



IMPAACT 2018
**Randomized Phase I Study of the Infectivity,
Safety, and Immunogenicity of a Single Dose of
the Recombinant Live-Attenuated Respiratory
Syncytial Virus (RSV) Vaccines RSV
 Δ NS2/ Δ 1313/I1314L or RSV 276 or Placebo,
Delivered as Nose Drops to RSV-Seronegative
Infants 6 to 24 Months of Age**

DAIDS ES #38405

This file contains the current IMPAACT 2018 protocol,
which is comprised of the following documents,
presented in reverse chronological order:

- Letter of Amendment #3, dated 10 June 2020
- Clarification Memorandum #2, dated 27 March 2020
- Letter of Amendment #2, dated 18 July 2018
- Letter of Amendment #1, dated 15 March 2018
- Clarification Memorandum #1, dated 1 September 2017
- Protocol Version V1.0, dated 15 June 2017

Letter of Amendment #3 for:

IMPAACT 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV delta NS2/delta 1313/11314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

Version 1.0, dated 15 June 2017

**DAIDS Study ID #38405
IND #17587**

Letter of Amendment Date: 10 June 2020

Table of Contents

Information/Instructions to Study Sites from the Division of AIDS	1
Letter of Amendment Signature Page.....	2
Summary of Modifications and Rationale	3
Implementation	3
A. Updates of Protocol Team Roster	3
B. Operational Guidance from Protocol CM #2, dated 27 March 2020	5

Information/Instructions to Study Sites from the Division of AIDS

The information contained in this Letter of Amendment (LoA) affects the IMPAACT 2018 study and must be submitted to site Institutional Review Boards and/or Ethics Committees (IRBs/ECs) as soon as possible for their review and approval. Approval must also be obtained from other site regulatory entities if applicable per the policies and procedures of the regulatory entities. All applicable IRB/EC and regulatory entity requirements must be followed.

Upon obtaining all required IRB/EC approvals and any other applicable regulatory entity approvals, each site should immediately begin implementing this LoA. Sites are required to submit an LoA registration packet to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center. Sites will receive a registration notification for the LoA after the DAIDS PRO verifies that all required registration documents have been received and are complete. Sites should not await this notification before implementing this LoA.

Please file this LoA, all associated IRB/EC and regulatory entity correspondence, and all correspondence with the DAIDS PRO in your essential document files for IMPAACT 2018. If the IMPAACT 2018 protocol is amended in the future, applicable contents of this LoA will be incorporated into the next version of the protocol.

IMPAACT 2018
Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a
Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus
(RSV) Vaccines RSV delta NS2/delta 1313/I1314L or RSV 276 or Placebo,
Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

DAIDS Study ID #38405

Version 1.0, Letter of Amendment #3
Letter of Amendment Signature Page

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

Signature of Investigator of Record

Date

Name of Investigator of Record
(printed)

Summary of Modifications and Rationale

This LoA updates the protocol team roster and incorporates the contents of protocol Clarification Memorandum (CM) #2.

Section A of this LoA includes the protocol team and site roster updates.

Section B of this LoA incorporates the contents of CM #2, which was issued on 27 March 2020 to safeguard the health and well-being of study participants in the context of circulating SARS-CoV-2 and the associated COVID-19 pandemic. CM #2 provided operational flexibility for conducting study visits and procedures when needed to ensure continued close communications with participants' families and to prioritize the conduct of clinically and scientifically important evaluations when possible. Consistent with the instructions provided in CM #2, implementation of Section B of this LoA is expected to be time-limited in relation to the COVID-19 pandemic. In consultation with IMPAACT Network leadership and the study Sponsor, the IMPAACT 2018 Protocol Team will determine when, in the future, the guidance in Section B is no longer applicable. When such a determination is made, study sites will be formally notified and instructed to inform IRBs/ECs and other applicable regulatory entities.

Implementation

Updates of the protocol team roster are shown in Section A of this LoA, using strikethrough for deletions and bold type for additions where applicable. Operational guidance for conducting study visits and procedures during the COVID-19 pandemic is provided in Section B of this LoA; conventions for use of strikethrough and bolding do not apply in this section.

A. Updates of Protocol Team and Site Rosters

To reflect current membership, the protocol title page, protocol team roster, and site roster are updated as follows:

Protocol Title Page

Clinical Trials Specialists: Charlotte Perlowski, MSPH
 Jennifer Libous, MS, CCRP
 Shane Reynolds, MSHS

Protocol Team Roster

Clinical Trials Specialists
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Site Roster

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B. Operational Guidance from Protocol CM #2, dated 27 March 2020

This CM provides operational guidance to study sites from the IMPAACT 2018 Protocol Team. The Protocol Team acknowledges that the extent to which site operations may be disrupted by the COVID-19 pandemic may vary across sites and over time. **All sites should follow applicable government, health authority, and institutional policies with respect to conduct of study visits and procedures, with utmost importance placed on the health and well-being of study participants and study staff.** Site investigators should continue to follow current protocol specifications for communication with the Protocol Team and/or Protocol Safety Review Team (PSRT) and should contact the PSRT (impaact.psrt2018@fstfrf.org) with any questions or concerns regarding this CM or management of study participants.

Visit Scheduling

- Sites should continue to conduct weekly non-visit contacts per protocol with participants' families during the remainder of RSV Season Surveillance (i.e., through 31 March 2020).
- Sites should discontinue all in-person Illness Visits and instead use chart abstraction (i.e., from non-study medical visits associated with the illness) and non-visit contacts with participants' families to collect available data related to illnesses meeting protocol-specified illness criteria during the remainder of RSV Season Surveillance (i.e., through 31 March 2020). The Illness Visit nasal wash specimen will not be collected.
- Sites should conduct Post-RSV Season Study Visits; effective with the issuance of this CM, the target window for the Post-RSV Season Study Visit is broadened to include 1 April to 31 July 2020 and the allowable window for this visit is broadened to include up to 30 September 2020.
 - Sites should utilize the broadened visit window as needed to permit continued completion of in-person Post-RSV Season Study Visits.
 - Sites that anticipate operational disruptions or closures in the near future are advised to conduct visits early in the target window. Visits conducted prior to opening of the target window would also be preferred to completely missing a visit at a later date.
 - Sites that are currently experiencing operational disruptions or closures are advised to conduct visits late in the target window. Visits conducted after closing of the target window (i.e., after 31 July 2020) but prior to the closing of the allowable window (i.e., by 30 September 2020) would also be preferred to completely missed visits.
 - Sites with no ability to conduct Post-RSV Season Study Visits in-person during any of the windows noted above should notify the protocol team.

Prioritization of Post-RSV Season Study Visit Procedures

- Sites with full capacity to conduct Post-RSV Season Study Visits in-person at the study clinic during the month of April 2020 should continue to do so in full compliance with the current protocol language. If it is not possible to conduct visits in April, the broadened windows noted above may be used.
- Sites may also conduct in-person Post-RSV Season Study Visits off-site, in full or in part, if permitted by applicable government, health authority, and institutional policies during any of the windows noted above. Where this option is permitted, site staff should communicate with parents/guardians to determine in advance where and when such visits will take place, with adequate protections for safety, privacy, and confidentiality. Off-site visit procedures should be conducted by site staff who are adequately qualified and trained to conduct the procedures, as determined by the site Investigator of Record (IoR), with attention paid to occupational health, biohazard containment, and specimen and data chain of custody. These staff should also be adequately trained and qualified to immediately assess and/or manage any adverse events and/or social impacts that may occur during the visits. If adverse events requiring further evaluation or management are identified during an off-site visit, study staff conducting the visit should arrange for appropriate clinical management, in consultation with the IoR or designee, as needed.
- Sites with limited capacity to conduct Post-RSV Season Study Visits in-person during any of the windows noted above should prioritize collection of blood for immunologic assays/serum antibodies to RSV and then blood for cellular immune assay (viable PBMCs); a secondary priority is collection of nasosorption SAM strip specimens.

Documentation

- Site-specific contingency plans, and the implementation thereof, should be documented in essential document files for IMPAACT 2018.
- Documentation should be entered in participant study charts in real-time should any of the following occur:
 - Missed visits
 - Out-of-window visits
 - Off-site visits (document the location of the visit)
 - Incomplete or partial visits (document which procedures were performed and which were not)
 - Remote contacts (e.g., by telephone) performed in lieu of in-person visits (document method used to complete the contact and which procedures were performed)
 - Any other participant contacts
 - Non-standard laboratory assays
- In consultation with the Division of AIDS, the IMPAACT Network is developing comprehensive guidance for documenting and/or reporting protocol deviations that may occur due to limited site capacity to conduct study visits or procedures during the COVID-19 pandemic. Once this Network-level guidance is available, it will be provided in a separate communication to all sites.

Clarification Memorandum #2 for:

IMPAACT 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV delta NS2/delta 1313/11314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

Version 1.0, dated 15 June 2017

**DAIDS Study ID #38405
IND #17587 held by DAIDS**

Clarification Memo Date: 27 March 2020

Summary of Clarifications

This Clarification Memorandum (CM) is being issued to safeguard the health and well-being of IMPAACT 2018 study participants in the context of circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated the coronavirus disease (COVID-19) pandemic.

As the study Sponsor, the Division of AIDS (DAIDS) has determined that this CM should be implemented immediately upon issuance. Consistent with United States Food and Drug Administration guidance, institutional review board/ethics committee (IRB/EC) approval of this CM is not required by the Division of AIDS prior to implementation. However, given the context of the COVID-19 pandemic and the importance of the guidance provided in this CM, sites should submit this CM to IRBs/ECs for their information or, if required by the IRBs/ECs, for their review and approval.

The purpose of this CM is to provide operational flexibility for conducting study visits and procedures when needed to ensure continued close communications with participants' families and to prioritize the conduct of clinically and scientifically important evaluations when possible.

Implementation of this CM is expected to be time-limited in relation to the COVID-19 pandemic. In consultation with IMPAACT Network leadership and the study Sponsor, the IMPAACT 2018 Protocol Team will determine when, in the future, the guidance provided in this CM is no longer applicable. When such a determination is made, study sites will be formally notified and instructed to inform their IRBs/ECs.

Please file this CM and any applicable IRB/EC correspondence in your essential document files for IMPAACT 2018.

Implementation

This CM provides operational guidance to study sites from the IMPAACT 2018 Protocol Team. The Protocol Team acknowledges that the extent to which site operations may be disrupted by the COVID-19 pandemic may vary across sites and over time. **All sites should follow applicable government, health authority, and institutional policies with respect to conduct of study visits and procedures, with utmost importance placed on the health and well-being of study participants and study staff.** Site investigators should continue to follow current protocol specifications for communication with the Protocol Team and/or Protocol Safety Review Team (PSRT) and should contact the PSRT (impaact.psrt2018@fstrf.org) with any questions or concerns regarding this CM or management of study participants.

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- Sites should continue to conduct weekly non-visit contacts per protocol with participants' families during the remainder of RSV Season Surveillance (i.e., through 31 March 2020).
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study staff conducting the visit should arrange for appropriate clinical management, in consultation with the IoR or designee, as needed.

- Sites with limited capacity to conduct Post-RSV Season Study Visits in-person during any of the windows noted above should prioritize collection of blood for immunologic assays/serum antibodies to RSV and then blood for cellular immune assay (viable PBMCs); a secondary priority is collection of nasosorption SAM strip specimens.

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 - Any other participant contacts
 - Non-standard laboratory assays
- In consultation with the Division of AIDS, the IMPAACT Network is developing comprehensive guidance for documenting and/or reporting protocol deviations that may occur due to limited site capacity to conduct study visits or procedures during the COVID-19 pandemic. Once this Network-level guidance is available, it will be provided in a separate communication to all sites.

Letter of Amendment # 2 for:

IMPAACT 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV Δ NS2/ Δ 1313/I1314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

Version 1.0, dated 15 June 2017

DAIDS Document ID 38405
IND # 17587 held by DAIDS

Letter of Amendment Date: 18 July 2018

Information/Instructions to Study Sites from the Division of AIDS

The information contained in this Letter of Amendment (LoA) affects the IMPAACT 2018 study and must be submitted to site Institutional Review Boards (IRBs) as soon as possible for their review and approval. Approval must also be obtained from site regulatory entities if applicable per the policies and procedures of the regulatory entities. All IRB and regulatory entity requirements must be followed.

Upon obtaining IRB approval and any other applicable regulatory entity approvals, each site should immediately begin implementing this LoA. Sites are required to submit an LoA registration packet to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). Sites will receive a registration notification for the LoA after the DAIDS PRO verifies that all required registration documents have been received and are complete. Sites should not await this notification before implementing this LoA

Please file this LoA, all associated IRB and regulatory entity correspondence, and all correspondence with the DAIDS PRO in your essential documents files for IMPAACT 2018. If the IMPAACT 2018 protocol is amended in the future, the contents of this LoA will be incorporated into the next version of the protocol.

IMPAACT 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV Δ NS2/ Δ 1313/I1314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

DAIDS Document ID 38405

Version 1.0, dated 15 June 2017

Letter of Amendment #2, dated 18 July 2018

LETTER OF AMENDMENT SIGNATURE PAGE

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

Signature of Investigator of Record

Date

Name of Investigator of Record
(printed)

Summary of Modifications and Rationale

This Letter of Amendment:

1. *Updates the Protocol Team Roster:* Frederic Bone replaced Andee Fox as the Laboratory Data Manager.
2. *Updates the protocol-specified procedures for emergency unblinding:* These changes have been made for compliance with international requirements that blinded studies allow rapid emergency unblinding by the site Investigator of Record, independently from the sponsor or Protocol Team. Specifically, Sections 5.8 and 9.4.1 have been updated to remove requirements for non-site staff (e.g., Protocol Team members) to be involved in emergency unblinding. Instead, these sections refer to the IMPAACT Network Manual of Procedures (MOP), which will contain the most current guidance.
3. *Adjusts secondary and exploratory objectives and outcomes:* These revisions have been made to more accurately reflect planned analysis timelines. Specifically, three objectives and two outcomes formerly designated as secondary have been recategorized as exploratory, as the Protocol Team anticipates that data related to these objectives/outcomes may not be available within the timeframe specified for reporting results from secondary objectives/outcomes to ClinicalTrials.gov.

Implementation

The modifications included in this LoA are listed below in order of appearance in the protocol and will be incorporated into the next protocol amendment as specified below. Additions to the text are indicated in bold; deletions are indicated in strikethrough.

1. Updates to Protocol Team Roster, page 11:

Protocol Laboratory Data Manager

~~Andee Fox, MPH~~ **Frederic Bone**

~~Laboratory Data Manager~~

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Email: ~~fox@fstrf.org~~ **bone@fstrf.org**

2. Updates to emergency unblinding procedures

- a. Section 5.8, Study Product Accountability, page 43, starting with fourth sentence:

~~On a case-by-case basis. If needed to guide management of a serious illness or medical emergency affecting a study participant, or if knowing the participant's treatment assignment would otherwise affect decisions regarding the participant's immediate medical management as determined by the site Investigator of Record (IoR) or treating clinician, the randomization code may only be released at the written request of the Protocol Chair(s) and only after discussion with the senior protocol statistician and the MO to the site IoR at his/her request for rapid unblinding of the participant's treatment assignment, independently from the sponsor or Protocol Team, as described in protocol Section 9.4.1. Additional information is available in and the IMPAACT Network Manual of Procedures (MOP) Appendix I: Unblinding Procedures. [...]~~

- b. Section 9.4.1, Monitoring by the Protocol Team, Blinding/Unblinding, page 71, second paragraph:

~~If the need arises to unblind a specific participant's assignment in the event to guide management of a serious illness or medical emergency or if knowing the participant's treatment assignment~~

would otherwise affect decisions regarding the participant's immediate medical management as determined by the site Investigator of Record (IoR) or treating clinician prior to completion of the RSV Season Surveillance Period, ~~IMPAACT~~ the procedures for emergency unblinding specified in the IMPAACT Network Manual of Procedures (MOP) ~~will~~ must 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.~~

3. Adjustments to objectives and outcomes

- a. Schema, Secondary Objectives and Exploratory Objectives, page 15: note that, previously, exploratory objectives were not listed in the Schema per the IMPAACT protocol template. The template has since been updated to include exploratory objectives in the Schema; therefore, the three objectives that have been moved from secondary to exploratory, plus the existing exploratory objective from protocol Section 2.3, are now included in the Schema.

Secondary Objectives

[...]

- ~~4. To characterize the B cell response to vaccine and to characterize these responses in the vaccine and placebo recipients who experience natural infection to wt RSV during the subsequent RSV season~~
- ~~5. To characterize the mucosal antibody response to each vaccine~~
- ~~6. To determine differences in infectivity and immunogenicity between the vaccines~~

Exploratory Objectives

1. To characterize the B cell response to vaccine and to characterize these responses in the vaccine and placebo recipients who experience natural infection to wt RSV during the subsequent RSV season
2. To characterize the mucosal antibody response to each vaccine
3. To identify differences in infectivity and immunogenicity between the vaccines
4. Study samples may be used to compare to samples from other RSV vaccine studies initiated by the Laboratory of Infectious Diseases, NIAID, NIH or to evaluate other questions related to respiratory viral infections

- b. Section 1.4: Rationale, page 30, last paragraph, first sentence:

As an **exploratory** ~~secondary~~ objective, additional details of the B cell response to RSV will be studied.

- c. Section 2: Objectives, page 31:

2.2 Secondary Objectives

The secondary objectives of this study are to:

[...]

- ~~2.2.3 To characterize the B cell response to vaccine 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~~
- ~~2.2.5 To identify differences in infectivity and immunogenicity between the vaccines~~

2.3 Exploratory Objectives

- 2.3.1 To characterize the B cell response to vaccine and to characterize these responses in the vaccine and placebo recipients who experience natural infection to wt RSV during the subsequent RSV season
- 2.3.2 To characterize the mucosal antibody response to each vaccine
- 2.3.3 To identify differences in infectivity and immunogenicity between the vaccines
- 2.3.4 Study samples may be used to compare to samples from other RSV vaccine studies initiated by the Laboratory of Infectious Diseases, NIAID, NIH or to evaluate other questions related to respiratory viral infections

d. Section 9.2: Outcome Measures, page 66:

9.2.2 Secondary Outcome Measures

- Types and grades of symptomatic, medically attended respiratory and febrile illness adverse events in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season.
- Antibody titers in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season.
- ~~Frequency of B cell response to vaccine~~
- ~~Mucosal antibody titers to vaccine, in nasal wash or nasosorption samples~~

9.2.3 Other Outcome Measures

Additional outcomes may be added once results for primary and secondary outcomes are completed.

- **Frequency of B cell response to vaccine**
- **Mucosal antibody titers to vaccine, in nasal wash or nasosorption samples**

e. Section 9.5: Analyses, page 73:

9.5.2 Assessment of Secondary Objectives

[...]

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 study product group. To address the criterion “Post-vaccination surveillance during the RSV season following vaccination should reveal substantial rises in RSV-neutralizing serum antibodies in a subset of vaccine recipients in the absence of RSV associated medically attended acute respiratory illness (RSV-MAARI), which would be indicative of exposure to wt RSV without illness,” the changes in median antibody titers between ~~day 56 the Pre-RSV Season Visit (or the Day 56 Visit if this visit was conducted in lieu of the Pre-RSV Season Visit)~~ and the Post-RSV ~~surveillance time point~~ **Season Visit** will be summarized in the subset of vaccine recipients who do not experience RSV-MAARI.

