely study evaluation and planning for appropriate next steps with respect to RSV candidate vaccine development. Unblinding will occur after all the relevant data that are needed to make decisions have been entered into the database and analyzed.

9.4.2 Monitoring by the NIAID Intramural Data and Safety Monitoring Board

The NIAID Intramural DSMB is constituted to review the safety data of Intramural NIAID clinical studies that require DSMB oversight. The NIAID Intramural DSMB includes independent experts in infectious diseases, biostatistics, and clinical research that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The DSMB will review the protocol prior to opening the study to enrollment. The DSMB will meet at least twice a year or on a schedule specified by the DSMB to review the completeness of the study data, the adherence to the protocol, and AE data. Cumulative safety data (pooled across arms, with the vaccine and placebo arms presented together) will be submitted to the DSMB Executive Secretary for DSMB review. The DSMB will be notified in the event of unintentional or intentional unblinding and consulted in the event that pausing or halting criteria are met. The DSMB Executive Secretary will provide the Protocol Chair with DSMB recommendations promptly, and the official DSMB Report will then be provided in a timely fashion through the office of the NIAID Clinical Director. The Protocol Chair will submit the written DSMB recommendations to the sites' IRBs upon receipt. All SAEs, LRIs, and all IND Safety Reports as specified in Section 7.2 will be reported by the Protocol Chair to the DSMB at the same time they are submitted to the IND sponsor or FDA. The Protocol Chair will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The Protocol Chair will notify the Board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study.

9.5 Analyses

9.5.1 Assessment of Primary Objectives

Safety data from all study participants who have been inoculated will be summarized, including data from participants who discontinue study early or have some missed visits. In the immunogenicity analyses, those who do not provide data for the Day 56 Visit follow-up (due to early discontinuation or missed visit) will be treated as "failures". Sensitivity analyses will be performed to check if the results are consistent with those when these participants are excluded. Participants who receive any of the disallowed treatments listed in Section 5.11 after inoculation may be excluded from the immunogenicity evaluations after the time of the treatment. These participants will, however, be included in the safety evaluations for the duration of the study.

These participants will not be replaced. Details of the analyses listed below will be included in the Statistical Analysis Plan.

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

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

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

Where appropriate, a 1-tailed Wilcoxon rank sum test will be used to test the hypothesis that the vaccinated group will exhibit greater peak viral titers and antibody titers following vaccination compared to the placebo group. A 1-tailed Fisher's exact test will be used to test the hypothesis that the vaccinated group will exhibit a greater proportion of participants who develop fourfold or greater rises in RSV-neutralizing antibody titer following vaccination compared to the placebo group.

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

The study results will be compared with the criteria listed in Section 1.1 to determine if this vaccine is a promising candidate for further evaluation in expanded Phase I studies or Phase II studies.

9.5.2 Assessment of Secondary Objectives

The summary of the frequency and severity of symptomatic, medically attended respiratory and febrile illness in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season will be presented. A line listing of the individual RSV antibody titer pre- and post- RSV Season Surveillance Period will be prepared. In addition, the geometric mean and median antibody titers will be provided for each treatment group. The B cell responses to vaccine as well as the quality and epitope specificity of RSV F specific antibody will be summarized for each treatment group. A line listing of the mucosal antibody response detected in nasal wash specimens will be prepared.

10 DATA HANDLING AND RECORD KEEPING

10.1 Data Management Responsibilities

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

Study sites must maintain adequate and accurate research records containing all information pertinent to the study for all screened and enrolled participants, including CRFs and supporting source data. In maintaining these records, sites must comply with the standards of source documentation specified in the DAIDS policy on Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials.
All DAIDS policies referenced in this section are available at:
https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures

CRFs are completed by study site staff and, following quality control and quality assurance reviews, are entered using a remote data entry system and transferred electronically to the DMC. Selected laboratory data are transferred electronically to the DMC through the LDMS.

At the DMC, computerized checks are applied to the transferred data and, when required, data queries are issued for resolution by study site staff. All data must be transferred to the DMC within timeframes specified in the forms instructions; queries must also be resolved in a timely manner.

Further information on the study CRFs and IMPAACT data management procedures, including a Forms Manual: Policies and Procedures for Forms Completion for DAIDS-Sponsored Clinical Trials, a comprehensive Computing Manual, and a User Manual for the Subject Enrollment System, are available on the DMC portal at www.fstrf.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/sites/default/files/documents/daids-sourcedocpolicy.pdf

Study-related documentation will be completed as required by the IRB, the sponsor, and regulatory authorities. Continuing review documentation will be submitted by the site investigator to the IRB as specified by the IRB. An annual report will be submitted by the sponsor to the FDA based on the anniversary date that the IND for the D46/NS2/NΔM2-2-HindIII vaccine went into effect. These reports will provide a brief description of the progress of the investigation as outlined in 21 CFR 312.33 and will include any revisions of the protocol not previously submitted to the FDA.

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, site drug regulatory authorities, site IRBs/IBCs, OHRP, and other applicable regulatory entities. Records must be kept on-site throughout the period of study implementation; thereafter, instructions for off-site storage may be provided by NIH. No study records may be removed to an off-site location or destroyed prior to receiving approval from NIH.

