Protocol C3671002

A PHASE 1/2, PLACEBO-CONTROLLED, RANDOMIZED, OBSERVER-BLIND, DOSE-FINDING, FIRST-IN-HUMAN STUDY TO DESCRIBE THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF AN ADJUVANTED RESPIRATORY SYNCYTIAL VIRUS (RSV) VACCINE IN HEALTHY OLDER ADULTS

Statistical Analysis Plan (SAP)

Version: 2

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1. VERSION HISTORY

This statistical analysis plan (SAP) for Study C3671002 is based on the protocol (Amendment 3) dated 22 Aug 2019.

Table 1. Summary of Major Changes in SAP Amendments

Version/Date	Associated Protocol Amendment	Rationale	Specific Changes
1/15 Feb 2019	Original (09 Oct 2018)	Not Applicable	Not Applicable
2/09 Jun 2020	3 (22 Aug 2019)	Changes to maintain consistency with the 2015 version of the SAP template. Updates according to protocol amendment 3.	• Added a document footer specifying the 2015 version of the SAP template, which does not include estimands. • Added text to clarify that the existing objectives and endpoints apply to the Primary Study Cohort - Stage 1. • Added objectives, study design, endpoints, analysis sets and analyses, and summaries for the newly added 2 cohorts: Primary Study Cohort - Stage 2, which will evaluate revaccination of the Stage 1 subjects with the RSV vaccine and concomitant SIIV, and the RSV Vaccine Month-0, Month-2 Cohort, which will evaluate a 240-µg dose of the RSV vaccine with CpG/Al(OH)3, but without SIIV (Section 2.1, Section 2.2, Section 3, Section 4, and Section 6). • Added text regarding the initiation of Stage 2 vaccination (Section 2.2). • Added text to explain safety data and blood sample collection (Section 2.2). • Added a new algorithm for calculating fold rise (Section 3.2.1 and Section 5.3.2).

Table 1. Summary of Major Changes in SAP Amendments

Version/Date	Associated Protocol Amendment	Rationale	Specific Changes
			• Added analysis related to H3N2 assay, including the definitions of seroprotection and seroconversion and the rationale for the definitions (Section 3.3.1 and Section 6.3.5). • Added lower limit of quantitation (LLOQ) tables for assay data (Section 5.3.2). • Added text to explain the structure of Section 6. • Added text to explain that the inclusion of each applicable blood sampling visit will not always follow the protocol-required visit window (Section 6.2.1.2). • Added text to describe 3 interim analyses for the 3 different cohorts and to explain that the 3 interim analyses may span multiple reporting events (Section 7). • Removed Table 2 (randomization) • Added Section 3.1.1 and Section 3.1.1.2 detailing local reactions and systemic events. • Added Section 3.1.4 detailing AEs. • Expanded information throughout Section 3.2, Section 3.3, and Section 3.4 regarding endpoints. • Added to Section 3.5 and added Section 5.2 regarding study conduct and general methods. • Expanded Section 4 on study populations.

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C3671002. A brief description of the study design and the study objectives is given below. A list of tables, listings, and figures, mock-up tables, listings, and figures, and programming rules are prepared separately based on the methods described in this document. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

2.1. Study Objectives

2.1.1. Primary Objective

Primary Study Cohort - Stage 1:

- To describe the safety and tolerability of an adjuvanted respiratory syncytial virus (RSV) vaccine given concomitantly with seasonal inactivated influenza vaccine (SIIV).

2.1.2. Secondary Objectives

Primary Study Cohort - Stage 1:

- To describe the immune responses elicited by an adjuvanted RSV vaccine given with SIIV.
- To describe the immune responses elicited by SIIV given alone or with an adjuvanted or unadjuvanted RSV vaccine.

2.1.3. Exploratory Objectives

Primary Study Cohort - Stage 1:

- To more fully characterize the immune response to an adjuvanted RSV vaccine and to any concurrent RSV infection.
- To more fully characterize the immune response to SIIV.

Primary Study Cohort - Stage 2:

- To describe the safety and tolerability of a second dose of adjuvanted RSV vaccine given concomitantly with SIIV.
- To describe the immune responses elicited by a second dose of an adjuvanted RSV vaccine given concomitantly with SIIV.
- To more fully characterize the immune response to SIIV when given concomitantly with a second dose of RSV vaccine.

RSV Vaccine Month-0, Month-2 Cohort:

- To describe the safety and tolerability of the first and second dose of an adjuvanted RSV vaccine administered 2 months apart.

- To describe the immune responses elicited by the first and second dose of adjuvanted RSV vaccine administered 2 months apart.

2.2. Study Design

This is a Phase 1/2, multicenter, randomized, placebo-controlled, observer-blind, dose- and formulation-finding study.

The study will evaluate the safety, tolerability, and immunogenicity of up to 7 different RSV vaccine candidates with bivalent formulations (RSV A and RSV B) at 3 dose levels of 60 µg of the prefusion RSV F antigens CCI 120 µg CCI and 240 µg CCI formulated with aluminum hydroxide ($Al[OH]_3$) or $CpG/Al(OH)_3$, or a 240-µg RSV vaccine CCI alone, when administered concomitantly with SIIV, or 240 µg formulated with $CpG/Al(OH)_3$ on a Month-0 and Month-2 schedule without SIIV.

The study is organized in the following cohorts:

1. The Primary Study Cohort - Stage 1 will evaluate 7 RSV vaccine doses/formulations given concomitantly with SIIV at Month 0.

2. The Primary Study Cohort - Stage 2 will evaluate revaccination of the Stage 1 subjects with the RSV vaccine and concomitant SIIV, administered at Month 12.

3. The RSV Vaccine Month-0, Month-2 Cohort will evaluate a 240-µg dose of the RSV vaccine with $CpG/Al(OH)_3$ given at Months 0 and 2. No concomitant SIIV will be given.

Male and female subjects 65 to 85 years of age will be enrolled. Subjects will be randomized equally across all dose groups in the primary cohorts. The RSV Vaccine Month-0, Month-2 Cohort will be randomized 1:1 to receive RSV vaccine or placebo.

Primary Study Cohort - Stage 1. Enrollment in the Primary Study Cohort - Stage 1 commenced in April 2019 and completed in June 2019. Approximately 250 subjects 65 to 85 years of age were equally randomized at Visit 1 to receive one of the 7 RSV vaccine doses/formulations or placebo (a total of 8 parallel groups), concomitantly with SIIV.

Primary Study Cohort - Stage 2. Prior to completion of Stage 1, subjects may be invited to participate in Stage 2. Safety and immunogenicity data obtained from the C3671001 and C3671002 studies will be used to determine whether Stage 2 will proceed and, if so, whether all or some of the RSV formulations will be evaluated.

Two protocol administrative change letters (PACLs) were distributed to the sites regarding initiation of Stage 2 vaccination. The first PACL, dated in January 2020, was to instruct the sites that all subjects would be revaccinated in Stage 2. The second PACL, dated in March 2020, was to instruct sites to postpone the initiation of Stage 2 until further notice because of the coronavirus disease 2019 (COVID-19) pandemic.

RSV Vaccine Month-0, Month-2 Cohort. Approximately 62 RSV vaccine–naïve subjects were to be randomized 1:1 to receive either 240 µg of RSV vaccine with CpG/Al(OH)$_3$ or placebo at Visit 1 (Month 0). At Visit 3 (Month 2), subjects will receive a second dose of RSV vaccine or placebo as randomized at Visit 1. No concomitant SIIV will be given.

Key safety data collected for all study subjects in all cohorts include electronic diary (e-diary) reports of local reactions and systemic events that occur in the 14 days after each vaccination visit (RSV vaccine or placebo). Serious adverse events (SAEs) are being collected for all subjects from informed consent until study completion. Adverse events (AEs) are collected from informed consent until 1 month after the Vaccination 1 visit (for Stage 1); from reconsent until 1 month after the Vaccination 2 visit (for Stage 2); and from informed consent until 1 month after the Vaccination 2 visit (for the RSV Vaccine Month-0, Month-2 Cohort). Medically attended AEs (MAEs) are collected after the AE collection period. AEs occurring up to 48 hours after blood draws are also collected. An internal review committee (IRC) and an external data monitoring committee (E-DMC) will monitor safety in this study.

