Protocol VAX31-101

A Phase 1/2, Randomized, Observer-Blind, Dose-Finding, Active-Controlled, Parallel-Group, Clinical Study to Evaluate the Safety, Tolerability, and Immunogenicity of a 31-Valent Pneumococcal Conjugate Vaccine (VAX-31) in Healthy Adults Aged 50 Years and Older

Protocol Number: **(Version Date)**	VAX31-101 v2.0 (Amendment 1) / 08-DEC-2023
Name of Test Drug:	VAX-31 (31-valent Pneumococcal Conjugate Vaccine)
Phase:	1 / 2
Methodology:	Randomized, Observer-Blind, Dose-Finding, Active-Controlled, Parallel-Group
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Sponsor Representative:	[redacted]
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SIGNATURE PAGE

Protocol Title:	A Phase 1/2, Randomized, Observer-Blind, Dose-Finding, Active-Controlled, Parallel-Group, Clinical Study to Evaluate the Safety, Tolerability, and Immunogenicity of a 31-Valent Pneumococcal Conjugate Vaccine (VAX-31) in Healthy Adults Aged 50 Years and Older
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Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned analyses described herein. I agree that the planned analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the methodology described in the protocol, clinical development plan, and all applicable regulatory guidance's and guidelines.

I have discussed any questions I have regarding the contents of this document with the author.

I also understand that any subsequent changes to the planned analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report (CSR).

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[redacted]

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LIST OF IN-TEXT TABLES

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ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BLQ	Below limit of quantification
CI	Confidence Interval
CBER	Center for Biologics Evaluation and Research
CRF	Case Report Form
CSR	Clinical Study Report
DBL	Database Lock
DMC	Data Monitoring Committee
EXP	Exposed Population
GMC	Geometric Mean Concentration
GMT	Geometric Mean Titer
GMFR	Geometric Mean Fold Rise
ICH	International Conference on Harmonisation
iDBL	Interim Data Base Lock
IEP	Immunogenicity Evaluable Population
IgG	Immunoglobulin G
IP	Investigational Product
LLOQ	Lower Limit Of Quantification
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary of Regulatory Activities
NOCI	New Onset of Chronic Illnesses
OPA	Opsonophagocytic antibody
PCV20	20-valent pneumococcal conjugate vaccine (Prevnar 20®)
PD	Protocol Deviation
PT	Preferred Term
RND	Randomized Population
SAE	Serious Adverse Event
SAF	Safety Population
SAP	Statistical Analysis Plan
SI	International System of Units
SOC	System organ class

TEAE	Treatment Emergent Adverse Event
TLFs	Tables, listings and figures
ULOQ	Upper Limit of Quantification
VAX-31	31-valent investigational pneumococcal conjugate vaccine
WHO	World Health Organization

1. INTRODUCTION AND OBJECTIVES OF ANALYSIS

1.1. Introduction

This statistical analysis plan (SAP) is designed to outline the methods to be used in the analysis of study data to answer the study objective(s). Analysis sets, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the CSR for this trial.

Analyses planned for the Data Monitoring Committee (DMC) meeting at the end of Stage 1 were described in the separate document "VAX31-101 DMC Charter v1.0" dated 31-OCT-2023.

1.2. Objectives

The primary objectives are:

- To evaluate the safety and tolerability of a single injection of VAX-31 at 3 dose levels administered to healthy adults aged 50 years and older.
- To compare the safety of VAX-31 to that of Prevnar 20® (PCV20) administered to 4 age groups:
    - Subjects 50 years of age and older (i.e. Overall)
    - Subjects 50 to 59 years of age
    - Subjects 60 years of age and older
    - Subjects 65 years of age and older

The secondary objectives are:

- Safety: To assess laboratory value abnormalities and/or potentially clinically significant laboratory values following the administration of VAX-31 at 3 dose levels compared to control groups receiving PCV20 for subjects 50 years and older.
- Immunogenicity: To assess the induction of antibody responses to the 20 serotypes in common in PCV20 and VAX-31, as well as to the additional 11 non-PCV20 serotypes in VAX-31 for the following subpopulations:
    - Subjects 50 years of age and older (i.e. Overall)
    - Subjects 50 to 59 years of age
    - Subjects 60 years of age and older
    - Subjects 65 years of age and older

The exploratory objective is to further characterize the immune response for each treatment group.

2. STUDY DESIGN

2.1. Introduction

This Phase 1/2 parallel-group, randomized, 2-stage observer-blind study is to be conducted in 3 populations of healthy adults, aged 50 to 59, 60 to 64, and 65 years and older. Subjects will be randomly assigned in a 1:1:1:1 ratio to receive either VAX-31 at 1 of 3 dose levels or a single dose of PCV20, the active comparator.

In Stage 1 of the study, 64 subjects (16 for each VAX-31 dose group and 16 for the comparator group) 50 to 64 years of age will be enrolled initially. If the DMC agrees that the study may continue after reviewing data from Stage 1, approximately 736 additional adults aged 50 to 64 years as well as a minimum of 200 adults aged 65 years and older will be concurrently enrolled in Stage 2. Subjects will have screening procedures (physical examination, vital signs, urine pregnancy test for females of childbearing potential), and if eligible, will be enrolled into the study and receive VAX-31 or PCV20 on Day 1. All subjects will have a urine sample collected as well as blood samples drawn for immunogenicity analysis (OPA and IgG) and safety laboratory analysis at Day 1 (before vaccination) and Month 1 (30 days after vaccination). Solicited Adverse Events (AEs) will be collected for 7 days after vaccination and unsolicited safety information for 30 days after vaccination, with Serious Adverse Events (SAEs), New Onset of Chronic Illnesses (NOCIs), and Medically Attended Adverse Events (MAAEs) collected up to 6 months after vaccination.

