IMPAACT 2018 and CIR 321

ClinicalTrials.gov Identifier: NCT03227029

PRIMARY STATISTICAL ANALYSIS PLAN

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

This is IMPAACT 2018 SAP Version 2.0 with names of authors,
names of publication writing team members and analysis timeline redacted.

Version 2.0

September 9, 2020

Protocol Version 1.0, CM #2, LOA#3

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Version History:

Version	Changes Made	Date Finalized
1	Original Version	31 January 2018
1.1	Updated to protocol Version 1.0, incorporating CM # 2 and LOA #3. No changes are needed based on these documents.	28 August 2020
2.0	Updated to protocol Version 1.0, incorporating CM # 1-2 and LOA #1-3. Minor changes are needed based on these documents. Changes made: Sections 3.3 and 5.4: removed secondary objectives 3-5 as they are now exploratory.	9 September 2020

1 Introduction

This Primary Statistical Analysis Plan (SAP) outlines the general statistical approaches that will be used in the primary statistical analysis of IMPAACT 2018. The focus is on analyses that address the study's primary and secondary objectives, as well as summaries of the primary and secondary outcome measures that will be posted on ClinicalTrials.gov. It will facilitate discussion of the statistical analysis components among the study team and provide agreement between the team and statistician regarding the analyses to be performed and presented in the primary statistical analysis report. Detailed outlines of tables and coding descriptions that will be included in the primary statistical analysis report are included in the Analysis Implementation Plan (AIP).

Analyses for the primary statistical report will be initiated once the last participant has completed the last study visit, all laboratory data are available, all queries have been resolved, and the database frozen for analysis. This report will be used for submission of results to ClinicalTrials.gov. Results are required to be submitted within one year of the primary completion date (PCD), which is the date the last participant is examined for the purposes of data collection for the primary outcome measure. For this study, the PCD is based on the last study visit.

2 Key Updates to the SAP

Up-version to version 1.1 to acknowledge CM#1-2 and LOA#1-3. In Sections 3.3 and 5.4, the last three secondary objectives were removed, as they are now exploratory.

3 Protocol Overview

3.1 Study Design

DESIGN: A double-blind, randomized, placebo-controlled study to evaluate the safety and immunogenicity of two vaccines in RSV-seronegative infants and children. Seronegativity refers to RSV antibody status, which is defined as a serum RSV-neutralizing antibody titer <1:40 (as determined within 42 days prior to inoculation).

SAMPLE SIZE: Approximately 80 healthy RSV-seronegative infants and children ≥6 months (180 days) to <25 months (750 days) of age will be randomized at a ratio of 2:2:1 to receive one of the two vaccines or placebo, respectively.

REGIMEN: A single dose of RSV ΔNS2/Δ1313/I1314L vaccine, RSV 276 vaccine, or placebo at study entry.

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

*plaque forming units

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

This protocol is a companion study to CIR 321, a study being conducted by the Center for Immunization Research (CIR, Johns Hopkins, Baltimore), and the Laboratory of Infectious Diseases (LID, NIAID, NIH, Bethesda). The protocols have identical primary and secondary objectives; inoculation schedules; evaluation assays and schedules; safety monitoring and reporting.

3.2 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 wild-type (wt) RSV without illness.

3.3 Study Objectives and Outcome Measures

Primary objectives and outcome measures:

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

    - Outcome measures:
        - Grade 1 or higher solicited study product-related AEs occurring from Study Day 0 through Study Day 28
        - Grade 1 or higher unsolicited study product-related AEs occurring from Study Day 0 through Study Day 28

2. Safety: To assess study product-related serious adverse events (SAEs) from Day 0 through midnight on the 56th day following inoculation

    - Outcome measure: study product-related SAEs occurring from Study Day 0 through Study Day 56

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 $\log_{10}$

    - Outcome measures:
        - 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

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

    - Outcome measure: 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

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)

    - Outcome measures:
        - ≥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-PRNT 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

Secondary objectives and outcome measures:

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

    - Outcome measure: 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

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 serum RSV-neutralizing antibodies are present in a subset of vaccine recipients in the absence of RSV-MAARI, which would be indicative of exposure to wt RSV without illness

    - Outcome measure: Antibody titers in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season

4 Definitions

4.1 Baseline

Study Day 0 is defined as the inoculation date. The values used for baseline summaries will be the last evaluation on or before the inoculation date.

