Clinical Study Protocol VAX31-101

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
Protocol Number:	VAX31-101
Investigational Product:	VAX-31 (31-valent Pneumococcal Conjugate Vaccine)
Sponsor:	Vaxcyte, Inc. 825 Industrial Road, Suite 300 San Carlos, CA 94070 USA Tel: 650-837-0111
Version:	2.0
Date:	08 December 2023

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Protocol Signature Page
Protocol Number VAX31-101

This Clinical Study Protocol document and all information provided to you, the Principal Investigator, related to this protocol are the confidential and proprietary information of Vaxcyte, Inc. ("Sponsor") and are subject to the terms of the Confidential Disclosure Agreement between you and Vaxcyte, Inc.

By signing below, I hereby confirm the following:

I agree to abide by the terms of the Vaxcyte, Inc. Confidential Disclosure Agreement.

I have read this protocol entitled, 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, in its entirety, and I agree to conduct the study according to this protocol. Any changes in procedure will only be made if necessary to protect the safety, rights, or welfare of subjects.

I agree to comply with the current International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice in addition to the appropriate FDA Code of Federal Regulations (CFR) and applicable state and local regulations.

I agree to conduct the study in person or to supervise the study.

I agree to ensure that all who assist me in the conduct of the study have access to the study protocol, including any amendments thereto, and are also made aware of their responsibilities in meeting the foregoing obligations.

_____
Principal Investigator (Print Name)

_____
Title

_____ _____
Signature Date

Site to send signed copy to Vaxcyte, Inc. and to keep original for files.

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List of Abbreviations

ACIP	Advisory Committee on Immunization Practices
AE	Adverse event(s)
ALCOA-C	Attributable, legible, contemporaneous, original, accurate, and complete
ALPO	Aluminum phosphate
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
CBC	Complete blood count
CFR	Code of Federal Regulations
CI	Confidence interval(s)
CRF	Case report form(s)
CRO	Contract research organization(s)
CRP	C-reactive protein
CRM	Cross-reactive material
CSR	Clinical Study Report
DMC	Data Monitoring Committee
DP	Drug Product(s)
eCRF	Electronic Case Report Form(s)
eCRM	Proprietary nontoxic diphtheria toxin carrier protein used in VAX-31
EDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ePRO	Electronic patient reported outcomes
ET	Early Termination
FDA	Food and Drug Administration
FIH	First-in-Human
GCP	Good Clinical Practice
GMC	Geometric Mean Concentration
GMFR	Geometric Mean Fold Rise
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation

IEP	Immunogenicity evaluable population
IgG	Immunoglobulin G
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
LLOQ	Lower Limit of Quantitation
MAAE	Medically attended adverse event(s)
MOPA	Multiplexed opsonophagocytic assay
MSD	Meso Scale Discovery
NOCI	New onset of chronic illness(es)
NZW	New Zealand White (species of rabbits)
OPA	Opsonophagocytic assay
pAMF	Para-azidomethyl-L-phenylalanine
PCV	Pneumococcal conjugate vaccine(s)
PCV13	13-valent pneumococcal conjugate vaccine (Prevnar 13®)
PCV15	15-valent pneumococcal conjugate vaccine (Vaxneuvance™)
PCV20	20-valent pneumococcal conjugate vaccine (Prevnar 20®)
PD	Pneumococcal disease
PFS	Prefilled syringe(s)
PPV	Pneumococcal polysaccharide vaccine
PPV23	23-valent pneumococcal polysaccharide vaccine (Pneumovax® 23)
PS	Polysaccharide
S. pneumoniae	*Streptococcus pneumoniae*
SAE	Serious adverse event(s)
SAP	Statistical analysis plan
SMMP	Safety medical management plan
SOC	System organ class
SST	Serum Separator Tube
SUSAR	Suspected unexpected serious adverse reaction(s)
TEAE	Treatment emergent adverse event(s)
VAX-31	31-valent investigational pneumococcal conjugate vaccine
US	United States
WHO	World Health Organization
WHODrug	World Health Organization Drug (medicinal information dictionary)

