Protocol C3671016

A PHASE 1, OPEN-LABEL, AGE-DESCENDING, DOSE-FINDING STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF RESPIRATORY SYNCYTIAL VIRUS PREFUSION F SUBUNIT VACCINE (RSVpreF) IN CHILDREN 2 TO <18 YEARS OF AGE

Statistical Analysis Plan
(SAP)

Version: 3

Date: 06 Mar 2024

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APPENDICES

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 13 Mar 2023	Original 31 Jan 2023	N/A	N/A
2 25 Sep 2023	Original 31 Jan 2023	1. Updated the Phase 1 safety analysis based on FDA feedback on multiple programs 2. Added details of the Phase 2/3 safety analysis based on the new e-diary and CRF 3. Added information on AESIs 4. Added the Tier 1 analysis per FDA BLA review comments (Phase 2/3) 5. Clarified the Phase 1 immunogenicity analysis 6. Added additional clarification on analysis	1. Section 3.1.2, Section 5.3.1.1, Section 6.1.2, Section 6.1.4.3 2. Section 3.1.3, Section 6.1.3, Section 6.1.5 3. Section 3.1.2.3, Section 3.1.3.2 4. Section 3.5.2, Section 6.6.1 5. Section 2.2.3 6. Section 6.5.2
3 06 Mar 2024	Protocol amendment 1 09 Feb 2024	1. Removed the Phase 2/3 content 2. Updated the AESI terms per new CRF 3. Updated the other endpoints with seroresponse 4. One reference was removed (Miettinen O, Nurminen M)	1. Throughout the SAP 2. Section 3.1.1.3 3. Section 3.3.1, Section 6.3 4. Section 8

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C3671016.

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. Modifications to the Analysis Plan Described in the Protocol

There is no change in analysis from the plan specified in the protocol.

2.2. Study Objectives, Endpoints, and Estimands

Type	Objective	Endpoint	Estimand
Primary ***Safety***	To describe the safety and tolerability of RSVpreF at each dose level in children 5 to <18 years of age and children 2 to <5 years of age.	Local reactions (pain at the injection site, redness, and swelling). Systemic events (fever, vomiting, diarrhea, headache, fatigue/tiredness, muscle pain, and joint pain). AEs. SAEs. NDCMCs.	In participants receiving 1 dose of study intervention at each dose level, for each age stratum: • The percentage of participants reporting local reactions within 7 days after vaccination. • The percentage of participants reporting systemic events within 7 days after vaccination. • The percentage of participants reporting AEs from vaccination through 1 month after vaccination. • The percentage of participants reporting SAEs throughout the study. • The percentage of participants reporting NDCMCs throughout the study.
Secondary ***Immunogenicity***	To describe the immune responses elicited by RSVpreF at each dose level in children 5 to <18 years of age and children 2 to <5 years of age.	RSV A and RSV B NTs.	In participants in compliance with the key protocol criteria (evaluable immunogenicity population): • GMTs of NT for RSV A and RSV B 1 month after vaccination. • GMTs of NT for RSV A and RSV B before vaccination. • GMFRs of NT for RSV A and RSV B from before vaccination to 1 month after vaccination.

Type	Objective	Endpoint	Estimand
Secondary ***Immunogenicity***	To describe the cell-mediated immune response in children 5 to <18 years of age and children 2 to <5 years of age.	RSV F antigen–specific CD4+ T cells secreting IFNγ. RSV F antigen–specific CD4+ T cells secreting IL-4.	In participants in compliance with the key protocol criteria (evaluable immunogenicity population): • Median frequencies of RSV F antigen–specific CD4+ T cells expressing IFNγ before vaccination and 1 month after vaccination. • Median frequencies of RSV F antigen–specific CD4+ T cells expressing IL-4 before vaccination and 1 month after vaccination.

2.2.1. Primary Estimand(s)

2.2.1.1. Primary Safety Estimands

2.2.1.1.1. Reactogenicity Estimands

Reactogenicity estimands have the following 5 attributes:

- **Treatment condition:** Up to 2 dose levels received on Day 1 from each cohort are applicable for safety estimands. Therefore, up to 6 dose/age groups are applicable to treatment condition: 1) RSVpreF 120 µg in the 5- to <18-year-old healthy cohort; 2) RSVpreF 120 µg in the 5- to <18-year-old cohort with chronic medical conditions; 3) RSVpreF 120 µg in the 2- to <5-year-old cohort; 4) RSVpreF 60 µg in the 5- to <18-year-old healthy cohort; 5) RSVpreF 60 µg in the 5- to <18-year-old cohort with chronic medical conditions; and 6) RSVpreF 60 µg in the 2- to <5-year-old cohort.

- **Population:** Pediatric participants as defined by the study inclusion/exclusion criteria in the Phase 1 cohort.

- **Variables:** Each item included in the e-diary from Days 1 through 7 after vaccination for each treatment condition (refer to Section 3.1.1.1 and Section 3.1.1.2).

- **Intercurrent events:** All data collected after the intercurrent event will be included.

