VAX24-102

Statistical Analysis Plan: A Phase 2, Randomized, Observer-Blind, Dose-Finding, Controlled Clinical Study to Evaluate the Safety, Tolerability, and Immunogenicity of a 24-Valent Pneumococcal Conjugate Vaccine (VAX-24) in Healthy Adults 65 Years and Older

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Protocol VAX24-102

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Protocol Number: **(Version Date)**	VAX24-102 v.2.0 / 31-MAY-2022
Name of Test Drug:	VAX-24 (24-valent Pneumococcal Conjugate Vaccine)
Phase:	2
Methodology:	Randomized, Observer-Blind, Dose-Finding, Controlled, Parallel-Group
Sponsor:	Vaxcyte, Inc. 825 Industrial Road Suite 300 San Carlos, CA 94070 USA Tel: 650 837 0111
Sponsor Representative:	[redacted] Medical Monitor
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SIGNATURE PAGE

Protocol Title:	A Phase 2, Randomized, Observer-Blind, Dose-Finding, Controlled Clinical Study to Evaluate the Safety, Tolerability, and Immunogenicity of a 24-Valent Pneumococcal Conjugate Vaccine (VAX-24) in Healthy Adults 65 Years and Older
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[redacted]

Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned pharmacokinetic analyses described herein. I agree that the planned pharmacokinetic analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the pharmacokinetic 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 pharmacokinetic author.

I also understand that any subsequent changes to the planned pharmacokinetic 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).

[redacted]

[redacted]

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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
EDC	Electronic Data Capture
GMC	Geometric Mean Concentration
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
GMFR	Geometric Mean Fold Rise
ICH	International Conference on Harmonisation
IEP	Immunogenicity Evaluable Population
IgG	Immunoglobulin G
IP	Investigational Product
LLOQ	Lower Limit Of Quantification
MAAE	Medically Assisted Adverse Event
MedDRA	Medical Dictionary of Regulatory Activities
NOCI	New Onset of Chronic Illnesses
OPA	Opsonophagocytic antibody
PCV20	20-valent Pneumococcal Conjugate Vaccine
PD	Protocol Deviation
PT	Preferred Term
QA	Quality Assurance
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System organ class
TEAE	Treatment Emergent Adverse Event
TLFs	Tables, listings and figures

ULOQ	Upper Limit Of Quantification
VAX-24	24-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 clinical study report (CSR) for this trial.

1.2. Objectives

The primary objective is:

- To evaluate the safety and tolerability of a single injection of VAX-24 at three dose levels administered to healthy adults 65 years and older.

The secondary objective is:

- To assess the induction of antibody responses by VAX-24 dose levels compared to control groups receiving PCV20

[redacted]

2. STUDY DESIGN

2.1. Introduction

This Phase 2, randomized, observer-blind study is to be conducted in healthy adults aged 65 and older. Subjects may screen for up to 30 days prior to randomization; however, they may be screened and randomized within the same visit. Demographics, medical history, and concomitant medication use will be collected. Subjects will be randomly assigned in a 1:1:1:1 ratio to receive either VAX-24 at one of three dose levels or the active comparator, Prevnar 20 (PCV20).

Subjects will receive VAX-24 or PCV20 on Day 1. Solicited Adverse Events (AEs) will be collected for 7 days postvaccination via diary and unsolicited safety information for 28 days post-vaccination, with Serious Adverse Events (SAEs), new onset of chronic illnesses (NOCIs), and medically attended adverse events (MAAEs) collected up to 6 months post- vaccination. All subjects will have blood samples drawn for immunogenicity analysis for opsonophagocytic assay (OPA) and Immunoglobulin G (IgG) at Days 1 and 29. A Data Monitoring Committee (DMC) will be assembled for the study. If the study meets a pre-defined stopping criterion, the study will be paused, and data reviewed by the DMC prior to re-starting the enrollment.

The schedule of events is in Section 9.1, further details available in the study protocol.

2.2. Sample Size and Power

The sample size is not driven by statistical assumptions for formal hypothesis testing but was based on the safety objective for the study. A total of 200 subjects should provide sufficient information to assess safety and immunogenicity.
The sample size will also be adequate to establish variance of immune response in this target population and plan future studies.

2.3. Randomization Methodology

A total of 200 subjects are planned to be randomized in a 1:1:1:1 allocation ratio i.e. 50 subjects in each VAX-24 dose arm and 50 in the PCV20 group. There will be no stratification factors.
Treatment will be assigned automatically by the IBM EDC (Electronic Data Capture) system during the study, using a Randomization List generated and uploaded before the enrollment of the first subject.