10.3 Clinical Investigator's Brochure

Investigators will receive the current version of the Clinical Investigator's Brochure (IB) that comprehensively describes all the available preclinical experience with the 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 policy on Requirements for Clinical Quality Management Plans, which is available at: https://www.niaid.nih.gov/sites/default/files/documents/qmppolicy.pdf

11 CLINICAL SITE MONITORING

Site monitors under contract to NIAID or NICHD will visit study sites to inspect study facilities and review participant study records including consent forms, CRFs, 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. The 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.

The trial will be conducted in compliance with this protocol, ICH GCP guidelines, and any applicable regulatory requirement(s). The study site monitoring will be conducted according to the "NIAID/DAIDS and NICHD Clinical Research Site Monitoring Guidelines".

The site investigator or designee will make study documents (e.g., consent forms, CRFs) and pertinent medical or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NIAID staff for confirmation of study data. The sponsor will retain originals of the Form FDA 1572 and copies of other study documents as deemed necessary.

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 CRF 46; subsequent to initial review and approval, IRBs/IBCs must review the study at least annually. Site investigators must also promptly report to the IRB/IBC any changes in the study and any unanticipated problems involving risks to participants or others.

All IRB/IBC policies and procedures must be followed and complete documentation of all correspondence to and from the 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 (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, the site investigator is responsible for providing the IRB 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 the IRB by the investigator in accordance with IRB guidelines and government regulations.

12.2 Vulnerable Participants

The NIH is mandated by law to ensure that children be included in clinical research when appropriate (32, 33). This study responds to that mandate and will provide clinical research data to inform RSV vaccine infectivity, safety and immunogenicity in children. Nonetheless, the infants who take part in this study are considered vulnerable participants per the US Code of Federal Regulations, and site 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). IRBs/IBCs 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 with the requirements of the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research, which is available at: https://www.niaid.nih.gov/sites/default/files/documents/enrollingchildrenrequirements.pdf

12.3 Informed Consent

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 IRB prior to its use.

Written informed consent for infant 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 infant 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 are not expected to receive any direct study product-related benefit from enrollment in this study. 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

12.5.1 Venipuncture

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

12.5.2 Nasal Wash

Risks occasionally associated with nasal wash include pain or discomfort and occasionally epistaxis. Nasal washes are not standard care in well children and are not usually performed on ill children, although many parents/guardians are advised to use saline nose drops and nasal bulb suction (the 2 components of the nasal wash procedure used in this study) to clear a young child's congested nostrils during a URI.

12.5.3 Receipt of Study Product

If the D46/NS2/NΔM2-2-HindIII vaccine 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 we have no present knowledge. Parents/guardians will be informed of any such risks should further data become available.

12.6 Reimbursement/Compensation

Compensation will be provided to the participant's parent/guardian based on each site's standard. The amount must be reviewed and approved by each sites' IRB. Compensation will be in accordance with each institution's IRB policies and will be specified in site-specific ICFs or other materials if applicable per IRC/EC 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, CRFs, 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 policies 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 obtained for this study from 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. Because the certificate cannot be enforced outside of the US, however, it applies only to US sites and 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 tests. 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 product 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 independent clinical site monitors who will perform monitoring visits as described in Section 11. As part of these visits, monitors will inspect study-related documentation to ensure compliance with all applicable US and local 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, as appropriate, by their local IRB/IBC, local IBC, and any other applicable regulatory entity. 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 IRB/IBC 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:
http://rsc.tech-res.com/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 and local 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.

13.4 Protocol Deviation Reporting

Per the policy for Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials, 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.

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

Deviations should be reported to site 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 1 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 Network MOP.

13.5 ClinicalTrials.gov

This protocol is not subject to the United States Food and Drug Administration Amendments Act of 2007 (FDAAA). However, it will be registered in ClinicalTrials.gov to meet International Committee of Medical Journal Editors requirements.

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, and NIAID policies. Any presentation, abstract, or manuscript will be made available for review by the pharmaceutical and NIAID sponsors prior to submission. Publication or presentation approval will conform to any CRADA or other collaborative agreement in place.

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APPENDICES

Appendix I: Tables Referenced in the Background Section

Table 9: Comparison of Genomic Sequence D46/NS2/N/ΔM2-2-HindIII, Lot RSV#011B, to recombinant wt RSV A2 D46

Gene Region	RSV Nucleotide[1]					Encoded Amino Acid, Position in ORF			Motif; rationale for mutation
	nt position[1]	rRSV A2 D46 (KT992094)	D46/NS2/N/ΔM2-2-HindIII			rRSV A2 D46 (KT992094)	D46/NS2/N/ΔM2-2-HindIII		
			cDNA, Seed Lot	CTM			cDNA, Seed Lot	CTM	
NS2	779	A	G	G		K51	R51	R51	NS2 (K51R)
N	1209	A	G	G		T24	A24	A24	N (T24A)
N	1938	A	G	G		S266	S266	S266	
P	2485	G	G	G/A		D47	D47	D47N	dimorphism, 40% adenosine 40% asparagine residue
SH	4537-39	TTT	TTT	TTT/ TTTT[3]		ncr	ncr	ncr	30% of population with insertion of T residue[3]
M2-2	8199	T	A	A					HindIII restriction site
M2-2	8200	C	G	G					
M2-2	8202-434		234 nt deletion	234 nt deletion					Deletion of M2-2 ORF
L	15,064 -68	A_5	A_5	A_5, A_6, A_7 [3]		ncr	ncr	ncr	30% of population with insertion of A residue; 70% of population with insertion of two A residues[3]

[1] Genomic position in reference to wt recombinant RSV strain A2 D46 ((2); Genbank accession number KT992094). The term "D46" refers to a specific sequence and cDNA of strain A2. The numbering of all sequence positions is based on the complete sequence of wt recombinant RSV strain A2 D46 that was described by Collins et al. (2), which is the parent of the present vaccine strains. All sequences are positive-sense.