A defined safety review of data (solicited and unsolicited AE and SAE) through 7 days after vaccination of the 64 subjects in Stage 1 will be conducted by an independent Data Monitoring Committee (DMC) before proceeding with Stage 2 of the study.

The schedule of assessments is in Appendix A, further details available in the study protocol.

2.2. Sample Size and Power

For subjects enrolled in Stage 1 of the study, comprising 64 subjects 50 to 64 years of age, sample size is not driven by statistical assumptions for formal hypothesis testing but is based on the safety objective for the study. With 16 subjects, events that occur at a frequency of 15% or more will be detected with at least 92% probability and events that occur at a frequency of 10% or more will be detected with 81% probability. Therefore 16 subjects in each treatment group are proposed as the basis for the number of subjects in whom to conduct the first safety assessment.

For subjects enrolled in Stage 2 of the study, comprising approximately 736 subjects aged 50 to 64 years as well as a minimum of 200 subjects aged 65 years and older, sample size is not based on statistical justification. A total of approximately 800 subjects aged 50 to 64 (with a minimum of 200 subjects aged 60 to 64), as well as a minimum of 200 subjects aged 65 years and older, should provide sufficient information to assess safety and immunogenicity in the older age groups. The sample size will also be adequate to plan future studies.

2.3. **Randomization Methodology**

In Stage 1, a group of 64 subjects are planned to be randomized in a 1:1:1:1 allocation ratio i.e.16 subjects in each VAX-31 dose level group and 16 in the PCV20 group. There will be no stratification factors.

In Stage 2, a total of 736 subjects, with a minimum of 200 subjects aged 60 to 64, along with a minimum of 200 subjects aged 65 years and older, are planned to be randomized in a 1:1:1:1 allocation ratio. The randomization will be stratified by site.

2.4. **Blinding**

This is an observer-blind study in which all participants, Investigators, and study personnel involved in the conduct of the study, including data management, are blinded to treatment assignment except for:

- Unblinded independent statisticians who will prepare and have access to the randomization code
- Unblinded data manager (Randomization and Supply Management)
- Unblinded pharmacist or designee at site who will prepare Investigational Product (IP)
- Unblinded Administrator at site who will dispense IP
- Unblinded Sponsor Clinical Supply Manager
- Unblinded Clinical Research Associate/Study Monitor who will monitor pharmacy
- Unblinded Lead Clinical Research Associate and Clinical Trial Assistant who will review the unblinded Clinical Research Associate/Study Monitor's reports and manage IP supply orders and inventory
- Unblinded Sponsor Quality Assurance Consultant(s)

Although unblinded to treatment assignment, none of the above roles will have access to unblinded summaries of immunogenicity or safety data.

The following personnel will have access to unblinded data, including individual treatment assignment and individual immunogenicity data, in order to discharge their roles for data analysis and review during the planned Interim Analysis the end of Stage 1 and the Month 1 Analysis:

- The unblinded statistical programming team, and independent support statistician who will prepare unblinded reports for DMC meetings
- The unblinded data managers who will receive the unblinded data and prepare reconciliation reports and generate unblinded queries
- The DMC members who will review unblinded data reports

- The unblinded programming team and the unblinded statisticians who will prepare the unblinded report for Month 1 Analysis.
  The blinded programmers and initial blinded statistician might take these roles upon Sponsor approval following a formal unblinding process. In that case, a new blinded statistician will be designed, but no new blinded programmers will be needed. The newly unblinded programmers and newly unblinded statistician will stop interacting with the Sponsor and the rest of the blinded study team until final database lock (DBL).
- Unblinded Sponsor Reviewer Consultant

Other than the above-mentioned personnel, all other individuals involved in the study conduct, statistical analysis and reporting will remain blinded to individual treatment assignments and individual immunogenicity data until official study unblinding at the end of the study.

Sponsor personnel and limited blinded personnel will become treatment-group unblinded and have access to immunogenicity and safety summaries after the interim database lock (iDBL) for the Month 1 Analysis, as described in Section 2.6.

2.5. **Interim Analyses**

A defined safety evaluation will be conducted for the Stage 1 cohort of 64 subjects aged 50 to 64 years, based on the safety data collected through Day 8. The results will be reported to the DMC by treatment group preserving the double-blind status on the subject level, unless the DMC requests unblinded individual treatments. If the interim safety analysis supports and DMC concludes that no safety concerns exist, the study will resume enrollment of approximately 936 subjects aged 50 years and older.
For further information including the tables, listings and figures (TLF) to be provided for the DMC review, please refer to the VAX31-101 DMC Charter and associated DMC Mock TLFs document.

2.6. **Schedule of Analyses**

Statistical analyses described in this SAP will be carried out in two stages:

- Month 1 Analysis:
  An interim database lock will occur after all subjects have completed the Month 1 visit and their data up to Month 1 visit have been cleaned. Primary Safety and Immunogenicity endpoints will be prepared by a team of unblinded programmers with the support of an unblinded statistician using the live randomization and kit lists and the unblinded immunogenicity data. The exact scope and list of outputs for the Month 1 Analysis will be decided prior to the iDBL by the Sponsor and documented in a separate document.
  The results by treatment group will then be available to Sponsor and other study personnel. All blinded personnel, as indicated in Section 2.4, will remain blinded to individual treatment until final database lock. To that effect, no listing of individual data

will be part of the Month 1 Delivery and the remaining outputs will be reviewed first by the Unblinded Sponsor Reviewer to assess potential unblinding. The Unblinded Sponsor Reviewer will redact any results by treatment group that may be unblinding and provide the redacted outputs to the wider blinded team.

