Protocol C4801001

A PHASE 1, RANDOMIZED, OPEN-LABEL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF PNEUMOCOCCAL CONJUGATE FORMULATIONS IN HEALTHY ADULTS 18 THROUGH 49 YEARS OF AGE

Statistical Analysis Plan (SAP)

Version: 2

Date: 05 Dec 2022

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[redacted]

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APPENDICES

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1/ 18 Aug 2022	Original protocol 26 May 2022	N/A	N/A
2/ 05 Dec 2022	Original protocol 26 May 2022	[redacted]	[redacted]

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study C4801001. 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 are no modifications to the analysis plan described in the protocol.

2.2. Study Objectives, Endpoints, and Estimands

The estimands corresponding to each primary, secondary, [redacted] objective are described in Table 2. The estimands to evaluate the immunogenicity objectives are based on evaluable populations (see Section 4 for definition). These estimands estimate the study intervention effect in the hypothetical setting where participants follow the study schedules and protocol requirements as directed. Missing serology results will not be imputed.

Table 2. List of Primary, Secondary, and Exploratory Objectives, Endpoints, and Estimands

Objectives	Endpoints	Estimands
Primary:	**Primary:**	**Primary:**
To describe the safety profile of [redacted] candidates in adults 18 through 49 years of age	• Prompted local reactions (redness, swelling, and pain at the injection site) • Prompted systemic events (fever, headache, fatigue, muscle pain, and joint pain) • AEs • SAEs	In participants receiving the study intervention from each group, the percentage of participants reporting: • Local reactions within 7 days after administration • Systemic events within 7 days after administration • AEs within 1 month after administration • SAEs within 1 month after administration
Secondary:	**Secondary:**	**Secondary:**
To describe the immune responses elicited by the [redacted] candidates	• OPA titers [redacted]	In participants complying with the key protocol criteria (evaluable participants) from each group: • [redacted] OPA GMTs 1 month after administration
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

2.3. Study Design

C4801001 is a Phase 1, multicenter, randomized, open-label, active-control study designed to evaluate the safety and immunogenicity of different [redacted] candidates that include [redacted] This study will be conducted at investigator sites in the US. Approximately 405 adults 18 through 49 years of age with no history of pneumococcal administration will be enrolled. Participants will be enrolled and randomized equally to 1 of 9 groups (45 participants per group): 6 groups will receive 1 of 6 different [redacted] candidates [redacted] see Table 3).

The key control group will receive polysaccharide conjugate for [redacted] using the same [redacted] as contained in 13vPnC (Prevnar 13®) and 20vPnC (Prevnar 20®). In addition, PCV15 (Vaxneuvance [Merck]) and 13vPnC are both licensed in the US for use in adults and will be included as supplemental controls.

[redacted] Blood will be taken at Visit 1 prior to administration and Visit 2 approximately 1 month after administration regardless of investigational site.

The study will use site-based randomization and [redacted]

Table 3. Study Intervention Groups

Group	Carrier Protein	Chemistry	Polysaccharide Dose	Dose Volume	N
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	45
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	45
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	45
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	45
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	45
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	45
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	45

Table 3. Study Intervention Groups

Group	Carrier Protein	Chemistry	Polysaccharide Dose	Dose Volume	N
PCV15 (supplemental control)	N/A	N/A	[redacted]	0.5 mL	45
13vPnC (supplemental control)	CRM_{197}	RAC	[redacted]	0.5 mL	45

Abbreviations: CRM_{197} = cross-reactive material 197; RAC = reductive amination chemistry; [redacted]

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

3.1.1. Safety Endpoints

3.1.2. Primary Safety Endpoints

- Local reactions (redness, swelling, and pain at the injection site) within 7 days after administration
- Systemic events (fever, headache, fatigue, muscle pain, and joint pain) within 7 days after administration
- AEs within 1 month after administration
- SAEs within 1 month after administration

3.1.2.1. Local Reactions and Systemic Events

The local reactions, including redness, swelling, and pain at the injection site, and the systemic events, including fever, fatigue (tiredness), headache, muscle pain, and joint pain, are reported in the e-diary from Day 1 through Day 7 after administration, where Day 1 is the day of administration. The e-diary entries from the participant will be the primary data source for these events. However, any events related to administration that are considered local reactions or systemic events starting within 7 days after administration, that happen to be entered on the AE CRF, will be consolidated with e-diary data, and included in the reactogenicity summary. This section describes derivations with details for the assessment of the reactogenicity data: severity level, duration, and onset day.

Severity and Maximum Severity

The definitions for reactogenicity severity collected from the e-diary and from AEs are described in Appendix 2.