[2] ncr, non-coding region

[3] Insertions of additional nucleotides in noncoding regions or in runs of adenosine residues in gene end signals are frequently seen in clinical RSV isolates, and are considered inconsquential.

Differences between LID ΔM2-2 and D46/NS2/N/ΔM2-2-HindIII are indicated in light shading.

Table 10: Viral titers of nasopharyngeal swab samples from AGMs inoculated with LID ΔM2-2, LID ΔM2-2 1030s, or D46/NS2/N/ΔM2-2-HindIII[a]

RSV Vaccine candidate	AGM ID	NP virus titer (log_{10} PFU/mL) on indicated days[b]											Duration of shedding[c]	Peak virus titer	Sum of daily titers[d]
		1	2	3	4	5	6	7	8	9	10	12			
LID ΔM2-2 (Study 1)	7806	-	1.4	1.7	2.7	2.6	4.0	3.9	1.4	-	2.7	-	9	4.0	21.4
	7705	-	-	-	2.7	2.3	3.6	2.4	1.2	-	-	-	5	3.6	14.3
	7747	-	-	1.3	0.7	-	1.5	1.3	-	-	-	-	5	1.5	7.2
	7674	-	0.7	-	-	-	2.3	1.8	1.5	-	-	-	7	2.3	8.8
	Mean:												***6.5***	***2.9***	***12.9***
LID ΔM2-2 1030s (Study 1)	8033	-	-	-	-	-	-	-	-	-	-	-	0	0.35	3.9
	7720	-	-	-	-	-	-	-	-	-	-	-	0	0.35	3.9
	7844	-	-	-	-	-	-	-	-	-	-	-	0	0.35	3.9
	7847	-	-	-	-	-	-	-	-	-	-	-	0	0.35	3.9
	Mean:												***0***	***0.35***	***3.9***
D46/NS2/N/ΔM2-2-HindIII (Study 2)	8417	0.7	-	-	-	-	-	-	-	-	-	-	1	0.7	4.2
	8489	-	-	-	-	-	-	-	-	-	-	-	0	0.35	3.9
	8515	-	-	-	-	-	-	-	-	-	-	-	0	0.35	3.9
	8574	-	-	-	-	-	-	-	1.2	-	-	-	8	1.2	4.7
	Mean:												***2.3***	***0.7***	***4.2***

[a] *AGMs were inoculated by the combined intranasal and intratracheal routes with 10^6 PFU of the indicated virus in a 1 mL inoculum per site (total dose: $2x10^6$ PFU per animal). The AGM studies were approved by the Animal Care and Use Committee of NIAID, NIH.*

[b] *Combined nasopharyngeal (NP) swabs were placed in 2 mL of L-15 medium with sucrose phosphate buffer as stabilizer. Virus titrations were performed on Vero cells at 37°C. The lower limit of detection was 0.7 log_{10} PFU/mL. Samples with no detectable virus are represented as "-". Peak titers for each animal are underlined.*

[c] *The period of days from the first to the last day on which virus was detected, including negative days (if any) in between.*

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

Table 11: Viral titers of tracheal lavage samples from AGMs inoculated with LID ΔM2-2, LID ΔM2-2 1030s, or D46/NS2/N/ΔM2-2-HindIII[a]

RSV vaccine candidate	AGM ID	Tracheal lavage virus titer (log_{10} PFU/mL) on indicated days[b]						Duration of shedding[c]	Peak virus titer	Sum of daily titers[d]
		2	4	6	8	10	12			
LID ΔM2-2 (Study 1)	7806	2.5	3.4	4.6	-	-	-	7	4.6	12.6
	7705	1.6	-	3.3	1.5	-	-	9	3.3	8.5
	7747	1.8	1.0	6.0	2.3	-	-	9	6.0	12.5
	7674	-	1.3	2.7	2.3	1.0	-	9	2.7	8.7
	Mean:							***9***	***4.2***	***10.6***
LID ΔM2-2 1030s (Study 1)	8033	-	-	-	-	-	-	0	0.7	4.2
	7720	-	-	-	-	-	-	0	0.7	4.2
	7844	-	-	-	-	-	-	0	0.7	4.2
	7847	-	-	-	-	-	-	0	0.7	4.2
	Mean:							***0***	***0.7***	***4.2***
D46/NS2/N/ΔM2-2-HindIII (Study 2)	8417	-	2.3	2.5	1.6	1.3	-	7	2.5	9.1
	8489	1.0	2.7	3.2	3.2	-	-	9	3.2	11.5
	8515	1.3	2.0	-	1.7	-	-	9	2.0	7.1
	8574	1.8	2.1	2.2	1.7	-	-	9	2.2	9.1
	Mean:							***9***	***2.5***	***9.2***

[a] *AGMs were inoculated by the combined intranasal and intratracheal routes with 10^6 PFU of the indicated virus in a 1 mL inoculum per site (total dose: 2 x 10^6 PFU per animal).*