2.4. Stopping Rules

During the vaccination period(s), if any of the stopping rules are triggered, no further administration of study vaccine(s) will occur until safety data are reviewed by the DMC in communication with the Medical Director and the Investigator at the site where the event

occurred, as needed. Study dosing may be resumed if the DMC determines it is safe to proceed with no modifications or with modifications to the protocol plans.

[redacted]

Enrollment may be resumed following review of available safety data by the DMC. Vaxcyte clinical safety team will refer to the VAX24-102 Safety Medical Monitoring Plan for detailed procedures on stopping rule reporting and safety oversight for the study.

The study is planned to be completed after all subjects have completed the Month 6 visit (or Early Discontinuation, as appropriate), all necessary safety follow-up has been completed, and all data have been monitored and queries have been resolved. The Sponsor reserves the right to terminate the study prior to the planned study completion.

2.5. **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
- Third-Party Vendors (Supply Management)
- Unblinded pharmacist or designee at site who will prepare Investigational Product (IP)
- Unblinded Administrator at site who will dispense IP
- 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 (QA) 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 conduct of the study:

- The unblinded statistical programming team, the unblinded data manager, and independent support statistician who will prepare Safety unblinded reports for DMC meetings (excluding immunogenicity data)
- The DMC members who will review Safety unblinded data reports (excluding immunogenicity data)
- The unblinded programming team and the unblinded statistician who will prepare the unblinded report for the Final Analysis

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.

2.6. **Interim Analyses**

No Interim Analysis is planned for this study.

2.7. **Schedule of Analyses**

Only one Final Analysis is planned for this study. The final database lock (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 safety findings through Month 6 and immunogenicity through Day 29 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 including NOCI and MAAE, physical examinations, measurement of vital signs.

The primary safety endpoints are:

- Percentage of subjects reporting solicited local reactions within 7 days after vaccination (redness, swelling, and pain at injection site).
- Percentage of subjects reporting solicited systemic events within 7 days after vaccination (fever, headache, fatigue, muscle pain, and joint pain).
- Percentage of subjects reporting unsolicited AEs within 1 month after vaccination.
- 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.

3.3. Immunogenicity Variables

Immunogenicity samples will be collected for all subjects at Day 1, Day 29 and at the Early Discontinuation Visit for subjects discontinuing before Month 6 visit. Immunogenicity parameters to be determined include OPA and IgG assays.

The secondary endpoints related to Immunogenicity assessments are:

- 24 VAX-24 Pneumococcal serotype-specific OPA geometric mean titer (GMTs) at 1 month after vaccination (Day 29).
- 24 VAX-24 Pneumococcal serotype-specific IgG geometric mean concentration (GMCs) at 1 month after vaccination (Day 29).

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]

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: 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: Includes all subjects from the Screened Population who received at least one study vaccine administration. Each subject will be analyzed as treated.
- Safety Population : 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.
    - 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
        - Day 29: Day 26 through Day 34, inclusive

  Each subject will be analyzed as treated. Programming identification of patients excluded of the IEP is detailed in Section 4.2.

"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 Randomized Population will be used for the analysis of exposure.
The Safety Population will be the primary set for the analysis of the safety parameters.

The Immunogenicity Evaluable Population will be the primary set for the immunogenicity analysis, with the Exposed Population 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 Final DBL, [redacted] will download the 'dv' csv file from IBM 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. It is expected that 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.

The PD classification file will be finalized prior to the final DBL. All PDs and their classification will be presented in a data listing.

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

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 25.0 unless otherwise noted. Concomitant medications will be coded using B3 World Health Organization (WHO) Drug Global (March 2022) 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 Day 29 visit.
  - **Follow-up:** The period from Day 29 visit to Month 6 visit.
- **Visits:**
  - **Study day 1:** The date of study vaccine administration.
  - **End of Treatment Visit**: The Day 29 visit, or the early discontinuation visit for subjects who withdraw study prior to Day 29.
  - **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.
- **Conversion factors (for derived data calculations):**
  - 1 month = 30.4375 days
  - 1 week = 7 days
- **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 Evens (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 continuing solicited adverse event?'. The corresponding question in the diary page will not be checked programmatically as it is expected that both sources will be reconciled before DBL so that only the unsolicited page can be used for this analysis.
- **Immunogenicity:**
  - **GMC [or GMT]**: The geometric mean concentration (or titer) = antilog (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 = antilog (LSMeans [log10 x]) / antilog(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 Day 29 visit and the one at Day 1.
  - **GMFR**: The geometric mean fold ratio = antilog (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. General Statistical Methods

6.1.1. 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.