- **Population-level summary:** The rates of the variables (reporting each reactogenicity item) in each treatment condition (by dose/age/risk) separately.

2.2.1.1.2. AE Estimands

AE estimands have the same attributes (treatment condition, population, intercurrent events, population-level summary) as reactogenicity estimands (Section 2.2.1.1.1), except:

- **Variables:** AEs reported within 1 month after vaccination (Section 3.1.1.3).

2.2.1.1.3. SAE Estimands

SAE estimands have the same attributes (treatment condition, population, intercurrent events, population-level summary) as reactogenicity estimands (Section 2.2.1.1.1), except:

- **Variables:** SAEs reported throughout the study (Section 3.1.1.3).

2.2.1.1.4. NDCMC Estimands

NDCMC estimands have the same attributes (treatment condition, population, intercurrent events, population-level summary) as reactogenicity estimands (Section 2.2.1.1.1), except:

- **Variables:** NDCMCs reported throughout the study (Section 3.1.1.3).

2.2.2. Secondary Estimand(s)

All secondary estimands for immunogenicity objectives will use the hypothetical strategy and describe the RSVpreF immune response in participants without the intercurrent events. In other words, the immune response is estimated in the hypothetical setting where participants follow the study schedule and protocol requirements as directed. Each is defined in the subsections below.

2.2.2.1. Secondary Immunogenicity Estimands

2.2.2.1.1. Secondary RSV NT Immunogenicity Estimands

- **Treatment condition:** Up to 6 dose/age groups received on Day 1 from the study cohort are applicable to treatment condition: 1) RSVpreF 120 µg in the 5- to <18-year-old healthy cohort; 2) RSVpreF 120 µg in the 5- to <18-year-old high-risk cohort; 3) RSVpreF 120 µg in the 2- to <5-year-old cohort; 4) RSVpreF 60 µg in the 5- to <18-year-old healthy cohort; 5) RSVpreF 60 µg in the 5- to <18-year-old high-risk cohort; and 6) RSVpreF 60 µg in the 2- to <5-year-old cohort.

- **Population:** Pediatric participants as defined by the study inclusion/exclusion criteria for the Phase 1 cohort.

- **Variables:** RSV serum NTs for subgroup A and subgroup B.

- **Intercurrent events:** The following intercurrent events could impact the interpretation or the measurement of the immune response:

  1. The participant did not receive RSVpreF as randomized in each study.

  2. The participant did not meet the study inclusion criteria or did meet the exclusion criteria.

  3. Major protocol violations: The participant received a prohibited vaccine or treatment that may alter the immune response.

4. Blood was taken outside an acceptable window for immunogenicity evaluation (<27 days or >42 days after RSVpreF).

The clinical question of interest is based on the comparison the immune response elicited from RSVpreF in pediatric participants from this study's cohort, without any influence from any other immune-modifying drugs or vaccines and measured within a homogeneous time window. Therefore, all data after intercurrent events 1, 2, and 3, as well as all data at intercurrent event 4, if collected, will be excluded. Major protocol violations will be determined by clinical review.

- **Population-level summary**: GMT of NT before vaccination and 1 month after vaccination, and GMFR of NT from before vaccination to 1 month after vaccination, in each treatment condition (by age/with or without chronic medical condition group/dose level) separately.

2.2.2.1.2. Secondary PBMC Immunogenicity Estimands

PBMC immunogenicity estimands have the same attributes (treatment condition, population, and intercurrent events) as RSV immunogenicity estimands (Section 2.2.2.1.1), except:

- **Variables:** RSV F antigen–specific CD4+ T cells secreting IFNγ and IL-4.

- **Population-level summary**: Median frequency before vaccination and 1 month after vaccination, in each treatment condition (by age/risk group) separately.

2.2.3. Additional Estimand(s)

Additional estimands, as supplemental analyses to support the primary and secondary immunogenicity objectives, are defined. The table below lists the strategies for addressing intercurrent events, which are listed in Section 2.2.1 and Section 2.2.2 for the immunogenicity objectives. The remaining estimand attributes are the same for each objective.

Immunogenicity Objective	Intercurrent Event Handling Strategy
Secondary RSV NT immunogenicity	Treatment policy
Secondary PBMC immunogenicity	Treatment policy

For the immunogenicity objectives, the treatment policy will be based on actual age, risk condition, and the vaccine received, as the 6 groups are not randomized.

2.3. Study Design

This is a Phase 1 open-label, age-descending, dose-finding study of the safety, tolerability, and immunogenicity of RSVpreF in RSV-seropositive children 2 to <18 years of age. Up to 120 participants will receive RSVpreF with up to 2 dose levels across 2 age strata (2 to <5 years and 5 to <18 years) and 2 risk substrata in the older group (5 to <18 years of age). Approximately 60 to 120 participants are expected to be enrolled.

Age Stratum (Years)	Number of Participants
2 to <5	~20/~20[a]
5 to <18	~40/~40[a,b]
Total	~60-120

a. Up to 2 dose levels of RSVpreF (120 µg and 60 µg) will be tested in participants.
b. Approximately 40 participants 5 to <18 years old will be enrolled, with ~20 healthy participants and ~20 participants with high-risk chronic medical conditions receiving each dose level.