[b] *On days 2, 4, 6, 8, 10, and 12, tracheal lavage was performed with 3 mL of PBS. Virus titrations were performed on Vero cells at 37°C. The lower limit of detection was 1.0 log_{10} PFU/mL of lavage solution. Samples with no detectable virus are represented as "-". Peak titers for each animal are underlined.*

[c] *The period of days from the first to the last day on which virus was detected, including negative days (if any) in between.*

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

Table 12: Neutralizing antibody titers of AGMs inoculated with LID ΔM2-2, LID ΔM2-2 1030s, or D46/NS2/N/ΔM2-2-HindIII[a]

RSV Vaccine candidate	AGM ID	Neutralizing antibody titers ($PRNT_{60}$, reciprocal log_2) on indicated days[b]		
		0	21	28
LID ΔM2-2 (Study 1)	7806	<3.3	7.2	7.2
	7705	<3.3	8.8	8.2
	7747	<3.3	8.3	8.4
	7674	<3.3	6.7	6.2
	Mean:	<3.3	7.8	7.5
LID ΔM2-2 1030s (Study 1)	8033	<3.3	5.4	6.6
	7720	<3.3	<3.3	<3.3
	7844	<3.3	<3.3	4.3
	7847	<3.3	6.8	6.8
	Mean:	<3.3	4.7	5.2
D46/NS2/N/ΔM2-2-HindIII (Study 2)	8417	<3.3	8.8	10.4
	8489	<3.3	6.6	8.0
	8515	<3.3	6.1	6.4
	8574	<3.3	9.4	8.2
	Mean:	<3.3	7.7	8.3

[a] AGMs were inoculated i.n. and i.t. with 10^6 PFU of the indicated virus in a 1 mL inoculum per site (total dose = $10^{6.3}$ PFU per animal).
[b] On days 0, 21, and 28 p.i., serum was obtained. Neutralizing antibody titers were determined in a 60% plaque reduction neutralization assay. The lower limit of detection was 3.3 (1:10).

Table 13: Viral Titers of Nasopharyngeal Swab Samples from AGMs Inoculated with CTM D46/NS2/N/ΔM2-2-HindIII, Lot RSV#011B

Virus Test Article[a]	ID	NP virus titer (log_{10} PFU/mL) on indicated days[b]											Peak virus titer	Sum of daily titers [d]
		1	2	3	4	5	6	7	8	9	10	12		
D46/NS2/N/ ΔM2-2-HindIII Lot RSV#011B[c]	9041	-	-	-	-	-	-	-	-	-	-	-	0.35	3.9
	8938	-	-	-	-	1.3	1.3	1.3	1.9	1.0	-	-	1.9	8.9
	8926	-	-	-	-	-	0.7	-	1.4	1.4	-	-	1.4	6.3
	8911	-	-	-	0.7	1.0	1.0	2.2	2.3	1.0	-	-	2.3	10.0
	***Mean*:**	**-**	**-**	**-**	**0.4**	**0.8**	**0.8**	**1.1**	**1.5**	**0.9**	**-**	**-**	**1.6**	**7.3**

[a] *Monkeys were inoculated i.n. and i.t. with $10^{6.0}$ PFU of D46/NS2/N/ΔM2-2-HindIII in a 1 mL inoculum per site (total dose =2x10^6 PFU/AGM).*
[b] *Virus titrations were performed on Vero cells. The lower limit of detection was 0.7 log_{10} PFU/mL. RSV could not be detected in any of the samples, represented as "-".*
[c] *CTM vial numbers 0019 to 0021, 1267 to 1269, 2483, 2484, and 2486*

Table 14: Viral Titers of Tracheal Lavage samples from AGMs Inoculated with CTM D46/NS2/N/ΔM2-2-HindIII, Lot RSV#011B

Virus Test Article[a]	ID	TL virus titer (log_{10} PFU/mL) on indicated day[b]						Peak virus titer	Sum of daily titers [d]
		2	4	6	8	10	12		
D46/NS2/N/ ΔM2-2-HindIII Lot RSV#011B[c]	9041	2.0	1.9	2.3	0.7	-	-	2.3	8.3
	8938	1.0	1.6	2.5	2.5	-	-	2.5	9.0
	8926	0.7	0.7	2.6	1.7	-	-	2.6	7.1
	8911	2.2	2.6	0.7	3.0	-	-	3.0	9.8
	***Mean*:**	***1.5***	***1.7***	***2.0***	***2.0***			***2.6***	***8.6***

[a] *Monkeys were inoculated i.n. and i.t. with $10^{6.0}$ PFU of D46/NS2/N/ΔM2-2-HindIII in a 1 mL inoculum per site (total dose =2x10^6 PFU/AGM)*
[b] *Virus titrations were performed on Vero cells at 37°C. The lower limit of detection was 1.0 log_{10} PFU/mL. Samples with no detectable virus are represented as "-". D46/NS2/N/ΔM2-2-HindIII is strongly restricted in the LRT of AGMs.*
[c] *CTM vial numbers 0019 to 0021, 1267 to 1269, 2483, 2484, and 2486.*
[d] *The sum of daily titers is used as an estimate for the magnitude of shedding (area under the curve). A value of 0.7 was used for samples with no detectable virus.*

Table 15: Serum $PRNT_{60}$ Titers from AGMs Inoculated with the CTM D46/NS2/N/ΔM2-2-HindIII, Lot RSV#011B

Virus Test Article	ID	RSV Neutralization Titer (Log_2 of reciprocal) on days		
		0	21	28
D46/NS2/N/ ΔM2-2-HindIII Lot RSV#011B[c]	9041	< 3.3	6.9	6.4
	8938	< 3.3	7.1	7.2
	8926	< 3.3	6.7	6.1
	8911	< 3.3	7.2	7.2
	***Mean*:**	**-**	***7.0***	***6.7***

[a] *CTM vial numbers 0019 to 0021, 1267 to 1269, 2483, 2484, and 2486*. On days 0, 21, and 28, serum was obtained. Neutralizing antibody titers were determined in a 60% plaque reduction neutralization assay. The lower limit of detection was 3.3 (1:10). Samples below the lower limit of detection are recorded as "-".