~~The B cell responses to vaccine will be summarized for each study product group. A line listing of the mucosal antibody response detected in nasal wash specimens will be prepared.~~

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

9.5.3 Assessment of Exploratory Objectives

The B cell responses to vaccine and the mucosal antibody responses detected in nasal wash specimens will be summarized for each study product group.

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

Additional analyses may be added once results for primary and secondary outcomes are completed.

Letter of Amendment # 1 for:

IMPAACT 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV Δ NS2/ Δ 1313/I1314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

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Information/Instructions to Study Sites from the Division of AIDS

The information contained in this Letter of Amendment (LoA) affects the IMPAACT 2018 study and must be submitted to site Institutional Review Boards (IRBs) as soon as possible for their review and approval. Approval must also be obtained from site regulatory entities if applicable per the policies and procedures of the regulatory entities. All IRB and regulatory entity requirements must be followed.

Upon obtaining IRB approval and any other applicable regulatory entity approvals, each site should immediately begin implementing this LoA. After the IRB-approved updated site-specific informed consent form (ICF) is made available, all study participants still in follow-up must be re-consented at their next scheduled study visit, and all new participants must be consented using the updated site-specific ICF.

Sites are required to submit an LoA registration packet to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). Sites will receive a registration notification for the LoA after the DAIDS PRO verifies that all required registration documents have been received and are complete. Sites should not await this notification before implementing this LoA

Please file this LoA, all associated IRB and regulatory entity correspondence, and all correspondence with the DAIDS PRO in your essential documents files for IMPAACT 2018. If the IMPAACT 2018 protocol is amended in the future, the contents of this LoA will be incorporated into the next version of the protocol.

IMPAACT 2018

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

DAIDS Document ID 38405

**Version 1.0, Letter of Amendment #1
Dated 15 March 2018**

LETTER OF AMENDMENT SIGNATURE PAGE

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

Signature of Investigator of Record

Date

Name of Investigator of Record
(printed)

Summary of Modifications and Rationale

This Letter of Amendment:

1. *Updates the Team Roster and Site Roster:* Contact information has been updated for Clinical Trials Specialist Jennifer Libous, and Ellen Chadwick has replaced Ram Yogev as the site investigator at site 4001.
2. *Specifies the inclusion of additional individuals in early unblinding:* The NIAID RSV vaccine development program involves a Cooperative Research and Development Agreement (CRADA) between NIAID and Sanofi Pasteur, and the program includes a CRADA Scientific Advisory Board (SAB) comprising a number of experts from the field. The Protocol Team has obtained permission from the IMPAACT Management Oversight Group (MOG) and the NIAID Intramural Data and Safety Monitoring Board (DSMB) to confidentially share unblinded study data—which have already been provided to a subset of Protocol Team members per protocol Section 9.4.1—with several members of the SAB and Sanofi Pasteur as needed to facilitate planning for the vaccine program. Protocol Section 9.4.1 has been updated accordingly and has also been revised to correctly refer to Ruth Karron as the Companion Protocol Chair, rather than the Protocol Vice Chair (her former title was inadvertently carried over from a prior IMPAACT RSV protocol).
3. *Updates language regarding regulatory entities that may review study records:* Per ICH GCP E6 4.8.10(n) and DAIDS requirements, it is mandatory that all DAIDS-sponsored and/or supported trials include language that informs participants that other US, local, and international regulatory entities may also review study records. Protocol Section 10.2 and the sample ICF have been updated accordingly. As noted in the instructions above, after the IRB-approved updated site-specific informed consent form (ICF) is made available, all study participants still in follow-up must be re-consented at their next scheduled study visit, and all new participants must be consented using the updated site-specific ICF.

Implementation

The modifications included in this LoA are listed below in order of appearance in the protocol and will be incorporated into the next protocol amendment as specified below. Additions to the text are indicated in bold; deletions are indicated in strikethrough.

4. Roster updates

a. Team Roster, page 9:

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[...]

Jennifer Libous, MS, CCRP

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b. Site Roster, page 12:

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5. Section 9.4.1, Monitoring by the Protocol Team, Blinding/Unblinding, page 71, third paragraph:

A subset of protocol team members limited to the Protocol Statistician, **the Companion 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~~ **To request scientific advice, the subset of protocol team members may share the unblinded data with a limited number of representatives from Sanofi Pasteur and the CRADA Scientific Advisory Board. This unblinding will** enable more efficient and timely study evaluation and planning for appropriate next steps with respect to RSV candidate vaccine development.

6. Section 10.2, Essential and Source Documents and Access to Source Data, page 75, fourth paragraph, first sentence:

All study records must be accessible for inspection, monitoring, and/or auditing during and after the conduct of the study by authorized representatives of the study sponsors and their contracted monitors, IMPAACT, the US Food and Drug Administration, **the European Medicines Agency (EMA)**, site drug regulatory authorities, site IRBs/IBCs, OHRP, and other applicable **US, local, and international** regulatory entities.

7. Appendix VI: Sample Informed Consent Form, What about confidentiality?, page 101, sixth paragraph:

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, **and the European Medicines Agency (EMA)**
- The sponsor of the study and people with whom the sponsor may contract for the study, such as study monitors.
- **Other US, local, and international regulatory groups**

Clarification Memorandum #1 for:

IMPAACT 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV Δ NS2/ Δ 1313/11314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

Version 1.0, dated 15 June 2017

**DAIDS ES # 38405
IND # 17587 Held By DAIDS**

Clarification Memorandum Date: 1 September 2017

Summary of Clarifications and Rationale

This Clarification Memorandum (CM) adds the IND number to the protocol, specifies that Lactated Ringer's will be used as the vaccine diluent and placebo following observation of precipitate in the 2X L-15 Leibovitz medium that was intended to be used for this purpose, and clarifies a reference to infection with wild-type RSV.

Implementation

This CM has been approved by the NIAID and NICHD Medical Officers. Institutional Review Board (IRB) approval of this CM is not required by the study sponsor prior to implementation. However, sites may submit this CM to the responsible IRBs for their information or, if required by the IRBs, for their approval prior to implementation.

The content of the CM does not impact the sample informed consent forms for the study or the benefit-to-risk ratio for study participants.

This CM should be maintained in each site's essential documents file for IMPAACT 2018. It is the responsibility of the Investigator of Record to ensure that all study staff are made aware of this CM. The content of this CM will be incorporated into any future amendment of the IMPAACT 2018 protocol.

The modifications included in this CM are listed below in order of appearance within a specified clarification in the protocol. Additions to the text are indicated in bold; deletions are indicated in strikethrough.

1. Addition of the IND number to the title page. The IND number (17587) has been added as follows.

DAIDS ES # 38405
IND # ~~[TBD]~~ **17587** Held by DAIDS

2. Updates to the product to be used as the vaccine diluent and placebo. Following observation of precipitate in the 2X L-15 Leibovitz medium that was intended for use as the vaccine diluent and placebo, the Protocol Team determined that Lactated Ringer's Solution for Injection, USP should instead be used for this purpose. (Note that Lactated Ringer's is also used for the protocol-specified nasal washes.) A pause in enrollment/inoculation was initiated prior to enrollment of any participants in IMPAACT 2018; thus, no participants have received vaccine diluent or placebo containing L-15 Leibovitz medium through this study. The protocol has been updated as follows.

- a. ABBREVIATIONS AND ACRONYMS, page 8.

[...]
~~L-15~~ ——— ~~Leibovitz L-15 medium~~
[...]
~~SWFI~~ ——— ~~Sterile Water For Injection~~
[...]

- b. Section 5.1, Study Products, third bullet point, page 39.

- Placebo for RSV vaccine will be ~~1X L-15 Leibovitz medium~~ **(1X L-15) Lactated Ringer's Solution for Injection, USP 0.5ml**

- c. Section 5.3.2, Diluent for RSV Δ NS2/ Δ I1313/I1314L and RSV 276, page 40.

The diluent for RSV Δ NS2/ Δ I1313/I1314L and RSV 276 is ~~1X Leibovitz L-15 medium~~
Lactated Ringer's Solution for Injection, USP.

~~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.~~

- d. Section 5.3.3, Placebo for RSV Δ NS2/ Δ I1313/I1314L and RSV 276, page 40.

The placebo for RSV Δ NS2/ Δ I1313/I1314L and RSV 276 is ~~1X Leibovitz L-15 medium~~
Lactated Ringer's Solution for Injection, USP.

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

- e. Section 5.4, Study Product Storage, second paragraph, page 40.

~~Leibovitz L-15 medium will be stored in a secure refrigerator at 2°C to 8°C. Lactated Ringer's Solution for Injection, USP should be stored at room temperature in accordance with the manufacturer's recommendation and transferred to a secure 2°C to 8°C refrigerator at least 24 hours before use. Vaccine diluent/placebo will be prepared from new, unopened containers for each use.~~

~~Sterile Water for Injection, USP (SWFI) must also be stored in the refrigerator at 2°C to 8°C.~~

- f. Section 5.5.1, Preparation of Diluent for RSV ΔNS2/Δ1313/I1314L and RSV 276, page 41.

~~The diluent is prepared by mixing concentrated 2X Leibovitz L-15 medium with sterile water for injection in 1:1 ratio. Lactated Ringer's Solution for Injection, USP. The prepared vaccine diluent will be 1X Leibovitz L-15 medium.~~

~~Please follow the MOP for detailed instructions on diluent preparation.~~

- g. Section 5.5.2, Preparation of Placebo for RSV ΔNS2/Δ1313/I1314L and RSV 276, page 41.

~~Placebo for RSV ΔNS2/Δ1313/I1314L and RSV 276 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. Lactated Ringer's Solution for Injection, USP.~~

~~Placebo will be drawn up to a volume of 0.5 mL in a sterile 1 mL oral syringe and labeled per instructions in MOP. An auxiliary label stating "FOR INTRANASAL ADMINISTRATION ONLY" will be affixed to the syringe or outside bag. The labeled, filled syringe(s) will be transported in a cooler monitored and maintained at 2°C to 8°C with ice or cold packs to the clinical site for administration. Placebo must be administered within 4 hours of concentrated 2X Leibovitz L-15. Lactated Ringer's Solution for Injection, USP being removed from the refrigerator.~~

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

- h. Section 5.5.3, Preparation of Live Recombinant Respiratory Syncytial Virus RSV ΔNS2/Δ1313/I1314L and RSV 276, page 41.

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

[...]

~~At least two vials of undiluted vaccine are needed to prepare the vaccine dose. When manipulating the undiluted vaccine, use as small a gauge needle as possible to avoid loss of vaccine in the needle and syringe hub. The frozen vaccine will be thawed and diluted with 1X L-15. Lactated Ringer's Solution for Injection, USP to the indicated dose of either 10⁶ (RSV ΔNS2/Δ1313/I1314L) or 10⁵ PFU (RSV 276) in 0.5 mL prior to administration.~~

~~The diluted vaccine will be drawn up [...] However, the expiration time is assigned based on the time the concentrated 2X Leibovitz L-15. Lactated Ringer's Solution for Injection, USP is removed from the refrigerator in order to maintain the blind.~~

[...]

i. Section 5.7, Study Product Acquisition, page 42.

The clinical lots of RSV ΔNS2/Δ1313/I1314L and RSV 276 were generated by Charles River Laboratories using the seed virus provided by the National Institutes of Health (NIH).

~~A specific lot concentrated 2X L-15 Leibovitz Medium that~~ **Lactated Ringer's Solution for Injection, USP** will be used ~~to prepare as 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),~~ **diluent/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 shipment of the ~~vaccines and diluent/placebo~~ **study products.**

j. Section 5.8, Study Product Accountability, page 43.

The site pharmacist is responsible for maintaining an accurate inventory and accountability record of vaccine and ~~Leibovitz L-15 medium~~ **diluent/placebo** supplies for this study. [...] Partially used vials of vaccine and ~~Leibovitz L-15 medium components~~ **diluent/placebo** may not be saved and reused at a later time.

3. Clarification of definition of infection with the study vaccine virus. In their initial review of IMPAACT 2018, the DSMB recommended that the team use consistent terminology throughout the protocol for the definition of infection with vaccine virus. The team confirmed that a consistent definition of infection with vaccine virus is already used throughout the protocol; however, it was noted that a reference to infection with wild-type (wt) RSV in the protocol could be updated to clarify that a fourfold or greater rise in RSV-specific antibody titer would indicate infection with wt RSV.

Section 6.12.2, Specimen Preparation, Testing, Storage, and Shipping, Virologic and Immunologic Assays, first paragraph, third sentence, page 54.

These samples will be used to determine whether a fourfold or greater rise in **RSV-specific serum** antibody titer has occurred during the RSV season, which would signify infection with wt RSV.

IMPAACT 2018

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**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 # 38405
IND # [TBD] Held by DAIDS**

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**Version 1.0
15 June 2017**

IMPAACT 2018
Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a
Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus
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TABLE OF CONTENTS

TABLE OF CONTENTS	2
LIST OF TABLES	5
LIST OF FIGURES	5
PROTOCOL SIGNATURE PAGE	6
ABBREVIATIONS AND ACRONYMS	7
PROTOCOL TEAM ROSTER	9
SITE ROSTER	12
SCHEMA	14
1 INTRODUCTION	17
1.1 Overview	17
1.2 Background	21
1.3 Prior Research	24
1.3.1 Experimental Vaccines against Respiratory Syncytial Virus	24
1.3.2 Preclinical Studies	24
1.3.3 Previous Clinical Experience	26
1.4 Rationale	29
1.5 Hypotheses	30
2 OBJECTIVES	31
2.1 Primary Objective	31
2.2 Secondary Objectives	31
2.3 Exploratory Objective	32
3 STUDY DESIGN	32
4 STUDY POPULATION	34
4.1 Inclusion Criteria	34
4.2 Exclusion Criteria	35
4.3 Co-Enrollment Considerations	37
4.4 Recruitment, Screening, and Enrollment Process	37
4.5 Participant Retention	38
4.6 Participant Withdrawal or Termination from the Study	38
5 STUDY PRODUCT CONSIDERATIONS	39
5.1 Study Products	39
5.2 Study Product Regimens	39
5.3 Study Product Formulation	39
5.3.1 Vaccines	39
5.3.2 Diluent for RSV ΔNS2/Δ1313/11314L and RSV 276	40
5.3.3 Placebo for RSV ΔNS2/Δ1313/11314L and RSV 276	40
5.4 Study Product Storage	40
5.5 Study Product Preparation	41
5.5.1 Preparation of Diluent for RSV ΔNS2/Δ1313/11314L and RSV 276	41
5.5.2 Preparation of Placebo for RSV ΔNS2/Δ1313/11314L and RSV 276	41
5.5.3 Preparation of Live Recombinant Respiratory Syncytial Virus RSV ΔNS2/Δ1313/11314L and RSV 276	41
5.6 Study Product Administration/ Inoculation Procedure	42
5.7 Study Product Acquisition	42

5.8	Study Product Accountability	43
5.9	Disposition of Used/Unused Study Product.....	43
5.10	Final Disposition of Study Products	43
5.11	Concomitant Medications	43
5.11.1	Prohibited Concomitant Medications.....	43
5.11.2	Precautionary Concomitant Medications.....	43
6	STUDY VISITS AND PROCEDURES.....	44
6.1	Screening Visit.....	44
6.2	Enrollment Visit.....	45
6.3	Acute Phase Visits and Contacts	48
6.3.1	Acute Phase Visits: Study Days 3, 5, 7, 10, 12, 14, 17, and 28 (all are ± 1 day).....	48
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).....	49
6.4	Day 29 Contact (+ 1 day)	49
6.5	Post-Acute Phase (Days 30 to 56)	50
6.5.1	Day 56 Visit (+7 Days)	50
6.6	Period after Day 56 Visit until October 31 st or as specified in the MOP	51
6.7	Pre-RSV Season Study Visit (October 1 st to 31 st or as specified in the MOP).....	51
6.8	RSV Season Surveillance (November 1 st through March 31 st following inoculation or as specified in the MOP).....	51
6.9	Post-RSV Season Study Visit (April 1 st to 30 th)	52
6.10	Illness Visit	52
6.11	Early Discontinuation Study Visit.....	53
6.12	Additional Considerations for Laboratory Procedures	53
6.12.1	Specimen Collection	53
6.12.2	Specimen Preparation, Testing, Storage, and Shipping.....	54
6.12.3	Biohazard Containment.....	55
7	SAFETY ASSESSMENT, MONITORING, AND REPORTING	55
7.1	Safety-Related Roles and Responsibilities.....	56
7.1.1	Site Investigators.....	56
7.1.2	Protocol Safety Review Team.....	56
7.1.3	Data Safety Monitoring Board	56
7.1.4	Sponsor Reporting	56
7.2	Safety-Related Recording on Case Report Forms	57
7.3	Expedited Adverse Event (EAE) Reporting	59
7.3.1	Adverse Event Reporting to DAIDS	59
7.3.2	Reporting Requirements for this Study	59
7.3.3	Expedited AE Reporting Period.....	59
7.4	Grading Severity of Events.....	60
8	PARTICIPANT MANAGEMENT	62
8.1	Management of Adverse Events	62
8.1.1	Solicited Adverse Events	62
8.1.2	Serious Adverse Event.....	63
8.2	Pausing and Stopping Rules	64
9	STATISTICAL CONSIDERATIONS	65
9.1	General Design Issues	65
9.2	Outcome Measures	65
9.2.1	Primary Outcome Measures.....	65
9.2.2	Secondary Outcome Measures.....	66
9.3	Sample Size and Accrual	66
9.3.1	Sample Size and Randomization	66
9.3.2	Sample Size Limitations with Respect to Safety	66
9.3.3	Sample Size Limitations with Respect to Infectivity and Immunogenicity	67

9.4	Monitoring.....	70
9.4.1	Monitoring by the Protocol Team	70
9.4.2	Monitoring by the NIAID Intramural Data and Safety Monitoring Board	71
9.5	Analyses.....	72
9.5.1	Assessment of Primary Objectives.....	72
9.5.2	Assessment of Secondary Objectives.....	73
10	DATA HANDLING AND RECORD KEEPING	74
10.1	Data Management Responsibilities	74
10.2	Essential and Source Documents and Access to Source Data	74
10.3	Clinical Investigator's Brochure	75
10.4	Quality Control and Quality Assurance.....	75
11	CLINICAL SITE MONITORING	76
12	HUMAN SUBJECTS PROTECTIONS	76
12.1	Institutional Review Board/Ethics Committee Review and Approval	76
12.2	Vulnerable Participants.....	77
12.3	Informed Consent.....	77
12.4	Potential Benefits.....	78
12.5	Potential Risks.....	78
12.5.1	Venipuncture	78
12.5.2	Nasal Wash.....	78
12.5.3	Receipt of Study Product	78
12.6	Reimbursement/Compensation	78
12.7	Privacy and Confidentiality	78
12.8	Management of Incidental Findings.....	79
13	ADMINISTRATIVE PROCEDURES	79
13.1	Regulatory Oversight.....	79
13.2	Protocol Registration	79
13.3	Study Implementation.....	80
13.4	Protocol Deviation Reporting.....	80
13.5	ClinicalTrials.gov	81
14	PUBLICATIONS	81
15	REFERENCES.....	82
	APPENDICES.....	85
	Appendix I: Tables Referenced in the Background Section	85
	Appendix II: Schedule of Events: Screening, Acute Phase, and Post-Acute Phase.....	91
	Appendix III: Schedule of Events: RSV Pre-season Sampling, seasonal surveillance, and Post-season Sampling.....	92
	Appendix IV: Definitions of Solicited Adverse Events	93
	Appendix V: RSV Seasonality in Baltimore	94
	Appendix VI: Sample Informed Consent Form	95