For all participants enrolled, local reactions (redness, swelling, and pain at the injection site) and systemic events (fever, vomiting, diarrhea, headache, fatigue/tiredness, muscle pain, and joint pain) are collected from Day 1 (vaccination day) through Day 7 via the e-diary. AEs are collected from consent through the 1-month postvaccination visit. Additionally, AEs occurring up to 48 hours after blood draws that are related to study procedures are reported in the CRF. SAEs and NDCMCs will be collected from the time the participant's parent(s)/legal guardian provide informed consent through the duration of the study.

All participants 2 to <5 years of age must be seropositive. RSV serostatus will be determined before vaccination. Blood samples are collected before vaccination and at the 1-month postvaccination visit to assess RSV A and RSV B NT, as well as cell-mediated immune response through measuring RSV F antigen–specific CD4+ T cells secreting IFNγ and RSV F antigen–specific CD4+ T cells secreting IL-4.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

3.1.1. Primary Safety Endpoints

Primary safety endpoints include both reactogenicity data collected from the e-diary and AE data collected from the CRF.

Based on feedback from the FDA on multiple vaccine programs, reactogenicity data will utilize both e-diary data (prompted local reactions and systemic events) and reactogenicity events (related or unrelated) reported in the AE CRF during the e-diary collection period. Since the AE CRF does not designate a specific page to collect reactogenicity data for an untransmitted e-diary, Pfizer has adopted a process of providing a listing of AEs reported within 7 days after vaccination to the clinical team to review and determine ("flag") which

PTs should be considered reactogenicity events before the database lock. AEs reported on the same day of vaccination but missing a start time are defaulted to AEs reported after vaccination. Following these review steps, those AEs reported within 7 days after vaccination that match flagged PTs (either related or unrelated) will be considered reactogenicity data. If the same reactogenicity event is reported on the same day from both the e-diary and the AE CRF, the highest grade from the 2 data sources will be used for that specific day for analysis.

It should be noted that the data collection in the AE CRF is different from that of the e-diary:

- For redness, swelling, and fever, the measured size of redness and swelling at the injection site and temperature are recorded in the e-diary, but not in the AE CRF. As the missing e-diary entries are monitored with ongoing review of the prompted reactions reported in the AE CRF, any measurement recorded in the query response will be taken into consideration for the primary analysis using the data handling memo for analysis purposes. For the 7 days, only the maximum grading from both sources will be used for the aggregated severity analysis.

- For pain at the injection site and all other systemic events, the severity grading algorithm for the e-diary data and the AE CRF may not be the same. Pfizer will choose the highest-severity grade.

If a participant did not have any e-diary data transferred within 7 days after vaccination, the AE CRF data will not be used for derivation, because doing so may inflate the denominator and bias the analysis, since participants who did not transfer e-diary data may be less likely to report reactogenicity data. If a participant did not report any e-diary data, the participant will not be included in the analysis of reactogenicity data.

The subsections below describe how to derive each safety endpoint.

3.1.1.1. Local Reactions Within 7 Days After Vaccination

The local reactions include redness, swelling, and pain at the injection site from Day 1 through Day 7 after vaccination, where Day 1 is the day of vaccination with RSVpreF. Any reported reactogenicity events in the AE CRF during the e-diary collection period are included in the derivation discussed below.

The following 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 local reactions on each day, for each vaccine group as mentioned above.

3.1.1.1.1. Presence of Local Reactions Within 7 Days After Vaccination

For the summary of the presence (yes or no) of a local reaction during the interval from Day 1 through Day 7 after vaccination, the following 2 variables are derived for each participant included in the reactogenicity subset:

1. Presence (yes or no) of each local reaction on any day (Day 1 through Day 7).

   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 1-7)	The participant reports the reaction as "yes" on any day (Days 1-7).	The participant reports the reaction as "no" on all 7 days or as a combination of "no" and missing on all 7 days.	The participant reports the reaction as missing on all 7 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 7).

   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 1-7)	The participant reports any local reaction as "yes" on any day (Days 1-7).	The participant reports all reactions as "no" on all 7 days or as a combination of "no" and missing on all 7 days for all 3 local reactions.	The participant reports all local reactions as missing on all 7 days.

3.1.1.1.2. Maximum Severity of Local Reactions Within 7 Days After Vaccination

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	Mild grading from the e-diary per Table 2 in the protocol or mild from the AE CRF	Moderate grading from the e-diary per Table 2 in the protocol or moderate from the AE CRF	Severe grading from the e-diary per Table 2 in the protocol or severe from the AE CRF	Necrosis or exfoliative dermatitis
Swelling	Mild grading from the e-diary per Table 2 in the protocol or mild from the AE CRF	Moderate grading from the e-diary per Table 2 in the protocol or moderate from the AE CRF	Severe grading from the e-diary per Table 2 in the protocol or severe from the AE CRF	Necrosis

Table 4. Grading Scale for Local Reactions

	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Grade 4[a]
Pain (at the injection site)	Does not interfere with activity (mild from the e-diary or AE CRF)	Interferes with activity (moderate from the e-diary or AE CRF)	Prevents daily activity (severe from the e-diary or AE CRF)	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 CRF and thus not reported from the e-diary.