Blood samples are collected from all subjects for antibody testing. For Stage 1 subjects, blood samples were taken before Vaccination 1 and 1, 3, 6, and 12 months after Vaccination 1. For Stage 2 subjects, blood samples for antibody testing are collected before Vaccination 2 and 1, 3, 6, and 12 months after Vaccination 2. For the RSV Vaccine Month-0, Month-2 Cohort, blood samples for antibody testing are collected before Vaccination 1, 1 month after Vaccination 1, before Vaccination 2, and 1, 6, and 12 months after Vaccination 2.

A subset of study subjects in all cohorts will have additional blood taken for cellular assays (peripheral blood mononuclear cells [PBMCs]). For Stage 1 subjects in the PBMC subset, the blood samples were taken before Vaccination 1 and 1 week, 1 month, and 6 months after Vaccination 1. For Stage 2 subjects in the PBMC subset, the blood samples are taken before Vaccination 2 and 1 week, 1 month, and 6 months after Vaccination 2. For the RSV Vaccine Month-0, Month-2 Cohort PBMC subset, the blood samples are taken before Vaccination 1, 1 week and 1 month after Vaccination 1, 2 months after Vaccination 1 (or before Vaccination 2), and 1 week and 1, 6, and 12 months after Vaccination 2.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

Primary Study Cohort - Stage 1:

- Local reactions within 14 days after Vaccination 1.
- Systemic events within 14 days after Vaccination 1.
- AEs within 1 month after Vaccination 1.
- MAEs and SAEs through 12 months after Vaccination 1 (from Visit 1 through Visit 5).

3.1.1. Local Reactions

The local reactions reported in the e-diary are redness, swelling, and pain at the injection site, from Day 1 through Day 14 after each vaccination, where Day 1 is the day of vaccination. This section describes derivations with details for the assessment of local reactions: any presence, maximum severity, duration, and onset day of local reactions, in addition to presence of severe local reactions on each day.

3.1.1.1. Presence of Local Reactions

For the summary of the presence (yes or no) of a local reaction during the interval from Day 1 through Day 14 after each vaccination, where Day 1 is the day of vaccination, the following 2 variables are required in order to compute the proportions:

1. Presence (yes or no) of each local reaction on any day (Day 1 through Day 14).

   The derivation is described in Table 2.

Table 2. Derived Variables for Each Local Reaction

Variable[a]	Yes (1)	No (0)	Missing (.)
Any day (Days 1-14)	Subject reports the reaction as "yes" on any day (Days 1-14).	Subject reports the reaction as "no" on all 14 days or as a combination of "no" and "missing" on all 14 days.	Subject reports the reaction as "missing" on all 14 days.

a. The variable will be defined for each of the 3 local reactions.

2. Presence (yes or no) of any local reaction on any day (Day 1 through Day 14).

   For any local reaction on any day, a similar definition can be applied as given in Table 3.

Table 3. Derived Variables for Any Local Reaction

Variable	Yes (1)	No (0)	Missing (.)
Any day (Days 1-14)	Subject reports any local reaction as "yes" on any day (Days 1-14).	Subject reports the reaction as "no" on all 14 days or as a combination of "no" and "missing" on all 14 days for all 3 local reactions.	Subject reports all local reactions as "missing" on all 14 days.

3.1.1.2. Maximum Severity of Local Reactions

The grading of local reactions is listed in Table 4.

Table 4. Grading Scale for Local Reactions

	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Grade 4[a]
Redness	2.5 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (>20 measuring device units)	Necrosis or exfoliative dermatitis
Swelling	2.5 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (>20 measuring device units)	Necrosis
Pain (at the injection site)	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization for severe pain at the injection site

a. Grade 4 assessment should be made by the investigator using the AE severity grading scale. The assessment will be collected on the AE case report form and thus not reported from the e-diary.

The following 2 variables are required in order to compute the proportions of subjects reporting maximum severity:

1. Maximum severity of each local reaction on any day (Day 1 through Day 14).

The maximum severity (highest grading) of each local reaction within 14 days after vaccination will be derived. The maximum severity will be derived as follows:

= "Missing," if values are missing for all days (Days 1-14);

= 0, if the subject reports all reactions as "no" or a combination of "missing" and "no" for all days (Days 1-14);

= Highest grade (maximum severity) within 14 days after vaccination, if the answer is not "no" for at least 1 day.

2. Maximum severity of any local reaction on any day (Day 1 through Day 14).

The maximum severity for any local reaction will be derived as follows:

= "Missing," if values are missing for all days (Days 1-14) across all 3 local reactions;

= 0, if the subject reports all reactions as "no" or a combination of "missing" and "no" for all days (Days 1-14) for any individual location reaction;

= Highest grade (maximum severity) within 14 days after vaccination, if the answer is not "no" for at least 1 day for at least 1 local reaction.

3.1.1.3. Duration of Each Local Reaction

The duration of each local reaction will be calculated in days as (resolution date of reaction – start date of reaction + 1). Resolution of the event is the last day on which the event is recorded in the e-diary or the date the event ends (according to the grading scale definitions in Table 4) if it is unresolved during the subject diary recording period (end date collected on the case report form [CRF]), unless chronicity is established. For this multiple vaccination study, the date the reaction ended for one vaccination should not be after the beginning of the next vaccination. If there is no known end date, the duration will be considered unknown and set to "missing." Subjects with no reported reaction (including redness and swelling of less than 2.5 cm [5 measuring device units] per Table 4) have no duration.

3.1.1.4. Onset Day of Each Local Reaction

The onset day of each local reaction will be derived. Onset day is defined as the first day of reporting any severity.

For the onset day of each local reaction, if a subject reports changes in severity of the local reaction, only the first day of reporting that specific local reaction will be counted.

3.1.1.5. Presence of Severe Local Reaction on Each Day

Presence (yes or no) of any severe local reaction on each of the 14 days (Day 1 through Day 14) follows the derivation as described in Table 5.

Table 5. Derived Variables for Any Severe Local Reaction on Each Day

Variable	Yes (1)	No (0)	Missing (.)
Any severe local reaction on a specific day	Subject reports the severe reaction as "yes" on a specific day for any of the 3 local reactions.	Subject reports the reaction as a combination of any of the following: "no," "mild," "moderate," or missing on that specific day.	Subject reports all 3 local reactions as "missing" on that specific day.

3.1.2. Systemic Events

Systemic events, including fever, fatigue, headache, vomiting, nausea, diarrhea, muscle pain, and joint pain, are reported via e-diary from Day 1 through Day 14 after each vaccination, where Day 1 is the day of vaccination. The derivations below for systemic events will be handled similarly to the way local reactions are handled for presence, severity level, duration, onset day, and severe systemic events on each day.

1. Presence (yes or no) of each systemic event on any day (Day 1 through Day 14).
2. Presence (yes or no) of any systemic event on any day (Day 1 through Day 14).
3. Maximum severity of each systemic event on any day (Day 1 through Day 14).
4. Maximum severity of any systemic event on any day (Day 1 through Day 14).
5. Duration of each systemic event.
6. Onset day of each systemic event.
7. Presence (yes or no) of any severe local reaction on each of the 14 days.

The systemic events gradings are provided in Table 6.

Table 6. Grading Scale for Systemic Events

	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Grade 4[a]
Fatigue (= tiredness in diaries)	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe fatigue
Headache	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe headache
Vomiting	1 to 2 times in 24 hours	>2 times in 24 hours	Requires intravenous hydration	Emergency room visit or hospitalization for severe vomiting
Nausea	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe nausea
Diarrhea	2 to 3 loose stools in 24 hours	4 to 5 loose stools in 24 hours	6 or more loose stools in 24 hours	Emergency room visit or hospitalization for severe diarrhea

Table 6. Grading Scale for Systemic Events

	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Grade 4[a]
Muscle pain	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe muscle pain
Joint pain	Does not interfere with activity	Some interference with activity	Prevents daily routine activity	Emergency room visit or hospitalization for severe joint pain

a. Grade 4 assessment should be made by the investigator using the AE severity grading scale. The assessment will be collected on the AE case report form and thus not reported from the e-diary.