LIST OF TABLES

Table 1: Overview of recent RSV vaccine candidates with M2-2 deletion.....	19
Table 2: Overview of RSV vaccine candidates with NS2 or M2-2 deletion to be studied in IMPAACT 2018.....	20
Table 3: AE eCRF Recording Requirements.....	58
Table 4: EAE Reporting	60
Table 5: Grading Table for Solicited AEs.....	61
Table 6: Fever Grading*.....	61
Table 7: The Probability of Observing LRI events in Vaccinees	67
Table 8: Percent of Participants Experiencing AEs with Exact 90% Confidence Intervals	67
Table 9: Magnitude of Difference in Responses Detectable with 80% Power	68
Table 10: Percent of Vaccinees who are Infected with Vaccine Virus with Exact 90% Confidence Intervals.....	70
Table 11: Viral Titers of Nasopharyngeal Swab Samples from African Green Monkeys Inoculated with the CTM RSV Δ NS2/ Δ 1313/I1314L, Lot#006A, or with recombinant wt RSV rA2	85
Table 12: Viral Titer of Tracheal Lavage samples from African Green Monkeys Inoculated with the CTM RSV Δ NS2/ Δ 1313/I1314L, Lot RSV#006A, or with recombinant wt RSV rA2 ^a	86
Table 13: Neutralizing antibody titers of AGMs inoculated with CTM RSV Δ NS2/ Δ 1313/I1314L, Lot RSV#006A ^a , or with recombinant wt RSV rA2 ^b	87
Table 14: Viral titers of nasopharyngeal swab samples from African green monkeys (AGMs) inoculated with recombinant wildtype RSV A2, MEDI/ Δ M2-2, or RSV 276 ^a	88
Table 15: Viral titers of tracheal lavage samples from AGMs inoculated with recombinant wildtype RSV A2, MEDI/ Δ M2-2, or RSV 276 ^a	89
Table 16: Neutralizing antibody titers of AGMs inoculated with recombinant wildtype RSV A2, MEDI/ Δ M2-2, or RSV 276 ^a	90

LIST OF FIGURES

Figure 1: Study Overview.....	16
Figure 2: Study Phases and Periods of Evaluation.....	33
Figure 3: Investigational Product Label (Enlarged Sample).....	40
Figure 4: Power curves for comparisons between responses to either vaccine to responses to placebo.....	69

IMPAACT 2018
Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a
Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus
(RSV) Vaccines RSV ΔNS2/Δ1313/11314L or RSV 276 or Placebo, Delivered as
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Version 1.0
Dated 15 June 2017

DAIDS Study ID #38405

PROTOCOL SIGNATURE PAGE

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

Name of Investigator of Record

Signature of Investigator of Record

Date

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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
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
eCRF	electronic case report form
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
RSV-MAARI	RSV-associated medically attended acute respiratory illness
SAE	Serious Adverse Event
SAM	synthetic adsorptive matrix
SDAC	Statistical & Data Analysis Center, Harvard School of Public Health
SDMC	Statistical and Data Management Center
SES	Subject Enrollment System
SFM	serum-free medium
SID	Study Identification Number
SOP	standard operating procedure
SPG	sucrose-phosphate-glutamate buffer
SUSAR	Serious and unexpected suspected adverse reactions
SWFI	Sterile Water For Injection
TL	Tracheal lavage
URI	upper respiratory illness
URT	upper respiratory tract
US	United States
VAR	vaccine administration record
wt	wild-type

IMPAACT 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV ΔNS2/Δ1313/11314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

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IMPAACT 2018
Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a
Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus
(RSV) Vaccines RSV ΔNS2/Δ1313/I1314L or RSV 276 or Placebo, Delivered as
Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

SCHEMA

- Purpose:** To assess whether the RSV ΔNS2/Δ1313/I1314L and RSV 276 vaccines are safe, infectious and immunogenic in this age group.
- Design:** A double-blind, randomized, placebo-controlled study design will be used to evaluate the safety and immunogenicity of the vaccines in RSV-seronegative infants. Participants will be randomized to receive RSV ΔNS2/Δ1313/I1314L vaccine, RSV 276 vaccine, or placebo in a 2:2:1 ratio. This protocol, IMPAACT 2018, is a companion protocol to the Johns Hopkins University, Center for Immunization Research (CIR) protocol CIR 321.
- 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 80 (IMPAACT 2018 and CIR 321 combined)
- Study Product:** Eligible RSV-seronegative infants will receive a single dose of RSV ΔNS2/Δ1313/I1314L vaccine, RSV 276 vaccine, or placebo intranasally at entry.

N	Product	Dose
32	RSV ΔNS2/Δ1313/I1314L Vaccine	10 ⁶ PFU**
32	RSV 276 Vaccine	10 ⁵ PFU**
16	Placebo	0

**plaque-forming units (PFU)

- Study Duration:** Approximately 20 months total. Accrual is expected to be completed in approximately 14 months from first enrollment, and all 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 outside of RSV season, i.e., between April 1st and October 14th for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP. All participants will remain on study until they complete the post-RSV season visit between April 1st and April 30th in the calendar year following

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.
2. Safety: To assess the frequency of study product-related serious adverse events (SAEs) from Day 0 through midnight on the 56th day following inoculation
3. Infectivity: To determine the peak titer of vaccine virus shed and duration of virus shedding by each participant, where the primary aim is to check if the mean peak titer of shed virus in nasal washes is approximately $2.5 \log_{10}$
4. Infectivity: To assess the proportion of vaccinated infants infected* with study vaccine, where the primary aim is to check whether >90% of vaccinees are infected with vaccine virus
5. Immunogenicity: To characterize antibody responses (Day 56) to the study product in each treatment group, where the primary aim is to check if the RSV-neutralizing antibody titers in the vaccine groups are similar to or better than MEDI/ΔM2-2 (geometric mean titer of 1:97)

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

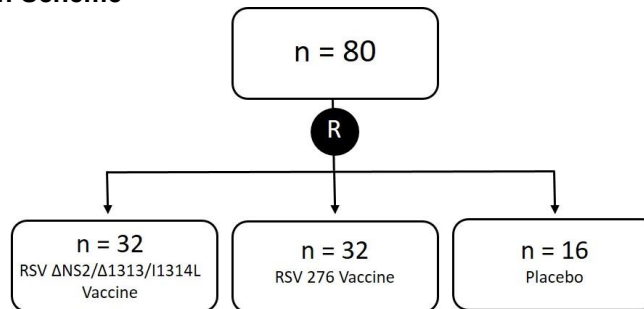
Secondary Objectives

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, where the primary aim is to check if substantial rises in RSV-neutralizing serum antibodies are present in a subset of vaccine recipients in the absence of RSV-associated medically attended acute respiratory illness (RSV-MAARI), which would be indicative of exposure to wt RSV without illness
3. To characterize the B cell response to vaccine 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 each vaccine
5. To determine differences in infectivity and immunogenicity between the vaccines

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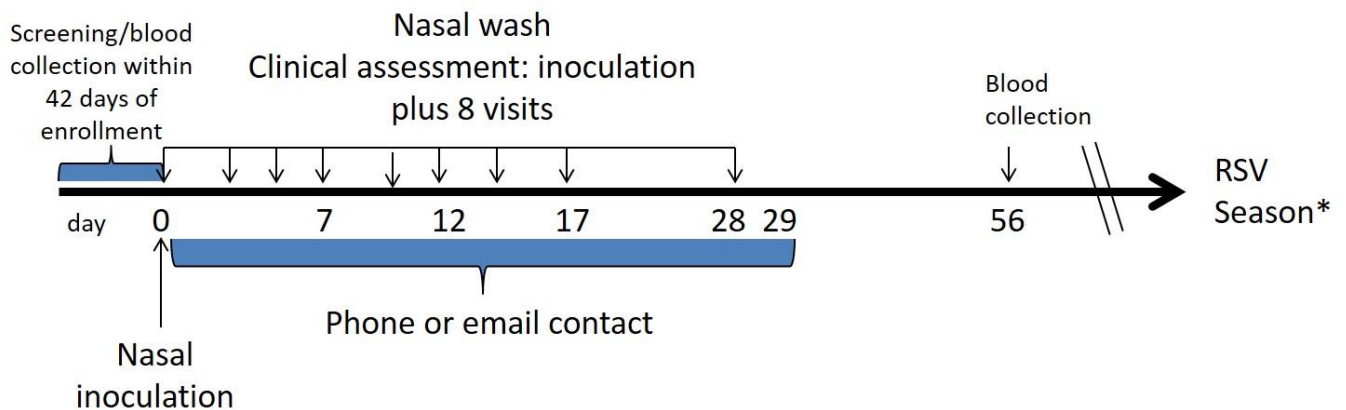
Figure 1: Study Overview

Panel A: Randomization Scheme

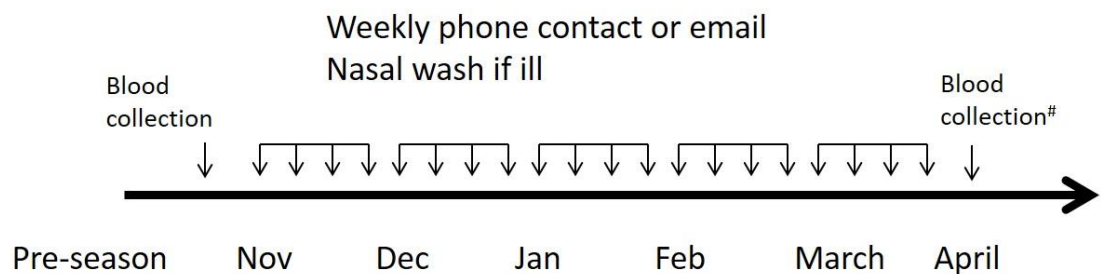


Panel B: Study Procedures (All Study Product Arms)

ACUTE AND POST-ACUTE PHASE



RSV SEASON SCHEDULE



Unblinding will occur per Section 9.4.1:

**A subset of team members will be unblinded at the completion of Day 56 for the last participant enrolled. Other team members will be unblinded at the completion of follow-up after all study data have been entered.*

**Participants' families will be unblinded during the Post-RSV Season Study Visits in April of the calendar year following enrollment.*

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 similar to responses induced by wild type infection (3). This protocol is part of a multi-year development plan aimed at identifying a candidate RSV vaccine that is sufficiently attenuated but still immunogenic.

One attenuation strategy that has shown great promise is the deletion of the viral interferon antagonist NS2, resulting in an NS2 deletion mutant. The most promising NS2 deletion candidate, RSV Δ NS2/ Δ I1313/I1314L, contains a second attenuating element, a deletion of a single codon of the polymerase ORF, in addition to the NS2 deletion. The resulting vaccine candidate is attenuated and moderately temperature sensitive. In a Phase I trial (NCT01893554), this candidate vaccine was evaluated in RSV-seropositive children 12-59 months of age at a dose of 10^6 PFU [10 vaccinees (V), 5 placebo recipients (P)], and no shedding or immune response was detected, indicative of attenuation. Next, it was evaluated in seronegative children 6-24 months of age at two sequential dose levels. At the lower dose of 10^5 PFU (15V, 7P), the vaccine was poorly infectious (7% and 80% of recipients shed virus detected by culture and RT-qPCR, respectively) and immunogenicity was low. At the higher dose of 10^6 PFU (20V, 10P), 80% and 90% of recipients shed virus detected by culture and RT-qPCR, respectively, and 94% had an F-ELISA serum antibody response greater or equal to a 4-fold rise in titer.

Another very promising strategy is deletion of a large section of the M2-2 open reading frame, 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/ Δ M2-2, was studied sequentially in adults, seropositive children, and then seronegative infants and children (age 6 to 24 months) (5). This study found that the vaccine had excellent immunogenicity, associated with very low viral replication; at a 10^5 PFU dose, only 60% of RSV-seronegative vaccinees shed vaccine virus (detectable by viral culture in nasal wash (NW) samples) at a low mean peak titer of only 1.5 log₁₀ PFU/ml NW. MEDI/ Δ M2-2 had a very promising phenotype; it is possible that a dose of 10^6 PFU might increase infectivity and possibly replication and further enhance immunogenicity. However, MEDI/ Δ M2-2 was evaluated as part of a Collaborative Research and Development Agreement (CRADA) with Medimmune, LLC, that has been terminated, and this material was not available for further study. Therefore, a close facsimile, RSV 276, was generated to replace MEDI/ Δ M2-2, as described in greater detail below.

Based on infectivity, safety, and immunogenicity data to date, these two candidate vaccines—RSV Δ NS2/ Δ I1313/I1314L and RSV 276, a facsimile of RSV MEDI/ Δ M2-2—appear to be the strongest candidates to move into an expanded Phase IB trial. This expanded Phase IB study is being planned to initiate in 2018 and will be designed to evaluate safety and immunogenicity of these two lead candidates. However, before the Phase IB trial can be designed, there are some remaining unanswered questions about these candidates that require the present Phase I study. The gaps in data necessitating the proposed trial include:

- RSV Δ NS2/ Δ 1313/I1314L requires additional safety data. While initial data are promising, only 20 participants received vaccine at the dose to be used. Evaluation of additional participants will be needed to evaluate immunogenicity and safety.
- The Δ M2-2 vaccine candidate, MEDI/ Δ M2-2, is not available for further study. A close facsimile of this virus (differing at the cDNA level by only 2 marker nucleotides in a restriction site in a non-coding region), called RSV 276, has now been prepared. RSV 276 will be studied in parallel to RSV Δ NS2/ Δ 1313/I1314L. It is anticipated that RSV 276 will be equivalent to MEDI/ Δ M2-2. This first-in-human study of RSV 276 is needed to confirm infectivity, safety and immunogenicity of RSV 276.

Table 1 includes an overview about recent RSV studies involving related candidates. The candidates of IMPAACT 2018 are described in Table 2.

Table 1: Overview of recent RSV vaccine candidates with M2-2 deletion

IMPAACT Protocol	Vaccine name	Attenuating mutations, key features	Expected effect	Study status
CIR protocol CIR 275	MEDI/ Δ M2-2	<ul style="list-style-type: none"> • Deletion of the RSV RNA regulatory M2-2 protein ΔM2-2 • Contains SH noncoding region that is deleted in “LID” versions; • differs from LID versions by one point mutation each in the NS2 and N proteins 	<ul style="list-style-type: none"> • Attenuation • Increased immunogenicity 	<ul style="list-style-type: none"> • Achieved target enrollment (n=30) • Highly attenuated • Highly immunogenic (Karron et al., 2015)
2000	LID Δ M2-2	<ul style="list-style-type: none"> • Deletion of the RSV RNA regulatory M2-2 protein ΔM2-2 	<ul style="list-style-type: none"> • Attenuation • Increased immunogenicity 	<ul style="list-style-type: none"> • Closed to accrual (n=29) • Manuscript in preparation • Peak titers higher than expected, > MEDI/ΔM2-2
2011	LID Δ M2-2 1030s	<ul style="list-style-type: none"> • Deletion of the RSV RNA regulatory M2-2 protein ΔM2-2 • genetically stable "1030" attenuating point mutation 	<ul style="list-style-type: none"> • Attenuation • Temperature sensitivity • Increased immunogenicity 	<ul style="list-style-type: none"> • Closed to accrual • Achieved target enrollment (n=33) • Analysis pending
2012	LID cp Δ M2-2	<ul style="list-style-type: none"> • Deletion of the RSV RNA regulatory M2-2 protein ΔM2-2 • five point mutations (derived from cold-passaged RSV) in the RSV nucleoprotein, fusion protein, and polymerase protein 	<ul style="list-style-type: none"> • Attenuation (increased by addition of cp mutations) • Increased immunogenicity 	<ul style="list-style-type: none"> • Closed to accrual • n = 17 • Interim analysis: vaccine will not meet at least one of the desired characteristics
2013	D46/NS 2/N/ Δ M2-2- HindIII	<ul style="list-style-type: none"> • Deletion of the RSV RNA regulatory M2-2 protein ΔM2-2; design based on MEDI/ΔM2-2. • Contains SH noncoding region that is deleted in “LID” versions; • one point mutation in NS2, N proteins; identical on amino acid level to MEDI/ΔM2-2 	<ul style="list-style-type: none"> • Attenuation • Lower level of replication than LID ΔM2-2 due to the presence of SH non-coding region • Increased immunogenicity 	<ul style="list-style-type: none"> • Planned accrual: n=33 • Projected to complete enrollment in summer 2017 based on prior experience

Table 2: Overview of RSV vaccine candidates with NS2 or M2-2 deletion to be studied in IMPAACT 2018

IMPAACT Protocol	Vaccine name	Attenuating mutations, key features	Expected effect	Study status
2018	RSV 276	<ul style="list-style-type: none"> • Deletion of the RSV RNA regulatory M2-2 protein ΔM2-2; design based on MEDI/ΔM2-2. • Contains the SH noncoding region that is deleted in “LID” versions; • RSV 276 replaces MEDI/ΔM2-2, differing only by two nucleotides in the 5’ noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ΔM2-2; CG in RSV 276) 	<ul style="list-style-type: none"> • Attenuation • Lower level of replication than LID ΔM2-2 due to the presence of SH non-coding region • Increased immunogenicity 	<ul style="list-style-type: none"> • Planned accrual: n=80 • Projected to open in July 2017
	RSV Δ NS2/ Δ I1313/I1314L	<ul style="list-style-type: none"> • Deletion of the RSV interferon antagonist NS2 (ΔNS2) • Deletion of codon 1313 of the polymerase gene (ΔI1313), Codon 13114 was genetically stabilized (I1314L) 	<ul style="list-style-type: none"> • Attenuation • Temperature sensitivity • Increased immunogenicity 	

In the proposed study, the lead candidates from each attenuation strategy, RSV Δ NS2/ Δ I1313/I1314L and RSV 276, will be evaluated side-by-side, and the results will guide the design of the planned Phase 1B study. A placebo group is included because of the high background of respiratory/febrile illness in this age group in RSV-seronegative infants.

It is hoped that one or both candidates will have the following characteristics:

- >90% of vaccinees should be infected with vaccine virus as defined by shedding vaccine virus, detected by infectivity assay and/or RT-qPCR and/or ≥ 4 -fold rise in RSV-specific serum antibodies, detected by ELISA against the RSV F protein and/or an RSV plaque reduction neutralization assay (RSV-PRNT);
- The vaccines will be safe;
- 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/ Δ M2-2 (geometric mean titer of $>1:97$); and
- Post-vaccination surveillance during the RSV season following vaccination should reveal substantial rises in RSV-neutralizing serum antibodies in a subset of vaccine recipients in the absence of RSV-associated medically attended acute respiratory illness (RSV-MAARI), which would be indicative of exposure to wt RSV without illness.

RSV Δ NS2/ Δ I1313/I1314L was previously evaluated at both 10^5 PFU and 10^6 PFU; both doses were well tolerated, but only the 10^6 PFU dose was sufficiently infectious and immunogenic. For this reason, RSV Δ NS2/ Δ I1313/I1314L will only be tested at the 10^6 dose level. RSV 276 will be evaluated at a dose of 10^5 PFU, the dose used in the previous study of MEDI/ Δ M2-2. The results from this Phase I study will guide the design of the Phase 1B study.

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 (6), and worldwide, RSV infects at least 34 million children under 5 years, resulting in 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 (7, 8).