- Final Analysis:
  The final DBL will occur when all subjects have completed the study, and all data through the last Month 6 visit have been cleaned. Individual Treatment unblinding will be available to all study personnel after the final DBL. The Final Analysis will include all analyses described in this SAP. A single completed CSR will summarize immunogenicity data and safety findings through Month 6 for all subjects.

3. STUDY ENDPOINTS

3.1. Efficacy Variables

No Efficacy endpoint has been defined for this study.

3.2. Safety Variables

Safety assessments performed during the study included solicited AE up to Day 8 and monitoring of unsolicited adverse events, physical examinations, measurement of vital signs, clinical laboratory evaluations including hematology, serum chemistry, and urinalysis. See Section 6.2.5 for the definition of age group.

The primary endpoints are:

- Percentage of subjects reporting solicited local reactions within 7 days after vaccination (redness, swelling, and pain at injection site) in each age group.
- Percentage of subjects reporting solicited systemic events within 7 days after vaccination (fever, headache, fatigue, muscle pain, and joint pain) in each age group.
- Percentage of subjects reporting unsolicited AE within 1 month after vaccination in each age group.
- Percentage of subjects reporting SAEs within 6 months after vaccination.
- Percentage of subjects reporting NOCIs within 6 months after vaccination.
- Percentage of subjects reporting MAAEs within 6 months after vaccination.

The secondary endpoint related to Safety assessments is:

- Safety: Percentage of participants with laboratory value abnormalities and/or potentially clinically significant laboratory values at 1 month after vaccination.
- Safety: Change from baseline in laboratory parameters at 1 month (30 days) after vaccination

3.3. Immunogenicity Variables

Immunogenicity samples will be collected for all subjects at Day 1, Month 1 and at the Early Discontinuation Visit for subjects discontinuing before Month 1 visit. Immunogenicity parameters to be determined include OPA and IgG assays.

The secondary endpoints related to Immunogenicity assessments are:

- 31 VAX-31 Pneumococcal serotype-specific OPA geometric mean titers (GMT) at 1 month after vaccination.

- 31 VAX-31 Pneumococcal serotype-specific IgG geometric mean concentrations (GMC) at 1 month after vaccination.

The exploratory endpoints are all evaluated at 1 month after vaccination:

- GMFR in serotype-specific OPA
- GMFR in serotype-specific IgG
- Percentage of participants with fold rise ≥ 4 in serotype-specific OPA
- Percentage of participants with fold rise ≥ 4 in serotype-specific IgG
- Geometric mean ratio (GMR) of serotype-specific OPA and IgG
- Proportion achieving an OPA titer of at least the serotype-specific LLOQ of the assay
- Reverse cumulative distribution curves for OPA titers and IgG concentrations

4. ANALYSIS SETS

4.1. Analysis Set Definitions

The following Analysis sets will be evaluated and used for presentation and analysis of the data:

- Screened Population: Includes all screened subjects who provided informed consent and were assigned a study subject number, regardless of whether the subject was randomized or not. This population will be used to account fully for subject disposition, starting with the informed consent. The screened population will not be analyzed as such but will be available in the clinical database.
- Randomized Population (RND): Includes all subjects from the Screened Population who consented, provided demographic and other Baseline Screening measurements, and were randomized. Each subject will be analyzed as randomized.
- Exposed Population (EXP): Includes all subjects from the Screened Population who received at least one study vaccine administration. Each subject will be analyzed as treated.
- Safety Population (SAF): Includes all subjects in the Exposed Population but excluding subjects lost to follow up at Day 1 reporting no solicited or unsolicited AEs. Each subject will be analyzed as treated.
- Immunogenicity Evaluable Population (IEP): Includes all subjects in the Exposed Population who:
    - Had no major protocol deviation that would impact immunogenicity assessment or other reason to be excluded as defined prior to unblinding or analysis.
    - Had not received a prohibited medication or vaccine. Identification of subjects receiving prohibited medications will be done via a medical review from the Sponsor of a listing of all concomitant medications, and PD will be requested to be added by the Investigator if they were not entered yet.
    - Provided evaluable serum sample results for baseline, the relevant post-vaccination time points, and within the required time frames:
        - Baseline: Day 1 or within 30 days before first study vaccine administration
        - Month 1: Day 25 through Day 35, inclusive

  Each subject will be analyzed as treated.

"As randomized" means according to the vaccine regimen to which the subject was randomized, while "as treated" means according to the vaccine regimen a subject received, rather than the vaccine regimen to which the subject may have been randomized.

The RND will be used for the analysis of exposure.
The SAF will be the primary set for the analysis of the safety parameters.
The IEP will be the primary set for the immunogenicity analysis, with the EXP set as supportive.

4.2. **Protocol Deviations**

All deviations from the protocol are documented in the study file. In addition, deviations are reported to the Institutional Review Board as applicable.