For each local reaction or systemic event after administration, the maximum severity grade will be derived for the collection period (Day 1 through Day 7, where Day 1 is the day of administration) as follows:

> maximum severity grade = highest grade (maximum severity) within 7 days after administration (Day 1 through Day 7) among severity grades reported for that local reaction or systemic event

Temperatures recorded in degrees Fahrenheit will be programmatically converted to degrees Celsius first for reporting. Fever will be grouped into ranges for the analysis according to Table 4. Maximum temperature range over the period from Day 1 through Day 7 will be mapped into the ranges described in Table 4 for summary of maximum temperature. For fever reported on the AE CRF within 7 days after administration, mild fever will be grouped in the ≥38.0°C to 38.4°C range, moderate fever will be grouped in the >38.4°C to 38.9°C range, and severe fever will be grouped in the >38.9°C to 40.0°C range (unless specifically noted to be >40.0°C in an unplanned visit contact or noted on the AE CRF).

Table 4. Ranges for Fever

≥38.0°C to 38.4°C
>38.4°C to 38.9°C
>38.9°C to 40.0°C[a]
>40.0°C[a]

Note: Fever is defined as temperature ≥38.0°C based on e-diary data or indicated on the AE CRF within 7 days after administration.

a. Participants reporting a fever >102.0°F (>38.9°C) will be prompted to contact the study site.

[redacted]

[redacted]

[redacted]

[redacted]

3.1.2.2. Adverse Events

AEs will be categorized according to MedDRA terms. AEs will be assessed from the time of informed consent signing through 1 month after study intervention administration.

AEs will be summarized by SOC and PT on a participant level.

The primary endpoints will be supported by summaries and listings of related AEs, severe AEs, and immediate AEs (within the first 30 minutes after administration).

AE reporting will be based on the specific reporting period. Standard algorithms for handling missing AE dates will be applied as described in the Pfizer Vaccine data standard rules as described in Section 5.3.

3.1.2.3. Serious Adverse Events

SAEs will be categorized according to MedDRA terms. SAEs will be collected from the signing of the ICD through the end of the study (ie, 1 month after study intervention administration).

The safety endpoint "SAEs from administration through 1 month after administration" will be summarized by SOC and PT on the participant level.

3.2. Secondary Endpoint

3.2.1. Secondary Immunogenicity Endpoint

[redacted] OPA GMT 1 month after study intervention administration

3.2.1.1. OPA Titers

OPA titers for [redacted] will be determined for all participants prior to study intervention administration at Visit 1 and at Visit 2 (approximately 1 month after Visit 1).

OPA titers above the LLOQ are considered accurate and their quantitated values will be reported. OPA 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.

[redacted]

[redacted]

3.4. Baseline and Other Variables

Measurements or samples collected prior to the study intervention administration at Visit 1 are considered the baseline data for the assessments.

3.4.1. Demographics, Medical History, and Physical Examination

The demographic variables are age at the study intervention administration (in years), sex (male or female), BMI, race (Black/African American, American Indian, or Alaska Native, Asian, Native Hawaiian or other Pacific Islander, White), and ethnicity (Hispanic/Latino, non-Hispanic/non-Latino, not reported). In cases where more than 1 category is selected for race, the participant would be counted under the category "multiracial" for analysis.

Age at the time of administration of study intervention (in years) will be derived based on the participant's birthday. For example, if the administration day is 1 day before the participant's 19th birthday, the participant is considered to be 18 years old. For participants who were randomized but not administered a study intervention, the randomization date will be used in place of the date of administration for the age calculation. If the randomization date is also missing, then the informed consent date will be used for the age calculation.

Medical history will be categorized according to MedDRA.

3.4.2. Concomitant Vaccines and Concomitant Medications

The name and date of administration for all concomitant nonstudy vaccinations received and the concomitant medications used to treat SAEs from the time of signing of the informed consent through Visit 2 will be recorded on the CRF. Concomitant vaccines and concomitant medications will be coded using the WHO Drug Dictionary.

3.5. Safety Endpoints

AEs and SAEs are described above in the Primary Safety Endpoints section (Section 3.1.2).

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Participant Analysis Set	Description
Enrolled	All participants who sign the ICD.
Randomized	All participants who are assigned a randomization number in the IRT system.
Evaluable immunogenicity	Any participants who: 1. are eligible and randomized, 2. receive the study intervention to which they are randomized, 3. have at least 1 valid OPA titer or IgG concentration within 27 to 49 days, inclusive, after administration, and 4. have no other major protocol deviations as determined by the clinician. The evaluable immunogenicity population will be the primary analysis population for OPA titer and IgG concentration [redacted]

Participant Analysis Set	Description
[redacted]	[redacted]
[redacted]	[redacted]
Safety	All participants who receive the study intervention.