RSV is an enveloped RNA virus that is a member of the newly organized *Pneumoviridae* family, genus *Orthoneumovirus* (9). RSV has a single negative-sense strand RNA genome of 15.2 kilobases encoding 10 mRNAs. Each mRNA encodes a single protein, with the exception of the M2 mRNA, which contains 2 overlapping open reading frames (ORFs). The 11 RSV proteins are: the viral RNA-binding nucleoprotein N, the phosphoprotein P, the large polymerase protein L, the attachment glycoprotein G, the fusion glycoprotein F, the small hydrophobic surface glycoprotein SH, the internal matrix protein M, the 2 nonstructural proteins NS1 and NS2, and the M2-1 and M2-2 proteins encoded by the M2 mRNA. The gene order is: 3'-NS1-NS2-N-P-M-SH-G-F-M2-L-5'. RSV transcription and genome replication take place exclusively in the cytoplasm, and virions form by budding from the apical plasma membrane of respiratory epithelial cells.

Currently, no licensed vaccine against RSV is available, although there is broad consensus that such a vaccine is urgently needed and should be a global health priority. Although passive immunoprophylaxis with the monoclonal RSV-neutralizing antibody palivizumab (Synagis®; MedImmune) is available for high-risk infants, this approach is not feasible for general use. A formalin-inactivated vaccine against RSV was evaluated clinically in the 1960s and did not confer protection; instead, disease enhancement occurred at a high rate following natural infection of vaccinees with wt RSV (10). 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 (11, 12).

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 four weeks of age (13).

Human RSV has a single serotype with two antigenic subgroups, A and B. The two subgroups exhibit a 3- to 4-fold reciprocal difference in neutralization by polyclonal convalescent serum. Analysis of glycoprotein-specific responses in infants by enzyme-linked immunosorbent assay (ELISA) with purified F and G glycoproteins showed that the fusion proteins (F proteins) were 50% related antigenically, and the G proteins were 7% related (14). 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 (15). Thus, the F protein is responsible for most of the observed cross-subgroup neutralization and protection, and a subgroup A vaccine virus is likely to induce a broad immune response against wt RSV of either subgroup. Antibodies to the F protein are one of the endpoints evaluated in this study.

The RSV vaccines to be evaluated in this study were derived using a recombinant deoxyribonucleic acid (DNA)-based technique called reverse genetics (16). The technique of reverse genetics has been used to produce a number of licensed vaccines; among them is FluMist® (MedImmune). This technique allows *de novo* recovery of infectious virus entirely from complementary DNA (cDNA) in a qualified cell substrate under defined conditions. Reverse genetics provides a means to introduce predetermined mutations into the RSV genome via the cDNA intermediate. Derivation of vaccine virus from cDNA minimizes the risk of contamination with adventitious agents and helps to keep the passage history brief and well documented. Once recovered, the vaccine virus is propagated in the same manner as a biologically derived virus. As a result of repeated passage and amplification, the drug substance (clinical trials material) does not contain any recombinant DNA. Both RSV vaccine candidates to be tested under this protocol are derivatives of strain A2, subgroup A.

Vaccine Description

In previous Phase I studies in RSV-seronegative infants and children, 6 to 24 months of age, the live-attenuated RSV strains RSV Δ NS2/ Δ 1313/I1314L and the close facsimile of RSV 276, MEDI/ Δ M2-2, have emerged as the most promising candidates.

RSV Δ NS2/ Δ 1313/I1314L contains two independent attenuating elements: (i) a 523 nt deletion of the viral interferon/apoptosis antagonist NS2 gene, and (ii) an amino acid deletion in the L protein (Δ 1313; deletion of S1313). In addition, RSV Δ NS2/ Δ 1313/I1314L contains the genetically stabilizing mutation I1314L (17). This stabilizing mutation was included to prevent the de-attenuating second site mutation I1314T (18). RSV with NS2 deletion is attenuated in animal models, including in chimpanzees (19). As shown in the bovine RSV/calf model (20), the deletion of an RSV interferon/apoptosis antagonist may increase immunogenicity. To study the attenuating effect of Δ 1313 alone, a recombinant RSV with deletion of codon 1313 of the L gene was evaluated (RSV Δ 1313). On its own, the deletion of codon 1313 of the polymerase gene had a substantial attenuating effect on RSV. Δ 1313 rendered RSV temperature sensitive (*ts*), with a shutoff temperature (T_{SH}) of 37°C, and was strongly attenuating in mice.

MEDI/ Δ M2-2 is not available for further study. RSV 276 was designed to be essentially identical to MEDI/ Δ M2-2, differing only by two nucleotides in the 5' noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ Δ M2-2; CG in RSV 276). Like MEDI/ Δ M2-2, RSV 276 contains a deletion of the RSV RNA-regulatory M2-2 protein, resulting in increased mRNA production and reduced RNA replication (4). 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 (21). M2-2 is expressed intracellularly at a low level (22), and it is not known whether it is packaged into the virion. Recombinant RSV with M2-2 deletion grows more slowly in vitro than wt RSV (4, 23). 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 (Δ M2-2) 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. The decreased RNA replication results in the delayed assembly of new virus particles, resulting in attenuation. Indeed, M2-2 deletion mutants were highly attenuated in pre-clinical and clinical studies (5, 23, 24).

As indicated above, RSV 276 was designed to be a close facsimile to MEDI/ΔM2-2, a vaccine candidate that was very highly restricted, well tolerated, safe, and immunogenic in a previous study in RSV-seronegative children and infants (5). RSV 276 contains the same 234 nt deletion of the M2-2 open reading frame as MEDI/ΔM2-2, and it is identical to MEDI/ΔM2-2, except for two marker nucleotides in the 5' noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ΔM2-2; CG in RSV 276, CC in wt RSV). It is anticipated that these two nucleotide differences between MEDI/ΔM2-2 and RSV 276 will not have any effect on the replication, attenuation, safety, and immunogenicity in humans because they are in a non-coding region adjacent to the deletion in M2-2.

RSV ΔNS2/Δ1313/I1314L and RSV 276 are cDNA derived live-attenuated RSV vaccine candidates. The seed viruses were generated at the Laboratory of Infectious Diseases (LID), NIAID (non-GMP), and transferred to Charles River Laboratories [CRL; Malvern, PA; operated under cGMP (current Good Manufacturing Practice)]. The seed viruses passed pre-production testing (Sterility, *Mycoplasma*, Bacteriostasis/Fungistasis, and testing for porcine circovirus types 1 and 2; testing performed at CRL under cGMP), and were accepted for manufacturing of the Drug Product under cGMP. For the production of the Drug Products at CRL, Vero cells (MF 11702) were grown in OptiPRO™ serum-free medium. On day 3 post-infection, OptiPro™ medium was removed, and replaced with fresh medium (Dulbecco's modified Eagle medium (DMEM) in case of RSV 276; OptiPro serum-free medium (SFM) in case of RSV ΔNS2/Δ1313/I1314L). Antibiotics were not used in any stage of cell passage, virus growth, or vaccine development. The virus-containing supernatant was harvested on day 7 (RSV ΔNS2/Δ1313/I1314L) or on day 5 (RSV 276), and clarified by centrifugation. Any residual intact cells were removed by filtration.

Clarified supernatant in 1X SPG (sucrose, 0.218 M; KH₂PO₄, 0.0038 M; K₂HPO₄, 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 -80°C ± 15°C.

The RSV ΔNS2/Δ1313/I1314L Drug Product is a concentrate of live recombinant RSV ΔNS2/Δ1313/I1314L Vero Grown Virus Vaccine (Lot RSV#006A) in OptiPro SFM with 1X SPG (sucrose, 0.218 M; KH₂PO₄, 0.0038 M; K₂HPO₄, 0.0072 M; L-Glutamic Acid, 0.0054 M). The Drug Product has a potency of about 7.3 log₁₀ PFU/mL and is diluted to dose on site.

The RSV 276 Drug Product is a concentrate of live recombinant RSV 276 Vero Grown Virus Vaccine (Lot RSV#014A) in DMEM without phenol red with 1X SPG (sucrose, 0.218 M; KH₂PO₄, 0.0038 M; K₂HPO₄, 0.0072 M; L-Glutamic Acid, 0.0054 M). The Drug Product has a potency of about 6.2 log₁₀ PFU/mL and is diluted to dose on site.

The Final Drug Products, RSV Δ NS2/ Δ I1313/I1314L, Lot RSV#006A and RSV 276, Lot RSV#014A, passed all in-vitro and in-vivo testing required for viral vaccines (Detection of Inapparent Viruses in a Viral Vaccine Product, *in vitro* Tuberculosis Testing, PCR-based Reverse Transcriptase Testing, Porcine Circovirus Testing, Sterility, *Mycoplasma*, Bacteriostasis/Fungistasis, Residual DNA testing, DNA Sizing, Endotoxin, Determination of the Sucrose Level, pH Determination, Intact Cell Assay, Potency/Infectivity, Identity, Purity, Toxicology and Pharmacology testing). Sequence analysis confirmed that the genomic sequences of the Drug Products, Lots RSV#006A and Lot RSV#014A, were identical to the cDNA that each was derived from. RSV Δ NS2/ Δ I1313/I1314L, Lot RSV#006A, and RSV 276, Lot RSV#014A, were released by CRL for use as investigational vaccines.

1.3 Prior Research

1.3.1 Experimental Vaccines against Respiratory Syncytial Virus

Efforts have been directed toward the development of a live-attenuated RSV vaccine because of the advantages of live-attenuated vaccines over inactivated or subunit vaccines. These advantages include the ability to (i) induce the full spectrum of protective immune responses including serum and local antibodies as well as CD4⁺ and CD8⁺ T cells and innate immunity, (ii) infect and replicate in the presence of maternal antibody permitting immunization of young infants, and (iii) produce an acute, self-limited, attenuated infection that is well tolerated and readily eliminated from the respiratory tract. Another important advantage is the absence of vaccine-related enhanced disease, as has been confirmed in clinical studies (2).

Several live-attenuated RSV vaccines have been evaluated in clinical trials in adult and pediatric populations as part of NIAID's ongoing RSV vaccine development program (5, 13, 25-27). RSV Δ NS2/ Δ I1313/I1314L and RSV 276 represent the lead vaccine candidates from these two attenuation strategies. Prior to RSV Δ NS2/ Δ I1313/I1314L, three RSV vaccine viruses with NS2 deletion (rA2cp Δ NS2, rA2cp248/404 Δ NS2, and rA2cp530/1009 Δ NS2) had been evaluated in clinical studies (28). Prior to evaluation of RSV 276 under the present protocol, several RSV candidates with M2-2 deletion have been evaluated. RSV 276 differs from the lead Δ M2-2 candidate, MEDI/ Δ M2-2, only by two marker nucleotides in a noncoding region. A short summary of preclinical studies involving these vaccine candidates is included below, and a description of the previous human experience with the prior candidates that are most closely related to the candidates of the present protocol can be found in Section 1.3.3.

1.3.2 Preclinical Studies

1.3.2.1 Genetic Stability of RSV Δ NS2/ Δ I1313/I1314L

RSV Δ NS2/ Δ I1313/I1314L is temperature sensitive, with a shut-off temperature for virus replication of 38°C-39°C. This phenotype is associated with the deletion of a single codon of the polymerase ORF, codon 1313 (17, 18). To prevent a de-attenuating second-site mutation that had been observed in an in-vitro genetic stability stress test, the neighboring codon I1314 was genetically stabilized by changing it to I1314L. In further in-vitro stability stress tests, this I1314L change indeed stabilized the 1313 site of the RSV polymerase ORF. The genetic stability of RSV Δ NS2/ Δ I1313/I1314L was confirmed by sequence analysis of RSV Δ NS2/ Δ I1313/I1314L isolates from a previous Phase 1 study. This recent pediatric vaccine study showed that RSV Δ NS2/ Δ I1313/I1314L is a genetically stable RSV vaccine candidate.

1.3.2.2 Evaluation of the Attenuation Phenotype of RSV Δ NS2/ Δ 1313/I1314L and RSV 276 in Experimental Animals

Recombinant RSV with deletion of Δ NS2 and codon 1313 of the polymerase ORF was evaluated for its ability to replicate in the upper and lower respiratory tract (URT and LRT, respectively) of mice. While wt RSV rA2 replicated to about 4.0 log₁₀ PFU and 4.5 log₁₀ PFU per g of tissue in the upper and lower respiratory tract of mice, replication of the deletion Δ NS2/ Δ 1313 mutant was reduced to below the level of detection, a surrogate for attenuation.

Replication and immunogenicity of RSV Δ NS2/ Δ 1313/I1314L was evaluated in nonhuman primates (African green monkeys, AGM). AGMs are semi-permissive for RSV. AGMs which were seronegative for RSV were inoculated intranasally and intratracheally with RSV Δ NS2/ Δ 1313/I1314L; a dose of 1 x 10⁶ PFU of vaccine in a 1 mL volume was administered per site to sedated juvenile male and female AGMs (total dose per animal: 2 x 10⁶ PFU). Nasopharyngeal (NP) swabs were collected daily on Days 0 through 10 and Day 14, tracheal lavage (TL) samples were collected every other day from Day 2 through Day 10 and on Day 14, and virus shedding was analyzed by plaque assay. Serum RSV neutralizing antibody titers were determined by a complement-enhanced 60% plaque reduction neutralization assay. Results from studies following the same protocol, performed in animals from the same group and origin, inoculated with recombinant wtRSV A2 at the same dose, were included for comparison (Appendix I, Table 11, Table 12, and Table 13). Studies were approved by the Animal Care and Use Committee of NIAID, NIH. Virus shedding was analyzed by immunoplaque assay. Substantial shedding from the upper and lower respiratory tract over several days of the RSV A2 control virus was detected by plaque assay, with mean peak titers of 3.5 log₁₀ PFU per mL in the URT and in the LRT (Appendix I, Table 11, Table 12). Compared to RSV A2, mean peak titers of RSV Δ NS2/ Δ 1313/I1314L were slightly reduced in the URT (3.2 log₁₀ PFU per mL), and greatly reduced in the LRT (1.4 log₁₀ PFU per mL), with detectable shedding in TL samples of only three of four animals. The sum of daily titers (area under the curve) of shedding of RSV Δ NS2/ Δ 1313/I1314L from the upper and lower respiratory tract was substantially lower than that of RSV A2. Despite the lower level of shedding, RSV Δ NS2/ Δ 1313/I1314L was immunogenic in AGMs (Table 13).

RSV 276 also was evaluated for its ability to replicate in AGMs in two independent non-GLP studies. The first NHP study was done to evaluate an Experimental Lot of RSV 276 (total dose administered: 2 x 10⁶ PFU per animal), and the second study (total dose administered: 2 x 10⁶ PFU per animal) was done to test the clinical safety of the clinical trial material (CTM) RSV 276 in nonhuman primates (Appendix I, Table 14, Table 15, and Table 16). As described above, AGMs, seronegative for RSV, were inoculated intranasally and intratracheally with 1 x 10⁶ PFU of RSV 276 in a 1 mL volume per site (total dose per animal: 2 x 10⁶ PFU). NP and TL samples were collected as described above, and virus shedding was analyzed by plaque assay. Serum RSV neutralizing antibody titers were determined by a complement-enhanced 60% plaque reduction neutralization assay (PRNT₆₀). Results from studies following the same protocol, performed in animals from the same group and origin, inoculated with MEDI/ΔM2-2 and recombinant wt RSV A2 at the same dose, were included for comparison (Appendix I, Table 14, Table 15, and Table 16). Substantial shedding from the upper and lower respiratory tract over several days of the RSV A2 control virus was detected by plaque assay, with mean peak titers of 4.2 log₁₀ PFU per mL in the URT, and 4.1 log₁₀ PFU per mL in the LRT (Appendix I, Table 14, Table 15). RSV 276 was infectious for AGMs, and except for a single animal with undetectable shedding from the upper respiratory tract, all animals from two independent studies shed RSV 276 over several days from the upper and lower respiratory tract. However, compared to RSV A2, shedding of RSV 276 was reduced in the upper and lower respiratory tract. Mean peak titers of the experimental lot and of

the CTM of RSV 276 were not significantly different from those of MEDI/ΔM2-2 in a previous study. Both lots of RSV 276 induced serum neutralizing antibody titers that were higher than those to MEDI/ΔM2-2, and comparable to those to the RSV A2 control virus (Table 16). These results show that at a total dose of 2×10^6 PFU, administered intranasally and intratracheally, RSV 276 is attenuated to a similar degree as MEDI/ΔM2-2, and very immunogenic in AGMs.

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

1.3.3 Previous Clinical Experience

RSV ΔNS2/Δ1313/I1314L has recently been evaluated in a first-in-human Phase I study. The live-attenuated recombinant RSV 276 vaccine virus is being evaluated for the first time in humans in the present study. The RSV 276 vaccine is a close facsimile of the live-attenuated recombinant RSV MEDI ΔM2-2 vaccine virus, which has been studied in RSV-seropositive cohorts, and in RSV-seronegative children.

1.3.3.1 RSV Vaccine Candidates with NS2 Deletion

Prior to RSV ΔNS2/Δ1313/I1314L, three RSV vaccine viruses with NS2 deletion (rA2cpΔNS2, rA2cp248/404ΔNS2, and rA2cp530/1009ΔNS2) had been evaluated in clinical studies (28).

rA2cpΔNS2 contains the NS2 deletion as well as a set of 5 amino acid substitutions in the N, F, and L proteins that were identified in a cold-passaged (cp) RSV [V267I in N; E218A and T523I in F; and C319Y and H1690Y in L (29, 30)]. rA2cp248/404ΔNS2 contains all of the mutations in rA2cpΔNS2 as well as two additional point mutations: "248," an amino acid substitution in the L protein (Q831L) (29, 31, 32), and "404," a nucleotide substitution in the M2 gene start signal (31, 33). rA2cp530/1009ΔNS2 also contains all of the mutations in rA2cpΔNS2 as well as two additional point mutations in L: "530" (F521L) and "1009" (M1169V) (34, 35).

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

rA2cp248/404ΔNS2 was evaluated in adults (n=16 vaccinees), 15- to 59-month-old RSV-seropositive children (n=16 vaccinees), and 6- to 24-month-old RSV-seronegative children (n=10

vaccinees). rA2cp248/404ΔNS2 was not shed by adults. Only one seropositive child shed vaccine virus (peak titer of $10^{0.4}$ PFU/mL of nasal wash), and four seronegative children shed vaccine virus (mean peak titer of $10^{2.3}$ PFU/mL of nasal wash).

rA2cp530/1009ΔNS2 was also evaluated in adults (n=19 vaccinees), 15-to-59-month-old RSV-seropositive children (n=14 vaccinees), and 6- to 24-month-old RSV-seronegative children (n=15 vaccinees). rA2cp530/1009ΔNS2 was shed by one adult (peak titer of $10^{0.4}$ PFU/mL of nasal wash), none of the seropositive children, and three seronegative children (mean peak titer of $10^{1.3}$ PFU/mL of nasal wash). In summary, deletion of the NS2 gene attenuates RSV. rA2cpΔNS2 was over-attenuated for adults but under-attenuated for use in young children, whereas both rA2cp248/404ΔNS2 and rA2cp530/1009ΔNS2 were over-attenuated and insufficiently immunogenic for seronegative children (28).