Subject-specific deviations are recorded in the subject's source documents. The Sponsor will review all protocol deviations (PDs) on an ongoing basis and will be responsible for categorizing protocol deviations as exclusionary from IEP. At the time of iDBL and Final DBL, [redacted] will download the 'dv' csv file from Zelta including all deviations entered in the system and will send it to the Sponsor. The Sponsor will use this source data to produce a classification Excel file by adding 2 new columns:

- IEPEXCL ("Leads to Exclusion from IEP?"). This column will be filled for each PD with:
  - 'Yes' if the PD leads to exclusion of the subject from the IEP
  - 'No' if the PD does not lead to exclusion of the subject from the IEP
- IEPEXCLREAS ("Reason for leading to exclusion from IEP"). This column will be filled for each row where IEPEXCL=Yes, with some categorical reasons of exclusions. Since prohibited medication/vaccine, immunogenicity samples taken out of window, and other major PDs will be recorded as PD and classified as exclusionary from IEP by the Sponsor, only the variable IEPEXCL will be used by [redacted] to identify subjects excluded from IEP.

The PD classification file will be finalized prior to iDBL for the Month 1 Analysis, and prior to the final DBL for the Final Analysis.

In addition to the PD classification by the Sponsor, out of window immunogenicity samples will be identified programmatically, and corresponding subjects excluded from the IEP, even if there was not an associated exclusionary PD.

All PDs and their classification will be presented in a data listing.

5. DATA HANDLING

5.1. Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software (Version 9.4), unless otherwise noted. Medical History and adverse events will be coded using Medical Dictionary of Regulatory Activities (MedDRA) version 26.1 unless otherwise noted. Concomitant medications will be coded using B3 World Health Organization (WHO) Drug Global (September 2023) unless otherwise noted.

5.2. Data Conventions

The following conventions will be used:

- **Period definition:**
  - **Screening Period:** The period prior to Day 1 visit.
  - **Treatment and Observation:** The period from date of vaccine administration to Month 1 visit (included).
  - **Follow-up:** The period from Month 1 visit (excluded) to Month 6 visit.
- **Visits:**
  - **Study day 1:** The date of study vaccine administration.
  - **End of Treatment Visit:** The Month 1 visit, or the early discontinuation visit for subjects who withdraw study prior to Month 1 visit.
  - **End of Study Visit:** The last recorded visit date.
  - **Unscheduled visits:** Unscheduled visits results will be listed, but not included in tables or graphs. Rules to map unscheduled visit to analysis visit are defined in Section 5.5.
- **Baseline characteristics and change from baseline:**
  - Weight values recorded in pounds will be converted to kilograms using the following formula: kilograms = pounds/2.2046.
  - Height values recorded in inches will be converted to centimeters using the following formula: centimeters = inches*2.54.
  - Duration on study (weeks) = (Last visit date – randomization date + 1) / 7.
  - (Absolute) Change from baseline = Value at the time point – Baseline value.
- **Adverse events:**
  - **Solicited Adverse Events** will be analyzed using the nominal visit (i.e. the page) on which they were recorded and not according to the date they were entered.

- **Solicited Adverse Events continuing beyond 7 days**: Solicited AEs continuing beyond 7 days will be reported in the "Adverse Events (Unsolicited)" page as per Case Report Form (CRF) Completion guidelines and will be identified programmatically as all unsolicited adverse events with answer 'Yes' to the question 'Is this a Prolonged Solicited Adverse Event?'. The corresponding question in the diary page ('Were any of the above symptoms continuing after Day 7?') will not be used as it is expected that both sources will be reconciled before iDBL so that only the unsolicited page can be used for the analysis.

- **Immunogenicity:**
  - OPA results are reported as titer and IgG results are reported as concentration
  - **GMC [or GMT]**: The geometric mean concentration (or titer) = antilog10 (LSMeans [log10 x]), where x is the assay result and LSMeans is the least square means calculated by the model.
  - **GMR**: The geometric mean ratio = antilog10 (LSMeans [log10 x]) / antilog10 (LSMeans [log10 y]), where x and y are the 2 assays results and LSMeans is the least square means calculated by the model.
  - **Fold Ratio**: The ratio between titer/concentration at Month 1 visit and the one at Day 1.
  - **GMFR**: The geometric mean fold ratio = antilog10 (LSMeans [log10 y]), where y is the assay fold ratio and LSMeans is the least square means calculated by the model.
  - **4-fold increase**: A subject achieved 4-fold increase if fold ratio ≥ 4
  - **BLQ**: Below lower limit of quantification (LLOQ). Per Center for Biologics Evaluation and Research (CBER) criterion, BLQ titer/concentration will be analyzed as:
    - 0.5*LLOQ for calculations of GMT/GMC and GMT/GMC Ratios (GMRs)
    - 1*LLOQ for calculations of fold ratio.
  - **ULOQ**: Upper limit of quantification. Titer/Concentration above ULOQ will be analyzed as:
    - 1*ULOQ for all calculations (GMT/GMC, GMRs, fold-ratio)

5.3. **Methods of Pooling Data**

Not Applicable.

5.4. **Withdrawals, Dropouts, Loss to Follow-up**

Subjects who undergo Early Discontinuation after randomization and before Day 1 vaccine administration may be replaced by randomizing an additional subject at the Sponsor's discretion. Subjects who undergo Early Discontinuation after study vaccination will not be replaced.