1. Protocol Synopsis

Sponsor	Vaxcyte, Inc.
Name of Investigational Product	VAX-31 (31-valent Pneumococcal Conjugate Vaccine)
Names of Comparator Products	Prevnar 20® (20-valent Pneumococcal Conjugate Vaccine; PCV20)
Study 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
Study Number	VAX31-101
Study Phase	1/2
Study Sites	Approximately 25 investigative sites in the US

Study Objectives and Endpoints

Primary:

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 PCV20 administered to 4 age groups:
  - Subjects 50 to 59 years of age
  - Subjects 50 years of age and older
  - Subjects 60 years of age and older
  - Subjects 65 years of age and older

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 adverse event (AE) within 1 month after vaccination in each age group
- Percentage of subjects reporting serious adverse event (SAE) within 6 months after the vaccination
- Percentage of subjects reporting new onset of chronic illness (NOCI) within 6 months after the vaccination
- Percentage of subjects reporting medically attended adverse event (MAAE) within 6 months after vaccination

Study Objectives and Endpoints cont.

Secondary:

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 to 59 years of age
  - Subjects 50 years of age and older
  - Subjects 60 years of age and older
  - Subjects 65 years of age and older

The secondary endpoints include the following:

- Safety: Percentage of participants with laboratory value abnormalities and/or potentially clinically significant laboratory values at 1 month (30 days) after vaccination
- Safety: Change from baseline in laboratory parameters at 1 month (30 days) after vaccination
- Immunogenicity: 31 VAX-31 Pneumococcal serotype-specific opsonophagocytic assay (OPA) geometric mean titer (GMT) at 1 month (30 days) after vaccination
- Immunogenicity: 31 VAX-31 Pneumococcal serotype-specific IgG geometric mean concentration (GMC) at 1 month (30 days) after vaccination

[redacted]

Study Design

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. In Stage 2, 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. 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. 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 (predose) and Month 1 (30 days after vaccination). Subjects will receive VAX-31 or PCV20 on Day 1. Solicited AE will be collected for 7 days after vaccination and unsolicited safety information for 30 days after vaccination, with SAE, NOCI, and MAAE 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.

If the DMC agrees that the study may continue after reviewing data from Stage 1, Stage 2 will commence and 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. 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. 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 (predose) and Month 1 (30 days after vaccination). Subjects will be randomly assigned in a 1:1:1:1 ratio to receive either VAX-31 at 1 of 3 dose levels or PCV20. Solicited AE will be collected for 7 days post-vaccination and unsolicited safety information for 30 days post-vaccination, with safety data (limited to SAE, NOCI, and MAAE) collected up to 6 months post-vaccination.

Study Population

The study population will comprise approximately 1000 healthy US adults: up to 600 eligible subjects aged 50 to 59, a minimum of 200 subjects aged 60 to 64, as well as a minimum of 200 subjects aged 65 years and older.

Main Criteria for Inclusion

Healthy adult subjects aged 50 years and older without a previous history of pneumococcal disease and who have never received a licensed or investigational pneumococcal vaccine.

Test Product, Dose, and Mode of Administration

VAX-31 will be administered once by intramuscular (IM) injection at 1 of 3 dose levels based on the amount of polysaccharide present: 1.1 mcg (Low), 2.2 mcg (Mid), and 3.3 mcg (High) for all serotypes with the exception of serotypes 1, 5, and 22F which will be 1.65 mcg, 3.3 mcg, and 4.4 mcg for the 3 dose levels, respectively.