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

		ACUTE PHASE																					POST-ACUTE PHASE			
	Screening	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18-27 (contact each day)	Day 28	Day 29	Day 30-55	Day 56	Illness Visit	Early DC
In person visit	X	X			X		X		X			X		X		X			X		X			X	X	X
Non-visit contact			X	X		X		X		X	X		X		X		X	X		X		X	Per 6.5			
Informed consent	X																									
History	X																									
Interim History		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Per 6.5	X	X	X
Physical exam (full)	X																									
Clinical assessment (focused PE)		X			X		X		X			X		X		X			X		X				X	
Administer study product		X																								
Blood for: immunologic assays	5mL																							5mL		5mL
Blood for: cellular immune assay (viable PBMCs)	3mL																							3mL		3mL
Nasal wash for: RSV antibody		X																			X			X		X
Nasal wash for: viral detection & quantification		X			X		X		X			X		X		X			X		X				X	X
Request adventitious agent assay																									X	
Total blood volume	8mL	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	8mL	--	8mL

Appendix III: Schedule of Events: RSV Pre-season Sampling, seasonal surveillance, and Post-season Sampling

	Pre-RSV season	Weekly contact	Post-RSV season	Illness Visit	Early DC
Visit Period	Oct 1st to Oct 31st	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		5 mL
Blood for: cellular immune assay (viable PBMCs)	3 mL		3 mL		3 mL
Nasal wash for antibody	X		X		
Nasal wash for: viral detection & quantification				X	
Request adventitious agent assay				X	
TOTAL BLOOD VOLUME	8 mL	--	8 mL	--	8 mL

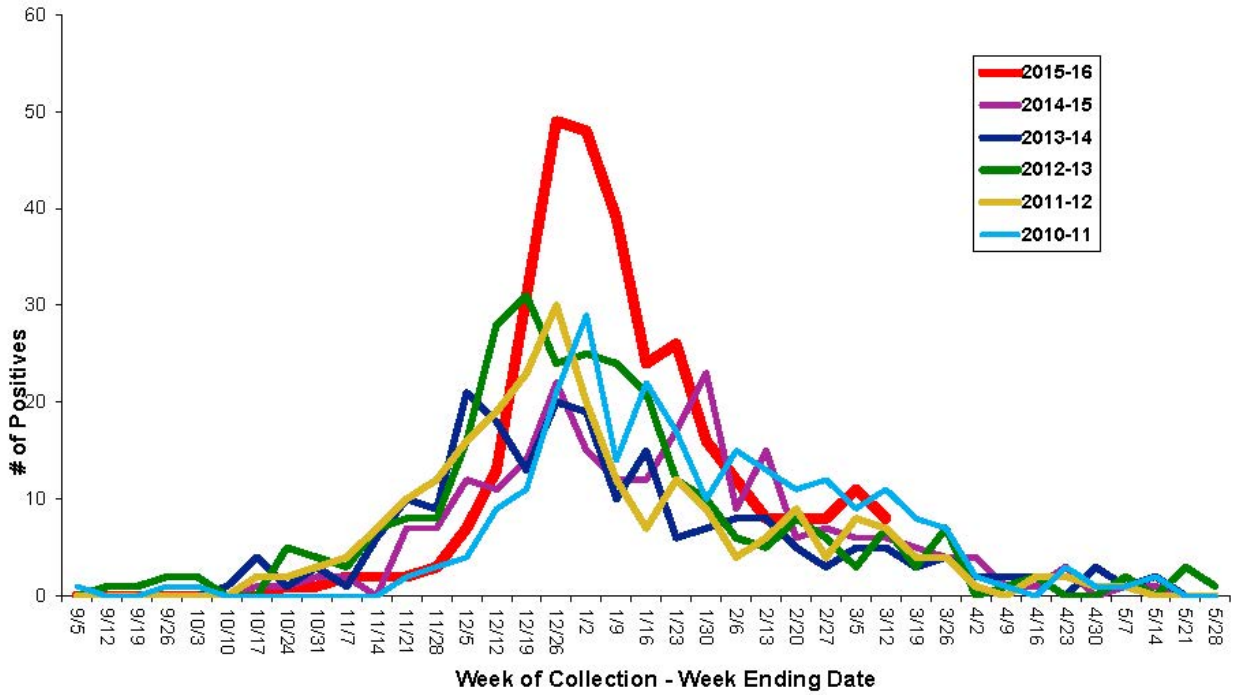
Appendix IV: Definitions of Solicited Adverse Events

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) [1,2,3]	Barking cough, hoarseness, and inspiratory stridor
Rhonchi[2,3]	Coarse breath sounds which are not transmitted noises from the upper airway and do not clear with cough.
Rales[2,3]	Abnormal lung sound heard through a stethoscope. Rales may be sibilant (whistling), dry (crackling) or wet (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.