[redacted]

6. STATISTICAL METHODS

6.1. Sample Size Justification

For subjects enrolled in Stage 1 of the study, comprising 64 subjects 50 to 64 years of age, sample size sample size is not driven by statistical assumptions for formal hypothesis testing but is based on the safety objective for the study. With 16 subjects, events that occur at a frequency of 15% or more will be detected with at least 92% probability and events that occur at a frequency of 10% or more will be detected with 81% probability. Therefore 16 subjects in each treatment group are proposed as the basis for the number of subjects in whom to conduct the first safety assessment.

For subjects enrolled in Stage 2 of the study, comprising approximately 736 subjects aged 50 to 64 years as well as a minimum of 200 subjects aged 65 years and older, sample size is not based on statistical justification. A total of approximately 800 subjects aged 50 to 64 (with a minimum of 200 subjects aged 60 to 64), as well as a minimum of 200 subjects aged 65 years and older, should provide sufficient information to assess safety and immunogenicity in the older age groups. The sample size will also be adequate to plan future studies.

6.2. General Statistical Methods

6.2.1. General Methods

All outputs will be incorporated into Microsoft Word files, sorted, and labeled according to the International Conference on Harmonization (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, safety, and immunogenicity parameters. For categorical variables, summary tabulations of the number and percentage within each category (with the number of subjects with missing data) of the parameter will be presented. For continuous variables, the mean, median, standard deviation, minimum and maximum values will be presented.

Statistical hypothesis testing will be performed on immunogenicity parameters with all tests conducted at the 2-sided, 0.05 level of significance. Summary statistics will be presented, as well as 95% confidence intervals (CI) on selected parameters, as described in the sections below.

6.2.2. Definition of Baseline

For the following endpoints, baseline is defined as the assessment collected the closest to the study vaccine administration on Day 1 or prior to study vaccine administration (up to 30 days before Day 1):

- Laboratory Parameters
- Immunogenicity

Laboratory and immunogenicity results from samples collected on Day 1 after the vaccine injection can still be considered as baseline results.

For the following endpoint, baseline is defined as the last non-missing assessment prior to study vaccine administration:

- Vital signs: Day 1 prior to study vaccine administration (up to 30 days before Day 1)

6.2.3. Adjustments for Covariates

The randomization for Stage 1 will not be stratified.

The randomization for Stage 2 will be stratified by site.

Immunogenicity analyses:

- For the Analysis of variance (ANOVA), study site will be included as fixed effect in the model.
- For the Analysis of covariance (ANCOVA) and the logistic regression, study site will be included as fixed effect and the log baseline titer will be included as covariate in the model.
  The rationale for including baseline values as a covariate is that prior data have shown vaccine response is correlated with baseline antibody levels.

6.2.4. Multiple Comparisons/Multiplicity

Since there are no formal hypothesis tests in this study, no adjustment for multiplicity will be made.

[redacted]

6.2.6. **Missing, Unused, and Spurious Data**

Binary endpoints / Continuous data:

Missing data are assumed to be missing at random and ignorable. Missing data will not be estimated or imputed. Denominators for percentages will be based only on the number of subjects with non-missing values.
The only exception are some derived Day 1 to Day 7 endpoints for solicited AEs. Additional details for handling of missing data in these cases can be found in Section 6.5.1.1.

Dates:

For partial or missing AE start dates the following imputation rules will be applied:

1. If year is not missing and is after the year of first dose:
    a. If month is missing, then month will be imputed as January.
    b. If day is missing, then day will be imputed as the first of the month.
2. If year is not missing and is the same as the year of the first dose:
    a. If month is missing, then impute the month as the month of the first dose date.
    b. If day is missing, and the month is the same as the month of the first dose date, then impute day as the day of the first dose date.
    c. If day is missing but month is after the month of first dose date, then impute day as the first day of the month.
3. If year is missing, then impute the year as the year of the first dose date:
    a. If month is missing, then impute the month as the month of the first dose date.
    b. If day is missing, then impute the day as the day of the first dose date.
4. If the start date is completely missing, but the AE is either ongoing (i.e. AE stop date is missing) or the stop date is after the first dose date then impute the start date as the first dose date.
5. For any cases involving the rules above, if the AE end date is before the AE start date, then do not impute the AE start date and assume that the AE is treatment emergent/concomitant for the purpose of the analysis. Further, if the AE stop date occurs prior to the first dose date, do not impute the AE start date, and assume that the AE is not treatment emergent.

No imputations will be applied to AE stop dates or other dates. As indicated above, AEs with missing stop date are considered ongoing.

6.3. **Study Population**

6.3.1. **Subject Disposition**

Subject disposition will be presented on the Screened Population by treatment group, by age group and overall, including the number screened, the number of screen failures, the number randomized and the number who received study vaccine administration. The study disposition will also be presented along with the reasons for early study withdrawal.
The summary will be repeated on the Exposed Population.

The number of subjects in the RND will be presented on the Randomized Population by treatment group, by age group and overall.
The number of subjects in EXP, SAF and IEP will be presented on the Exposed Population by treatment group, by age group and overall.

The following by-subject listings will be presented.

- Study completion information, including the reason for premature study withdrawal
- Visit dates
- Inclusion in study Analysis Sets
- Reason for exclusion from the IEP
- Protocol deviations

6.3.2. Demographic and Baseline Characteristics

Demographic and baseline characteristics will be presented. Summary will include the following: age, sex, ethnicity, race, height, weight, body mass index.

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and summarized alphabetically by System Organ Class (SOC) and Preferred Term (PT).

Childbearing potential and method of contraception will be presented in Listings only.

Table summaries to be produced by treatment group and overall and repeated for the following:

Populations: RND, SAF, IEP

Subgroups: Overall (50+), 50-59, 60+, 65+

Demographic, baseline characteristics and medical history data will also be provided in data listings on the Randomized Population.