Based on these results, RSVΔNS2/Δ1313/I1314L was developed. In a recent Phase I trial (NCT01893554), this candidate vaccine was evaluated in RSV-seropositive children 12-59 months of age at a dose of 10^6 PFU (10V, 5P), and no shedding or immune response was detected, indicative of attenuation. Next, it was evaluated in seronegative children 6-24 months of age at two sequential dose levels. At the lower dose of 10^5 PFU (15V, 7P), the vaccine was poorly infectious (7% and 80% of recipients shed virus detected by culture and RT-qPCR, respectively) and immunogenicity was low. At the higher dose of 10^6 PFU (20V, 10P), 80% and 90% of recipients shed virus detected by culture and RT-qPCR, respectively, and 94% had an F-ELISA serum antibody response defined by at least a 4-fold rise in titer. Assessment of neutralizing antibodies for this study will occur when the samples after RSV surveillance have been collected.

1.3.3.2 RSV MEDI/ΔM2-2

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

RSV MEDI/ΔM2-2 was subsequently evaluated at a 10^5 PFU dose in RSV-seronegative children. RSV MEDI/ΔM2-2 replicated at low titers yet induced substantial RSV neutralizing antibody responses in RSV-seronegative children. Vaccine virus was detected by culture in only 60% (12 of 20) RSV-seronegative vaccinees, at a low mean peak titer of $1.5 \log_{10}$ PFU/ml. 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. Thus, while the levels of infectivity and replication of RSV MEDI/ΔM2-2 were similar to what might be seen with an over-attenuated vaccine virus, the levels of RSV-specific serum antibodies, indicated that this virus was surprisingly immunogenic. Respiratory illnesses were observed in 85% of vaccinees and 70% of placebo recipients, including fever (20%

vs. 30%), rhinorrhea (85% vs. 50%), cough (35% vs. 30%), and otitis media (5% vs. 0%). Lower respiratory tract illness (LRI) was not detected in any participant. Transmission of vaccine virus occurred in a set of 13-month-old twin study participants; both were minimally symptomatic and vaccine virus shed retained the M2-2 deletion and remained very highly restricted in the secondary infection. When data on vaccine virus infectivity and immunogenicity in RSV-seronegative children were compared to those achieved with rA2 cp248/404/1030/ΔSH, a live-attenuated RSV vaccine candidate that was well tolerated and immunogenic in previous pediatric Phase I studies (13), it was found that the MEDI/ΔM2-2 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 medically attended illness, suggesting that the vaccine was protective yet primed for strong anamnestic responses to RSV. The M2-2 deletion was stable in all shed vaccine virus samples that were tested (5).

Thus, MEDI/ΔM2-2 phenotype appears to be very promising for an RSV vaccine. However, because of the overall low titer of shedding, and the lack of shedding in a number of vaccinees, it was possible that a virus that replicates somewhat more efficiently might be more immunogenic, provided it is suitably attenuated. Therefore, several other ΔM2-2 candidates with differences affecting replication and temperature sensitivity were also evaluated.

1.3.3.3 RSV LID ΔM2-2

A closely genetically related ΔM2-2 vaccine virus, called RSV LID ΔM2-2, has been developed. RSV LID ΔM2-2 was evaluated in RSV-seronegative children (IMPAACT 2000/CIR 291, ClinicalTrials.gov identifiers NCT02237209, NCT02040831), 20 of whom received vaccine and nine 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 $10^{5.9}$ log₁₀ copies/mL by qRT-PCR. Overall, the level of replication of RSV LID ΔM2-2 in seronegative children was greater than expected based on previous study of 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 than the targeted 51 participants.

Compared to RSV MEDI/ΔM2-2, LID ΔM2-2 replicated to higher viral titers, as measured in nasal washes from RSV-seronegative vaccinees. MEDI/ΔM2-2 and LID ΔM2-2 differ in three general ways: (i) they were derived from two different laboratory lineages of strain A2 and have 19 point mutations scattered through the genome that result in two amino acids coding changes; (ii) they have differences in the construction of the ΔM2-2 deletion that result in 24 nucleotide differences, and (iii) the LID/ΔM2-2 virus has a 112 nt deletion and five coding changes in the 5' noncoding region in the LID ΔM2-2 SH gene. This latter mutation in particular may confer increased replication of LID ΔM2-2 in vivo.

Thus, a vaccine candidate was desirable with a slightly lower level of replication, similar to that of MEDI/ΔM2-2. As described above, RSV 276 was designed to be similar to MEDI/ΔM2-2. In pre-clinical nonhuman primate studies in AGMs, the most permissive RSV animal model

currently available, the level of replication of RSV 276 was similar to MEDI/ΔM2-2. Based on the non-clinical testing results, it is anticipated that the investigational RSV 276 live vaccine candidate will be similar to the previously studied RSV vaccine candidate MEDI/ΔM2-2, and will be safe and immunogenic upon intranasal application in RSV-seronegative children and infants.

RSV 276 differs from MEDI/ΔM2-2 only by two nucleotides in the 5' noncoding region of the M2 gene, and therefore it is reasonable to expect that these two viruses will have similar levels of replication and attenuation in humans, and likely will be phenotypically indistinguishable. MEDI/ΔM2-2 was evaluated in RSV-seropositive adults and RSV-seropositive infants and children at a dose of 10⁶ PFU, and virus shedding was undetectable at this dose by culture or RT-qPCR. The results showed that RSV MEDI/ΔM2-2 is highly attenuated and does not infect RSV-experienced individuals. At a dose of 10⁵ PFU, vaccine shedding in RSV-seronegative infants and children also was very low, showing that this vaccine virus is very highly attenuated in seronegative children. Since RSV MEDI/ΔM2-2 did not infect RSV-seropositive individuals at the dose level of 10⁶ PFU, testing RSV 276 in RSV-seropositive individuals would not be expected to yield any relevant safety information.

1.4 Rationale

In previous Phase I studies in RSV-seronegative infants and children, 6 to 24 months of age, the two live-attenuated RSV strains RSV ΔNS2/Δ1313/I1314L and RSV MEDI/ΔM2-2, based on two different attenuation strategies, have emerged as the most promising candidates. Based on infectivity, safety, and immunogenicity data, these two candidate vaccines appear to be the strongest candidates to move into expanded Phase IB trials. However, before the Phase IB trials can be designed, there are some remaining unanswered questions about these candidates that require a Phase I study. The gaps in data necessitating the proposed trial include:

- The initial ΔM2-2 vaccine candidate, MEDI/ΔM2-2, is not available for further study. A close facsimile of this virus, called RSV 276, has now been prepared. RSV 276 differs from MEDI/ΔM2-2 only by two marker nucleotides in the 5' noncoding region of the M2 gene (nt 8198/99; GC in MEDI/ΔM2-2; CG in RSV 276). A first-in-human study of RSV 276 is needed to confirm its safety and immunogenicity.
- Additional safety data are needed regarding RSVΔNS2/Δ1313/I1314L prior to the larger Phase IB study, due to the small number of infants who have received this product to date.
- This study will monitor vaccine shedding at several time points during the acute phase. The detailed virus shedding data obtained under this protocol will be used to refine the design and the most suitable evaluation time points for a Phase IB study with a less intensive sampling schedule.

This protocol monitors safety, infectivity, replication, and immunogenicity of both candidates, with particular attention to vaccine virus infectivity and replication (i.e., percentage of participants shedding virus, peak vaccine virus titer in nasal washes, as well as duration of shedding), which are the most quantifiable metrics for the level of attenuation. Based on results from this study, a comparison of infectivity and immunogenicity of these candidates will be possible. It is anticipated that the two lead candidate from the different attenuation strategies will move forward to an expanded Phase IB study in 2018 to further evaluate safety and immunogenicity.

Rationale for first in human testing of RSV 276 in RSV-seronegative infants. MEDI/ΔM2-2, a candidate vaccine that differs from RSV 276 by only two marker nucleotides in the 5' noncoding

region of the M2 gene, has been studied in seronegative infants and found to be very highly restricted and well tolerated. Given that the only difference in the two vaccines is in a non-coding region, it is expected that RSV 276 will have identical attenuation compared to MEDI/ΔM2-2.

The primary immunogenicity endpoints to be evaluated in this study are serum RSV neutralizing antibody titers, and serum antibody titers to the RSV F protein (by ELISA). Neutralizing antibody is a well-established and important surrogate marker of protection from RSV disease. Antibodies to the F protein are also associated with cross-subgroup neutralization and protection (15). 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, additional details of the B cell response to RSV will be studied. RSV neutralizing serum antibody levels represent the most reliable correlate of protection from RSV LRI; the protective role of antibody has been established in infants over years of preventive use of palivizumab (36). Recent findings suggest that antibodies to the pre-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. The team plans 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

RSV ΔNS2/Δ1313/I1314L and RSV 276 will be safe, infectious, and immunogenic in RSV-seronegative infants 6 to 24 months of age. The vaccines will be good candidates to move forward in a Phase IB study by meeting the following criteria:

- >90% of vaccinees should be infected with vaccine virus as defined by shedding vaccine virus, detected by infectivity assay and/or RT-qPCR and/or ≥ 4 -fold rise in RSV-specific serum antibodies, detected by ELISA against the RSV F protein and/or an RSV plaque reduction neutralization assay (RSV-PRNT);
- The vaccines will be safe;
- 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 $>1:97$); and
- Post-vaccination surveillance during the RSV season following vaccination should reveal substantial rises in RSV-neutralizing serum antibodies in a subset of vaccine recipients in the absence of RSV-associated medically attended acute respiratory illness (RSV-MAARI), which would be indicative of exposure to wt RSV without illness.

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
- 2.1.2** Safety: To assess the frequency of study product-related serious adverse events (SAEs) from Study Day 0 through midnight on the 56th day following inoculation
- 2.1.3** Infectivity: To determine the peak titer of vaccine virus shed and duration of virus shedding by each participant, where the primary aim is to check if the mean peak titer of shed virus in nasal washes is approximately 2.5 log₁₀
- 2.1.4** Infectivity: To assess the proportion of vaccinated participants infected* with study vaccine, where the primary aim is to check whether >90% of vaccinees are infected with vaccine virus
- 2.1.5** Immunogenicity: To characterize antibody responses (Day 56) to the study product in each treatment group, where the primary aim is to check if the RSV-neutralizing antibody titers in the vaccine groups are similar to or better than MEDI/ΔM2-2 (geometric mean titer of 1:97)

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

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, where the primary aim is to check if substantial rises in RSV-neutralizing serum antibodies are present in a subset of vaccine recipients in the absence of RSV-associated medically attended acute respiratory illness (RSV-MAARI), which would be indicative of exposure to wt RSV without illness
- 2.2.3** To characterize the B cell response to vaccine 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

2.2.5 To identify differences in infectivity and immunogenicity between the vaccines.

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 or to evaluate other questions related to respiratory viral infections

3 STUDY DESIGN

IMPAACT 2018 is a companion study to the Johns Hopkins University (JHU) Center for Immunization Research (CIR) protocol 321. The CIR 321 and IMPAACT 2018 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 321.

The study will be conducted in infants at the JHU CIR and selected IMPAACT sites in the United States. The vaccines 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 (13, 26, 37). In these previous studies, live-attenuated RSV vaccines were highly restricted in replication and poorly immunogenic in children with titers $\geq 1:40$ 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. Approximately 80 RSV-seronegative participants will be randomized at a ratio of 2:2:1 to the three arms of the study; two active vaccine arms and one placebo arm. Placebo recipients are needed in pediatric studies to distinguish the background respiratory and febrile illnesses that occur in infants and children from those attributable to study vaccine. These numbers were chosen based upon experience with Phase I evaluation of other live-attenuated respiratory virus candidate vaccines (25-27) and statistical considerations (see Section 9).

Enrollment will occur between April 1st and October 14th for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP to avoid the time during which wt RSV typically circulates in the community. Specific study phases are described in the paragraphs that follow.

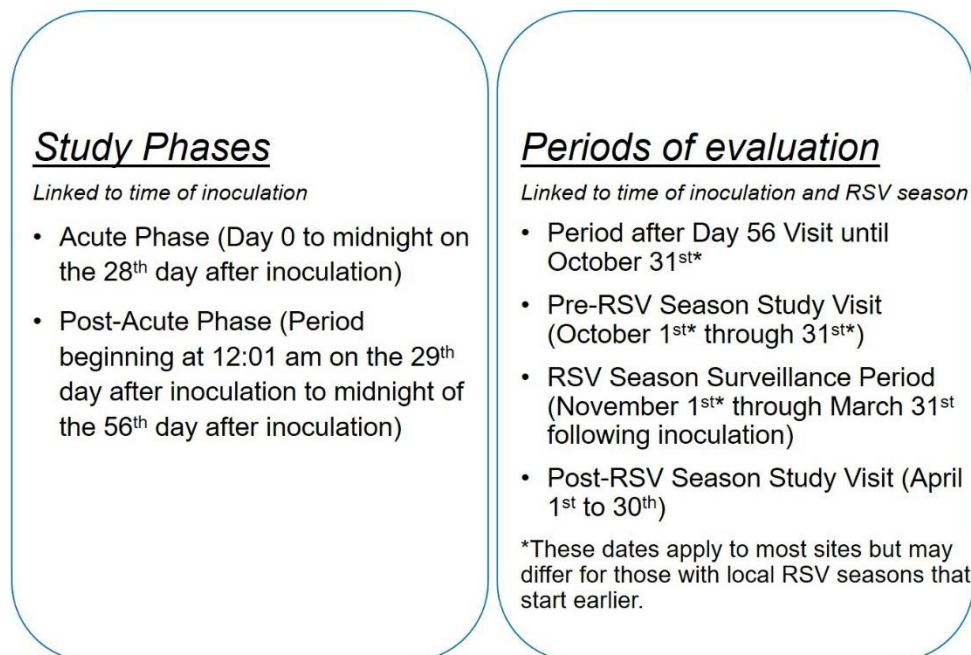
Duration of participation in the initial phase of the study is 56 days, which consists of an Acute and a Post-Acute Phase. During the Acute Phase (Study Day 0 to midnight on the 28th day following inoculation), participants will be contacted daily. These contacts will consist either of: 1) in-person 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 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP, site study staff will make weekly contact with the participants to identify medically attended episodes of fever, URI or LRI, or otitis media. 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 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP) 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. All 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 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP.

Figure 2: Study Phases and Periods of Evaluation



4 STUDY POPULATION

The vaccines will be evaluated in RSV-seronegative infants ≥ 6 months (180 days) to < 25 months (750 days) of age. Approximately 80 participants will be enrolled (32 per vaccine arm, 16 in the placebo arm) from US sites only. Infants will be selected for participation according to the criteria in Sections 4.1 and 4.2. The sections that follow describe study-specific co-enrollment considerations; the recruitment, screening, and enrollment process; and participant retention and withdrawal or termination. Sites are expected to obtain the participant's medical records from the participant'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** In good health based on review of the medical record, history, and physical examination, without evidence of chronic disease.
- 4.1.3** Parent/guardian is willing and able to provide written informed consent as described in protocol Section 12.3.
- 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** Growing normally for age (i.e., not downwardly crossing two major centiles on a standard growth chart) in the six months prior to enrollment 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** 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** 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** Any receipt of bone marrow/solid organ transplant.
- 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** Any previous anaphylactic reaction.
- 4.2.7** Any previous vaccine-associated adverse reaction that was Grade 3 or above. Note: if grading is not possible, determine if the reaction was considered severe or life threatening; if so, it is exclusionary.
- 4.2.8** Any 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/other children who is/are, or is/are scheduled to be, enrolled in IMPAACT 2018 AND the date of enrollment to IMPAACT 2018 will not be concurrent with the other participant(s) living in the household (i.e., all eligible children from the same household must be enrolled on the same date).
- 4.2.13** Member of a household that contains another child who is, or is scheduled to be, enrolled in another study evaluating an intranasal live-attenuated RSV vaccine, AND there has been or will be an overlap in residency during that other child's participation in the study's Acute Phase (Days 0 to 28).

4.2.14 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/mm³. CD4 T lymphocyte count must have been measured within 6 months prior to enrollment for individuals with detectable virus or within 12 months prior to enrollment for individuals with undetectable virus, 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/mm³ (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/mm³ (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.

Documenting the verbal report of CD4 T cell lymphocyte count is sufficient if the parent/guardian is confident of history. Individually identifiable information (e.g., name) pertaining to members of the household will not be collected or recorded. Confirmatory laboratory tests for members of the household will not be ordered or collected.

4.2.15 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.

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

- fever (rectal temperature of $\geq 100.4^{\circ}\text{F}$ (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.

4.2.17 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

4.2.18 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

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

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

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

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

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

4.2.22 Born at less than 34 weeks gestation.

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

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

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

4.3 Co-Enrollment Considerations

Co-enrollment to any 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 2018 is allowable for participants already enrolled in IMPAACT P1112, provided all eligibility criteria above are met; in particular, per criterion 4.2.19, the participant must not have received P1112 study product in the past 6 months. The P1112 and IMPAACT 2018 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 (e.g., hospital, community, and private pediatric clinics) where recruitment will occur as part of the site selection process, which will be reviewed and approved by the Protocol Team. All recruitment materials must be reviewed and approved by 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. Screening may occur no more than 42 days prior to enrollment during the dates specified in Section 6.2.

The IMPAACT Data Management Center (DMC) Subject Enrollment System (SES) will be used to track enrollment. When informed consent is obtained, a participant identification number (PID) will be assigned to the infant by the study staff. For infants found to be eligible, randomization and enrollment will occur upon successful entry of required eligibility data into the SES. Successful entry into the SES will generate a study identification number (SID) and blinded prescribing information for the study product arm to which the infant has been randomly assigned; this represents the effective point of enrollment. Note that any eligible children living in the same household will be randomized to the same arm per Section 9.1 and must be randomized/enrolled on the same date. 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, parents/guardians of infants participating in this study may voluntarily withdraw their infants from the study at any time. Any participant who has received study product will be encouraged to remain in study follow-up for the duration of the study even if sample collection is refused.

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

- 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 re-locates away from the study site or is otherwise determined to be lost-to-follow-up.
- Investigator or designee determines that continued participation in the study would be unsafe or otherwise not in the best interest of the participant, after consultation with the protocol team.
- Other – a category used when previous categories do not apply; requires an explanation.

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

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 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) Δ NS2/ Δ 1313/I1314L 10^6 PFU per 0.5ml vaccine
- Live Recombinant Respiratory Syncytial Virus RSV 276 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 the indicated 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

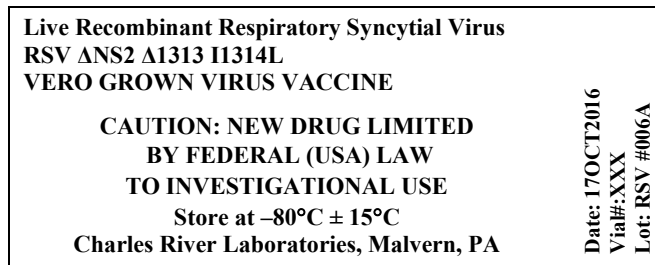
5.3.1 Vaccines

The RSV Δ NS2/ Δ 1313/I1314L vaccine is provided in a sterile 2.0 mL cryovial, each containing 0.6 mL of Vaccine (Lot RSV #006A), with a titer of approximately $10^{7.3}$ PFU/mL. The vaccine virus concentrate is diluted to an appropriate dose by designated licensed pharmacy personnel to prepare a dose of 10^6 PFU in a 0.5 mL volume. The vaccine vial is labeled as shown below in Figure 3.