Fever is defined as an oral temperature of ≥100.4°F (≥38.0°C). The highest temperature for each day will be recorded in the e-diary. Any temperature recorded as <95.0°F (35.0°C) or >107.6°F (42.0°C) will be treated as a data entry error and excluded from the analyses. For reporting purposes, fever will be analyzed using the following temperature ranges:

- 38.0°C to 38.4°C (100.4°F – 101.1°F)
- 38.5°C to 38.9°C (101.2°F – 102.0°F)
- 39.0°C to 40.0°C (102.1°F – 104.0°F)
- >40.0°C (>104.0°C)

3.1.3. Use of Antipyretic/Pain Medication

Antipyretic/pain medications used to treat symptoms are recorded in the e-diary. This variable will be presented along with systemic events summary, but not counted as a systemic event. The following variables will be derived:

1. Use of antipyretic/pain medication on "any day (Days 1-14)" after vaccination.
2. Duration of use of antipyretic/pain medication after vaccination.
3. Onset day of antipyretic/pain medication use after vaccination.

3.1.4. Adverse Events

AE reporting will be based on the specific reporting period. Standard algorithms for handling missing AE dates and missing AE severity will be applied as described in the Pfizer Vaccine data standard rules.

As this is a Phase 1/2 study, with limited sample size included in each vaccine group, the value of applying the 3-tier approach is limited. Therefore, because of a small number of

subjects in each dose/formulation group, safety assessment may be best carried out using descriptive statistics.

For AEs within 1 month after each vaccination, the interval calculation will be based on the study visit. For example, for the Primary Study Cohort - Stage 1, AEs within 1 month after Vaccination 1 will include any AE that occurred from the day of Vaccination 1 until the day before the 1-month post–Vaccination 1 visit (Visit 2). In general, this will be derived for each vaccination:

- Primary Study Cohort - Stage 1: AEs within 1 month after Vaccination 1.
- Primary Study Cohort - Stage 2: AEs within 1 month after Vaccination 2.
- RSV Vaccine Month-0, Month-2 Cohort – AEs within 1 month after Vaccination 1.
- RSV Vaccine Month-0, Month-2 Cohort – AEs within 1 month after Vaccination 2.

The following derivations/summaries will be included for each of the above:

1. Proportions of subjects reporting AEs during this interval summarized for each system organ class (SOC) and preferred term (PT) (and "any event"), separately.
2. Proportion of subjects reporting any AE during this interval.
3. Proportion of subjects reporting any MAE during this interval.
4. Proportion of subjects reporting any SAE during this interval.
5. Proportion of subjects reporting any related AE during this interval.
6. Proportion of subjects reporting any severe AE during this interval.
7. Proportion of subjects reporting any intermediate AE. Intermediate AE is defined as an AE reported within the first 30 minutes after vaccination.
8. Proportion of subjects reporting any AE leading to withdrawal during this interval.
9. Proportion of subjects reported any AE of specific interest (Refer to Section 8.4.2 of the protocol for details on the diseases/disorders included in this category).

3.1.5. Medically Attended AEs and Serious AEs

Proportions of subjects reporting SAEs and MAEs will be summarized for each SOC and PT (and "any event"), separately, for the following intervals:

- Primary Study Cohort - Stage 1: MAEs (or SAEs) through 12 months after Vaccination 1.

- Primary Study Cohort - Stage 2: MAEs (or SAEs) through 12 months after Vaccination 2.

- RSV Vaccine Month-0, Month-2 Cohort: MAEs (or SAEs) through 12 months after Vaccination 2.

3.2. Secondary Endpoint(s)

Primary Study Cohort - Stage 1:

- RSV A– and RSV B–neutralizing antibody titers measured before and 1 month after Vaccination 1.

- Hemagglutination inhibition assay (HAI) titers for all strains in the SIIV measured before and 1 month after Vaccination 1.

3.2.1. RSV A– and RSV B–Neutralizing Antibody Titers

RSV A– and RSV B– neutralizing antibody titers will be determined on all sera collected at applicable visits for antibody testing.

Titers above the lower limit of quantitation (LLOQ) are considered accurate and their quantitated values will be reported. Refer to Section 5.3.2 for LLOQs. Titers below the corresponding LLOQ or denoted as below the limit of quantitation (BLQ) will be set to 0.5 × LLOQ for analysis. Missing assay results will not be imputed. Calculations and derivations will be based on imputed values.

In addition to titer, the neutralizing titer (NT) fold rise from before vaccination will be derived for both RSV A and RSV B as detailed below:

1. For the Primary Stage Cohort - Stage 1: NT fold rise will be derived from before Vaccination 1 to each post–Vaccination 1 visit with NT assay performed.

2. For the Primary Stage Cohort - Stage 2:

    a. NT fold rise will be derived from before Vaccination 2 to each post–Vaccination 2 visit with NT assay performed.

    b. NT fold rise will be derived from before Vaccination 1 to each post–Vaccination 1 visit (including any post–Vaccination 2 visits and at the Vaccination 2 visit) with NT assay performed.

    c. NT fold rise will be derived from 1 month after Vaccination 1 to 1 month after Vaccination 2.

3. For the RSV Vaccine Month-0, Month-2 Cohort:

    a. NT fold rise will be derived from before Vaccination 1 to each applicable post–Vaccination 1 visit (note: 2 months after Vaccination 1 is the same as before Vaccination 2).

    b. NT fold rise will be derived from before Vaccination 2 to each post–Vaccination 2 visit with NT assay performed.

    c. NT fold rise will be derived from 1 month after Vaccination 1 to 1 month after Vaccination 2.

For calculating a fold rise, < LLOQ will be converted to 0.5 × LLOQ for a numerator, and < LLOQ will be converted to LLOQ for a denominator when only one of either the numerator or denominator is < LLOQ. If both the numerator and denominator are < LLOQ, then both will be converted in the same way.

3.2.2. HAI Titers

Hemagglutination inhibition antibody titers will be measured by the standard HAI for the 3 influenza vaccine strains contained in the SIIV. The assay will be performed by VisMederi (Siena, Italy), with LLOQ = 1:10 for each strain.

Titers above the LLOQ are considered accurate and their quantitated values will be reported. Titers below the corresponding LLOQ or denoted as BLQ will be set to 0.5 × LLOQ for analysis. Missing assay results will not be imputed. Calculations and derivations will be based on imputed values.

For the Primary Stage Cohort - Stage 1: HAI fold rise will be derived from before Vaccination 1 to 1 month after Vaccination 1 for each strain tested.

For the Primary Stage Cohort - Stage 2: HAI fold rise will be derived from before Vaccination 2 to 1 month after Vaccination 2 for each strain tested.

In addition to the HAI titer and HAI fold rise, 2 binary variables will be derived for each of the influenza strains tested for both cohorts:

1. HAI seroprotection before and after SIIV vaccination: HAI titer is ≥1:40.

2. HAI seroconversion after SIIV vaccination:

    a. If the HAI titer is <1:10 (LLOQ for HAI) before SIIV administration, seroconversion is achieved if the postvaccination titer is ≥1:40.

    b. If the HAI titer is ≥1:10 before SIIV administration, seroconversion is achieved if the fold rise from before SIIV to the postvaccination titer is ≥4.

3.3. Exploratory Endpoints

Primary Study Cohort - Stage 1:

- RSV A– and RSV B–neutralizing antibody titers measured at 1 week (PBMC subjects) and 3, 6, and 12 months after Vaccination 1.
- Plasmablast frequencies and T-cell phenotype and functional analysis before and 1 week after Vaccination 1 (PBMC subjects).
- Memory B-cell and T-cell frequencies and their functional analysis before vaccination and 1 and 6 months after Vaccination 1.
- Immunoglobulin G (IgG) titers against prefusion F (preF) before vaccination and 1, 3, 6, and 12 months after Vaccination 1.
- IgG titers to nonvaccine RSV antigens before vaccination and 1, 3, 6, and 12 months after Vaccination 1.
- H3N2-neutralizing antibody titers measured before vaccination and 1 month after Vaccination 1.