6.3.3. Prior and Concomitant Medication

Prior and concomitant medications will be coded using the WHO Drug dictionary.

Prior medications will be defined as any medications with a start date before the date of study vaccine administration.

Concomitant medications will be defined as any medications with a start date on or after the date of study vaccine administration, as well as medications taken prior to the study vaccine administration and continuing after.

If a medication date or time is missing, or partially missing, and it cannot be determined whether it was taken on or after start of treatment, it will be considered a concomitant medication.

Results will be tabulated by Anatomic Therapeutic Class level 2 and preferred term.

Table summaries to be produced by treatment group and overall and repeated for the following:

Population: SAF

Subgroups: Overall (50+)

Prior and concomitant medications will be included in a by-subject data listings.

6.3.4. **Exposure and Compliance**

Frequency and percentage of subjects with vaccinations will be summarized. For subjects who received a vaccine, the administered vaccine will be summarized.

Table summary to be produced by treatment group and repeated for the following:

Population: RND

Subgroups: Overall (50+), 50-59, 60+, 65+

6.4. **Efficacy Evaluation**

Not Applicable.

6.5. **Safety Evaluations**

All Safety summaries to be produced by treatment group, for the following:

Population: SAF

Subgroups: Overall (50+), 50-59, 60+, 65+

6.5.1. **Adverse Events**

6.5.1.1. Solicited Adverse Events

Solicited AEs are protocol-specified local and systemic symptoms/events that are proactively collected from the subject and evaluated by the Investigator or designee. Solicited AEs are collected for 7 days after each injection, starting on Day 1, within an electronic diary.

Solicited AEs for this study are:

- Local events: Pain, Erythema (redness) at the injection site, Edema (swelling) at the injection site
- Systemic events: Fever (oral temperature ≥ 100.4ºF), Fatigue, Headache, Muscle pain, Joint pain

The Subject and the Investigator will grade all AEs for severity from “mild” (grade 1) to “potentially life-threatening” (grade 4), except for severity of redness and swelling recorded as diameters (cm) and graded accordingly (see Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2007 for details on grading scale).

Only Solicited AEs collected in the eDiary CRF, within 7 days after vaccine administration (i.e. from Day 1 to Day 7) will be analyzed in this Section. Solicited AEs continuing beyond 7 days after injection will be combined with unsolicited AEs and described separately as specified in Section 6.5.1.3.

Occurrence of a solicited AE on a specific Day will be identified by a Grade≥1 for that AE on that day. Subject with no recorded grade on a specific day will be considered missing for that

day in the by-day summaries. For overall summaries (i.e. Solicited AEs within 7 days post-injection), only non-missing grades are considered to derive endpoints, i.e. a subject will be considered missing in these summaries, only if their grade is missing for all 7 days.

The following endpoints will be calculated:

- Maximum severity (Investigator):

  For each solicited AE collected for a subject, the maximum severity will be the highest grade recorded, as per Investigator, for all occurrences of this AE within 7 days of vaccine administration (i.e., from Day 1 post-administration to Day 7) for the overall summary, or within a single day for the summary by day. There will be no replacement in case of missing severity.

- Maximum relationship (Investigator):

  For each unsolicited AE collected for a subject, the maximum relationship will be the strongest relationship, as per Investigator, for all occurrences of this AE. There will be no replacement in case of missing relationship.

- Time of first onset of first event (days):

  Day of onset of the first solicited AE, as per the nominal visit where the first solicited AE was first reported. This will range from 1 (Day 1 = Day of study vaccine administration) to 7 (Day 7).

- Duration of solicited AEs (days):

  Number of days between the onset of the solicited AE (i.e. nominal visit of first occurrence of Grade≥1) and the end of the solicited AE (i.e. nominal visit when Grade is back to 0). If there are different occurrences of a same solicited AE within 7 days, the durations will be added up. For solicited AEs continuing beyond 7 days, the end day of the continuing unsolicited adverse event will be used as the end day for the calculation. If the unsolicited AE is ongoing (i.e. stop date is missing), then duration will be the Number of days between the onset of the solicited AE and the study discontinuation date.
  Example 1: If a subject has a solicited event Pain starting on Day 1 and finishing on Day 4, the duration is 4.
  Example 2: If a subject has a solicited event Fatigue starting on Day 2 and finishing on Day 3. And a new solicited event Fatigue starting on Day 7 and ending on Day 9. The Duration will be 2+3=5.

Frequencies and percentages of subjects experiencing each solicited AE will be presented overall and by severity. For each of the time points or time intervals presented in the summaries, only subjects with at least one observation (ie, any non-missing values but excluding "Not done/unknown") for the solicited AEs will be summarized.

The following summaries of subjects will be performed:

- Solicited AEs within 7 days post-injection, for each event and for any event, overall and by maximum severity. Details by category (local, systemic) will also be presented. (Severity as per Investigator)
- Possibly or probably related solicited AEs within 7 days post-injection, for each event and for any event, by maximum severity (Severity and Relatedness as per Investigator) This summary will only be presented on the Overall (50+) population.
- Not related solicited AEs within 7 days post-injection, for each event and for any event, by maximum severity (Severity and Relatedness as per Investigator) This summary will only be presented on the Overall (50+) population.
- Solicited AEs by day post-injection, for each event and for any event, overall and by maximum severity on that day. (Severity as per Investigator)
- Solicited AEs by day post-injection, for each event and for any event, overall and by maximum severity on that day. (Severity as per Subject)
- Time of first onset of solicited AEs within 7 days post-injection for each event and any event. Duration of solicited AEs, after injection, for each event. Maximum duration of any event. (Severity as per Investigator)

Listings of all solicited AEs will be provided by subject.