A major protocol deviation is a protocol deviation that, in the opinion of the sponsor's clinician, would materially affect assessment of immunogenicity, eg, participant 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 clinician will identify those participants with major protocol deviations before any unblinded analysis.

5. GENERAL METHODOLOGY AND CONVENTIONS

Methodology for summary and statistical analyses of the data collected in this study is described here.

In general, the study data will be summarized by study intervention group. CIs for all endpoints in the statistical analysis will be presented as 2-sided at the 95% level.

For all the OPA/IgG immunogenicity endpoints, the analysis will be primarily based on the evaluable immunogenicity population. [redacted]

Participants will be summarized according to the study intervention group to which they were randomized. Missing laboratory results will not be imputed.

[redacted]

The safety analyses are based on the safety population. Participants will be summarized according to the study interventions they received.

5.1. Hypotheses and Decision Rules

5.1.1. Estimands

The estimands corresponding to each primary, secondary, [redacted] objective are described in Table 2 in Section 2.2.

The estimands to evaluate the immunogenicity objectives are based on the evaluable immunogenicity population (Section 4). These estimands estimate the study intervention effect in the hypothetical setting where participants follow the study schedules and protocol requirements as directed. [redacted] Missing immunogenicity results will not be imputed.

5.1.2. Statistical Hypotheses

This is a Phase 1 study, and all the analyses are descriptive. No hypothesis tests are planned for this study.

5.2. General Methods

5.2.1. Analyses for Binary Endpoints

Descriptive statistics for categorical variables (eg, proportions) are the percentage (%), the numerator (n) and the denominator (N) used in the percentage calculation, and the 95% CIs where applicable.

The exact 95% CI for binary endpoints for each group will be computed using the F distribution (Clopper-Pearson method).[1]

5.2.2. Analyses for Continuous Endpoints

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

5.2.2.1. Geometric Means

For immunogenicity results of OPA titers and IgG concentrations, the GMs will be computed along with associated 95% CIs. The GM and the 95% CI will be calculated as the mean and the CI of the assay results on the natural logarithm scale and then exponentiating the results. Two-sided 95% CIs will be calculated based on the t distribution.

[redacted]

[redacted]

5.3. Methods to Manage Missing Data

A partial AE start date (missing day or missing both month and day) will be imputed by assigning the earliest possible start date using all available information, such as the stop date of the AE and the administration date(s) from the same participant, following the Pfizer standard of handling incomplete AE start dates. A complete missing start date for an AE is not allowed in the data collection.

The LLOQ for each assay will be provided by Vaccine Research and Development as part of the electronic data transfer or within the Clinical Testing Completion Memo prior to statistical analysis. Assay results above the LLOQ will be reported, and values below the LLOQ, denoted as BLQ, will be imputed as 0.5 × LLOQ for analysis.

No additional imputation will be applied to other missing data.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

6.1.1. Local Reactions and Systemic Events

6.1.1.1. Main Analysis

- Estimands:
    - The percentage of participants reporting local reactions (redness, swelling, and pain at the injection site) within 7 days after administration (Section 2.2).
    - The percentage of participants reporting systemic events (fever, headache, fatigue, muscle pain, and joint pain) within 7 days after administration (Section 2.2).
- Analysis set: Safety population (Section 4).
- Analysis time point: Within 7 days after administration.
- Analysis methodology: For percentages of each group, the 2-sided Clopper-Pearson CIs will be calculated (Section 5.2.1).
- Reporting results: Count and percentage of participants with the indicated endpoint and the associated 2-sided 95% CI for each and any local reaction or systemic event after administration in each group will be provided by maximum severity across severity levels.

[redacted]

Figures:

Bar charts with the proportions of participants for each local reaction or systemic event on each day (Day 1 through Day 7) and any day (Day 1 through Day 7) will be plotted for each group, with different patterns displayed in the bar charts for different severity levels (each day) and different maximum severity levels (any day), respectively.

6.1.2. Adverse Events

6.1.2.1. Main Analysis

- Estimand: The percentage of participants reporting AEs from study intervention administration through 1 month after administration (Section 2.2).
- Analysis set: Safety population (Section 4).
- Analysis time point: From the study intervention administration through 1 month after administration.
- Analysis methodology: Descriptive statistics (Section 5.2.1).
- Reporting results: AEs will be categorized according to MedDRA terms. Descriptive summary statistics (counts, percentages, and associated Clopper-Pearson 95% CIs) will be provided for any AEs, by each SOC and each PT within the SOC for each group.