4.2 Analysis Populations

Any children found to be ineligible and who the study team determine should not be included in any analyses, will be included in accrual and eligibility summaries only. Protocol deviations will be summarized but should not result in any infants being excluded from analyses.

Safety population: Safety analyses will be intent-to-treat and include all eligible infants randomized including all multiplets.

Infectivity and immunogenicity population: The primary infectivity and immunogenicity analyses will use an as-treated approach, limited to infants who were inoculated. If more than one child from a household is enrolled, one child will be randomly selected to be included in the primary infectivity and immunogenicity analyses.

5 Statistical Methods

Baseline characteristics will be summarized by treatment group but with no statistical comparisons comparing arms.

Statistical tests will not be adjusted for interim monitoring or multiple comparisons. The statistical tests will be 1-tailed to test the hypothesis that a vaccinated group will exhibit greater values following vaccination compared to the placebo group. The statistical tests will be one-sided with a nominal significance level of 0.05. Categorical data will be summarized using N (%), and continuous data using N, min, Q1, median, Q3, max, and mean (STD) (when appropriate). Any modifications to outcome measures after the team has seen data collected after entry will be identified as *post hoc*.

5.1 Visit and Evaluation Schedule

Study visits are conducted on Days 0, 3, 5, 7, 10, 12, 14, 17, and 28 (each with a window of ±1 day), and on Day 56 (with a window of +7 days). Non-clinic contacts (via phone or e-mail) are conducted on all other days during the Acute Phase (each with a window of ±1 day)**,** and on Day 29 (with a window of +1 day). A Pre-RSV season visit takes place between October 1st and October 31st, and then weekly contacts are made during Nov 1st and March 31st. These dates apply to most sites, but may differ for those with local RSV seasons that start earlier. A Post-RSV season visit takes place between April 1st and April 30th.

5.2 Safety Primary Objectives

The number and proportion of children experiencing at least one primary safety outcome measure will be summarized by treatment group with exact 90% confidence intervals (CIs). The types of primary outcome measures that occur will be summarized.

5.3 Infectivity and Immunogenicity Primary Objectives

In the case of multiplets, one child will be chosen at random for the primary analysis.

This is an as-treated analysis including the children who have been inoculated. Summaries will include (i) the proportion and 90% CI of participants infected with vaccine virus; ii) summaries of peak titer and of duration of virus shed for each of the two vaccine groups; iii) the proportion and 90% CI of participants who develop a four-fold or greater rise in RSV-neutralizing antibody titer

following vaccination, at day 56; iv) summaries of antibody titers to the RSV F protein and to the RSV-PRNT for each of the two vaccine groups. 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 four-fold 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.

Sensitivity analyses will repeat these summaries in the event that multiplets are enrolled in the study, re-doing the analysis using the other sibling(s).

5.4 Secondary Objectives

Summaries will include: i) number of participants experiencing symptomatic, medically attended respiratory and febrile illness (MAARI) during the RSV surveillance, and the subset of those who were infected with RSV during this time; ii) summaries of antibody titers to the RSV F protein and to the RSV-PRNT pre and post RSV surveillance by vaccine group; iii) summaries of change in antibody titers to the RSV F protein and to the RSV-PRNT between pre and post RSV surveillance for each of the two vaccine groups in the subset of vaccine recipients who do not experience RSV-MAARI.

6 Report Components

Detailed descriptions of the content of each of the following sections are given in the AIP.

1. Accrual
2. Baseline characteristics
3. Study status
4. Protocol deviations
5. Data timeliness and completeness
6. Safety:
    a. Primary safety outcomes
    b. Deaths
    c. Hospitalizations
    d. Concomitant medications
7. Primary infectivity and immunogenicity outcomes
    a. Primary infectivity and immunogenicity outcomes
    b. Sensitivity analyses
8. Secondary outcomes