6.5.1.2. Unsolicited Adverse Events

An unsolicited AE is an AE that is spontaneously reported by the subject or discovered by the Investigator. SAEs, NOCIs and MAAEs would be recorded as part of the collection of unsolicited AEs as specified in the visit procedures. Unsolicited AEs are collected separately from solicited AEs.

Solicited AEs continuing beyond 7 days will be identified using rules given in Section 5.2. They will not be presented in the summaries of unsolicited Adverse Events, but only in a Listing.

The definition of a treatment emergent AE (TEAE) is an event that occurs after vaccination and within the 30 days after vaccination (i.e., excluding those after a subject has given informed consent, but before vaccination):

- Onset (days) = Start date of AE – Date of Study Vaccine Administration + 1
- An AE is a TEAE if Onset≥1 and Onset≤30 and is not a continuing solicited AE

Adverse events are summarized by subject, therefore, in any tabulation, a subject contributes only once to the count for a given adverse event (SOC or preferred term).

Unsolicited AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by decreasing frequency of SOC and preferred term within SOC in the control group, as follows:

- Unsolicited TEAEs
- Possibly or probably related unsolicited TEAEs
- Unsolicited TEAEs leading to study withdrawal

- SAEs
- Possibly or probably related SAEs
- NOCIs
- Possibly or probably related NOCIs
- MAAEs
- Possibly or probably related MAAEs
- AEs leading to death (only presented on the Safety Population overall)

Following summaries will be presented on the Safety Population overall only by decreasing frequency of PT, with an additional column "VAX-31" combining all subjects vaccinated with any dose of VAX-31:

- Unsolicited TEAEs
- Possibly or probably related unsolicited TEAEs

Following by-subject Listings will be provided:

- Pre-vaccination AEs (non-emergent)
- All unsolicited TEAEs
- All Serious post-vaccination AEs, NOCIs and MAAEs

6.5.1.3. Combined Solicited and Unsolicited Adverse Events

Solicited AEs continuing beyond 7 days will be recorded by the sites and flagged using rules given in Section 5.2. They will be coded by MedDRA and following summary by decreasing frequency of SOC and preferred term within SOC will be provided:

- Solicited TEAEs continuing beyond 7 days
- Combined solicited TEAEs continuing beyond 7 days and unsolicited TEAEs

Following by-subject Listings will be provided:

- All solicited TEAEs continuing beyond 7 days

6.5.2. Laboratory Data

- A summary describing pregnancy tests results at Baseline will be presented on the overall population only. The list of Baseline test results to be described is given in *Table 2*.

Table 2 Baseline Laboratory Tests

Panel	Sample	Parameter
Urinalysis	Urine	Pregnancy (urine)

- The lists of laboratory parameters analyzed can be found in *Table 3*. Only the parameters in this table will be presented in the following summaries:
    - The actual value and change from Baseline to Month 1 will be summarized for each clinical laboratory parameter, including hematology, clinical chemistry, and urinalysis. For subjects discontinuing early before Month 1, the value at Early Study Discontinuation will be presented in Listings only.
    - A shift table from Baseline to Month 1 presenting abnormal values for each parameter will also be presented. For subjects discontinuing early before Month 1, the abnormal values at Early Study Discontinuation will be used instead of Month 1.
      Clinical laboratory values will be expressed using conventional international system of units (SI). In the event of repeat values, the last non-missing value per study day/time will be used. Clinically significant out-of-range lab results will be reported as adverse events by Investigators. Clinical significance will therefore not be reported in the laboratory outputs.

Table 3 Laboratory Parameters Collected and Summarized in Tables

Panel	Sample	Parameter
Hematology	Blood	Eosinophils (absolute)
Hematology	Blood	Hemoglobin
Hematology	Blood	Neutrophils (absolute)
Hematology	Blood	Platelet count
Hematology	Blood	White blood cell count (absolute)
Chemistry	Serum	Albumin
Chemistry	Serum	Alkaline phosphatase
Chemistry	Serum	Alanine aminotransferase (ALT)
Chemistry	Serum	Aspartate aminotransferase (AST)
Chemistry	Serum	Bicarbonate
Chemistry	Serum	Bilirubin (Total)
Chemistry	Serum	Bilirubin (Direct)
Chemistry	Serum	Blood urea nitrogen (BUN)
Chemistry	Serum	Calcium
Chemistry	Serum	Chloride
Chemistry	Serum	Cholesterol
Chemistry	Serum	Creatinine
Chemistry	Serum	Gamma-glutamyl transferase (GGT)
Chemistry	Serum	Glucose (random)
Chemistry	Serum	Lactate dehydrogenase (LDH)

Chemistry	Serum	Phosphorus
Chemistry	Serum	Potassium
Chemistry	Serum	Protein total
Chemistry	Serum	Sodium
Chemistry	Serum	Triglycerides
Chemistry	Serum	Uric acid
Urinalysis	Urine	Bilirubin
Urinalysis	Urine	Blood
Urinalysis	Urine	Glucose
Urinalysis	Urine	Ketones
Urinalysis	Urine	Leucocyte Esterase
Urinalysis	Urine	Nitrite
Urinalysis	Urine	pH
Urinalysis	Urine	Protein (Total)
Urinalysis	Urine	Specific Gravity (SG)
Urinalysis	Urine	Urobilinogen

All laboratory data will be provided in data listings.