NOTE: Solicited AEs will only be recorded on CRFs according to criteria defined in Section 7.2

Appendix V: RSV Seasonality in Baltimore



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

Appendix VI: Sample Informed Consent Form

DIVISION OF AIDS
INTERNATIONAL MATERNAL PEDIATRIC ADOLESCENT AIDS CLINICAL TRIALS (IMPAACT) NETWORK

For protocol: 2013
Phase I Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-Attenuated Respiratory Syncytial Virus Vaccine, D46/NS2/N/ΔM2-2-HindIII, Lot RSV#011B, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age
Version 1.0, dated 14 February 2017

SHORT TITLE FOR IMPAACT 2013: Safety and Immunogenicity of a Single Dose of the D46/NS2/N/ΔM2-2-HindIII Vaccine

INTRODUCTION

You are being asked to allow your baby to take part in this research study to test a vaccine to prevent Respiratory Syncytial Virus (RSV) illness in infants. This study is sponsored by the National Institutes of Health (NIH). The doctor in charge of this study at this site is: [*Site: insert name of site investigator*]. Before you decide if you want your baby 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 baby to take part in this study, you will be asked to sign 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 fighters) response of infants to a single dose of a vaccine against a virus called respiratory syncytial virus, or RSV. The study will measure the amount of vaccine virus that sheds (comes) from each infant receiving the vaccine. It will tell us how stable and strong the vaccine was during the study. This research study is testing an experimental vaccine. This vaccine has not been licensed by the U.S. Food and Drug Administration (FDA). Your baby was chosen to be in this study because your baby is at least 6 months (180 days) old and less than 25 months (750 days) old, has not had RSV, and is otherwise healthy.

RSV is a virus (germ) that can cause breathing problems in infants and 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 a live virus vaccine 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 vaccine in this study contains a live, weakened form of RSV and is given as nose drops. This vaccine has not been tested in humans. However, other RSV vaccines very similar to this one have 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 baby to participate in this study. If you agree, we will give your baby either 1 dose of vaccine or 1 dose of placebo. The placebo is made of water, salt, vitamins, and sugar that is gentle on the inside of the nose. It is sterile and approved for use on people. The placebo has no vaccine in it. Up to 33 babies who have not already had an illness caused by RSV virus will take part in the study.

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

The next few paragraphs provide an overview of the study procedures. After that, there are lists of the specific procedures that will be completed at different visits during the study.

At the first visit, your baby's blood will be tested to see if your baby has had RSV in the past. You will be told the result of the test. If your baby goes on the study, you will be told whether he/she got vaccine or placebo after the end of the study in the spring. You will not receive information about your baby's response to the vaccine, but you will receive a summary of the overall response to the vaccine for everyone in the study.

If you agree to allow your baby to take part in this study, you will be asked some questions to be sure he/she can be in this study.

Your baby cannot take part in this study if he/she already has antibodies against RSV, which means they already had the RSV illness, lives with people who have weak immune systems, or is not well. Your baby cannot take part in this study if he/she lives with or is in a daycare room with babies younger than 6 months of age, unless you are able to keep your baby out of daycare for 28 days after he/she receives vaccine or placebo. Your baby 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 baby gets any routine vaccines for the 28 days after the study vaccine or placebo. We ask that your baby does not take part in any other experimental vaccine or drug studies for 8 weeks after they receive vaccine or placebo. We will ask you to review and sign this study consent prior to administering the vaccine/placebo to your baby. At that time, we will ask you to answer questions to see how well you understand the study.

The vaccine/placebo will be given to your baby by gently squirting it inside his/her nose like nose drops. The amount is very small (a few drops). Your baby will need to lie down on his/her back for one minute after getting the vaccine/placebo. About 2 of each 3 enrolled babies will get the RSV vaccine and approximately 1 of each 3 enrolled babies will get nose drops without vaccine (placebo). Whether your baby gets the vaccine or nose drops without the vaccine (placebo) will be decided randomly by computer, like flipping a coin. Neither you nor the study doctors or study nurses will know whether your baby got the vaccine or placebo until the study ends, but this information can be made available to the study doctor if needed.

Your baby will be in this study until April of the year after he/she started the study. For the first 8 weeks after getting vaccine or placebo, your baby will be followed closely. During this part of the study, there will be about 9 days when your baby is seen by the clinical research staff and 21 days when your baby

will not be seen but you will be contacted by telephone or email by the clinical research staff. Your baby will also be followed from November 1st until March 31st (the winter season after your baby gets the vaccine or placebo). During this winter time, we will contact you [*site: insert the methods of contact*] each week to ask about your baby's health and arrange for follow-up visits if needed.

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

- If your baby 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 baby gets the vaccine or placebo, may take place at your home, at your baby's pediatric practice, or at one of the research sites. The visit where your baby receives vaccine or placebo must take place at your baby's pediatric practice or at one of the research sites where emergency equipment is available.
- For temperature measurements, you will be asked to use a temporal thermometer, which is used on your baby'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 baby has a forehead temperature ≥100.0°F, you will be asked to check your baby's rectal temperature within 20 minutes. Forehead and rectal thermometers will be given to you for use during the study.