Primary Study Cohort - Stage 2:

- Local reactions within 14 days after Vaccination 2.
- Systemic events within 14 days after Vaccination 2.
- AEs within 1 month after Vaccination 2.
- MAEs and SAEs through 12 months after Vaccination 2.
- RSV A– and RSV B–neutralizing antibody titers measured at 1 week (PBMC subjects) and 1, 3, 6, and 12 months after Vaccination 2.
- Plasmablast frequencies and T-cell phenotype and functional analysis before Vaccination 2 and 1 week after Vaccination 2 (PBMC subjects).
- Memory B-cell and T-cell frequencies and their functional analysis before Vaccination 2 and 1 and 6 months after Vaccination 2.
- IgG titers against preF before Vaccination 2 and 1, 3, 6, and 12 months after Vaccination 2.
- IgG titers to nonvaccine RSV antigens before Vaccination 2 and 1, 3, 6, and 12 months after Vaccination 2.

- HAI titers for all strains in the SIIV measured before and 1 month after Vaccination 2.

- H3N2-neutralizing antibody titers measured before Vaccination 2 and 1 month after Vaccination 2.

RSV Vaccine Month-0, Month-2 Cohort

- Local reactions within 14 days after Vaccinations 1 and 2.

- Systemic events within 14 days after Vaccinations 1 and 2.

- AEs within 1 month after Vaccinations 1 and 2.

- AEs from Vaccination 1 through 1 month after Vaccination 2.

- MAEs and SAEs through 12 months after Vaccination 2.

- RSV A– and RSV B–neutralizing antibody titers measured at scheduled time points before and after each vaccination scheduled.

- Plasmablast frequencies and T-cell phenotype and functional analysis before each vaccination and 1 week after Vaccinations 1 and 2 (PBMC subjects).

- Memory B-cell and T-cell frequencies and their functional analysis before each vaccination and 1 and 6 months after Vaccinations 1 and 2.

- IgG titers against preF at scheduled time points before and after each vaccination.

- IgG titers to nonvaccine RSV antigens at scheduled time points before and after each vaccination.

3.3.1. H3N2-Neutralizing Titers

H3N2-neutralizing antibody titers will be performed by VisMederi (Siena, Italy). The LLOQ for this assay is 1:10.

Titers above the LLOQ are considered accurate and their quantitated values will be reported. Titers below the corresponding LLOQ or denoted as BLQ will be set to 0.5 × LLOQ for analysis. Missing assay results will not be imputed. Calculations and derivations will be based on imputed values.

For the Primary Stage Cohort - Stage 1: H3N2 NT fold rise will be derived from before Vaccination 1 to 1 month after Vaccination 1.

For the Primary Stage Cohort - Stage 2: H3N2 NT fold rise will be derived from before Vaccination 2 to 1 month after Vaccination 2.

In addition to the H3N2 NT and fold rise, 2 binary variables will be derived:

1. Seroprotection for H3N2 NTs is defined as follows: H3N2 NT is ≥1:80.

2. Seroconversion for H3N2 NTs after vaccination is defined based on the NT before SIIV vaccination:

- When the NT is <1:20 before SIIV, seroconversion is achieved if the postvaccination NT is ≥1:80.

- When the NT is ≥1:20 after SIIV, seroconversion is achieved if the fold rise from the prevaccination to the postvaccination NT is ≥4.

Rationale:

Although no widely agreed cutoff protective level for NTs is available, estimates in the literature for neutralizing protective levels against H3N2 viruses range from 1:40 to 1:160. However, maximal protection against H3N2 was achieved at an HAI titer of 1:160 and an NT of 1:320, about twice as high a protective titer for H3N2 with neutralization assay as with HAI. Considering LLOQ is the same for HAI and neutralization assay, we chose 1:80 to define seroprotection, which is consistent with existing literature.[1,2]

3.3.2. Anti-RSV Prefusion F IgG

IgG levels will be determined against both RSV A and RSV B preF antigens in a direct-binding Luminex immunoassay. Refer to Section 5.3.2 for LLOQs.

Titers above the LLOQ are considered accurate and their quantitated values will be reported. Titers below the corresponding LLOQ or denoted as BLQ will be set to 0.5 × LLOQ for analysis. Missing assay results will not be imputed. Calculations and derivations will be based on imputed values.

In addition to IgG concentration, the anti-RSV preF IgG fold rise will be derived. These will be similar to NT (Section 3.2.1).

3.3.3. Ratio of Combined Neutralizing Titer A/B Fold Rise to Combined Anti-RSV Prefusion F IgG A/B Fold Rise

This endpoint will be derived by combining individual level assay data – NT and anti-RSV preF IgG.

- Combined NT A/B fold rise: For each individual, the combined RSV A/B–50% neutralizing fold rise is the geometric mean of the fold rise of RSV A–50% and the fold rise of RSV B–50%.

- Combined anti-RSV preF IgG A/B fold rise: For each individual, the combined RSV A/B–IgG fold rise is the geometric mean of the fold rise of RSV A–IgG and the fold rise of RSV B–IgG.

- Ratio: For each individual, the ratio is calculated using the individual combined RSV A/B–50% NT fold rise as numerator and using the individual combined RSV A/B–IgG fold rise as denominator.

This should be derived for all applicable visits with NT and IgG results.

3.3.4. Anti-RSV Nonvaccine Antigen Immunoglobulin

Four nonvaccine antigens (NVAs) will be assessed: matrix, nucleoprotein, Ga peptide, and Gb peptide. In the analysis, Ga and Gb are considered as 1 antigen, though there are 2 different assays. Refer to Section 5.3.2 for LLOQs.

Titers above the LLOQ are considered accurate and their quantitated values will be reported. Titers below the corresponding LLOQ or denoted as BLQ will be set to 0.5 × LLOQ for analysis. Missing assay results will not be imputed. Calculations and derivations will be based on imputed values.

The following binary variables will be derived:

1. Subjects with ≥4-fold rise on matrix.

2. Subjects with ≥4-fold rise on matrix and ≥4-fold rise on G (either Ga or Gb).

3. Subjects with ≥4-fold rise on all 3 antigens: matrix, G (either Ga or Gb), and nucleoprotein.

4. Subjects with ≥4-fold rise on any of the 3 antigens.

5. Seroconversion for possible RSV infection will be defined later.

Specifically, the above variables will be derived for each cohort:

- For the Primary Stage Cohort - Stage 1, these will be derived from before Vaccination 1 to each applicable post–Vaccination 1 visit in Stage 1.

- For the Primary Stage Cohort - Stage 2, these will be derived from before Vaccination 2 to each applicable post–Vaccination 2 visit in Stage 2.

- For the RSV Vaccine Month-0, Month-2 Cohort, these will be derived from before Vaccination 2 to each applicable post–Vaccination 2 visit.

3.3.5. Cellular Assays

PBMCs isolated from whole blood will be used for evaluation of cellular immune responses to the RSV vaccine formulations. Polyfunctional CD4 and CD8 T cells will be analyzed. Exploratory analyses may also include characterization of memory B-cell, plasmablast, and T-cell immune responses, and repertoire analyses. Refer to Section 5.3.2 for a list (not inclusive) of these exploratory assays.

3.4. Baseline Variables

3.4.1. Baseline Definition

Day 1 is defined as the day of Vaccination 1. Measurements or samples collected prior to Vaccination 1 on Day 1 are considered the baseline data for the assessments.

3.4.2. Demographics, Smoking History, and Medical History

Demographic variables collected include sex, race, ethnicity, and date of birth. Age at the time of vaccination (in years) will be derived based on birthday. For example, if the vaccination date is 1 day before the subject's 86th birthday, the subject is 85 years old.

Medical history of clinical significance will be collected and categorized according to the current version (at the time of reporting) of the Medical Dictionary for Regulatory Activities (MedDRA).

Subject smoking history is also collected at Visit 1.

3.4.3. E-Diary Completion

An e-diary will be considered transmitted if any data for the local reactions, systemic events, or use of antipyretic/pain medication are present for any day. If all data are missing for all items on the e-diary for all 14 days (local reactions, systemic events, and use of antipyretic/pain medication) after vaccination, then the e-diary will be considered not transmitted. An e-diary will be considered transmitted for a given day if any data are present for that day.