The following variables are derived for each participant:

1. Maximum severity of <u>each</u> local reaction on any day (Day 1 through Day 7).

   The maximum severity (highest grading) of each local reaction within 7 days after vaccination will be derived. The maximum severity will be derived as follows:

   = Missing, if values are missing for all days (Days 1-7);

   = 0, if the participant reports all reactions as "no" or a combination of missing and "no" for all days (Days 1-7);

   = *Highest grade* (maximum severity either from the e-diary or in the AE CRF) within 7 days after vaccination if the answer is not "no" for at least 1 day.

2. Maximum severity of <u>any</u> local reaction on any day (Day 1 through Day 7).

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

   = Missing, if values are missing for all days (Days 1-7) across all 3 local reactions;

   = 0, if the participant reports all reactions as "no" or a combination of missing and "no" for all days (Days 1-7) for all individual local reactions;

   = *Highest grade* (maximum severity) within 7 days after vaccination, if the answer is not "no" for at least 1 day for at least 1 local reaction.

3.1.1.1.3. Duration of Each Local Reaction

The duration of each local reaction will be calculated in days as the 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 AE CRF) or the date the event ends if it is unresolved during the participant e-diary recording period (end date collected on the CRF) or AE stop date, whichever is longer, unless chronicity is established.

If there is no known end date, the duration will be considered unknown and set to "missing". Participants with no reported reaction have no duration.

3.1.1.1.4. 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 the participant reports changes in severity of the local reaction, only the first day of reporting that specific local reaction will be counted.

3.1.1.1.5. Presence of Each and Any Local Reaction on Each Day and Any Day

Presence (yes or no) of each and any local reaction on each of the 7 days (Day 1 through Day 7) follows the derivation as described in Table 5.

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

Variable	Yes (1)	No (0)	Missing (.)
Each local reaction on a specific day	The participant reports the reaction on a specific day for a specific local reaction.	The participant reports the reaction as "no" on that specific day.	The participant reports the specific local reaction as missing on that specific day.
Any local reaction on a specific day	The participant reports the reaction on a specific day for any of the 3 local reactions.	The participant reports the reactions as "no" or a combination of "no" or missing on that specific day across all 3 local reactions.	The participant reports all 3 local reactions as missing on that specific day.
Each local reaction on any day	The participant reports that local reaction on any day (Days 1-7).	The participant reports the local reaction as "no" or a combination of "no" and missing across all 7 days.	The participant reports the local reaction as missing on all 7 days.
Any local reaction on any day	The participant reports any local reaction on any day (Days 1-7).	The participant reports the reactions as "no" or missing across all 7 days and across all 3 local reactions.	The participant reports all 3 local reactions as missing on all 7 days.

3.1.1.2. Systemic Events Within 7 Days After Vaccination

Systemic events, including fever, fatigue/tiredness, headache, vomiting, diarrhea, muscle pain, and joint pain, are reported from Day 1 through Day 7 after vaccination, where Day 1 is the day of vaccination with RSVpreF or placebo. The derivations for the systemic events as described below will be handled similarly to the way that local reactions are handled for the presence for each participant, severity level, duration, onset day, and presence of systemic event on each and any day.

1. Presence (yes or no) of each systemic event on any day (Day 1 through Day 7) after vaccination.

2. Presence (yes or no) of any systemic event on any day (Day 1 through Day 7) after vaccination.

3. Maximum severity of each systemic event on any day (Day 1 through Day 7) after vaccination.

4. Maximum severity of any systemic event on any day (Day 1 through Day 7) after vaccination.

5. Duration of each systemic event after vaccination.

6. Onset day of each systemic event after vaccination.

7. Presence (yes or no) of each systemic event on each and any of the 7 days after vaccination.

8. Presence (yes or no) of any systemic event on each and any of the 7 days after vaccination.

The grading scale for systemic events is provided in the protocol. However, the derivation of severity of each systemic event on each day should be based on the maximum severity reported from the e-diary or AE CRF, if data are reported from both sources; or the e-diary alone if not reported from the AE CRF.

Fever is defined as a temperature of ≥100.4°F (≥38.0°C). The highest temperature for each day will be recorded in the e-diary. For reporting purposes, fever will be analyzed using the following temperature ranges:

- Mild (≥38.0 to 38.4°C from the e-diary or mild grade from the AE CRF);
- Moderate (>38.4 to 38.9°C from the e-diary or moderate grade from the AE CRF);
- Severe (>38.9 to 40.0°C from the e-diary or severe grade from the AE CRF);
- Grade 4 (>40.0°C from the e-diary or severe grade from the AE CRF, plus documented >40.0°C via CRF query or other sources).

If a participant reports a fever (or severity of fever) by accident, the correct temperature will be transcribed in a data handling memo to be included in the analysis, and the temperature that is confirmed as incorrect will not be included in the analysis.