6.1.2.2. Supplementary Analyses

Related AEs, severe AEs, and immediate AEs (within the first 30 minutes after administration) will also be summarized for each group. Any AEs reported after informed consent signing and prior to the study intervention administration will not be included in the analyses but will be listed.

6.1.3. Serious Adverse Events

6.1.3.1. Main Analysis

- Estimand: The percentage of participants reporting SAEs from administration through 1 month after administration (Section 2.2).
- Analysis set: Safety population (Section 4).
- Analysis time point: From administration through 1 month after administration.
- Analysis methodology: Descriptive statistics (Section 5.2.1).
- Reporting results: SAEs will be categorized according to MedDRA terms. Counts, percentages, and the associated Clopper-Pearson 95% CIs of SAEs will be provided for each group.

6.2. Secondary Endpoint

6.2.1. [redacted] OPA Titers 1 Month After Administration

6.2.1.1. Main Analysis

- Estimand: [redacted] OPA GMT 1 month after administration (Section 2.2).
- Analysis set: Evaluable immunogenicity population and mITT population (as appropriate) (Section 4).
- Analysis time point: 1 Month after administration.
- Analysis methodology: GMT and the 2-sided 95% CI from each group will be derived based on the t distribution (Section 5.2.2.1).
- Reporting results: The GMT and 95% CI for [redacted] OPA will be provided for each group.

[redacted]

[redacted]

[redacted]

[redacted]

6.4. Baseline and Other Summaries and Analyses

6.4.1. Baseline Summaries

6.4.1.1. Demographic Characteristics

Demographic characteristics, including age, sex, race, and ethnicity, will be summarized for the safety population for each group and overall.

6.4.1.2. Medical History

Each reported medical history term will be mapped to an SOC and PT according to MedDRA. The number and percentage of participants having at least 1 diagnosis, overall, and at each SOC and PT level, will be summarized by group for the overall safety population.

6.4.2. Study Conduct and Participant Disposition

6.4.2.1. Participant Disposition

The number and percentage of randomized participants will be included in the participant disposition summary. In addition, the numbers and percentages of participants who received the study administration, who completed the follow-up visits, and who withdrew from the study, along with the reasons for withdrawal, will be tabulated by group (according to randomized group assignment). The reasons for withdrawal will be those specified in the database.

Participants excluded from each analysis population will also be summarized separately, along with the reasons for exclusion, by group.

6.4.3. Study Administration Exposure

6.4.3.1. Administration Timing and Administration

The number and percentage of participants randomized and receiving the study intervention will be tabulated for each group and overall, for all randomized participants. The denominator for the calculation of percentages is the total number of randomized participants in the given group or overall.

6.4.4. Concomitant Vaccinations and Concomitant Medications

Each concomitant vaccine will be summarized according to the ATC fourth-level classification. The number and percentage of participants receiving each concomitant vaccine after the study intervention will be tabulated by group. The safety population will be used. Concomitant medications will be summarized in a similar way as concomitant vaccines.

6.5. Safety Summaries and Analyses

AE and SAE summaries and analyses are described in the Primary Endpoints section (Section 6.1.2 and Section 6.1.3).

7. INTERIM ANALYSES

No interim analysis is planned in this study.

7.1. Introduction

Not applicable.

7.2. Analysis Timings

Statistical analyses will be carried out as specified below:

- An analysis will be conducted when safety data through approximately 1 month after study intervention administration are available.
- Immunogenicity data of OPA and IgG results through Visit 2 (1 month after study intervention administration) may be analyzed, when available, for 1 or more immunogenicity endpoints [redacted]
- Final analysis of safety and immunogenicity will be conducted when complete OPA and IgG results become available after the completion of the study. [redacted]

This is an open-label Phase 1 exploratory study. Immunogenicity data reviews by the sponsor may be conducted at any time the data are available to aid in decision-making for the sponsor programs.

Certain analyses may be combined if the data become available around the same time. Additional analyses may be conducted or combined if required for regulatory purposes or for further clinical evaluation of the study intervention.

8. REFERENCES

1. Collett D. Statistical inference for binary data. Chapter 2. In: Modelling binary data. 1st ed. London, England: Chapman & Hall; 1991:17-42.