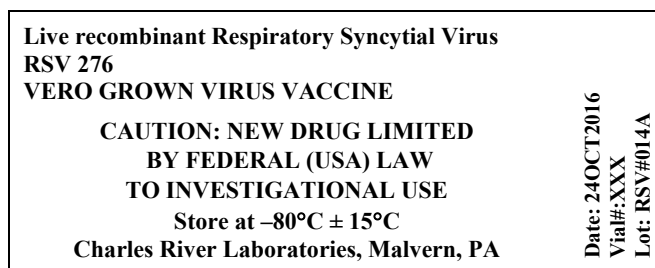
The RSV 276 vaccine is provided in a sterile 2.0 mL cryovial, each containing 0.6 mL of Vaccine (Lot RSV #014A), with a titer of approximately $10^{6.2}$ PFU/mL. The vaccine virus concentrate is diluted to an appropriate dose by designated licensed pharmacy personnel to prepare a dose of 10^5 PFU in a 0.5 mL volume. The vaccine vial is labeled as shown below in Figure 3.

Figure 3: Investigational Product Label (Enlarged Sample)

RSV ΔNS2/Δ1313/I1314L Vaccine:



RSV 276 Vaccine:



5.3.2 Diluent for RSV ΔNS2/Δ1313/I1314L and RSV 276

The diluent for RSV ΔNS2/Δ1313/I1314L and RSV 276 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 RSV ΔNS2/Δ1313/I1314L and RSV 276

The placebo for RSV ΔNS2/Δ1313/I1314L and RSV 276 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 vaccines, the placebo for the vaccines, and the RSV vaccines 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 Preparation of Diluent for RSV Δ NS2/ Δ 1313/I1314L and RSV 276

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 Preparation of Placebo for RSV Δ NS2/ Δ 1313/I1314L and RSV 276

Placebo for RSV Δ NS2/ Δ 1313/I1314L and RSV 276 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 syringe(s) will be transported in a cooler monitored and maintained at 2°C to 8°C 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 Preparation of Live Recombinant Respiratory Syncytial Virus RSV Δ NS2/ Δ 1313/I1314L and RSV 276

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

If the -80°C freezer where the RSV vaccines are stored is not in close proximity to where the preparation is being done, the vaccine vials should be transported on dry ice from the freezer to the 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.

At least two vials of undiluted vaccine are needed to prepare the vaccine dose. When manipulating the undiluted vaccine, use as small a gauge needle as possible to avoid loss of vaccine in the needle and syringe hub. The frozen vaccine will be thawed and diluted with 1X L-

15 to the indicated dose of either 10^6 (RSV Δ NS2/ Δ 1313/I1314L) or 10^5 PFU (RSV 276) in 0.5 mL prior to administration.

The diluted vaccine will be drawn up to a volume of 0.5 mL in a sterile 1 mL oral syringe and labeled per instructions in MOP. An auxiliary label stating “FOR INTRANASAL ADMINISTRATION ONLY” will be affixed to the syringe or outside bag. The labeled, filled syringe(s) will be transported in a cooler monitored and maintained at 2°C to 8°C 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 $-80^{\circ}\text{C} \pm 15^{\circ}\text{C}$ 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 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 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 lots of RSV Δ NS2/ Δ 1313/I1314L and RSV 276 were generated by Charles River Laboratories using the seed virus provided by the National Institutes of Health (NIH).

A 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 shipment of the vaccines and diluent/placebo.

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 vaccines will be prepared as instructed with the site pharmacist serving as the unblinded dispenser. A copy of the randomization code will be retained by the site pharmacist. On a case-by-case basis, the randomization code may only be released at the written request of the Protocol Chair(s) and only after discussion with the senior protocol statistician and the MO. Additional information is available in the IMPAACT Network Manual of Procedures (MOP) Appendix I: Unblinding Procedures. 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 vaccine vials and place it in the accountability log. Please see the IMPAACT 2018 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 immunogenicity results, the following treatments should be avoided after inoculation unless urgently clinically indicated or after consultation with the Protocol Safety Review Team (PSRT) via email at impaact.psrt2018@fstrf.org:

- 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

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 eCRFs. Refer to Section 7 for information on expedited adverse event (EAE) reporting, which is required at specified times during follow-up.

In addition to the protocol-specified procedures listed in this section, study staff may complete other tasks consistent with site SOPs, including but not limited to collecting, reviewing, and updating demographic and locator information; reviewing elements of informed consent; scheduling telephone contacts and visits; providing instructions for contacting study staff between visits; providing visit reminders; and following up on missed visits. All such tasks should be documented consistent with site SOPs. Clinical evaluations must be performed by a medical professional. Study staff should inform 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 spring enrollment period (i.e., screening may begin on March 18 and proceed through early October with results received by October 14 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP). 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.

iron works 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 (13, 25-27), 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 (38).

Screening Visit Procedures*		
Administrative and Regulatory		<ul style="list-style-type: none"> • 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		<ul style="list-style-type: none"> • 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	<p><i>Collect blood for:</i></p> <ul style="list-style-type: none"> • 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 2018; it is not necessary to collect another sample. • <i>Only from participants who are at sites with capacity for processing viable PBMCs:</i> 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 2018, if it was collected within the 42-day enrollment window for IMPAACT 2018.
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.

*No more than 42 days prior to enrollment; not prior to March 18 and through early October with results received by October 14 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP.

6.2 Enrollment Visit

Study enrollment and inoculation must occur outside of the RSV season, i.e., between April 1st and October 14th inclusive for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP, 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 $\geq 100.4^{\circ}\text{F}$ (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.psrt2018@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 to still be 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 <i>Must be no more than 42 days from screening and within 3 days of randomization</i>		
Administrative and Regulatory		<ul style="list-style-type: none"> • 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		<ul style="list-style-type: none"> • Obtain interim history • Clinical examination: <ul style="list-style-type: none"> ○ Perform focused clinical examination including temperature, heart rate, respiratory rate, EENT, lung, heart, and lymph nodes. ○ Record temperature, pulse, and respirations <p>Note: Confirm eligibility including clinical examination prior to administering study product.</p>
Laboratory	Blood	<p><i>If insufficient volume collected at screening, collect blood:</i></p> <ul style="list-style-type: none"> • To ensure that back-up aliquot is available for comparing pre- and post sera.
	Nasosorption SAM Strip	<p><i>Collect nasosorption strip for RSV antibody assays</i></p> <p>Note: The nasosorption must be performed prior to the nasal wash.</p>
	Nasal Wash	<p><i>Collect nasal wash for:</i></p> <ul style="list-style-type: none"> • RSV antibody assays • RSV viral detection and quantification <p>Note: The nasal wash must be performed prior to administering the study product.</p>
Study Product		<ul style="list-style-type: none"> • 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		<ul style="list-style-type: none"> • 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 (39). The parent/guardian will measure temporal artery temperatures following the manufacturer's directions. If any temporal artery temperature is $\geq 100.0^{\circ}\text{F}$, parents/guardians will be asked to measure a rectal temperature within 20 minutes (39). For study-specific management and grading of temperatures, see Section 8.1.1 and Table 6.

6.3 Acute Phase Visits and Contacts

Refer to 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 (29, 39-41). 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 (all are ± 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		<ul style="list-style-type: none">• 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	Nasosorption SAM strip	Day 28 visit only: Collect nasosorption strip for RSV antibody assays Note: The nasosorption must be performed prior to the nasal wash.
	Nasal Wash	Collect nasal wash for: <ul style="list-style-type: none">• RSV viral detection and quantification
		Day 28 visit only: Collect nasal wash for: <ul style="list-style-type: none">• RSV antibody assays• RSV viral detection and quantification
Prepare for follow-up		<ul style="list-style-type: none">• 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 (\pm 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 \pm 1 day)	
Interim History	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 Contact Procedures (+ 1 day)	
Interim History	<ul style="list-style-type: none"> Obtain interim history through midnight of the 28th day following inoculation Document highest temperature (temporal or rectal)
Prepare for follow-up	<ul style="list-style-type: none"> 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 56th 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		<ul style="list-style-type: none"> Obtain interim history since last contact Record temperature, pulse, and respirations.
Laboratory	Blood	<i>Collect blood for:</i> <ul style="list-style-type: none"> Serum antibodies to RSV <i>Only from participants who are at sites with capacity for processing viable PBMCs:</i> Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasosorption SAM strip	Collect nasosorption strip 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: <ul style="list-style-type: none"> 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).

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

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

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 eCRFs or reported under this protocol except for data as outlined in Table 3 in Section 7.2.

6.7 Pre-RSV Season Study Visit (October 1st to 31st or as specified in the MOP)

The Pre-RSV Season Study Visit is not required if the Day 56 Visit is conducted on or after October 1st for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP (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 nasosorption strip for RSV antibody assays.

Pre-RSV Season Study Visit (October 1 st * to 31 st *)		
Laboratory	Blood	<i>Collect blood for:</i> <ul style="list-style-type: none">• Serum antibodies to RSV• <i>Only from participants who are at sites with capacity for processing viable PBMCs:</i> Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasosorption SAM strip	Collect nasosorption strip for RSV antibody assays
Prepare for follow-up		<ul style="list-style-type: none">• Review plans for weekly contact during the RSV Season Surveillance Period (see Section 6.8).

*These dates apply to most sites but may differ for those with local RSV seasons that start earlier.

6.8 RSV Season Surveillance (November 1st through March 31st following inoculation or as specified in the MOP)

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 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP. Note, RSV Season Surveillance may overlap with the Study Acute and Post-Acute Phase. In this case, evaluations required for each of the relevant phases of the study will be conducted. During the RSV season 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 1 st * through March 31 st following inoculation)	
Clinical	<ul style="list-style-type: none"> Obtain interim history
Prepare for follow-up	<ul style="list-style-type: none"> 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

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

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

There will be a single in-person visit between April 1st and 30th. This window applies to all sites.

Post-RSV Season Study Visit (April 1 st to 30 th)		
Administrative and Regulatory		<ul style="list-style-type: none"> Inform participant's parent/guardian of study randomization, if known.
Laboratory	Blood	<i>Collect blood for:</i> <ul style="list-style-type: none"> Serum antibodies to RSV. <i>Only from participants who are at sites with capacity for processing viable PBMCs:</i> Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasosorption SAM strip	Collect nasosorption strip 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 7.4 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 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP): 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		<ul style="list-style-type: none"> • 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	<i>Collect nasal wash for:</i> <ul style="list-style-type: none"> • 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		<ul style="list-style-type: none"> • 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		<ul style="list-style-type: none"> • Obtain interim history
Laboratory	Blood	<i>Collect blood for:</i> <ul style="list-style-type: none"> • Serum antibodies to RSV • <i>Only from participants who are at sites with capacity for processing viable PBMCs:</i> Cryopreservation of PBMCs to characterize the B cell response. Remaining plasma to be stored.
	Nasosorption SAM strip	<i>If Early Discontinuation Visit is conducted within 56 Days of inoculation (Appendix II), collect nasosorption strip for RSV antibody assays</i> <ul style="list-style-type: none"> • Note: The nasosorption must be performed prior to the nasal wash.
	Nasal Wash	<i>If Early Discontinuation Visit is conducted within 56 Days of inoculation (Appendix II), collect nasal wash for:</i> <ul style="list-style-type: none"> • 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/sites/default/files/laboratorypolicy1.pdf>

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.impactnetwork.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 for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP). Laboratory testing will be performed by personnel that are not involved with clinical assessment to maintain the blinding of the study.

6.12.2 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. Screening serum samples will be shipped real time to JHU for RSV serology. All other serum will be shipped to JHU within approximately two weeks of obtaining the sample.

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 adsorption (nasosorption) 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. Synthetic adsorptive matrix (SAM) strips will be applied to the mucosa of the lower turbinate for 30 seconds. Mucosal antibody assays will be performed at LID, NIAID.

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.

Nasal wash samples for viral detection will be shipped within two weeks after the Acute Phase (Day 28) to JHU. Nasal adsorption specimens and nasal wash samples for antibody detection will be shipped within two weeks after the Post-Acute Phase (Day 56) to LID, NIAID. If a participant experiences an illness meeting protocol-specified Pausing/Stopping Rules, nasal wash specimens for adventitious PCR from the Illness Visit will be shipped real time to JHU. Plasma and PBMC samples will be held on-site until the end of the study and then shipped to LID, NIAID.

Plan for Future Use and Storage of Biological Samples

All specimens collected as part of this study may, with the parent/guardian's permission, be stored for future research as part of JHU CIR's approved biospecimen repository for vaccine research or at the LID, NIAID. These samples may be used for future screening for respiratory virus vaccine studies and to learn more about RSV infection and other diseases. These samples will not be sold or used to make commercial products. 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 eCRFs as indicated in Section 7.2 and complete expedited adverse event (EAE) reporting as indicated in Section 7.3. Site investigators are also responsible for prompt reporting 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 3), 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 eCRFs 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. 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 eCRFs, 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 eCRF recording requirements. In addition, there may be a period when no AEs are recorded on eCRFs if the Day 56 Visit is conducted in advance of the start of the RSV Season Surveillance Period (November 1st for most sites or—for sites with local RSV seasons that start earlier—as specified on a site-by-site basis in the MOP). The adverse events (solicited and unsolicited; and SAEs) to be recorded on eCRFs and the study phase and the calendar dates during which they are to be reported are defined in Table 5.

Adverse events identified in this study will be recorded on eCRFs as signs, symptoms, laboratory test results, and diagnoses, as described in Table 3. Expectations regarding the recording of Concomitant Medications are also detailed in this table.

Table 3: AE eCRF Recording Requirements

Study Phase at the time of event onset	Calendar Date	AEs to record on eCRFs	Concomitant Medications to record on eCRFs
Days 0 through midnight of 28 th day following inoculation (Acute Phase)	ANY	<ul style="list-style-type: none"> 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. <p>Note: SAEs and LRIs must be reported via DAIDS Adverse Experience Reporting System (DAERS; see Section 7.3.33). If randomization and Day 0 do not occur on the same day and AEs occur in the interim, record these AEs.</p>	<p>Record these medications on eCRFs regardless of whether the related event is recorded on eCRFs:</p> <ul style="list-style-type: none"> All cough and cold remedies including decongestants, cough suppressants, expectorants All nasal sprays (except saline spray) All antihistamines All antipyretics All prescription medications <p>For SAE and LRIs:</p> <ul style="list-style-type: none"> All concomitant medications related to the recorded event
From 12:01 am on 29 th day after inoculation to midnight of the 56 th day following inoculation (Post-Acute Phase)	ANY	<ul style="list-style-type: none"> All SAEs <p>Note: SAEs must be reported via DAERS (see Section 7.3.3).</p>	<ul style="list-style-type: none"> All concomitant medications related to the recorded event
After Day 56 Visit and until RSV Season Surveillance Period	Up to October 31 st * in year of inoculation	<ul style="list-style-type: none"> Grade ≥ 3 AE or SAE that is deemed related to Pre-RSV Season Study Visit procedures. 	<ul style="list-style-type: none"> All concomitant medications related to the recorded event
RSV Season Surveillance Period	November 1 st * to March 31 st following inoculation	<ul style="list-style-type: none"> Fever, LRI, URI, and/or otitis media that are medically attended All SAEs <p>Note: these events do not need to meet the Appendix IV criteria. SAEs and Grade ≥ 3 LRIs must be reported via DAERS (see Section 7.3.3).</p>	<p>For SAE and LRIs (all grades):</p> <ul style="list-style-type: none"> All concomitant medications related to the recorded event <p>Medications related to recorded medically attended illness should be documented in source notes.</p>
Post-RSV Season	April 1 st -April 30 th in the year after the inoculation	<ul style="list-style-type: none"> Grade ≥ 3 AE or SAE that is deemed related to Post-RSV Season Study Visit procedures. 	<ul style="list-style-type: none"> All concomitant medications related to the recorded event
Throughout study	ANY	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> All concomitant medications related to the recorded event

*These dates apply to most sites but may differ for those with local RSV seasons that start earlier.

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 SAE Reporting Category identified above, other AEs that must be reported in an expedited manner are LRIs occurring during the periods specified in Table 4.
- The study agents for which expedited reporting is required are recombinant live-attenuated respiratory syncytial virus vaccines RSV ΔNS2/Δ1313/I1314L and RSV 276 and 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).

7.3.3 Expedited AE Reporting Period

Table 4 details the events that must be reported in an expedited fashion via DAERS during specific periods of the study.

Table 4: EAE Reporting

Study Phase at the time of event onset	Calendar Date	Events to Report via DAERS
Days 0 through midnight of 28 th day following inoculation (Acute Phase)	ANY	<ul style="list-style-type: none"> • SAEs • LRIs
From 12:01 am on 29 th day after inoculation to midnight of the 56 th day following inoculation (Post-Acute Phase)	ANY	<ul style="list-style-type: none"> • SAEs
After Day 56 Visit and until RSV Season Surveillance Period	Up to October 31 st * in year of inoculation	<ul style="list-style-type: none"> • Follow-up related to SAEs/LRIs already reported
Pre-RSV Season	October 1 st * to October 31 st * in the year of inoculation	<ul style="list-style-type: none"> • SAEs related to Pre-RSV Season Study Visit procedures
RSV Season Surveillance Period	November 1 st * to March 31 st following inoculation	<ul style="list-style-type: none"> • SAEs • Grade ≥ 3 LRIs
Post-RSV Season	April 1 st to April 30 th in the year after the inoculation	<ul style="list-style-type: none"> • SAEs related to Post-RSV Season Study Visit procedures

*These dates apply to most sites but may differ for those with local RSV seasons that start earlier.

7.4 Grading Severity of Events

All solicited AEs and fever will be graded following protocol-defined grading system outlined in Table 5 and Table 6. 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 2.1, dated March 2017. In the event that this table is updated following protocol implementation, events will continue to be evaluated per this version of the DAIDS AE Grading Table. The DAIDS AE Grading Table is available on the RSC website at <http://rsc.tech-res.com/docs/default-source/safety/daids-ae-grading-table-mar2017.pdf?sfvrsn=6>.

Solicited AE Grading

Table 5: 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 6: Fever Grading*

Severity	Defined
Grade (0)	$\geq 100.0^{\circ}\text{F}$ but $< 100.4^{\circ}\text{F}$ ($\geq 37.8^{\circ}\text{C}$ but $< 38^{\circ}\text{C}$)
Grade (1)	$\geq 100.4^{\circ}\text{F}$ but $\leq 101.4^{\circ}\text{F}$ ($\geq 38^{\circ}\text{C}$ but $< 38.6^{\circ}\text{C}$)
Grade (2)	$\geq 101.5^{\circ}\text{F}$ but $\leq 102.4^{\circ}\text{F}$ ($\geq 38.6^{\circ}\text{C}$ but $\leq 39.1^{\circ}\text{C}$)
Grade (3)	$\geq 102.5^{\circ}\text{F}$ but $\leq 104.8^{\circ}\text{F}$ ($\geq 39.2^{\circ}\text{C}$ but $\leq 40.4^{\circ}\text{C}$)
Grade (4)	$\geq 104.9^{\circ}\text{F}$ ($\geq 40.5^{\circ}\text{C}$)

*Applies to any modality of temperature measurement

The expedited AE reporting period for this study is defined in Section 7.3.33.

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 product. 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. This definition of “adverse event” will be applied to all enrolled infants beginning at the time of randomization.

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 7.4) 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:

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

There are two categories of AEs specific to IMPAACT 2018: 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 eCRFs 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 eCRFs. During the Acute Phase of this study, Days 0 to 28, solicited AEs meeting the criteria for reporting will be recorded on eCRFs, assigned a severity grade (Section 7.4 and Table 5 and Table 6), 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 eCRFs if a clinical assessment done on the day of the event(s) does/did not confirm their presence. For example, if a parent/guardian reports rhinorrhea on the day of visit, and there is/was no rhinorrhea upon exam, then the participant is considered to not have rhinorrhea that day.