3.1.1.3. Adverse Events and Serious Adverse Events

Standard algorithms for handling missing AE dates and missing AE severity will be applied as described in the Pfizer vaccine data standard rules. Completely missing AE start dates will not be imputed.

The following derivations will be included for each participant:

1. Any AE reported within 1 month after vaccination – If the AE started on the same day of vaccination, if the AE start time is before the vaccination time, this AE would not be counted. Otherwise, if the AE start time is missing or after the vaccination time, the AE is included. Any AE reported after the 1-month visit will not be included in the analysis.

2. Any related AE reported within 1 month after vaccination – Similar to above except only a related AE is included (but excluding related reactogenicity reported within 7 days of vaccination) (Section 3.1.1).

3. Any immediate AE (AE start time is within 30 minutes after vaccination) reported after vaccination – Only include AEs that started on the same day of vaccination and with nonmissing AE start time that is within 30 minutes of vaccination. If the immediate AE is a related reactogenicity event, this would not be included as an immediate AE.

4. Any severe AE reported within 1 month after vaccination.

5. Any life-threatening AE reported within 1 month after vaccination.

6. Any AE leading to study withdrawal after vaccination.

7. Any SAE reported throughout the study.

8. Any NDCMC reported throughout the study.

9. Any AESI reported throughout the study.

Variables 1 through 6 listed above will be derived from excluding any reactogenicity (but not excluding reactogenicity SAEs) reported in the AE CRF during the e-diary collection period. AESIs are flagged in the CRF.

3.2. Secondary Endpoint(s)

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

RSV A– and RSV B–neutralizing antibody titers will be determined before vaccination and at 1 month after the vaccination visit.

Titers above the LLOQ are considered accurate and their quantitated values will be reported. Refer to Section 5.3.2 for LLOQ details. 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.

RSV A and RSV B NTs at each blood sampling time point are included in the assay result data, thus no derivation is needed. The following variables will be derived for each participant:

1. RSV A/B at each blood sampling time point: This will be derived as the geometric mean of RSV A and RSV B NTs measured at each blood sampling time point for each participant.

2. RSV A, RSV B, and RSV A/B NT fold rise: This will be derived from before vaccination to each applicable postvaccination visit. The numerator is the postvaccination value and the denominator is the prevaccination value.

   When calculating a fold rise, if assay results are < LLOQ, the assay results will be converted to 0.5 × LLOQ, except when the prevaccination assay result is < LLOQ while the postvaccination result is ≥ LLOQ, in which case the prevaccination value will be set to LLOQ.

3.2.2. PBMCs

Only fold rise from before vaccination to 1 month after vaccination will be derived for each of the PBMC endpoints (RSV F antigen–specific CD4+ T cells secreting IFNγ and RSV F antigen–specific CD4+ T cells secreting IL-4).

3.3. Other Endpoint(s)

3.3.1. RSV Seroresponse

RSV seroresponse after vaccination will be defined for each participant for Subgroup A and Subgroup B respectively:

- If prevaccination results are ≥ LLOQ, seroresponse is achieved if there is a ≥4-fold rise from prevaccination results.

- If prevaccination results are below LLOQ, seroresponse is achieved if the postvaccination titer is ≥4 × LLOQ.

3.4. Baseline Variables

3.4.1. Baseline Definition

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

3.4.2. Demographics, Baseline, and Medical History

The demographic variables that will be 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 participant's 6th birthday, the participant is 5 years old. Weight (kg) and height (cm) will be recorded for all participants.

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

For participants 2 to <5 years of age, only seropositive participants are included. All participants 5 years of age and older will be considered RSV seropositive and will not require a screening test.

Participants 2 to <18 years of age should either be healthy or be considered by the investigator to be at high risk of RSV disease based on the presence of 1 of the following chronic medical conditions:

- Cystic fibrosis
- Medically treated asthma
- Other chronic respiratory diseases and malformations of the lung
- Down syndrome
- Neuromuscular disease
- Cerebral palsy
- Hemodynamically significant or symptomatic congenital heart disease

3.4.3. E-Diary Completion

An e-diary will be considered transmitted if any data for the local reactions and systemic events are present for any day. If all data are missing for all items (local reactions and systemic events) on the e-diary for all 7 days 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 received from 28 days prior to study enrollment through the conclusion of study participation will be collected.

Nonstudy vaccinations will be categorized according to the latest version (at the time of reporting) of the WHODrug Dictionary.

3.5. Safety Endpoints

3.5.1. Vital Sign Data

The temperature collected at baseline will only be used to assess any potential protocol deviation for vaccination temporary delay. Therefore, it will not be included as a baseline variable.

3.5.2. Adverse Events

Tier 1 events: As this is a Phase 1 study, only Tier 1 AEs requested by FDA will be analyzed (Section 6.6.1). These are prespecified events of clinical importance and are maintained in a list in the product's safety review plan.