Formal 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.

6.1.2. Definition of Baseline

For all endpoints, baseline is defined as the last non-missing assessment prior to study vaccine administration. Specifically, as per the protocol, baseline evaluations are expected as follows:

- Vital signs: Day 1 prior to study vaccine administration
- Immunogenicity: Day 1 prior to study vaccine administration

[redacted]

6.1.3. Adjustments for Covariates

The randomization in this study will not be stratified.

The following variables will be used for adjustment in 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. In case the model does not converge, the log10 baseline titer will be removed from the model and a footnote explicating the change will be added.

6.1.4. Multiple Comparisons/Multiplicity

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

6.1.5. Subgroups

There is no plan for subgroup analysis at this stage.

6.1.6. 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 is:

- Systemic Solicited AEs with missing "Relationship to study vaccine" will be analyzed as "Possibly related".

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.

6.2. Study Population

6.2.1. Subject Disposition

Subject disposition will be presented by treatment group and overall, including the number screened, the number of screen failures and the number randomized. The study disposition will also be presented along with the reasons for early study withdrawal.

The number of subjects in each analysis set will be presented on the Randomized Population by treatment 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 and reason for exclusion from the IEP
- Protocol deviations

6.2.2. Demographic Characteristics

Demographic characteristics at enrollment (i.e., at the time of informed consent) 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).

Table summaries to be produced by treatment group and overall and repeated for the Randomized Population, the Safety Population and the Immunogenicity Evaluable Population.

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

6.2.3. 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 strictly 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 on the Safety Population.

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

6.2.4. 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 on the Randomized Population.

6.3. Efficacy Evaluation

Not Applicable.

6.4. Safety Evaluations

All Safety summaries to be produced by treatment group on the Safety Population.

6.4.1. Adverse Events

6.4.1.1. 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 ≥ 100.4°F), 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 diary CRF, within 7 days after vaccine administration (i.e. from Day 1 to Day 7) will be analyzed in this Section. Occurrence of a solicited AE on a specific Day will be identified by a Grade≥1 for that AE on that day.

Solicited AEs continuing beyond 7 days after injection will be combined with unsolicited AEs and described separately as specified in Section 6.4.1.3.

The following characteristics 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 (as per Investigator). 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 event will be used as end day for the calculation.
  Example: If a subject has a Fatigue starting on Day 2 and finishing on Day 3. And a new Fatigue starting on Day 7 and ending on Day 9. The Duration will be 1+2=3.
  If the solicited 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.

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 (i.e., 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)
- Solicited AEs by day post-injection, for each event and for any event, overall and by severity. (Severity as per Investigator)
- Solicited AEs by day post-injection, for each event and for any event, overall and by severity. (Severity as per Subject)

- Time of first onset of solicited AEs within 7 days post-injection for each event and any event. Duration of each solicited AEs and maximum duration for any event. (Severity as per Investigator)

Listings of all solicited AEs will be provided by subject.

6.4.1.2. 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 flagged using rules given in Section 5.2 and described in separate summaries as explained in Section 6.4.1.3.

The definition of a treatment emergent AE (TEAE) is an event that occurs after vaccination and within the 28 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≤28

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:

- Any unsolicited TEAEs
- Possibly or probably related unsolicited TEAEs
- Unsolicited TEAEs leading to study withdrawal
- Any AE leading to death
- Any SAE within 6 months after the vaccination by maximum relationship to study treatment. This summary will include serious solicited AEs.
- Any NOCI within 6 months after the vaccination by maximum relationship to study treatment.
- Any MAAEs within 6 months after the vaccination by maximum relationship to study treatment.

Following by-subject Listings will be provided:

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

6.4.1.3. 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

6.4.2. Laboratory Data

Not Applicable.

6.4.3. Vital Signs and Physical Examinations

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, Oral Temperature, Respiratory Rate and Heart 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.

Physical examination results at Baseline will be summarized. All physical examination findings will be presented in by-subject data listing.

6.5. Immunogenicity Evaluations

All Immunogenicity summaries to be produced by treatment group, for the Immunogenicity Evaluable Population and the Exposed Population.