Screening Visit
The purpose of the screening visit is to find out if your baby 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 and signing the consent form.
- going over your baby's medical history and doing a physical examination. If the physical examination results are not normal, the clinical research staff will tell you and refer your baby for follow-up care with your baby's primary medical provider.
- answering questions about the health of your baby and people living in your house.
- collecting a small amount of blood (about 2 teaspoons) to test for antibodies (germ fighters) against RSV and how your baby's cells are reacting to RSV. If your baby 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 and your baby's medical records.
- if your baby is found to be eligible for the study, your baby will be asked to return for a series of study visits, beginning with the visit when we will give your baby the vaccine or placebo.

Day Vaccine/Placebo is Given

- We will confirm that your baby has not been ill recently and will check your baby's temperature, pulse, and how fast your baby is breathing.
- Your baby will have a nasal wash. To do this, we will gently squirt less than 2 tablespoons of salt water inside your baby's nose and then collect it when it comes back out of the other side of the nose. This is done before the vaccine or placebo is given to check for other viruses and to check for antibodies in the nose.
- Your baby will receive 1 dose of vaccine or placebo given as nose drops using a small medicine dropper. Your baby will be lying on his/her back while we give the nose drops and will remain lying down for about 1 minute afterwards. Your baby can be in your lap during this time.
- After the nose drops are given, we will watch your baby 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 card to record your baby's temperature daily for 29 days (including the day the vaccine/placebo is given to your baby), and at any other time you are concerned about fever.

Monitoring for 56 Days after Vaccine/Placebo is Given

- Your baby will have study visits on Days 3, 5, 7, 10, 12, 14, 17, and 28 (each ± 1 day), after the vaccine or placebo is given. Each visit will take about 30 minutes, and we will:
    - Check your baby's temperature, pulse, and breathing rate.
    - Do a brief clinical assessment.
    - Ask about your baby's health since the last visit.
    - Give your baby a nasal wash using less than 2 tablespoons of salt water, as described above, to check for the virus that's in the study vaccine and other viruses. On Day 28 only, the nasal wash will also be used to check for antibodies in the nose.
    - 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 baby becomes ill during the following month.
- The study nurse will contact you on Days 1, 2, 4, 6, 8, 9, 11, 13, 15, 16, and daily from Days 18 to 27, and on Day 29. The study staff will ask you to report your baby's temperatures and any illness your baby has had since the last visit or contact. The contact may be by telephone, text, or email, whichever you prefer.
- Your baby will have a follow-up visit about 56 days after the study nose drops were given. At this visit, we will ask about your baby's health since the last visit and take a small amount of blood (about 2 teaspoons) from your baby to measure antibodies (germ fighters) against RSV and how your baby's blood cells are making antibodies. We will also give your baby a nasal wash using less than 2 tablespoons salt water to check for antibodies in the nose.
- We also ask you to call us right away to tell us about any illness that your baby 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 baby receives the vaccine or placebo.
- If your baby becomes ill, you may be asked to bring him/her to the clinic for an examination, sometimes as quickly as within 24 hours. We may do a nasal wash at that time to look for the RSV vaccine virus or any other virus that may be in your baby's nose.

Monitoring Before, During, and After RSV Season

- Your baby will also be followed during the winter RSV season (November 1st - March 31st) after getting the study nose drops. We will be in contact with you each week to inquire about your baby's health. If your baby has a fever, a respiratory illness, or an ear infection that requires medical care, we will work with you to schedule a visit so that we can perform a nasal wash and clinical assessment.
- We will collect a small amount of blood (about 2 teaspoons) once in October before the winter RSV season and once in April after the winter RSV season to look at the antibodies (germ fighters) against natural RSV infection and how your baby's blood cells are making antibodies. We will also give your baby a nasal wash using less than 2 tablespoons salt water to check for antibodies in the nose.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

There will be up to 33 babies taking part in this study.

HOW LONG WILL MY BABY BE IN THIS STUDY?

Your baby will be in this study through next April, which is between 7 and 13 months from now, depending on which month of the year he/she started the study.

WHAT ARE THE RISKS OF THE STUDY?

Risks of the Vaccine

- If the vaccine is not weakened enough, it may cause a runny nose, sore throat, cough, or other signs of a cold. It is also possible that it may cause a sinus infection, croup, ear infection, fever, wheezing, or pneumonia (infection of the lungs). In another study with a similar vaccine, mild respiratory illnesses were observed frequently in babies who received either vaccine or placebo. Runny nose occurred more often in babies who got the vaccine than those who got the placebo.
- Study investigators have used the same placebo for studies of RSV, parainfluenza, and influenza 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 baby will receive prompt medical care.
- The vaccine was made in a way that was designed to minimize the possibility of other ingredients. However, as with all biological products, there is a small chance that it contains 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 vaccine that are not yet known. If new information about possible side effects of this vaccine becomes available, we will let you know.
- It is possible that the vaccine virus could be spread from your baby to other people in the home or daycare and may make them sick. It could be spread to young babies and people with weakened immune systems. We have not seen this type of spread when other vaccines like this one have been studied.
- The vaccine could cause a severe allergic reaction. A severe reaction can cause hives, throat swelling, rapid heart rate, weakness, difficulty breathing, or death. Such reactions are rare.

Risks of Nasal Washes

Nasal washes may cause brief discomfort or pain that is like the feeling of getting salt water in the nose 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 baby to feel lightheaded or to faint. It sometimes takes more than 1 try to get blood from a small baby.