6.5.3. **Vital Signs**

The actual value and change from before study vaccine administration to after study vaccine administration will be summarized for each vital sign parameter: Systolic Blood Pressure, Diastolic Blood Pressure, Heart Rate, Oral Temperature, Respiratory Rate. In case of multiple measurements on Day 1 before vaccination, the closest measure from the time of vaccination will be used. In case of multiple measurements on Day 1 after vaccination, the closest measure from the time of vaccination will be used. Vital sign measurements will be presented in by-subject data listings.

6.6. **Immunogenicity Evaluations**

All Immunogenicity summaries to be produced by treatment group, for the following:

Population: IEP, EXP

Subgroups: Overall (50+), 50-59, 60+, 65+

All immunogenicity data will be provided by-subject data listings on the Exposed Population.

6.6.1. **GMT/GMC, GMR and GMFR**

To estimate the immunogenicity response 1 month after vaccination, OPA titers (log10) and IgG concentrations (mcg/mL) measured at Month 1 visit will be logarithmically transformed for analysis and GMTs/GMCs will be computed with 95% CI for each assay using ANOVA and ANCOVA.

- First model in an ANOVA with log10-transformed concentrations/titers at Month 1 as the dependent variable and treatment group and study site as the fixed effects in the model.
- Second model in an ANCOVA with log10-transformed concentrations/titers at Month 1 as the dependent variable, treatment group and study site as the fixed effects and log10 baseline concentration/titer as the covariate

The least squares means, and their 95% CIs calculated based on the ANOVA and ANCOVA will be back transformed and reported as the group GMT and GMC values. The p-value for the mean difference between groups, calculated using the F-test from the ANOVA/ANCOVA model, will be reported as well.

Comparisons between relevant groups will be done using GMRs. They will be calculated using the estimated adjusted GMTs/GMCs measured at Month 1 visit for all 31 serotypes in VAX-31 (of those 20 in PCV20), and mean square error calculated from the ANOVA and ANCOVA models using contrast statements. The main comparison of interest will be the three VAX-31 dose level groups versus the PCV20 (20 serotypes) group. However, the three VAX-31 dose level groups will also be compared in a pairwise fashion on a serotype-by-serotype basis. No adjustment for multiplicity will be applied and missing data will not be imputed.

The analysis of GMFR at Month 1 relative to Day 1 will also be computed using similar models:

- First model in an ANOVA with log10-transformed fold ratio at Month 1 as dependent variable and treatment group and study site as the fixed effects in the model.
- Second model in an ANCOVA with log10-transformed fold ratio at Month 1 as dependent variable, treatment group and study site as the fixed effects and log10 baseline concentration/titer as the covariate

The least squares means, and their 95% CIs calculated based on the ANOVA and ANCOVA will be back transformed and reported as the group GMFR value. No comparison between groups will be made.

The following Figures will be provided on the IEP only:

- A reverse cumulative distribution curve of OPA titers on Day 1 and Month 1 by treatment groups for each serotype
- A reverse cumulative distribution curve of IgG concentrations on Day 1 and Month 1 by treatment groups for each serotype
- Bar Plot of OPA GMTs at Month 1 from ANOVA model by treatment group and serotype
- Bar Plot of IgG GMCs at Month 1 from ANOVA model by treatment group and serotype

[redacted]

7. CHANGES TO PLANNED ANALYSES

There is no change between the protocol-defined statistical analyses and those presented in this statistical plan.

8. REFERENCES

1. International Council on Harmonization, Statistical Principles for Clinical Trials (ICH E9)
2. Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2007

9. APPENDICES

9.1. Schedule of Assessments

APPENDIX A: SCHEDULE OF EVENTS

	Visit Number:	--	1	2	3	4	5 - 9	--
	Study Visit:	Screening[a]	Day 1[a]	Day 8	Day 15 (Phone)	Month 1	Months 2, 3, 4, 5, 6 (Phone)	Early Termination
Study Event	Window:	-30 days		+3 days	+3 days	±3 days	±5 days	
Informed Consent		X						
Demographics, Medical History		X	X[b]					
Concomitant Medications		X	X	X	X	X[d]	X[d]	
Physical Exam(s), targeted		X	X[b]					
Vital Signs		X	X[c]					
Confirmation of Eligibility		X	X					
Randomization			X					
Study Vaccine Administration			X					
Post-vaccination Observation (at least 30 min)			X					
Issue e-Diary instructions, Ruler, Thermometer; Conduct Training			X					
Review e-Diary Data				X				
Adverse Event Evaluation (Solicited and/or Unsolicited)			X	X	X	X	X[d]	
Clinical Labs[d]								
Hematology, Chemistry, Urinalysis			X[b]			X		
Urine Pregnancy		X[b]	X[b]					
Serum for Immunogenicity			X[b]			X		

Refer to Protocol for Procedures

[a] Screening and Day 1 Visit are encouraged to be combined; however, subjects may screen for up to 30 days prior to randomizing into the study, and a separate Screening visit may be conducted.

[b] Conduct or collect prior to study vaccination (if indicated by updated medical history or change in health status, as applicable)..

[c] Vitals to be taken before and ≥30 minutes after study vaccine administration.

[d] Only NOCI, MAAE, SAE, and associated concomitant medications collected after Month 1.