3.4.4. Nonstudy Vaccines

Any nonstudy vaccinations given will be recorded in the CRF from the time of signing of the informed consent document (ICD) to the 12-month follow-up visit (Visit 5 for Primary Study Cohort - Stage 1 subjects, Visit 9 for Primary Study Cohort - Stage 2 subjects, and Visit 6 for RSV Vaccine Month-0, Month-2 Cohort subjects).

Nonstudy vaccinations will be categorized according to the latest version (at the time of reporting) of the World Health Organization (WHO) Drug Dictionary.

3.5. Safety Endpoints

Refer to Section 3.1 and Section 3.3.

4. ANALYSIS SETS

Data for all subjects will be assessed to determine if subjects meet the criteria for inclusion in each analysis population prior to unblinding and releasing the database, and classifications will be documented per standard operating procedures.

Three analysis populations will be defined for each of the 3 study cohorts: Primary Study Cohort - Stage 1, Primary Study Cohort - Stage 2, and RSV Vaccine Month-0, Month-2 Cohort.

4.1. Primary Study Cohort - Stage 1

4.1.1. Safety Population

All subjects receiving at least 1 dose of the investigational products (respiratory syncytial virus stabilized prefusion F subunit vaccine [RSVpreF] or placebo) at Visit 1 will be included in the safety population for this cohort. For the safety analyses, subjects will be analyzed according to the investigational product received. The safety population will be the only analysis population for safety evaluation included in Stage 1.

4.1.2. Evaluable Immunogenicity Population

This analysis population will include subjects who:

- Are eligible (have signed informed consent and met all inclusion/not met any exclusion criteria) and were randomized into the study;
- Have received both the SIIV and RSV vaccine or placebo and SIIV as randomized;
- Have a Visit 2 (1 month after vaccination) blood draw for assay testing within 27 to 42 days after Visit 1 (after vaccine administration) (note: the 27- to 42-day interval is calculated as the Visit 2 date minus the Visit 1 date);
- Have at least 1 valid and determinate assay result for the 1-month post–Vaccination 1 visit;
- Have no major protocol violation as determined by the study clinician.

Major protocol violations will be determined by clinical review. A major protocol violation is a protocol violation that, in the opinion of the sponsor's study medical monitor, would materially affect assessment of immunogenicity, eg, subject receipt of a prohibited vaccine or medication that might affect immune response or a medication error with suspected decrease in potency of the vaccine. The sponsor's medical monitor will identify those subjects with protocol violations before any immunogenicity analysis is carried out.

The evaluable immunogenicity population will be the primary analysis population for the immunogenicity endpoints evaluated among this cohort. Subjects will be included in the vaccine group as randomized in the analysis, which, by the population definition, is equivalent to the vaccine group corresponding to the vaccine received.

The evaluable immunogenicity population will also be the primary analysis population for the cellular assay results reported from this cohort.

4.1.3. Modified Intent-to-Treat Population

All randomized subjects who have at least 1 valid and determinate assay result related to the proposed analysis during Stage 1 will be included in the modified-intent-to-treat (mITT) population. Immunogenicity results based on the mITT population will be summarized according to the vaccination as randomized.

Analysis of immunogenicity results based on the mITT population will be considered supportive and will be performed for critical endpoints, such as secondary endpoints, only if there is enough difference (eg, >10%) between the evaluable immunogenicity population and the mITT population.

4.2. Primary Study Cohort - Stage 2

4.2.1. Safety Population

All subjects who were invited for Stage 2 and received at least 1 dose of the investigational products (RSVpreF or placebo) at Vaccination 2 (Visit 5) will be included in the safety population for this cohort. For the safety analyses, subjects will be analyzed according to the investigational product received. The safety population will be the only analysis population for Stage 2 safety evaluation.

4.2.2. Evaluable Immunogenicity Population

This evaluable immunogenicity population will include subjects who:

- Are eligible (have signed informed consent and met all inclusion/not met any exclusion criteria) and were randomized into the study;
- Have received vaccines (concomitant with SIIV) at Vaccination 1 and Vaccination 2 as randomized;
- Have a Visit 6 (1 month after Vaccination 2) blood draw for assay testing within 27 to 42 days after Visit 5, inclusive (after vaccine administration) (note: the 27- to 42-day interval is calculated as the Visit 6 date minus the Visit 5 date);
- Have at least 1 valid and determinate assay result for the 1-month post–Vaccination 2 visit;
- Have no major protocol violation as determined by the study clinician.

Major protocol violations will be determined similar to the description in Section 4.1.2 by clinical review.

4.2.3. Modified Intent-to-Treat Population

A subject who was randomized in Stage 1, consented to participate in Stage 2, and has at least 1 valid and determinate assay result related to the proposed analysis during Stage 2 will be included in the mITT population. The immunogenicity results based on the mITT population will be summarized according to the vaccination as randomized. Since Stage 2 is entirely exploratory, analysis of immunogenicity results based on the mITT population will be considered supportive and will be performed only if requested.

4.3. RSV Vaccine Month-0, Month-2 Cohort

4.3.1. Safety Population

All subjects receiving at least 1 dose of the investigational products (RSVpreF 240 µg + CpG/Al(OH)$_3$ or placebo) at Visit 1 will be included in the safety population for this cohort. For the safety analyses, subjects will be analyzed according to the investigational product received. The safety population will be the only analysis population for safety evaluation included in this study cohort.

4.3.2. Evaluable Immunogenicity Population

This evaluable immunogenicity population will include subjects who:

- Are eligible (have signed informed consent and met all inclusion/not met any exclusion criteria) and were randomized into the study;
- Have received both vaccinations at Months 0 and 2 as randomized;
- Have a Visit 4 (1 month after Vaccination 2) blood draw for assay testing within 27 to 42 days after Visit 3, inclusive (after vaccine administration) (note: the 27- to 42-day interval is calculated as the Visit 4 date minus the Visit 3 date);
- Have at least 1 valid and determinate assay result for the proposed analysis;
- Have no major protocol violation as determined by the study clinician.

Major protocol violations will be determined as described in Section 4.1.2 by clinical review.

4.3.3. Modified Intent-to-Treat Population

All randomized subjects included in this cohort who have at least 1 valid and determinate assay result related to the proposed analysis will be included in the mITT population. The immunogenicity results based on the mITT population will be summarized according to the vaccination as randomized. Since this cohort is entirely exploratory, analysis of immunogenicity results based on the mITT population will be considered supportive and will be performed only if requested.

4.4. Other Analysis Sets

No other analysis sets will be defined in this study.

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

5.1.1. Statistical Hypotheses

This Phase 1/2 study is designed to describe the safety, tolerability, and immunogenicity of the RSV vaccine alone or formulated with $Al(OH)_3$ or $CpG/Al(OH)_3$, when administered concomitantly with SIIV, and a 240-µg dose of the RSV vaccine with $CpG/Al(OH)_3$, but without SIIV. No formal statistical hypothesis testing will be performed. An estimation approach will be used to assess the safety and immunogenicity objectives.

5.1.2. Statistical Decision Rules

Statistical decision rules will not be utilized in this study. All analyses are considered descriptive in nature.

5.2. General Methods

Safety data, immunogenicity data, and cellular assay data will be summarized by vaccine group for each cohort. All analyses will be descriptive in nature.

5.2.1. Analyses for Binary Data

Descriptive statistics for binary variables are the proportion (%) and the numerator (n) and the denominator (N) used in the proportion calculation. The 2-sided 95% CI for percentage, and for difference in percentages, will also be presented, where appropriate.

1. The 2-sided 95% CI for the proportion will be constructed by the Clopper-Pearson method described by Newcombe.[3] The 2-sided 95% CI will be presented in terms of percentage.

2. The 2-sided 95% CI for the difference in the proportions may be computed using the Chan and Zhang method.[4] The 2-sided 95% CI will be presented in terms of percentage. This may be used for some exploratory analyses.

5.2.2. Analyses for Continuous Data

Unless otherwise specified, descriptive statistics for continuous variables are n, mean, median, standard deviation, minimum, and maximum.