Reference Therapy, Dose, and Mode of Administration PCV20 will be administered once by IM injection at a dose of 0.5 mL.
Treatment and Duration of Treatment Treatment comprises a single IM injection of VAX-31 or a single IM injection of PCV20.
Safety Assessments Subjects will be evaluated for solicited and unsolicited AE, including injection site reactions following vaccine administration. Additionally, vital signs, changes in laboratory values, NOCI, and MAAE following administration of study vaccines will be evaluated.
Statistical Methods Various standard statistical methods will be employed to analyze the data, including descriptive statistics, linear models, and graphical displays. Summary statistics will comprise frequencies and percentages of responses in each category for discrete measures and of counts, means, medians, standard deviations, 95% confidence intervals (CI), and minimum and maximum values for continuous measures and will be presented by study treatment. All calculated fields will be listed in various data listings for review. Additional analyses may be performed by the Sponsor to supplement these results. Version 9.4M6 (TS1M6) or higher of the SAS® statistical software package will be used to provide all statistical analyses.

2. Introduction

2.1 Background and Rationale

Streptococcus pneumoniae (*S. pneumoniae*) is the pathogen that causes pneumococcal disease (PD), classified as either noninvasive (pneumonia, acute sinusitis, otitis media, and conjunctivitis) or invasive (meningitis, sepsis, endocarditis, and pericarditis). A persistent global incidence of PD, driven by emerging serotypes not covered by currently available vaccines, has resulted in high morbidity and mortality worldwide. Pneumococci cause over 50% of all cases of bacterial meningitis in the US (Gierke, 2021). Mortality rates for invasive pneumococcal disease in the United States (US) range from 11% to 30% in adults (CDC, 2019). The overall case-fatality rate for pneumococcal bacteremia is approximately 20% overall, increasing to 60% in older adults (CDC, 2022). Pneumococcal pneumonia case fatality rates are also quite significant, estimated to be 5% to 7% overall with much higher rates also among older adults (Gierke, 2021). In a prospective population-based cohort study of adult residents in Louisville, Kentucky, from 1 June 2014 to 31 May 2016, mortality during hospitalization for pneumonia was 6.5%, corresponding to an estimated 102,821 annual deaths in the US (Ramirez, 2017).

Despite substantial reductions in PD with the availability of pneumococcal conjugate vaccines (PCV) as well as a pneumococcal polysaccharide vaccine (PPV), due to the diversity of serotypes and the phenomenon of serotype replacement, a significant burden of PD remains which is currently not covered by the currently available pneumococcal vaccines PCV13, PCV15, PCV20, and PPV23.

For example, the invasive pneumococcal disease (IPD) incidence in 2017 in the population aged <5 years old was reported to be 1.7 cases per 100,000 due to PCV13 serotypes, while the incidence of IPD due to non-PCV13 serotypes was 5.3 cases per 100,000. Similarly, in the population aged 65 years and older IPD incidence in 2017 was reported to be 6.8 cases per 100,000 due to PCV13 serotypes, while the incidence of IPD due to non-PCV13 serotypes was 19 cases per 100,000 (Varghese, 2020). In all age groups in 2017, approximately 28.3% of IPD in the US was due to serotypes in PCV13, 41.3% was due to serotypes in PCV15, 59.5% was due to serotypes in PCV20, 69.2% was due to serotypes in VAX-24, and 93.7% was due to serotypes in VAX-31 (calculated from Table 1 in Varghese, 2020).

Additionally, it is important to note that PCV have been shown to elicit T-cell-dependent immune response, resulting in a more robust and longer-lasting immune response in both infants and the elderly compared to that elicited by PPV23 (Andrews, 2014; Matanock, 2019; Richter, 2013). Based on this information, the Advisory Committee on Immunization Practices (ACIP) recommended in October 2021 the use of PCV20 without a subsequent dose of PPV23 or the use of Vaxneuvance™ (PCV15) followed by PPV23 in adults at increased risk of pneumococcal

disease, including all adults ≥65 years of age. In 2023, the CDC recommended routine administration of PCV15 or PCV20 for all adults aged 65 years or older who have never received any pneumococcal conjugate vaccine or whose previous vaccination history is unknown (CDC, 2023).