Guillain-Barre syndrome, acute polyneuropathy, atrial fibrillation (occurring from vaccination through the 1-month follow-up visit), preterm delivery (occurring from vaccination through the end of the study), preterm birth (occurring from vaccination through the end of the study), and hypertensive disorders of pregnancy (occurring from vaccination through the end of the study) will be included as the Tier 1 event analysis for RSVpreF. The derivation for Guillain-Barre syndrome and acute polyneuropathy is from Day 1 through Day 43 after vaccination (Day 1 is the vaccination day). The RSV program Tier 1 list of MedDRA PTs is maintained by the safety risk lead in the CAETeLiSt and is referenced in the safety surveillance review plan for the program. The current list of Tier 1 events referenced by this study for RSVpreF should be confirmed to ensure that appropriate Tier 1 events will be used to produce final tables/graphs before conducting an analysis.

Note that the denominator for calculating the preterm delivery, preterm birth, and hypertensive disorders of pregnancy would be the number of pregnant women.

As this is a Phase 1 study, no additional tiered AEs are planned for any analysis.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if participants 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.

For purposes of analysis, the following analysis sets are defined:

Participant Analysis Set	Description	Applicable Analysis
Enrolled population	All participants who have a signed ICD.	Study conduct such as participant disposition
Randomized population	All enrolled participants who are assigned a randomization number in the IRT system.	Immunogenicity analysis population disposition
E-diary safety population	All participants who receive the study intervention have at least 1 day of e-diary data transferred.	Primary safety endpoint analysis
Safety population	All enrolled participants who receive the study intervention.	Primary safety endpoint analysis

Defined Analysis Set	Description	Applicable Analysis
Evaluable immunogenicity population	This population will be defined for all participants who meet the following criteria: • Are eligible for the study; • Receive the intervention; • Have the 1-month postvaccination blood collection 27 through 42 days after vaccination; • Have at least 1 valid and determinate assay result 1 month after vaccination; • Have no major protocol violations from vaccination through the 1-month postvaccination blood draw.	Primary analysis population for immunogenicity endpoints (secondary)
mITT immunogenicity population	All participants who are randomized and have at least 1 valid and determinate assay result at any time point after receiving the study intervention.	Supplemental analysis on secondary immunogenicity endpoints

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

There is no statistical hypothesis.

5.2. General Methods

Unless otherwise stated, "CI" refers to a 2-sided CI in this document for 95% CI.

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

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

The subsections below describe the analysis for different types of endpoints.

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 95% CI for percentage, and for the difference in percentages, will also be presented, where applicable.

1. The 95% CI for the proportion (within–vaccine group) will be constructed by the Clopper-Pearson method described by Newcombe.[1] The 95% CI will be presented in terms of percentage.

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 the Student t distribution.

5.2.2.1. Geometric Means

Continuous immunogenicity endpoints will be logarithmically transformed for analysis. Geometric means and the associated 2-sided 95% CIs will be derived by calculating group means and CIs on the natural log scale, based on the Student t distribution, and then exponentiating the results.

5.2.2.2. Geometric Mean Fold Rises

GMFRs will be calculated as the group mean of the difference of logarithm-transformed assay results (later time point minus earlier time point) and exponentiating the mean. GMFRs are limited to participants with nonmissing values at both time points. The associated 2-sided 95% CIs will be obtained by constructing CIs using the Student t distribution for the mean difference on the logarithm scale and exponentiating the confidence limits.

5.2.2.3. Reverse Cumulative Distribution Curves

Empirical RCDCs will plot proportions of participants 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 line first going down and then to the right to the next assay value.

5.3. Methods to Manage Missing Data

5.3.1. Safety Data

Standard algorithms for 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 detail in the corresponding endpoint sections.

5.3.1.1. Reactogenicity Data

For derived variables based on the reactogenicity data, if any day of the 7-day e-diary is available, the "any day (Days 1-7)" data will be considered nonmissing.

The reactogenicity data are collected in the e-diary, which does not allow participants to skip any questions. Therefore, for a specific day, if the e-diary data are transferred for that day, all of the reactogenicity data for the participant on that day are nonmissing. In general, for any participant with all 7 days of the e-diary data missing, this participant will not be included in the analysis (ie, assuming MCAR). If only 1 through 6 days of e-diary data are transferred, it is expected that any missed reactogenicity events would be entered in the AE CRF if any experienced reactogenicity was not reported in the e-diary due to missed days. Therefore, the primary analysis will use reactogenicity recorded in the AE CRF to impute the partially missed e-diary data to estimate the reactogenicity rate during the e-diary collection period. The AE CRF is designed as a log page, which means only events that occurred will be recorded and events that did not occur will not be recorded. Therefore, all remaining missing days are considered as "no." This imputation can reasonably estimate the reactogenicity event rates during the e-diary collection period.

5.3.2. Immunogenicity Data

Any assays above LLOQ are considered accurate, and their quantitated values will be reported. Values below the LLOQ, or denoted as BLQ, 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 1 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.

The LLOQs for each assay will be included in the final released assay data.

Values for sera that are designated as 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, as MCAR is assumed for immunogenicity data according to Scott and Hsu.[2]

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint(s)

6.1.1. Local Reactions and Systemic Events

Reactogenicity data will be summarized for each of the 6 age/risk/dose-level groups (Section 2.2.1.1.1). Data will be summarized by vaccine group, according to the study interventions the participants actually received.