If the parent/guardian report of a fever meets the “definition” column criteria in [Appendix IV](#) on a day on which there was a clinical assessment, the fever will be recorded on eCRFs regardless of whether the clinical assessment confirmed its presence.

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

- Recorded on the eCRFs 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 eCRF, 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.psrt2018@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). Note that this procedure will be performed every time such a pause is triggered in order to maintain the blind. The virology studies will determine if there is shedding of RSV and/or adventitious viral agent(s) at the time of the event.
- Study accrual will remain suspended while the virology studies are performed.
- The DSMB and the study sponsor will be informed of the event.
- The DSMB will receive an e-mail from the unblinded study statistician (*while the rest of the team remains blinded*) containing the following:
 - Pertinent, blinded, clinical description of the event, prepared by the clinical trials specialists in consultation with site staff and approved by the Protocol Chair.
 - Results of virology studies, prepared by the testing lab.
 - Study product assignment (vaccine vs. placebo), from the unblinded study statistician.
- Follow-up visits for participants already inoculated will continue as outlined in [Appendix II](#).

The event will be reviewed by the DSMB prior to resuming enrollment. The DSMB will review the information provided, including whether the event can be attributed to an etiology, a cause or a diagnosis unrelated to the study vaccine or if it is associated with shedding of vaccine virus at the time of the event (even if another pathogen is identified).

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

The goal of this Phase I, blinded, randomized, placebo-controlled vaccine trial is to assess the safety, infectivity, and immunogenicity of the RSV ΔNS2/Δ1313/I1314L and RSV 276 vaccine candidates in RSV-seronegative pediatric participants and to assess whether these two vaccines are good candidates to move forward into a Phase IB study (by meeting the criteria listed in the Schema). Approximately 80 participants will be randomized in a 2:2:1 ratio to receive either of the candidate vaccines or placebo. The Protocol Team is interested in only whether the vaccines are better than placebo with respect to infectivity and immunogenicity; thus, the statistical comparisons between vaccine and placebo groups will consist of one-tailed tests. The study will not be powered for the modest difference expected between the two vaccines; thus, the groups receiving vaccine will be compared by means of descriptive analyses. Both vaccines may be used in a subsequent trial aimed at assessing efficacy, provided that this is not counter indicated by safety results.

In an effort to promote rapid enrollment, eligible children living in the same household (including twins, siblings, or other non-related children) will be allowed to enroll and will be randomized to the same study product arm. The potential bias introduced by correlations among such children will be addressed by means of sensitivity analyses, as described in Section 9.5.1.

Data from this study and from a companion study of these vaccines 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).

9.2 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 this time period; Day 0 nasal wash will be counted as baseline) and/or 2) ≥ 4 -fold rise in RSV serum neutralizing antibody titer and/or serum ELISA titer to the RSV F protein from study entry to Study Day 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 serum neutralizing antibody titer from study entry to Study Day 56

- RSV serum neutralizing antibody titers assessed by 60% RSV plaque reduction neutralization assay at study entry and Study Day 56
- ≥ 4 -fold rise in serum antibody titers to RSV F glycoprotein as assessed by ELISA from study entry to Study Day 56
- serum antibody titers to RSV F glycoprotein as assessed by ELISA at study entry and Study Day 56

9.2.2 Secondary Outcome Measures

- Types and grades of symptomatic, medically attended respiratory and febrile illness adverse events in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season.
- Antibody titers in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season.
- Frequency of B cell response to vaccine
- Mucosal antibody titers to vaccine, in nasal wash or nasosorption samples

9.3 Sample Size and Accrual

9.3.1 Sample Size and Randomization

Approximately 80 RSV-seronegative infants will be enrolled in the study (IMPAACT 2018 and CIR 321 combined) and will receive RSV Δ NS2/ Δ 1313/11314L (10^6 PFU), RSV 276 (10^5 PFU), or placebo in a 2:2:1 ratio (32:32:16). Assuming an attrition rate of about 6%, approximately 30 vaccine recipients per vaccine arm and 15 placebo recipients will provide data for the primary objectives. In the case of children from the same household, all eligible children will be assigned to the same study product regimen. This will be done to reduce the potential cross-contamination that could result if children living together were to receive different study products. Permuted block randomization will be used to ensure that the 2:2:1 ratio of treated to control participants will be maintained across time.

The sample size was chosen based upon past experience with Phase I evaluation of other live-attenuated respiratory virus candidate vaccines (5, 26, 27) and on the number of participants feasible to enroll in the 2017 fall prior to RSV season. No formal power calculations have been used. The following paragraphs show the limitations of the sample sizes being used.

All enrolled infants will be included in safety analyses.

9.3.2 Sample Size Limitations with Respect to Safety

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 safety of the vaccines and in particular, occurrence of LRI, which occurs very infrequently in children who participate in these types of studies but which would be considered a sentinel safety event if observed in participants infected with vaccine virus.

Table 7 shows the probability of observing 0 events of LRI within the sample of 30 vaccinees receiving either vaccine, 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 an 82% chance of observing 2 or more events in a group of size 30, and a 96% chance of observing at least a single event in a sample of this size.

Table 7: The Probability of Observing LRI events in Vaccinees

True underlying probability of LRI or AEs	N=30		
	Pr (0 events)	Pr (1+ events)	Pr (2+ events)
.01	.74	.26	.04
.03	.40	.60	.23
.05	.21	.79	.45
.1	.04	.96	.82
.15	.01	.99	.95

Table 8 presents 90% CIs around potential rates of AEs that might be observed in the sample of 30 vaccinees. The CIs around similar rates in a sample of 15 placebo recipients are also presented. Note that if no AEs are detected among either group of 30 vaccinees, there is 90% confidence that the true probability of AEs in the population from which the sample is drawn is between 0 and 10%.

Table 8: Percent of Participants Experiencing AEs with Exact 90% Confidence Intervals

N	% LRI or AEs	90% CI
15	0%	0% -- 18%
30	0%	0% -- 10%
15	10%	1% -- 32%
30	10%	3% -- 24%
15	20%	6% -- 44%
30	20%	9% -- 36%
15	30%	12% -- 55%
30	30%	17% -- 47%

9.3.3 Sample Size Limitations with Respect to Infectivity and Immunogenicity

Criterion “The mean peak titer of shed virus in nasal washes should be approximately 2.5 log₁₀ PFU”

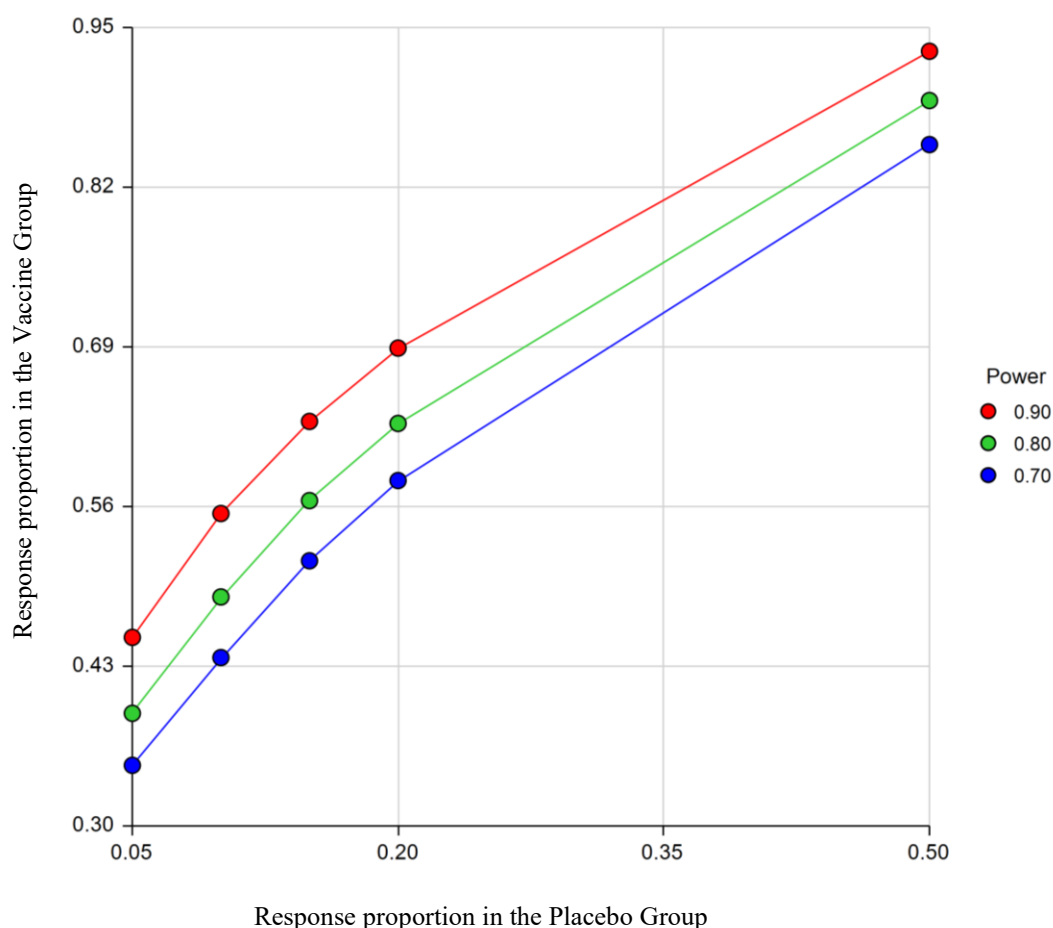
With a sample size of 30 vaccinees, the 90% CI around a targeted sample mean peak titer of shed virus of 2.5 log₁₀, assuming a SD of 1.5 (derived from IMPAACT 2000) is (2.04, 2.97). This ensures with 90% confidence that the true population mean peak titer of shed virus is between 2.04 and 2.97 log₁₀, and with 95% confidence that the true population mean of shed virus is not lower than 2.04 log₁₀.

With respect to infectivity and immunogenicity, group sample sizes of 30 in either of the vaccinated groups and 15 in the placebo group would achieve 80% power to detect a difference between the vaccine and placebo groups proportions of at least 0.34. The test statistic used is the one-sided Fisher's Exact test, in the direction that either vaccinated group will have a higher proportion than the placebo group. The alpha level of the test was targeted at 0.05. Table 9 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 9: Magnitude of Difference in Responses Detectable with 80% Power

Response Proportion in the Placebo Group	Response Proportion in Either Vaccinated Group	Difference
0.05	0.40	0.34
0.1	0.49	0.39
0.15	0.56	0.41
0.2	0.63	0.43
0.5	0.89	0.39

Figure 4: Power curves for comparisons between responses to either vaccine to responses to placebo



(Alpha=.05; N Vaccine=30; N Placebo=15; 1-Sided Exact Test)

Criterion “>90% of vaccinees should be infected with vaccine virus”

Table 10 presents 90% confidence intervals (CIs) around potential rates of infection with vaccine virus that might be observed in the sample of 30 vaccinees. For the target proportion of 93% (i.e. >90%), this ensures with 90% confidence that the true proportion of vaccinees who shed virus is between 80% and 98.7% and with 95% confidence that the true proportion of vaccinees who shed vaccine virus is not lower than 80%.

Table 10: Percent of Vaccinees who are Infected with Vaccine Virus with Exact 90% Confidence Intervals

N	% of vaccinees infected with vaccine virus	90% CI
30	90% (27/30)	76% -- 97%
30	93% (28/30)	80% -- 98.7%
30	97% (29/30)	86% -- 99.9%

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 will be submitted to the DSMB Executive Secretary for DSMB review. Two reports will be prepared: one that will preserve the blind and will include data pooled across arms (with the vaccine and placebo arms presented together) and a second one, in which the data will be broken down by masked treatment arm, and the codes identifying the arms will be provided to the

DSMB. The DSMB may choose not to unblind themselves if no events of major concern are observed. The DSMB will be 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

A detailed statistical analysis plan will be developed and finalized before the first distribution of safety data for DSMB review.

Where appropriate, a 1-tailed Wilcoxon rank sum test will be used to test the hypothesis that a 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 a 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. The statistical tests will be one-sided with a nominal significance level of 0.05. Statistical analyses will be based on the as-treated approach, unless otherwise specified. For safety, infectivity and immunogenicity analyses, the time starts at inoculation (Study Day 0).

In the event that twins or siblings are enrolled in the study, both siblings will be included in the safety analyses, to ensure that any serious adverse events is observed. In the infectivity and immunogenicity analyses, one of the siblings will be randomly selected to be included in the analyses. Sensitivity analyses will be performed in which the selected child will be replaced by the other sibling(s) to ensure that any choice would support the same overall results. Line listings will provide individual data from all children.

9.5.1 Assessment of Primary Objectives

The safety, infectivity and immunogenicity data will be summarized for each study product group. In addition, the criteria listed in Section 1.1 will be used to determine if these vaccines are promising candidates for further evaluation in a Phase IB study. The analyses for these criteria are described below.

Safety Analyses, addressing the criterion "The vaccines will be safe"

All enrolled infants will be included in safety analyses.

The frequency of study-product related solicited AEs and unsolicited AEs, along with 90% confidence intervals, during Study Days 0 to 28 and of study product-related SAE during Study Day 0 to the Day 56 will be summarized. A similar summary will include all AEs, regardless of relationship to study product. In addition, line listings 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.

Infectivity and Immunogenicity Analyses

The primary infectivity and immunogenicity analyses will be based on the as-treated approach, including the study participants who have been inoculated. In an additional analysis, those who do not provide data for the Study Day 28 or 56 (due to early discontinuation or missed visit) will be treated as “failures” in an intent-to-treat analysis. Sensitivity analyses will be performed to check whether the results are consistent with those observed 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 infectivity and immunogenicity evaluations after the time of the treatment.

Criterion “>90% of vaccinees should be infected with vaccine virus”

The proportion of participants infected with vaccine virus along with 90% confidence intervals, will be summarized. Infection with vaccine virus is defined by shedding vaccine virus, detected by infectivity assay and/or RT-qPCR, and/or fourfold or greater rise in RSV-specific serum antibodies, detected by ELISA against the RSV F protein and/or an RSV plaque reduction neutralization assay (RSV-PNRT).

Criterion “The mean peak titer of shed virus in nasal washes should be approximately 2.5 log₁₀ PFU”

The mean peak titer and mean duration of virus shed with 90% confidence intervals will be provided, for each study product group. In addition, line listing of the individual peak titer of vaccine virus shed and duration of virus shedding in nasal washes by each individual will also be prepared.

Criterion “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)”

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 study product 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.

9.5.2 Assessment of Secondary Objectives

Summaries 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 study product group. To address the criterion “Post-vaccination surveillance during the RSV season following vaccination should reveal substantial rises in RSV-neutralizing serum antibodies in a subset of vaccine recipients in the absence of RSV associated medically attended acute

respiratory illness (RSV-MAARI), which would be indicative of exposure to wt RSV without illness,” the changes in median antibody titers between day 56 and the post RSV surveillance time point will be summarized in the subset of vaccine recipients who do not experience RSV-MAARI.

The B cell responses to vaccine will be summarized for each study product group. A line listing of the mucosal antibody response detected in nasal wash specimens will be prepared.

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

10 DATA HANDLING AND RECORD KEEPING

10.1 Data Management Responsibilities

As described in Section 4.4, data on 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 eCRFs 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>

eCRFs 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 eCRFs 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/research/daids-clinical-research-policies-standard-procedures>

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 RSV ΔNS2/Δ1313/I1314L and RSV 276 vaccines went into effect. These reports will provide a brief description of the progress of the investigation as outlined in 21 CFR 312.33 and will include any revisions of the protocol not previously submitted to the FDA.

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

All study records must be accessible for inspection, monitoring, and/or auditing during and after the conduct of the study by authorized representatives of the study sponsors and their contracted monitors, IMPAACT, the US Food and Drug Administration, 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 vaccines. 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/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, eCRFs, medical records, laboratory records, and pharmacy records, to ensure protection of study participants, compliance with the IRB/IBC-approved protocol, and accuracy and completeness of records. 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, eCRFs) 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 CFR 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 (42, 43). 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/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. Parents/guardians will also be asked whether they agree to limited genetic testing, including future limited genetic testing; this testing 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 may not receive direct study product-related benefit from enrollment in this study. However, based on prior studies, there is the possibility of a reduced risk of disease from RSV for vaccine recipients. Placebo recipients will not receive any direct benefit from enrollment in this study. 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 a vaccine (RSV ΔNS2/Δ1313/I1314L or RSV 276) is insufficiently attenuated, participants could experience rhinorrhea, cough, fever, hoarseness, otitis media, or LRI. Immediate hypersensitivity reactions—which could be life threatening—including urticaria, anaphylaxis, or other Immunoglobulin E (IgE)-mediated responses are possible, as with any vaccine. There is a theoretical possibility, as with any investigational vaccine, of risks about which there is no present knowledge. Parents/guardians will be informed of any such risks should further data become available. A previous vaccine candidate, RSV MEDI/ΔM2-2, that is nearly identical to RSV 276, was previously evaluated in 20 RSV-seronegative children 6 to 24 months of age at a dose of 10^5 PFU and was very highly attenuated and appeared to be well tolerated.

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 site's 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, eCRFs, and other documents that may be transmitted off-site (e.g., EAE report forms, photographs of observed reactions) will be identified by PID only. Likewise, communications between study staff and Protocol Team members regarding individual participants will identify participants by PID only.

Study sites are encouraged but not required by DAIDS 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.

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 one or more study participants and/or significant impacts on the integrity of study data must also be reported within IMPAACT, following procedures specified in Section 12 of the IMPAACT Manual of Procedures.

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.

15 REFERENCES

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APPENDICES

Appendix I: Tables Referenced in the Background Section

Table 11: Viral Titers of Nasopharyngeal Swab Samples from African Green Monkeys Inoculated with the CTM RSV ΔNS2/Δ1313/I1314L, Lot#006A, or with recombinant wt RSV rA2

Group	Virus Test Article ^a	AGM ID	NP virus titer (log ₁₀ PFU/mL) on indicated days ^b											Peak virus titer	Sum of daily titers ^c
			1	2	3	4	5	6	7	8	9	10	14		
TM1	RSV ΔNS2/Δ1313 I1314L CTM RSV#006A ^d	7648	-	-	1.7	1.2	1.8	2.9	2.2	<u>4.0</u>	3.7	3.4	-	4.0	22.0
		7692	-	-	-	-	1.9	-	-	<u>3.3</u>	1.0	1.5	-	3.3	10.2
		7714	-	-	0.7	-	1.3	-	-	1.4	2.4	<u>2.8</u>	-	2.8	10.7
		7764	-	-	-	0.7	0.7	1.0	-	2.3	1.2	<u>2.9</u>	-	2.9	10.5
		Mean:												3.2	13.3
TM2	RSV rA2 ^e wt RSV	7638 ^f	-	-	-	-	-	-	-	-	-	-	-	0.7	3.9
		7744	-	0.7	2.1	3.3	<u>3.4</u>	<u>3.4</u>	2.5	<u>3.4</u>	2.4	1.2	-	3.4	23.1
		7781	1.0	-	1.0	2.1	2.6	1.8	<u>3.4</u>	2.9	1.6	0.7	-	3.4	17.1
		7799	-	1.5	<u>3.7</u>	3.3	2.5	3.0	3.3	2.9	2.6	1.2	-	3.7	24.7
		Mean:												3.5	21.7

^a Monkeys were inoculated intranasally and intratracheally, with 10⁶ PFU of the indicated virus in a 1 mL inoculum per site (total dose = 2x10⁶ PFU/AGM).