WHY WOULD THE DOCTOR TAKE MY BABY OFF THIS STUDY EARLY?

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

- It would be dangerous for your baby to continue;
- You do not follow study procedures as directed by the study doctors;
- New information becomes available regarding the safety of the vaccine;
- If it is in your baby's best interest;

- You do not consent to continue in the study after being told of changes in the research that may affect your baby;
- The study sponsor, the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT), the 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 BABY IS INJURED?

If your baby suffers physical injury from this study, the study doctor will provide or will refer your baby for immediate 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 baby the 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 legal rights by signing this consent form.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

- Your baby is not expected to receive any direct benefit from being in the study.
- 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 DO I/DOES MY BABY HAVE BESIDES THIS STUDY?

There are no licensed vaccines to protect against RSV illness at this time. There is no other similar study or licensed vaccine that we can offer your baby. You may choose to not allow your baby 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 baby'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 baby'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 baby's health information. These people may include the researchers, study and laboratory personnel, and other clinical research staff. Others who may see your baby's information are the groups of people who make sure that the study is being done as it should be: Hospital Institutional Review Boards (IRBs), 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 baby'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 baby's information are:

- The government agency "Office for Human Research Protections," that makes sure that we are conducting the research as planned, and the U.S. FDA
- The sponsor of the study and people with whom the sponsor may contract for the study, such as study monitors.

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

WHAT ARE THE COSTS TO ME?

There are no costs to you or your baby for him/her being in the study. The costs for 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 baby and your/your baby's insurance company if medical complications arise or if your baby's doctor decides extra tests are needed. In some cases, it is possible that your/your baby's insurance company will not pay for these costs, because your baby is taking part in a research study.

WILL MY BABY RECEIVE ANY COMPENSATION?

You will be paid for your baby's participation in this study at the following rate *[Site: insert payment schedule and amount.]*

You will also be paid during the winter RSV surveillance period as follows *[Site: insert payment schedule and amount.]*

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

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 BABY'S RIGHTS AS A RESEARCH PARTICIPANT?

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

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

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

At the end of the study, you will be told in writing whether your baby was given the vaccine or the placebo.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify your baby. At most, the website will include a summary of the results. You can search this website at any time.

WHAT ARE MY RESPONSIBILITIES?

- If you decide to withdraw your baby from the study early, we ask that you notify the study nurse or study doctor.
- If your baby comes off the study early, we will ask you to bring him/her into the clinic for an early discontinuation visit so that we can do a final blood draw (about 1 teaspoon) and nasal wash.
- Any baby 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 baby in other studies where your baby receives vaccines or medications for 8 weeks after he/she receives vaccine/placebo.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- *[Site: Insert name of the investigator or other study staff]*
- *[Site: insert telephone number of above]*

For questions about your baby's rights as a research participant, contact:

- *[Site: insert name or title of person on the Institutional Review Board (IRB) or other organization]*
- *[Site: insert telephone number of above]*

GENETIC TESTING

Some of the blood tests done for this study will look at how your baby's genes (DNA) affect his or her response to RSV. Researchers may use your baby's samples for limited genetic testing. For example, researchers may do "genetic variations" research. They may look at genes that affect how your baby fights infections. Our genes are passed to us from our birth parents. Genes are the basic "instruction book" for the cells that make up our bodies. The differences in people's genes can help explain why some people get a disease while others do not. The researchers will not contact you or your baby's regular health care provider with the results of these tests. This is because these tests are often done with experimental procedures and the results should not be used to make decisions about your baby's health care. However, if the researchers decide that a result is important information for your baby's health care, the study doctor will be notified. If you would like to be contacted with this sort of information, you must notify the study staff of any changes of your address and phone number. Your baby's name will not be available to the laboratory or to the scientists who may be doing limited genetic testing.

You may decide that you do not want your baby's blood used for limited genetic testing. Your baby can still be in this study even if you make this decision. Please read the following statement carefully and then mark your initials in the appropriate space provided.

I allow my baby's blood to be used for limited genetic testing, including future limited genetic testing, as part of this study.

Yes: Initials _____ Date _____

No: Initials _____ Date _____

STORAGE AND FUTURE USE OF UNUSED SPECIMENS

If you agree, any unused blood or nasal wash samples taken from your baby will be stored indefinitely (with protectors of identity) once this study is complete. These unused blood and nasal wash samples may be used for future laboratory studies to learn more about RSV and other viruses. This information may lead to other new virus vaccines in the future.

- Your baby's unused blood or nasal wash 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 be coded so that your baby's name cannot be easily identified.
- Reports about studies done with your baby's unused samples will not be put in your baby's health or study records.
- There will be no direct benefit to your baby in using the samples as noted above, but from studying the unused samples of babies 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 baby's unused samples may be included in medical papers and meeting reports, but your baby's name will not be used.

You can change your mind at any time about allowing your baby'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 baby's taking part in this study.

I will allow the use of my baby's unused blood or nasal wash samples to be stored indefinitely and to be used in future laboratory studies for the purposes described above. Your baby'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 baby's study samples will only be used for the testing described in this study.

SIGNATURE

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

_____
Study Participant's Name (print)

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

_____ _____
Clinical Research Staff Conducting Consent Discussion (print) Clinical Research Staff Signature and Date

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

_____ _____
Second Parent/Guardian's Name (As appropriate) Signature and Date