6.1.1.1. Main Analysis

- Estimand strategy: Treatment policy (Section 2.2.1.1.1).
- Intercurrent events and missing data: All data collected are included; partially missing e-diary data are imputed as "no" (Section 5.3.1.1); e-diary data that are confirmed as errors will not be used for analysis.
- Analysis set: Safety population (only participants with at least 1-day e-diary data transferred are included in the calculation) (Section 4).
- Analysis methodology: 95% CI of the proportion of participants reporting each event will be calculated using the Clopper-Pearson method (Section 5.2.1).
- Analysis timing: At primary analyses (when all primary endpoint data are available).
- Descriptive statistics, including the proportion (%), the numerator (n) and the denominator (N) used in the proportion calculation, and the 95% CI for percentage using the Clopper-Pearson method, will be presented for each group (Section 5.2.1).
- Bar charts with the proportions of participants for each and any local reaction and each and any systemic event throughout the 7 days will be plotted for each group. The bars will be divided into severity categories to highlight the proportions of participants by maximum severity.

6.1.1.2. Supplementary Analysis

To support the assessment of reactogenicity, the endpoints below, as specified in Section 3.1.1.1 and Section 3.1.1.2, will be summarized per the supplemental analysis with the same analysis population:

- Duration (days) of each local reaction and each systemic event after vaccination.
- Onset day of each local reaction and each systemic event after vaccination.
- Presence of each and any local reaction and each and any systemic event, on each of the 7 days and for "any day (Days 1-7)."

The presentation of the results will include a basic descriptive summary without the 95% CIs for each group.

6.1.1.3. Sensitivity Analysis of Reactogenicity

Maximum severity of reactogenicity is also assessed using the e-diary data only. The difference between the primary analysis and this sensitivity analysis will also be presented.

6.1.2. AEs, SAEs, and NDCMCs

AEs, SAEs, and NDCMCs will be summarized for each of the 6 age/risk/dose-level groups (Sections 2.2.1.1.2 through 2.2.1.1.4). Data will be summarized by vaccine group, according to the study interventions the participants actually received.

6.1.2.1. Main Analysis

- Estimand strategy: Treatment policy (Section 2.2.1.1.2).
- Intercurrent events and missing data: All data collected are included.
- Analysis set: Safety population (Section 4).
- Analysis timing: At primary analyses (when all primary endpoint data are available).
- Analysis methodology: 95% CIs of the proportion of participants reporting those events will use the Clopper-Pearson method (Section 5.2.1).
- Descriptive statistics, including the proportion (%), the numerator (n) and the denominator (N) used in the proportion calculation, and the 95% CI for percentage using the Clopper-Pearson method, will be presented for each vaccine group for each analysis interval (Section 5.2.1).
- Bar charts with the proportions of participants for each variable within the specified interval will be plotted for each group. The bars may be divided into relatedness categories to highlight the proportions of participants with related events.
- The main analysis will be based on AEs and will exclude the reactogenicity events that were reported in the AE CRF (except for SAEs) (Section 3.1.1.3).

6.1.2.2. Supplementary Analysis

To support the assessment of AEs, the endpoints below, as specified in Section 3.1.1.3, will be summarized with the same analysis population using the same presentation as specified in the main analysis:

- Immediate AEs after vaccination
- Related AEs reported within 1 month after vaccination
- Severe AEs reported within 1 month after vaccination
- Life-threatening AEs reported within 1 month after vaccination
- AEs leading to study withdrawal after vaccination
- AESIs reported throughout the study

6.1.2.3. Sensitivity Analysis of AEs

In addition, a sensitivity analysis will be conducted using all AEs (not excluding the reactogenicity events reported in the AE CRF during the e-diary collection period).

6.2. Secondary Endpoint(s)

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

NTs will be summarized for each of the 6 age/risk/dose-level groups (Section 2.2.2.1.1).

6.2.1.1. Main Analysis

- Estimand strategy: Hypothetical approach (Section 2.2.2.1.1).
- Intercurrent events and missing data: All data collected after or at intercurrent events will not be included; missing data will not be imputed.
- Analysis set: Evaluable immunogenicity population (Section 4).
- Analysis timing: At primary analyses (when all primary endpoint data are available). The data will be summarized after primary analysis with all time points presented.
- Analysis methodology: 95% CIs of GMTs and GMFRs (Section 5.2.2).
- Descriptive statistics, including sample size (n), RSV A NT, RSV B NT, and RSV A/B NT GMTs and GMFRs, and 95% CIs for GMTs and GMFRs at each applicable visit, will be presented for each vaccine group (Section 5.2.2).
- RCDCs for RSV A and RSV B will be plotted 1 month after RSV vaccination, by vaccine group.

6.2.1.2. Supplementary Analysis

To support the assessment of immunogenicity, estimands as specified in Section 2.2.3 using the treatment policy strategy will be summarized with the mITT immunogenicity population using the same presentation as specified in the main analysis, without the RCDCs.