^b Virus titrations were performed on Vero cells at 32°C. The lower limit of detection was 1.0 log₁₀ PFU/mL. Samples with no detectable virus are represented as "-". Peak titers for each animal are underlined. The results show that RSV ΔNS2/Δ1313/I1314L is strongly restricted in the URT of AGMs compared to RSV rA2.

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

^d CTM vial numbers 0004, 1377, 2505.

^e wt RSV, rA2 D53#51(4), 8/13/07

^f This AGM reacted to each anaesthesia by emesis. It is likely that the emesis interfered with the efficacy of the intranasal inoculation. Based on this assumption, NP shedding results from this animal were excluded from calculations of means and from statistical analysis.

Table 12: Viral Titer of Tracheal Lavage samples from African Green Monkeys Inoculated with the CTM RSV Δ NS2/ Δ I1313/I1314L, Lot RSV#006A, or with recombinant wt RSV rA2^a

Group	Virus Test Article ^a	AGM ID	TL virus titer (log ₁₀ PFU/mL) on indicated day ^b						Peak virus titer	Sum of daily titers ^c
			2	4	6	8	10	14		
TM1	RSV Δ NS2/ Δ I1313/I1314L CTM RSV#006A ^d	7648	-	-	-	<u>1.5</u>	-	-	1.5	5.0
		7692	-	-	-	1.0	<u>1.7</u>	-	1.7	5.5
		7714	-	-	-	-	-	-	-	4.2
		7764	-	-	-	<u>1.3</u>	-	-	1.3	4.8
		Mean:							1.4	4.9
TM2	RSV rA2 ^e wt RSV	7638	2.3	3.1	<u>3.6</u>	3.0	-	-	3.6	13.3
		7744	2.3	<u>2.7</u>	2.3	2.4	1.0	-	2.7	11.4
		7781	3.0	<u>3.2</u>	1.9	2.7	-	-	3.2	12.2
		7799	3.7	4.1	<u>4.7</u>	3.3	2.1	-	4.7	18.5
		Mean:							3.5	13.9

^a Monkeys were inoculated intranasally and intratracheally with 10⁶ PFU of the indicated virus in a 1 mL inoculum per site (total dose = 2x10⁶ PFU/AGM).

^b Virus titrations were performed on Vero cells at 32°C. The lower limit of detection was 1.0 log₁₀ PFU/mL. Samples with no detectable virus are represented as “-“. Underlined value indicates maximum titer for each animal. As expected, the highly temperature sensitive virus RSV Δ NS2/ Δ I1313/I1314L did not replicate in the lower respiratory tract of AGMs (body temperature: 39°C).

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

^d CTM vial numbers 0004, 1377, 2505

^e wt RSV, rA2 D53#51(4), 8/13/07

Table 13: Neutralizing antibody titers of AGMs inoculated with CTM RSV ΔNS2/Δ1313/11314L, Lot RSV#006A^a, or with recombinant wt RSV rA2^b

Group	Virus Test Article	AGM ID	RSV Neutralization Titer (Log ₂ of reciprocal) on days		
			0	21	28
TM1	RSV ΔNS2 Δ1313 I1314L Lot RSV #006A ^a	7648	<3.3	6.4	6.8
		7692	<3.3	6.6	6.4
		7714	<3.3	4.4	6.1
		7764	<3.3	5.5	6.0
		Mean:	<3.3	5.8	6.3
TM2	RSV rA2 ^b wt RSV	7638	<3.3	7.0	6.6
		7744	<3.3	6.5	7.9
		7781	<3.3	8.0	8.6
		7799	<3.3	7.6	7.8
		Mean:	<3.3	7.3	7.8

^a Lot RSV#006A, vial numbers 0004, 1377, 2505

^b wt RSV, rA2 D53#51(4), 8/13/07. The lower limit of detection of the 60% Plaque Reduction assay is 3.3 (Log₂ of the dilution reciprocal). Samples below the lower limit of detection are recorded as “-”.

Table 14: Viral titers of nasopharyngeal swab samples from African green monkeys (AGMs) inoculated with recombinant wildtype RSV A2, MEDI/ΔM2-2, or RSV 276^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 ^c
		1	2	3	4	5	6	7	8	9	10	12/14				
RSV A2 CTM RSV#004A	7209	-	-	3.1	4.4	4.4	<u>5.2</u>	3.6	4.1	3.4	2.0	-	8	5.2	31.1	
	7467	-	0.7	2.8	3.8	2.1	<u>3.9</u>	3.4	3.2	1.9	2.0	-	9	3.9	24.4	
	7468	-	1.7	2.6	<u>4.0</u>	3.9	3.7	3.6	2.7	3.6	2.6	-	9	4.0	29.0	
	7492	0.7	1.2	2.3	<u>3.9</u>	2.9	<u>3.9</u>	<u>3.9</u>	2.4	1.7	1.0	-	10	3.9	24.2	
Mean:													9	4.2	27.2	
RSV MEDI/ΔM2-2 CTM RSV#002A	Y655	-	-	-	<u>2.1</u>	1.2	1.2	1.5	1.5	1.2	-	-	6	2.1	10.3	
	Y684	-	-	0.7	0.7	1.0	-	<u>1.6</u>	-	-	-	-	5	1.6	6.5	
	Y609	-	-	-	0.7	<u>2.2</u>	1.6	<u>2.2</u>	2.1	1.0	-	-	6	2.2	11.1	
	Y658	-	-	-	<u>1.4</u>	-	-	-	-	-	-	-	1	1.4	4.9	
Mean:													5	1.8	8.3	
RSV 276 Exp. Lot	8918	-	-	-	-	-	-	-	-	-	-	-	0	-	3.9	
	8902	-	1.5	2.0	2.9	2.5	3.0	<u>3.1</u>	2.5	1.4	1.3	-	9	3.1	21.0	
	8913	-	-	-	-	-	0.7	<u>1.4</u>	1.0	-	-	-	3	1.4	6.0	
	8952	-	-	-	-	0.7	0.7	<u>1.5</u>	-	-	1.2	-	6	1.5	6.5	
Mean:													5	1.6	9.3	
RSV 276 CTM RSV#014A	8903	-	-	-	0.7	-	<u>1.3</u>	-	-	-	-	-	3	1.3	5.2	
	8900	-	-	0.7	-	1.0	1.0	<u>1.3</u>	1.0	1.0	1.0	-	8	1.3	8.4	
	9025	-	-	-	-	-	-	<u>1.2</u>	-	0.7	-	-	3	1.2	5.0	
	8986	-	-	-	<u>2.3</u>	1.0	1.0	1.2	-	0.7	-	-	6	2.3	8.3	
Mean:													5	1.5	6.7	

^a AGMs were inoculated by the combined intranasal and intratracheal routes with 10⁶ PFU of the indicated virus in a 1 mL inoculum per site (total dose: 2x10⁶ PFU per animal). Results from four individual studies are shown side-by-side. Results from a study of recombinant wildtype challenge virus lot (RSV A2 RSV#004A) are included for comparison. Studies were performed in AGMs from the same origin, following the same general sampling schedule for all studies. The AGM studies were approved by the Animal Care and Use Committee of NIAID, NIH. CTM, Clinical Trial Material; Exp. Lot, Experimental Lot. CTM RSV 275, RSV# 014A, vial numbers 0019-0022, 1269, 1271-1274, 2483-2487.

^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 log₁₀ PFU/mL. Samples with no detectable virus are represented as "-". Peak titers for each animal are underlined. Results of MEDI/ΔM2-2 and RSV 276 are not significantly different (ANOVA, Tukey post-hoc analysis).

^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 15: Viral titers of tracheal lavage samples from AGMs inoculated with recombinant wildtype RSV A2, MEDI/ΔM2-2, or RSV 276^a

RSV vaccine candidate ^a	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 ^c
		2	4	6	8	10	12/14			
RSV A2 CTM RSV#004A	7209	3.0	3.8	4.3	<u>4.5</u>	2.6	-	11	4.5	18.9
	7467	3.0	3.0	<u>4.3</u>	3.4	2.8	-	11	4.3	17.2
	7468	2.5	1.7	2.9	<u>3.0</u>	2.9	-	11	3.0	13.6
	7492	1.9	3.5	<u>4.7</u>	3.3	-	-	9	4.7	14.8
Mean:								11	4.1	16.1
RSV MEDI/ΔM2-2 CTM RSV#002A	Y655	-	0.7	1.0	<u>2.0</u>	-	-	7	2.0	5.8
	Y684	0.7	-	<u>2.3</u>	-	-	-	7	2.3	5.8
	Y609	-	2.3	<u>3.3</u>	1.3	-	-	7	3.3	9.1
	Y658	1.2	1.0	<u>2.9</u>	0.7	-	-	9	2.9	7.2
Mean:								8	2.6	7.0
RSV 276 Exp. Lot	8918	-	1.7	<u>2.3</u>	1.6	1.0	-	9	2.3	8.0
	8902	2.2	1.6	<u>2.9</u>	2.1	-	-	9	2.9	10.3
	8913	-	-	<u>3.2</u>	1.3	-	-	5	3.2	7.3
	8952	1.8	<u>2.1</u>	1.6	1.8	-	-	9	2.1	8.8
Mean:								8	2.6	8.6
RSV 276 CTM RSV#014A	8903	-	2.6	<u>3.8</u>	1.3	-	-	7	3.8	9.9
	8900	1.8	-	<u>2.4</u>	-	-	-	7	2.4	7.0
	9025	<u>2.6</u>	-	1.3	-	1.0	-	11	2.6	7.0
	8986	2.3	1.7	<u>3.9</u>	-	-	-	7	3.9	10.0
Mean:								8	3.2	8.5

^a AGMs were inoculated by the combined intranasal and intratracheal routes with 10⁶ PFU of the indicated virus in a 1 mL inoculum per site (total dose: 2 x 10⁶ PFU per animal). Results from four individual studies are shown side-by-side. Results from a study of recombinant wildtype challenge virus lot (RSV A2 RSV#004A) are included for comparison. Studies were performed in AGMs from the same origin, following the same general sampling schedule for all studies. The AGM studies were approved by the Animal Care and Use Committee of NIAID, NIH. CTM, Clinical Trial Material; Exp. Lot, Experimental Lot. CTM RSV 275, RSV# 014A, vial numbers 0019-0022, 1269, 1271-1274, 2483-2487.

^b On days 2, 4, 6, 8, 10, and 12 or 14, 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 log₁₀ PFU/mL of lavage solution, or log₁₀ PFU/mL for the MEDIΔM2-2 study. Samples with no detectable virus are represented as "-". Peak titers for each animal are underlined. Results of MEDI/ΔM2-2 and RSV 276 are not significantly different (ANOVA, Tukey post-hoc analysis).

^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 16: Neutralizing antibody titers of AGMs inoculated with recombinant wildtype RSV A2, MEDI/ΔM2-2, or RSV 276^a

RSV Vaccine candidate	AGM ID	Neutralizing antibody titers (PRNT ₆₀ , reciprocal log ₂) on indicated days ^b		
		0	21/22	28
RSV A2 CTM RSV#004A	7209	<3.3	7.1	6.7
	7467	<3.3	8.1	8.7
	7468	<3.3	5.9	5.9
	7492	<3.3	7.7	8.8
	Mean:	<3.3	7.2	7.5
RSV MEDI/ΔM2-2 CTM RSV#002A	Y655	<3.3	5.2	5.1
	Y684	<3.3	6.5	5.5
	Y609	<3.3	5.4	5.2
	Y658	<3.3	5.7	5.7
	Mean:	<3.3	5.7	5.4
RSV 276 Exp. Lot	8918	<3.3	6.1	6.2
	8902	<3.3	8.4	8.8
	8913	<3.3	8.8	8.9
	8952	<3.3	9.7	9.9
	Mean:	<3.3	8.3	8.5
RSV 276 CTM RSV#014A	8903	<3.3	9.0	10.1
	8900	<3.3	8.7	9.6
	9025	<3.3	6.4	6.9
	8986	<3.3	6.3	6.3
	Mean:	<3.3	7.6	8.2

^a AGMs were inoculated intranasally and intratracheally with 10⁶ 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 or 22, 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).

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

		ACUTE PHASE																		POST-ACUTE PHASE			Early DC				
		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
In person visit	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	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	
Nasosorption SAM strip for antibody		X																			X			X		X	
Nasal wash for: RSV antibody		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 1 st * to Oct 31 st *	Nov 1 st * to Mar 31 st	Apr 1 st to Apr 30 th		
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
Nasosorption SAM strip for antibody	X		X		X
Nasal wash for: viral detection & quantification				X	
Request adventitious agent assay				X	
TOTAL BLOOD VOLUME	8 mL	--	8 mL	--	8 mL

*These dates apply to most sites but may differ for those with local RSV seasons that start earlier.

Appendix IV: Definitions of Solicited Adverse Events

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

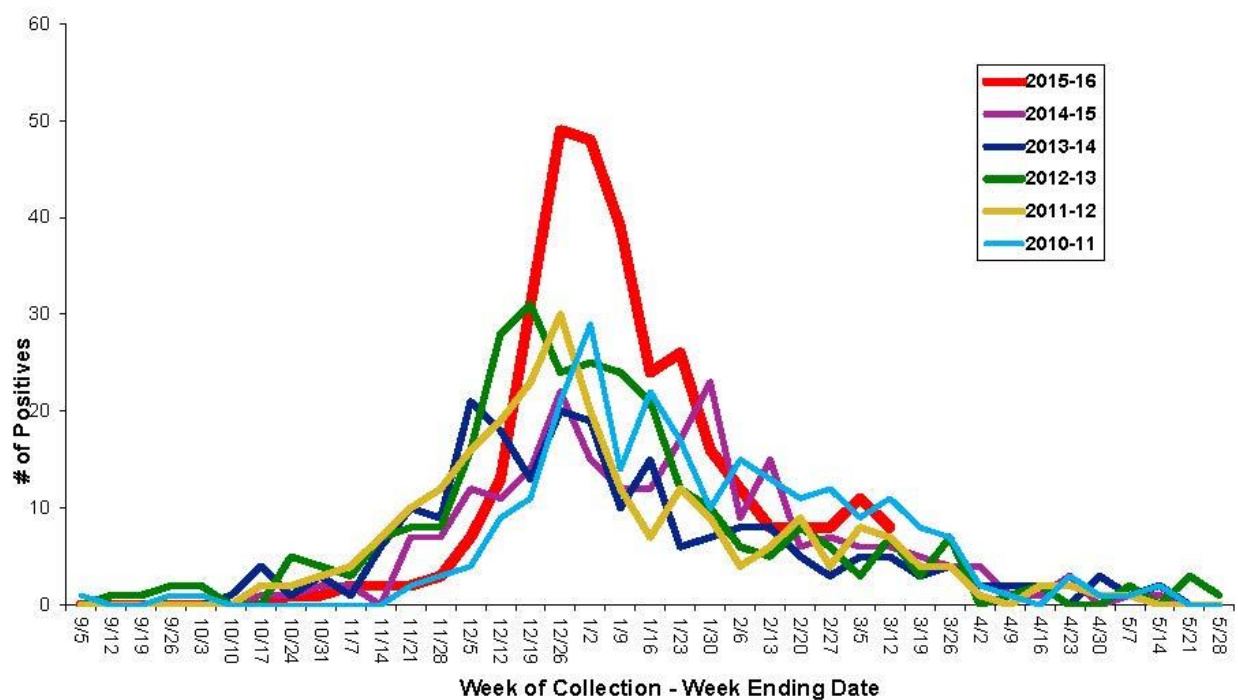
¹ Diagnosis must be made by a medical professional

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 eCRFs 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: 2018

Randomized Phase I Study of the Infectivity, Safety, and Immunogenicity of a Single Dose of the Recombinant Live-Attenuated Respiratory Syncytial Virus (RSV) Vaccines RSV Δ NS2/ Δ I1313/I1314L or RSV 276 or Placebo, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

Version 1.0, dated 15 June 2017

SHORT TITLE FOR IMPAACT 2018: Safety and Immunogenicity of a Single Dose of the RSV Δ NS2/ Δ I1313/I1314L or RSV 276 Vaccine

INTRODUCTION

You are being asked to allow your baby to take part in this research study to test vaccines 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 one of two vaccines against a virus called respiratory syncytial virus, or RSV. The study will look at the amount of vaccine virus that is in your child's nose over time. It will tell us how stable and strong the vaccine was during the study. This research study is testing two experimental vaccines. These vaccines have 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 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 live virus vaccines that may help prevent RSV illness in babies and children. A live virus vaccine contains a weakened, live virus that is made to help your body respond in a way that will protect you from getting sick from the virus. This is called an “immune response.” The investigational RSV vaccines in this study contain a live, weakened form of RSV and are given as nose drops. One of the vaccines has not been tested in humans. However, other RSV vaccines very similar to this one have been tested in both adults and children. The other vaccine has been tested in humans in one other research study. 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 one of the vaccines or 1 dose of placebo. The placebo has no vaccine in it. 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. Approximately 80 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 he/she 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 5 enrolled babies will get one of the RSV vaccines, and about 2 of each 5 enrolled babies will get the other RSV vaccine. Approximately 1 of each 5 enrolled babies will get nose drops without vaccine (placebo). Whether your baby gets one of the vaccines or nose drops without 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 one of the vaccines 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 [site: insert November 1st until March 31st unless other dates are specified in the MOP for your site] (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 or at one of the research sites/clinics. The visit where your baby receives vaccine or placebo must take place at one of the research sites/clinics 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 $\geq 100.0^{\circ}\text{F}$, 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. The physical examination will include checking your baby's temperature, pulse (heart rate), weight, length, and how fast your baby is breathing. 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 baby's medical records.
- if we think your baby may 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 confirm that he/she is eligible and then 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 (heart rate), 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. We will also use an absorbent strip to check for antibodies in the nose. The strip will be placed in your baby's nose for about 30 seconds and then removed.
- 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 chart 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, and we will also use an absorbent strip to check for antibodies in the nose. The strip will be placed in your baby's nose for about 30 seconds and then removed.
 - 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 check your baby's temperature, pulse, and breathing rate. We will also use an absorbent strip to check for antibodies in the nose. The strip will be placed in your baby's nose for about 30 seconds and then removed.
- 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 [*site: insert (November 1st until March 31st) unless other dates are specified in the MOP for your site*] 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 (a cold), 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 [site: insert October unless another month is specified in the MOP for your site] 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 use an absorbent strip to check for antibodies in the nose. The strip will be placed in your baby's nose for about 30 seconds and then removed. If your baby's Day 56 Visit occurred on or after [site: insert October 1 unless another date is specified in the MOP for your site], a separate visit in [site: insert October unless another month is specified in the MOP for your site] will not be required.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

There will be approximately 80 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 Vaccines

- If the vaccines are not weakened enough, they may cause a runny nose, sore throat, cough, or other signs of a cold. It is also possible that they 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 or colds 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 vaccines were 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 they contain unidentified material. There is a very small chance that such material may cause illness, including possibly serious illness.
- There may be other side effects of the vaccines that are not yet known. If new information about possible side effects of the vaccines 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 vaccines could cause a severe allergic reaction. A severe reaction can cause hives, throat swelling, rapid heart rate, weakness, difficulty breathing, or death. These reactions are rare.

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:

- For your baby's safety;
- 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 may not 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 one of the vaccines 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. At that visit, we will do a final blood draw (about 1 teaspoon) and collect a nasal wash and/or use an absorbent strip to check for antibodies in the nose. The strip will be placed in your baby's nose for about 30 seconds and then removed.
- 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. 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. 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. 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