9. APPENDICES

Appendix 1. List of Abbreviations

Abbreviation	Term
13vPnC	13-valent pneumococcal conjugate vaccine
20vPnC	20-valent pneumococcal conjugate vaccine
AE	adverse event
ATC	Anatomic Therapeutic Chemical (classification system)
BLQ	below the limit of quantitation
BMI	body mass index
[redacted]	[redacted]
CI	confidence interval
CRF	case report form
CRM_{197}	cross-reactive material 197 (nontoxic variant of diphtheria toxin)
e-diary	electronic diary
ELISpot	enzyme-linked immunosorbent spot
GM	geometric mean
GMC	geometric mean concentration
GMFR	geometric mean fold rise
GMT	geometric mean titer
ICD	informed consent document
IgG	immunoglobulin G
IRT	interactive response technology
LLOQ	lower limit of quantitation
LOD	limit of detection
MedDRA	Medical Dictionary for Regulatory Activities
[redacted]	[redacted]
[redacted]	[redacted]

Abbreviation	Term
[redacted]	[redacted]
[redacted]	[redacted]
N/A	not applicable
OPA	opsonophagocytic activity
[redacted]	[redacted]
PCV15	15-valent pneumococcal conjugate vaccine
PT	preferred term
RAC	reductive amination chemistry
[redacted]	[redacted]
SAE	serious adverse event
SAP	statistical analysis plan
[redacted]	[redacted]
SOC	system organ class
US	United States
WHO	World Health Organization

Appendix 2. Reactogenicity Data Consolidation

For reactogenicity collected in the e-diary, redness and swelling will be measured and recorded in measuring device (caliper) units, and then categorized during analysis as mild, moderate, or severe based on the grading scale in Table 5. Measuring device units can be converted to centimeters according to the following scale: 1 measuring device unit = 0.5 cm. Pain at the injection site will be assessed by the participant as mild, moderate, or severe according to the grading scale in Table 5. The systemic events of fatigue (tiredness), headache, muscle pain, and joint pain will be assessed by participants as mild, moderate, or severe according to the grading scale in Table 6. Grade 4 reactions and events, which can only be classified by an investigator, will be collected as an AE on the CRF. For reactogenicity collected in the AE CRF, the grading scales will be based on the AE intensity scale in Table 7. If a local reaction or systemic event is captured in more than 1 data source, eg, e-diary, unplanned assessments, and/or AE CRF, the highest grade will be used in the safety summary analysis.

Table 5. Grading Scales for Local Reactions Collected From the E-Diary

Local Reaction	Mild Grade 1	Moderate Grade 2	Severe Grade 3[a]	Grade 4[b]
Redness	5 to 10 measuring device units = >2.0 to 5.0 cm	11 to 20 measuring device units = >5.0 to 10.0 cm	>20 measuring device units = >10.0 cm	Necrosis or exfoliative dermatitis
Swelling	5 to 10 measuring device units = >2.0 to 5.0 cm	11 to 20 measuring device units = >5.0 to 10.0 cm	>20 measuring device units = >10.0 cm	Necrosis
Pain at injection site	Does not interfere with activity	Interferes with activity	Prevents daily activity[c]	Emergency room visit or hospitalization for severe injection site pain

Abbreviation: CRF = case report form.

Note: If the size of the redness and/or swelling falls between 2 measuring device units, the higher measuring device unit number will be recorded in the e-diary.

a. Participants experiencing Grade 3 local reactions are required to contact the investigator site. In the event that the participant does not call, the investigator will call the participant.
b. Grade 4 assessment should be made by the investigator; Grade 4 local reactions will not be collected in the e-diary but will be collected as AEs on the CRF. The intensity of the local reaction should be graded using the AE intensity grading scale.
c. Prevents daily activity, eg, results in missed days of school or work or is otherwise incapacitating.

Table 6. Grading Scales for Systemic Events Collected From the E-Diary

	Mild Grade 1	Moderate Grade 2	Severe Grade 3[a]	Grade 4[b]
Fatigue (tiredness)	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
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

Abbreviation: CRF = case report form.

a. Prevents daily routine activity, eg, results in missed days of school or work or is otherwise incapacitating; includes use of narcotics for analgesia.
b. Grade 4 assessment should be made by the investigator; Grade 4 systemic events will not be collected in the e-diary but will be collected as AEs on the CRF. The intensity of the systemic event should be graded using the AE intensity grading scale.

Table 7. Assessment of AE Intensity Grade

Grade	If required on the AE page of the CRF, the investigator will use the adjectives MILD, MODERATE, and SEVERE to describe the maximum intensity of the AE. For purposes of consistency, these intensity grades are defined as follows:	
1	MILD	Does not interfere with participant's usual function.
2	MODERATE	Interferes to some extent with participant's usual function.
3	SEVERE	Interferes significantly with participant's usual function.

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Signed By:	Date(GMT)	Signing Capacity
PPD		