The CI for the mean of the continuous variable will be constructed by the standard method based on Student's t distribution.

5.2.2.1. Geometric Mean Titer

Continuous immunogenicity endpoints will be logarithmically transformed for analysis. Geometric mean titer (GMT) or geometric mean concentration (GMC) and associated 2-sided 95% CI will be calculated at each available time point for each vaccine group and cohort. Geometric means and their 2-sided 95% CIs will be derived by calculating means and CIs on the natural log scale based on the t distribution, then exponentiating the results.

5.2.2.2. Geometric Mean Fold Rise

Geometric mean fold rises (GMFRs) will be limited to subjects with nonmissing values at both visits. Individual subject antibody-level fold rise from one time point to another time point will be transformed in natural logarithm scale for analysis. Means and their 2-sided 95% CIs on the natural log scale will be based on the 1-sample Student's t distribution. GMFRs and corresponding 2-sided 95% CIs will be calculated by exponentiating the results from the natural log scale.

5.2.2.3. Geometric Mean Ratio

The geometric mean ratio (GMR) will be calculated by transforming the group mean difference of the antibody levels in natural logarithm scale to original scale. Two-sided 95% CI is also computed by exponentiating the CIs from the natural log scales using 2-sample Student's t distribution for the mean difference of measures on the logarithmically transformed assay results.

5.2.2.4. Reverse Cumulative Distribution Curves

Empirical reverse cumulative distribution curves (RCDCs) will plot the proportion of subjects with values equal to or exceeding a specified assay value versus the indicated assay value, for all observed assay values. Data points will be joined by a step function with the data point on the left side of the step.

5.2.2.5. Nonparametric Comparison

As only a subset of subjects will be tested for cellular assays, small sample sizes are expected for each group. Therefore, nonparametric comparison between each RSVpreF dose/formulation group and the placebo group on each of the assay values at each applicable time point will be performed and exact p-values based on the 2-sample Wilcoxon test will be provided for these comparisons.

5.3. Methods to Manage Missing Data

5.3.1. Safety Data

Standard algorithms on handling missing AE dates and missing AE severity will be applied as described in the safety rulebook summary.

Missing data handling rules on the safety data are described in details the corresponding endpoint sections.

5.3.2. Immunogenicity Data

Any assay results above LLOQ are considered accurate and their quantitated values will be reported. Assay results noted as the upper limit of quantitation (ULOQ) will be set to the value of ULOQ for analysis. Values below the LLOQ or denoted as BLQ, or limit of detection (LOD), will be set to 0.5 × LLOQ for analysis. For calculating a fold rise, < LLOQ will be converted to 0.5 × LLOQ for a numerator, and < LLOQ will be converted to LLOQ for a denominator when only one of either the numerator or denominator is < LLOQ. If both the numerator and denominator are < LLOQ, then both will be converted in the same way.

CCI

Values for sera that are insufficient (QNS), indeterminate results, or values recorded as "not done" will be set to "missing." Additionally, any time point with no blood draws will not be included in the analysis. No imputation will be done for these missing values.

6. ANALYSES AND SUMMARIES

This section describes the analyses and summaries across all endpoints. In order to reconcile the protocol-specified endpoints (or estimands) and SAP template structure, the primary endpoints and secondary endpoints specified in the protocol are carried out in the "primary analysis" described in this section.

All exploratory endpoints specified in the protocol and the additional analyses for the protocol-specified primary and secondary endpoints are included in the "additional analysis," "additional summary," or "exploratory endpoints" sections in this document.

6.1. Primary Endpoints

6.1.1. Local Reactions

6.1.1.1. Primary Analysis

Endpoint: Proportions of subjects reporting prompted local reactions (pain at the injection site, redness, and swelling) within 14 days after vaccination.

- Analysis time points: Within 14 days after Vaccination 1.
- Analysis population: Safety population (Primary Study Cohort - Stage 1).
- Analysis methodology: Descriptive summary statistics.
- Supporting objective: Primary objective.

Reporting Results:

Proportions of subjects reporting prompted local reactions (each and any) after Vaccination 1 will be summarized by maximum severity level. Confirmed e-diary errors will be excluded from the analysis. The percentage (%), the numerator (n) and denominator (N) used in the percentage calculation, and the corresponding 95% Clopper-Pearson CI will be presented for each vaccine group.

6.1.1.2. Additional Summary for Assessing Local Reactions

To support the assessment of local reactions, similar endpoints will be summarized for the Primary Study Cohort - Stage 2 within 14 days after Vaccination 2; as well as for the RSV Vaccine Month-0, Month-2 Cohort within 14 days after Vaccination 1 and within 14 days after Vaccination 2.

To support the assessment of local reactions, additional endpoints (as defined in Section 3.1.1) will be summarized with all analysis time points (within 14 days after Vaccination 1 and within 14 days after Vaccination 2), analysis populations (Primary Study Cohort - Stage 1, Primary Study Cohort - Stage 2, and RSV Vaccine Month-0, Month-2 Cohort), analysis methodology, and appropriate reporting results. Confirmed e-diary errors will be excluded from these analyses.

Binary Endpoints

The percentage (%), the numerator (n) and denominator (N) used in the percentage calculation, and the corresponding 95% Clopper-Pearson CI will be presented for each vaccine group after each vaccination for the following endpoints:

- Subjects reporting any severe local reaction on each day and any day (Day 1 through Day 14).

Continuous Endpoints

The following endpoints will be summarized by displaying n, mean, median, standard deviation, minimum, and maximum for each vaccine group:

  - Duration of each local reaction after vaccination.
  - Onset day of each local reaction after vaccination.
  - Onset day of any local reaction after vaccination.

Figures: Bar charts with the proportions of subjects reporting each local reaction within 14 days after vaccination will be plotted for each vaccine group. The bars will be stacked by maximum severity to highlight the proportions of subjects by severity.

6.1.2. Systemic Events

6.1.2.1. Primary Analysis

Endpoint: Proportions of subjects reporting prompted systemic events (fever, fatigue, headache, vomiting, nausea, diarrhea, muscle pain, and joint pain) within 14 days after vaccination.

- Analysis time points: Within 14 days after Vaccination 1.
- Analysis population: Safety population (Primary Study Cohort - Stage 1).
- Analysis methodology: Descriptive summary statistics.
- Supporting objective: Primary objective.

Reporting Results:

Proportions of subjects reporting prompted systemic events (each and any) after Vaccination 1 will be summarized by maximum severity level. Confirmed e-diary errors will be excluded from the analysis. The percentage (%), the numerator (n) and denominator (N) used in the percentage calculation, and the corresponding 95% Clopper-Pearson CI will be presented for each vaccine group.

6.1.2.2. Additional Summary for Assessing Systemic Events

To support the assessment of systemic events, similar endpoints as above will be summarized for the Primary Study Cohort - Stage 2 within 14 days after Vaccination 2; as well as for the RSV Vaccine Month-0, Month-2 Cohort within 14 days after Vaccination 1 and within 14 days after Vaccination 2.

To support the assessment of systemic events, additional endpoints (as defined in Section 3.1.2) will be summarized with all analysis time points (within 14 days after Vaccination 1 and within 14 days after Vaccination 2), analysis populations (Primary Study Cohort - Stage 1, Primary Study Cohort - Stage 2, and RSV Vaccine Month-0, Month-2 Cohort), analysis methodology, and appropriate reporting results. Confirmed e-diary errors will be excluded from these analyses.

Binary Endpoints

The percentage (%), the numerator (n) and denominator (N) used in the percentage calculation, and the corresponding 95% Clopper-Pearson CI will be presented for each vaccine group after each vaccination for the following endpoints:

- Subjects reporting any severe systemic events on each day and any day (Day 1 through Day 14).

Continuous Endpoints

The following endpoints will be summarized by displaying n, mean, median, standard deviation, minimum, and maximum for each vaccine group:

  - Duration of each systemic event after vaccination.
  - Onset day of each systemic event after vaccination.
  - Onset day of any systemic event after vaccination.

Figures: Bar charts with the proportions of subjects reporting each systemic event within 14 days after vaccination will be plotted for each vaccine group. The bars will be stacked by maximum severity to highlight the proportions of subjects by severity.