Developing PCV with greater serotype coverage using conventional technologies has proven to be difficult for 2 reasons. First, data show that as more carrier protein/polysaccharide (PS) conjugates are added to existing PCV, immune responses to the PS often decrease due to the cumulative amount of carrier protein, which immunologically competes with the PS antigens (Dagan, 2010), as most recently seen with PCV20 (Pfizer, 2023). Second, conjugating additional PS to a carrier protein molecule may mask existing T-cell epitopes that are critically important in eliciting an immune response. In the PCV13 carrier protein CRM_{197}, approximately 20% of the 39 lysine residues border relevant T-cell epitopes (Choe, 1992; Diethelm-Okita, 2000; Raju, 1995). Since conventional conjugation chemistry heterogeneously conjugates PS to lysine residues independent of their proximity to T-cell epitopes, a bound PS may block the presentation of a T-cell epitope to the immune system, thus preventing the induction of a T-cell response. Meanwhile, the B-cell epitopes of both the carrier protein and the antigen are presented to the immune system, increasing B-cell competition for any available T-cell help. The masking of these critical epitopes prevents the conversion to a T-cell dependent immune response and negates the benefit of the carrier protein. This competition for T-cell help diminishes the immune response to the PS antigen and is believed to result in the phenomenon known as carrier suppression (Dagan, 2010). To address this issue, Vaxcyte is developing a 24-valent PCV (VAX-24), and a 31-valent PCV (VAX-31) using a novel process that combines site-specific conjugation to the carrier protein and a highly efficient conjugation chemistry. Vaxcyte employs a cell-free protein synthesis platform to insert the non-native amino acid para-azidomethyl-L-phenylalanine (pAMF) into eCRM, a recombinant version of CRM_{197}. These pAMF residues serve as PS conjugation sites and are inserted in place of purposefully selected lysines, such that critical T-cell epitopes remain consistently exposed upon conjugation to maximize the T-cell dependent immune response.

Vaxcyte's PCV clinical development program comprises 2 vaccines for active immunization against *S. pneumoniae*: VAX-24 and VAX-31. VAX-24 has been administered as a single dose to adults in 2 completed Phase 1/2 clinical trials. These clinical trials demonstrated that VAX-24 has an acceptable safety and tolerability profile similar to PCV20 and elicits an immune response meeting standard noninferiority criteria for all 20 serotypes shared with PCV20 and superiority criteria for the 4 serotypes not in PCV20. Additionally, VAX-24 reverses the historical trend of lower immune response with broader serotype coverage and elicits ~20% higher immune response across shared serotypes when compared with PCV20.

Safety data in adults are available from the VAX-24 clinical program, in which a total of 779 adults have received at least 1 dose of VAX-24. Two clinical studies of VAX-24 have been completed: VAX24-101, a Phase 1/2 study in adults 18 to 64 years of age and VAX24-102, a Phase 2 study in adults 65 years of age and older. The control vaccine in these studies was PCV20. In both studies, the safety and tolerability profile of VAX-24 was generally similar to that of licensed PCV. In both studies, the most commonly reported solicited adverse event (AE) in >10% of PCV20 recipients were pain at injection site (~70%), muscle pain (~50%), fatigue (~40%), headache (~30%), and arthralgia (~20%). All solicited AE were similarly distributed among the study groups. These events were typically mild to moderate and self-limiting.

The safety profile of VAX-24 was consistent across study groups with no apparent dose dependence, and the incidence of events was similar to that for the PCV20 control. In the VAX-24 groups, 12.3% of subjects experienced a Treatment Emergent Adverse Event (TEAE) within 1 month post-vaccination, 11.3% experienced a MAAE, 1.9% experienced NOCI, and 1.2% experienced SAE through 6 months post-vaccination. No NOCI or SAE were reported by the Investigators as related to VAX-24 and no clinically relevant abnormalities were observed in vital signs or laboratory parameters during either study. Additionally, protocol-specified safety laboratory assessments in the first-in-human study of VAX-24, VAX24-101, including baseline and Day 29 serum chemistry, hematology, and urinalysis panels, showed no clinically relevant post-vaccination changes or differences between treatment groups. Based on the favorable results from the VAX-24 clinical studies and the continued unmet need for vaccine-induced protection against additional serotypes not represented in currently approved PCV or VAX-24, Vaxcyte has initiated development of VAX-31.