6.2.2. PBMCs

6.2.2.1. Main Analysis

- Estimand strategy: Hypothetical approach (Section 2.2.2.1.2).
- Intercurrent events and missing data: All data collected after or at intercurrent events will not be included; missing data will not be imputed.
- Analysis set: Evaluable immunogenicity population (Section 4).

- Analysis timing: At primary analyses if data are available at that time. If data are available after primary analysis, data will be summarized after primary analysis.

- Descriptive statistics, including sample size (n), mean (standard deviation), median, Q1, Q3, range of RSV F antigen–specific CD4+ T cells secreting IFNγ, and RSV F antigen–specific CD4+ T cells secreting IL-4 at each applicable visit, will be presented for each group (Section 5.2.2).

- Descriptive statistics, including sample size (n), mean (standard deviation), median, Q1, Q3, range of RSV F antigen–specific CD4+ T cells secreting IFNγ, and RSV F antigen–specific CD4+ T cells secreting IL-4 fold rise at each applicable visit, will be presented for each group (Section 5.2.2).

6.3. Other Endpoint(s)

6.3.1. RSV NT Seroresponse

Descriptive statistics, including the proportion (%) of participants with RSV A NT seroresponse and RSV B NT seroresponse at 1 month after vaccination, the numerator (n) and the denominator (N) used in the proportion calculation, and the 95% CI for percentage using the Clopper-Pearson method, will be presented in the evaluable immunogenicity population.

6.4. Subset Analyses

Not applicable.

6.5. Baseline and Other Summaries and Analyses

Data will be summarized for each of the 6 age/risk/dose-level groups.

6.5.1. Baseline Summaries

For each group, descriptive summary statistics for demographic characteristics (age at vaccination, sex, race, and ethnicity) and baseline information (weight, height) will be generated, as well as for all participants in total, based on the safety population. The proportions of participants prespecified as being at risk (Section 3.4.2) will also be included.

Summary data will also be presented for the evaluable immunogenicity population.

Participant data listings for demography and baseline characteristics will also be generated.

6.5.2. Study Conduct and Participant Disposition

The number and proportion of randomized participants will be included in the participant disposition summary. In addition, vaccinated participants who completed the 6-month follow-up visit, and who withdrew before the follow-up visit, along with the reasons for withdrawal, will be tabulated by group and for all participants. The reasons for withdrawal will be those as specified in the database.

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

Participant study compliance such as e-diary completion, major protocol deviation, and blood draw within the protocol/analysis specified window will all be summarized.

Standard listings will all be generated, including, but not limited to, participants who withdrew during the study, participants excluded from analysis populations, and participants with major protocol violations.

6.5.3. Nonstudy Vaccines

Nonstudy vaccines recorded after signing the ICD through the end of the study will be categorized according to the WHODrug Dictionary and may be summarized by vaccine group and for all participants included in the safety population.

A listing may be used to replace the table.

6.6. Safety Summaries and Analyses

6.6.1. Adverse Events

For all of the AEs categorized in Section 3.1.1.3, each individual AE will be categorized by MedDRA and descriptively summarized by vaccine group.

For Tier 1 events, 2-sided 95% CIs for the events will be provided.

7. INTERIM ANALYSES

No interim analysis is planned.

7.1. Introduction

7.1.1. Analysis Timing

As this study is open-label, the sponsor may conduct reviews of the data during the course of the study for the purpose of decision making.

8. REFERENCES

1 Newcombe RG. Two-sided intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17(8):857-72.

2 Scott JA, Hsu H. Missing data issues at the FDA Center for Biologics Evaluation and Research. J Biopharm Stat. 2011;21(2):196-201.

9. APPENDICES

Appendix 1. List of Abbreviations

Abbreviation	Term
AE	adverse event
AESI	adverse event of special interest
BLQ	below the limit of quantitation
CAETeLiSt	Custom Adverse Event Term List System
CI	confidence interval
CRF	case report form
e-diary	electronic diary
FDA	Food and Drug Administration (United States)
GMC	geometric mean concentration
GMFR	geometric mean fold rise
GMT	geometric mean titer
ICD	informed consent document
IFNγ	interferon gamma
IL-4	interleukin 4
IRT	interactive response technology
LLOQ	lower limit of quantitation
MCAR	missing completely at random
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
N/A	not applicable
NDCMC	newly diagnosed chronic medical condition
NT	neutralizing titer
PBMC	peripheral blood mononuclear cell
PRR	patient-reported reactogenicity
PT	preferred term
Q1, Q3	first quartile, third quartile
QNS	quantity not sufficient
RCDC	reverse cumulative distribution curve
RSV	respiratory syncytial virus
RSV A	respiratory syncytial virus subgroup A
RSV B	respiratory syncytial virus subgroup B

Abbreviation	Term
RSVpreF	respiratory syncytial virus stabilized prefusion F subunit vaccine
SAE	serious adverse event
SAP	statistical analysis plan
SOC	system organ class
WHODrug Dictionary	World Health Organization Drug Dictionary

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