6.1.3. Use of Antipyretic/Pain Medication

Use of antipyretic/pain medication will be analyzed in a similar way as systemic events (Section 6.1.2).

6.1.4. AEs

6.1.4.1. Primary Analysis

Endpoint: Proportions of subjects reporting AEs.

- Analysis time points: Within 1 month after Vaccination 1.
- Analysis population: Safety population (Primary Study Cohort - Stage 1).
- Analysis methodology: Descriptive summary statistics.
- Supporting objective: Primary safety objective.

Reporting Results:

The percentage (%), the number of subjects, the denominator (N) used in the percentage calculation, and the corresponding 95% Clopper-Pearson CI for subjects reporting any AE, each SOC, and each PT within SOC will be presented for each vaccine group.

6.1.4.2. Additional Summary for Assessing Adverse Events

To support the assessment of AEs, similar endpoints as above will be summarized for the Primary Study Cohort - Stage 2 within 1 month after Vaccination 2; as well as for the RSV Vaccine Month-0, Month-2 Cohort within 1 month after each vaccination and from Vaccination 1 through 1 month after Vaccination 2.

To support the assessment of AEs, additional endpoints by category (related AE, severe AE, SAE, MAE, immediate AE, AE leading to withdrawal, AE of specific interest), as defined in Section 3.1.4, will be summarized with all analysis time points (within 1 month after Vaccination 1 and within 1 month after Vaccination 2), analysis populations (Primary Study Cohort - Stage 1, Primary Study Cohort - Stage 2, and RSV Vaccine Month-0, Month-2 Cohort), analysis methodology, and appropriate reporting results.

For each specific AE category (related AE, severe AE, SAE, MAE, immediate AE, AE leading to withdrawal), if the total number of subjects is >10% of the study population, the analysis summary by SOC and PT will also be provided for that category. Otherwise, only listings of these AEs will be provided by SOC, PT, and category designation (SAE, MAE, immediate AE, etc.).

If any nonserious AEs are reported to occur before vaccination or more than 1 month after vaccination (outside of the protocol-specified reporting window), they will not be summarized, but will be included in the AE listings.

Figures: Bar charts with the proportions of subjects reporting each AE category after vaccination will be plotted for each vaccine group.

6.1.5. SAEs

6.1.5.1. Primary Analysis

Endpoint: Proportions of subjects reporting SAEs.

- Analysis time points: Through 12 months after Vaccination 1.
- Analysis population: Safety population (Primary Study Cohort - Stage 1).
- Analysis methodology: Descriptive summary statistics.
- Supporting objective: Primary safety objective.

Reporting Results:

The percentage (%), the number of subjects (n), the denominator (N) used in the percentage calculation, and the corresponding 95% Clopper-Pearson CI for subjects reporting any SAEs, each SOC, and each PT within SOC will be presented for each vaccine group.

Figures: None.

6.1.5.2. Additional Summary for Assessing SAEs

To support the assessment of SAEs, similar endpoints as above will be summarized for the Primary Study Cohort - Stage 2 through 12 months after Vaccination 2; as well as for the RSV Vaccine Month-0, Month-2 Cohort through 12 months after Vaccination 2 and through 14 months after Vaccination 1.

Listings of these SAEs will be provided by SOC and PT.

6.1.6. MAEs

MAEs will be analyzed in a similar way as SAEs (Section 6.1.5).

6.2. Secondary Endpoint(s)

6.2.1. RSV A– and RSV B–Neutralizing Antibody Titers

6.2.1.1. Primary Analysis

Endpoint: RSV A– and RSV B–neutralizing antibody titers.

- Analysis time points: Before and 1 month after Vaccination 1.
- Analysis populations: Evaluable immunogenicity population (Primary Study Cohort - Stage 1).
- Analysis methodology: Descriptive summary statistics.

- Supporting objective: Secondary immunogenicity objective.

Reporting Results:

GMTs before and 1 month after Vaccination 1, and associated 2-sided 95% CIs, will be summarized by vaccine group for both RSV A– and RSV B–neutralizing antibody titers. Also, GMFRs from before to 1 month after Vaccination 1, and associated 2-sided 95% CIs, will be summarized by vaccine group for both RSV A– and RSV B–neutralizing antibody titers.

Figures:

RCDCs for RSV A– and RSV B–neutralizing antibody titers for the 2 time points (before Vaccination 1 and 1 month after Vaccination 1) will be generated by vaccine group.

6.2.1.2. Additional Analysis

For the Primary Study Cohort - Stage 1, GMTs at all other applicable visits after Vaccination 1 but before Vaccination 2 will be summarized along with associated 2-sided 95% CIs, by vaccine group, for both RSV A– and RSV B–neutralizing antibody titers.

Similarly, for the RSV Vaccine Month-0, Month-2 Cohort, GMTs at all applicable visits will be summarized with 2-sided 95% CIs.

For the Primary Study Cohort - Stage 2, GMTs at all applicable visits (including visits in Stage 1 and Stage 2) will be summarized with 2-sided 95% CIs.

It should be noted that the inclusion of each applicable blood sampling visit will not take the protocol-required visit window into consideration as long as subjects meet the evaluable immunogenicity population criteria.

Additionally, all fold rises specified in Section 3.2.1 will be summarized with GMFRs and associated 2-sided 95% CIs.

The following comparisons will be made in order to:

1. Assess adjuvant effects:

   For the Primary Study Cohort - Stage 1, GMTs at each post–Vaccination 1 visit will be compared between RSVpreF 240 µg + $Al(OH)_3$ + SIIV and RSVpreF 240 µg +SIIV; as well as between RSVpreF 240 µg + CpG/$Al(OH)_3$ + SIIV and RSVpreF 240 µg + SIIV. GMRs and associated 2-sided 95% CIs will be calculated for each post–Vaccination 1 visit in Stage 1.

2. Assess SIIV coadministration interference to immune response induced by RSVpreF 240 µg + CpG/Al(OH)$_3$:

   This will combine 1-month post–Vaccination 1 NT data from the **Primary Study Cohort - Stage 1** and the **RSV Vaccine Month-0, Month-2 Cohort.**

   GMTs at 1 month after Vaccination 1 for RSV A NT and RSV B NT will be compared between the RSVpreF 240 µg + CpG/Al(OH)$_3$ with SIIV group (**Primary Study Cohort - Stage 1**) and the RSVpreF 240 µg + CpG/Al(OH)$_3$–only group (**RSV Vaccine Month-0, Month-2 Cohort**). GMRs and associated 2-sided 95% CIs will be calculated.

For the 1-month postvaccination visit, GMT summaries from the **Primary Study Cohort - Stage 1** and the **RSV Vaccine Month-0, Month-2 Cohort** will be presented for each vaccine group together with pooled placebo data.

Analysis using the mITT population may be used if there is a large enough difference compared with the evaluable immunogenicity population.

Figures:

For the remaining 2 cohorts (Primary Study Cohort - Stage 2 and RSV Vaccine Month-0, Month-2 Cohort), RCDCs will be plotted for prevaccination and 1-month postvaccination visits only.

NT GMT kinetics will be plotted across all visits for each cohort.

6.2.2. HAI Titers

6.2.2.1. Primary Analysis

Endpoint: HAI titers for all strains tested.

- Analysis time points: Before and 1 month after vaccination.
- Analysis populations: Evaluable immunogenicity population (Primary Study Cohort - Stage 1).
- Analysis methodology: Descriptive summary statistics.
- Supporting objective: Secondary immunogenicity objective.

Reporting Results:

GMTs before and 1 month after Vaccination 1 and associated 2-sided 95% CIs will be summarized by vaccine group for each influenza strain tested. Also, GMFRs from before to 1 month after Vaccination 1 and associated 2-sided 95% CIs will be summarized by vaccine group for each strain tested.

Figures:

RCDCs for each influenza strain testing at the 2 time points (before Vaccination 1 and 1 month after Vaccination 1) will be generated by vaccine group.

6.2.2.2. Additional Analysis

For the Primary Study Cohort - Stage 1, the seroprotection rate before Vaccination 1 and 1 month after Vaccination 1, and seroconversion rates from before Vaccination 1 to 1 month after Vaccination 1, will be summarized with n, percentage (%), and 2-sided 95% CI, among the evaluable immunogenicity population.