6.5.1. GMT/GMC, GMR and GMFR

To estimate the immunogenicity response 1 month first vaccination, OPA titers (log10) and IgG concentrations (mcg/mL) measured at Day 29 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 Day 29 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 Day 29 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. Comparisons between relevant groups will be based on the estimated adjusted GMTs measured at Day 29 visit for 24 serotypes in VAX-24 (of those 20 in PCV20), and mean square error calculated from the basic ANOVA model using contrast statements. The main comparison of interest will be the three VAX-24 dose levels group versus the PCV20 (20 serotypes) group. However, the three VAX-24 dose 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. GMRs will also be calculated from the ANCOVA and ANOVA models.

The analysis of GMFR at Day 29 relative to Day 1 will also be computed using similar models:

- First model in an ANOVA with log10-transformed fold ratio at Day 29 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 Day 29 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.

6.5.2. Threshold analyses

For the 4 non-PCV20 serotypes that are included in VAX-24 (i.e., 2, 9N, 17F and 20B), the adjusted proportion of subjects with a 4-fold increase in OPA titer will be evaluated using the following model:

- Logistic regression, with an indicator variable for achieving a 4-fold increase in OPA titer at Day 29 as the dependent variable, and treatment group and study site as fixed effects and log10 baseline titer as a covariate. Proportion in each treatment group will be presented along their 95%CI. The difference of proportions in each of the three VAX-

24 dose levels group versus the PCV20 will also be presented along the 95% CI. If the lower bound of the 95% CI is >0.1, the VAX-24 dose will be deemed statistically superior to PCV20.

Following summaries will also be provided for all serotypes:

- OPA titers:
  - Percentages of subjects achieving a 4-fold increase at Day 29 and associated Wilson score 95% CI and difference of proportions between pair-wise treatment groups using Fisher's exact test.
  - Percentages of subjects with titer ≥ LLOQ at Day 29 and associated Wilson score 95% CI and difference of proportions between pair-wise treatment groups using Fisher's exact test.
- IgG concentrations:
  - Percentages of subjects achieving a 4-fold increase at Day 29 and associated Wilson score 95% CI and difference of proportions between pair-wise treatment groups using Fisher's exact test.
  - Percentages of subjects with titer ≥ LLOQ at Day 29 and associated Wilson score 95% CI and difference of proportions between pair-wise treatment groups using Fisher's exact test.

Following Figures will be provided on the IEP:

- A reverse cumulative distribution curve of OPA titers on Day 1 and Day 29 by treatment groups for each serotype
- A reverse cumulative distribution curve of IgG concentrations on Day 1 and Day 29 by treatment groups for each serotype
- Bar Plot of OPA GMTs at Day 29 from ANOVA model by treatment group and serotype
- Bar Plot of IgG GMCs at Day 29 from ANOVA model by treatment group and serotype
- Bar Plot of percentages of subjects achieving a 4-fold increase in OPA titers by serotype
- Bar Plot of percentages of subjects achieving a 4-fold increase in IgG concentrations by serotype
- Forest Plot of OPA GMRs and GMFRs from ANOVA model at Day 29 for VAX-24 compared with PCV20 by serotype
- Forest Plot of IgG GMRs and GMFRs from ANOVA model at Day 29 for VAX-24 compared with PCV20 by serotype

Finally, all immunogenicity data will be provided by-subject data listings on the Exposed Population.

7. CHANGES TO PLANNED ANALYSES

In the Section 9 of Protocol v2.0, there is a mention a data freeze and treatment group unblinding for the primary safety and secondary immunogenicity endpoints through Day 29 visits. But no data freeze, and no interim analysis are planned for the study. Treatment unblinding will occur only at the time of final database lock.

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. Appendix A: Schedule of Events

APPENDIX A: SCHEDULE OF EVENTS

	Study Visit:	Screening	Day 1[a]	Day 8	Day 29	Month 3 ar (Phone)	Early Termination
	Window:	-30d		+3d	±3d[e]	±5d	
Informed Consent		X					
Demographics, Medical History		X	X[b]				
Concomitant Medications		X	X	X	X[d]	X[d]	
Physical Exam(s), targeted		X	X[b]				
Vital Signs		X	X[c]				
Confirmation of Eligibility		X	X				
Serum for Immunogenicity			X[b]		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 Diary Data				X			
AE Evaluation (Solicited and/or Unsolicited)			X	X	X	X[d]	

Refer to Protocol for Procedures

Abbreviations: AE = adverse event; SAE = serious adverse event

[a] Subjects may screen for up to 30 days prior to randomizing into the trial; 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 prior and after study vaccine administration (≥ 30 min).

[d] New onset of chronic illnesses, MAAEs and SAEs, and associated concomitant medications collected after Day 29.

[e] Immunogenicity samples can be collected from Day 26 through Day 34, inclusive, for the subject to be included in the IEP.