For the Primary Study Cohort - Stage 2, GMTs before and 1 month after Vaccination 2 and GMFRs from before to 1 month after Vaccination 2 will all be descriptively summarized with associated 2-sided 95% CIs by vaccine group for each influenza strain tested. The seroprotection rate before Vaccination 2 and 1 month after Vaccination 2, and seroconversion rates from before Vaccination 2 to 1 month after Vaccination 2, will be summarized with n, percentage (%), and 2-sided 95% CI. RCDCs for each influenza strain testing at the 2 time points (before Vaccination 2 and 1 month after Vaccination 2) will be generated by vaccine group.

The following comparisons will be made in order to assess the interference of RSV vaccine's coadministration with the SIIV immune response:

> For the **Primary Study Cohort - Stage 1,** GMTs for the groups including SIIV coadministration with RSV vaccine dose/formulation at 1 month after Vaccination 1 will be compared with the SIIV-only (with placebo) group, for each influenza strain tested. GMRs and associated 2-sided 95% CIs will be calculated.
>
> For the **Primary Study Cohort - Stage 2,** GMTs for the groups including SIIV coadministration with RSV vaccine dose/formulation at 1 month after Vaccination 2 will be compared with the SIIV-only (with placebo) group, for each influenza strain tested. GMRs and associated 2-sided 95% CIs will be calculated.
>
> All analyses will be performed among the evaluable immunogenicity population.

6.3. Other Endpoint(s)

6.3.1. Plasmablast Frequencies and T-Cell Phenotype and Functional Analysis

For each cohort, descriptive statistics (median, n, and range [minimum-maximum]) will be summarized for each relevant assay at each applicable time point, among subjects included in the evaluable immunogenicity population.

Additionally, the exact p-value from the Wilcoxon 2-sample test will be used to compare each RSVpreF dose/formulation group and placebo group.

6.3.2. Memory B-Cell and T-Cell Frequencies and Their Functional Analysis Before Vaccination

Analyses will be the same as Section 6.3.1.

6.3.3. Anti-RSV Prefusion F IgG

For each cohort, the IgG A and IgG B will be descriptively summarized with GMC and associated 2-sided 95% CI, at each applicable visit among the evaluable immunogenicity population (Section 6.2.1).

Similarly, for each cohort, the fold rise of IgG A and IgG B (defined in Section 3.3.2) will be descriptively summarized with GMFR and associated 2-sided 95% CI, at each applicable visits among the evaluable immunogenicity population.

GMCs at 1 month after Vaccination 1 for IgG A and IgG B will be compared between the RSVpreF 240 µg + CpG/Al(OH)$_3$ with SIIV group (**Primary Study Cohort - Stage 1**) and the RSVpreF 240 µg + CpG/Al(OH)$_3$–only group (**RSV Vaccine Month-0, Month-2 Cohort**). GMRs and associated 2-sided 95% CIs will be calculated.

For the 1-month postvaccination visit, a GMC summary from the **Primary Study Cohort - Stage 1** and the **RSV Vaccine Month-0, Month-2 Cohort** will be presented for each vaccine group together with pooled placebo data.

6.3.4. Anti-RSV Nonvaccine Antigen Immunoglobulin

For each cohort, the endpoints defined in Section 3.3.4 will be summarized with n, percentage (%), and 2-sided 95% CI, for each applicable visit, among the evaluable immunogenicity population.

6.3.5. H3N2-Neutralizing Antibody Titer

All analyses included in Section 6.2.2 (for HAI titer) will be repeated for H3N2.

6.3.6. Ratio of Combined Neutralizing Titer A/B Fold Rise to Combined Anti-RSV Prefusion F IgG A/B Fold Rise

For each cohort, at each applicable visit, the ratio will be summarized with geometric mean, along with 2-sided 95% CI, among the evaluable immunogenicity population.

6.4. Subset Analyses

Subgroup analysis for immunogenicity results (NT) may be carried out by anti-RSV NVA immunoglobulin seroconversion at certain visits or across some visits. As the definition of NVA seroconversion was to estimate possible nonsymptomatic RSV infection, stratified analyses may help to elucidate the relationship between immune response induced by RSVpreF and possible infection.

6.5. Baseline and Other Summaries and Analyses

6.5.1. Baseline Summaries

6.5.1.1. Demographics and Smoking History

For each cohort, descriptive summary statistics for demographic characteristics (age at vaccination, sex, race, and ethnicity) will be generated for each vaccine group, as well as the total subjects, based on the safety population.

The proportions of subjects reporting as "current smoker," "ex-smoker," and "never smoked" will be summarized in a similar way.

Summary data may also be presented for the evaluable immunogenicity population if the population size difference is >10%.

Subject data listings for demography and baseline characteristics data will also be listed.

6.5.1.2. Medical History

Each reported medical history term will be mapped to a SOC and PT according to the current version (at the time of reporting) of MedDRA. The number and percentage of subjects with at least 1 diagnosis, overall and at each SOC and PT level, may be summarized by vaccine group and for overall subjects included in the safety population for each cohort.

However, as medical history was mainly collected to ensure subjects meet study eligibility, this information may only be listed.

6.5.2. Study Conduct and Subject Disposition

The number and proportion of randomized subjects will be included in the subject disposition summary. In addition, subjects who completed each vaccination visit and subjects who withdrew before the 12-month follow-up visit, along with the reasons for withdrawal, will be tabulated by vaccine group. The reasons for withdrawal will be those as specified in the database.

The number and proportion of subjects who were randomized, were vaccinated, and had blood drawn within the protocol-specified time frame, and before or after the specified window, may also be tabulated by vaccine group.

Subjects excluded from the evaluable immunogenicity and mITT populations will also be summarized with reasons for exclusion.

Standard listings, including subjects who withdrew during the study, subjects excluded from analysis populations, subjects with major protocol violations, subjects who did not receive the vaccine as randomized, etc., will all be included.

6.5.2.1. E-Diary Completion

For each cohort, the number and percentage of vaccinated subjects with e-diary data transmitted for each day (Days 1 through 14) and for all days after each vaccination in the required reporting period may be summarized by vaccine group and for all subjects included in the safety population.

However, e-diary transmission rate is relevant to monitoring study conduct. The summary may be omitted as the information is included in reactogenicity listings.

6.5.2.2. Nonstudy Vaccines

Nonstudy vaccines taken after signing the informed consent through the end of the study will be categorized according to the WHO Drug Dictionary and may be summarized by vaccine group and for all subjects included in the safety population.

A listing may be used to replace the table.

6.6. Safety Summaries and Analyses

All safety summaries are included in Section 6.1.

7. INTERIM ANALYSES

7.1. Introduction

Three interim analyses will be conducted before the final analysis at the completion of the study.

7.2. Interim Analyses and Summaries

- **Primary Study Cohort - Stage 1**

An analysis will be conducted when 1-month post–Vaccination 1 immunogenicity data from all subjects in the Primary Study Cohort - Stage 1 are available. All available safety, tolerability, and immunogenicity data from subjects will be included in the analysis.

- **Primary Study Cohort - Stage 2**

An analysis will be conducted when 1-month post–Vaccination 2 immunogenicity data from all subjects in the Primary Study Cohort - Stage 2 are available. All available safety, tolerability, and immunogenicity data will be included in the analysis.

- **RSV Vaccine Month-0, Month-2 Cohort**

An analysis will be conducted when 1-month post–Vaccination 2 immunogenicity data from all subjects in this cohort are available. All available safety, tolerability, and immunogenicity data will be included in the analysis.

Assays may come in batches; therefore, some interim analyses may be performed in batches. One interim analysis may span multiple reporting events. The results of these analyses will be used to select the most appropriate dose(s), formulation(s), and dosing schedule of the RSV vaccine for use in further studies in older adults.

Additional analyses may be conducted before the final analysis to support other internal program-level decisions as needed.

Safety data will be summarized on an ongoing basis.

No multiplicity adjustments will be applied for these analyses.

The final analysis will be performed after all the subjects complete the study and when all of the data are available.

8. REFERENCES

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