VAX-31 vaccine is a sterile liquid suspension of capsular pneumococcal polysaccharide antigens of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 31, 33F and 35B, each individually conjugated in a site-specific manner to a carrier protein referred to as eCRM, which is an amino acid variant of the detoxified version of the diphtheria toxin known as CRM_{197}.

VAX-31 contains the same 24 conjugated polysaccharides as VAX 24, plus 7 additional serotypes. The same manufacturing processes are used for VAX-31 and VAX-24, and they are supplied in the same formulation (295 mcg succinate, 4385 mcg sodium chloride, 1175 mcg sodium phosphate, 500 mcg polysorbate 80 at pH 5.8, with 0.125 mg of aluminum phosphate added per 0.5 mL dose). Each vial is filled to deliver a single 0.5 mL dose of vaccine for IM administration, with no preservative.

The current protocol, VAX31-101, is a dose-finding Phase 1/2 study designed to evaluate the safety, tolerability, and immunogenicity of the VAX-31 vaccine at 3 different dose levels in adult subjects aged 50 years and older. The VAX-31 vaccine is described in Section 5.

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2.1.2 Rationale for Dosage and Route of Administration

VAX-31 will be administered at 1 of 3 dose levels based on the amount of polysaccharide present: 1.1 mcg (Low), 2.2 mcg (Mid), and 3.3 mcg (High) for all serotypes with the exception of serotypes 1, 5, and 22F, which will be 1.65 mcg, 3.3 mcg, and 4.4 mcg for the 3 dose levels, respectively. This dose range was informed by the polysaccharide dose in PCV20 and the results of dose-ranging studies with VAX-24. Each Mid dose is formulated to contain a similar quantity of *S. pneumoniae* polysaccharides as that of PCV20, the current standard of care. Serotypes 1, 5, and 22F are increased in each of the dose levels because these 3 serotypes had the lowest immune responses in VAX24-101. Site-specific conjugation to the eCRM protein carrier increases the PS-to-eCRM ratio, enhancing overall T-cell epitope exposure. Consequently, VAX-31 Mid dose is consistent with current dosing of licensed PCV, such as PCV15 and PCV20. Vaxcyte implemented a Low dose and a High dose to characterize the dose response to VAX-31. Safety data from VAX-24 clinical studies support including the High dose in the initial dose-finding study for VAX-31. [redacted]

3. Objectives and Endpoints

3.1 Primary

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 50 years of age and older.
- To compare the safety of VAX-31 to that of PCV20 administered to 4 age groups:
  - Subjects 50 to 59 years of age
  - Subjects 50 years of age and older
  - Subjects 60 years of age and older
  - Subjects 65 years of age and older

Safety and tolerability will be assessed by measuring the incidence of solicited local and systemic AE, unsolicited AE, serious adverse events (SAE), new onset of chronic illness (NOCI), and medically attended adverse events (MAAE):

- 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 (30 days) after vaccination in each age group
- Percentage of subjects reporting SAE within 6 months after the vaccination
- Percentage of subjects reporting NOCI within 6 months after the vaccination
- Percentage of subjects reporting MAAE within 6 months after vaccination

3.2 Secondary

The secondary objectives are:

- Safety: To assess laboratory value abnormalities and/or potentially clinically significant laboratory values following 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 as well as to the additional 11 non-PCV20 serotypes in VAX-31 for the following subpopulations:
  - Subjects 50 to 59 years of age
  - Subjects 50 years of age and older
  - Subjects 60 years of age and older
  - Subjects 65 years of age and older

The secondary endpoints include the following:

- Safety: Percentage of participants with laboratory value abnormalities and/or potentially clinically significant laboratory values at 1 month (30 days) after vaccination
- Safety: Change from baseline in laboratory parameters at 1 month (30 days) after vaccination
- Immunogenicity: 31 VAX-31 Pneumococcal serotype-specific OPA geometric mean titer (GMT) at 1 month (30 days) after vaccination.
- Immunogenicity: 31 VAX-31 Pneumococcal serotype-specific IgG geometric mean concentration (GMC) at 1 month (30 days) after vaccination

[redacted]

[redacted]

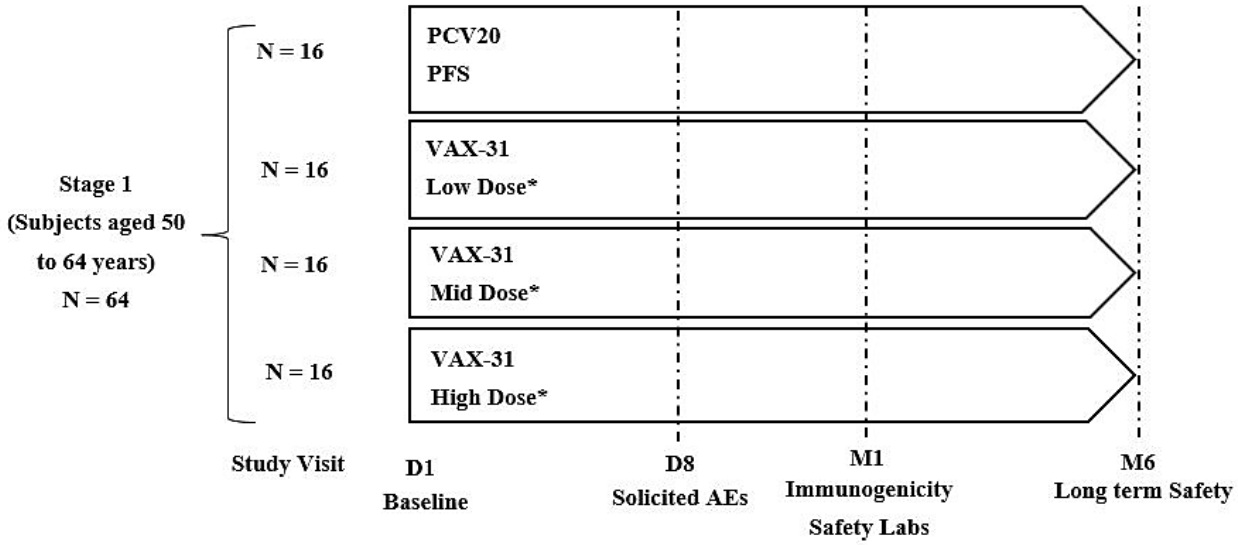
4. Study Plan

4.1 Study Design

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 years, 60 to 64 years, 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 (1.1 mcg [Low], 2.2 mcg [Mid], and 3.3 mcg [High], for all serotypes with the exception of serotypes 1, 5, and 22F which are 1.65 mcg, 3.3 mcg, and 4.4 mcg for the 3 dose levels, respectively), or the comparator (PCV20).

In Stage 1, a group of 64 subjects (16 from each VAX-31 dose group and 16 from the PCV20 group) aged 50 to 64 years will be enrolled initially (Figure 1). Females of childbearing potential will take a urine pregnancy test at screening and immediately prior to vaccination. Subjects will receive VAX-31 or PCV20 on Day 1. Solicited AE will be collected for 7 days post-vaccination and unsolicited safety information for 30 days post-vaccination, with safety data (limited to SAE, NOCI, and MAAE) collected up to 6 months post-vaccination. All subjects enrolled in Stage 1 will have a urine sample collected as well as blood samples drawn for safety lab and immunogenicity analysis (OPA and IgG) at Day 1 (predose) and Month 1 (refer to Section 6.6.3). A defined safety review of data (AE) through 7 days post-vaccination will occur by an independent Data Monitoring Committee (DMC) before proceeding with enrollment of the Stage 2 subjects.

Figure 1 Study Schema for Stage 1

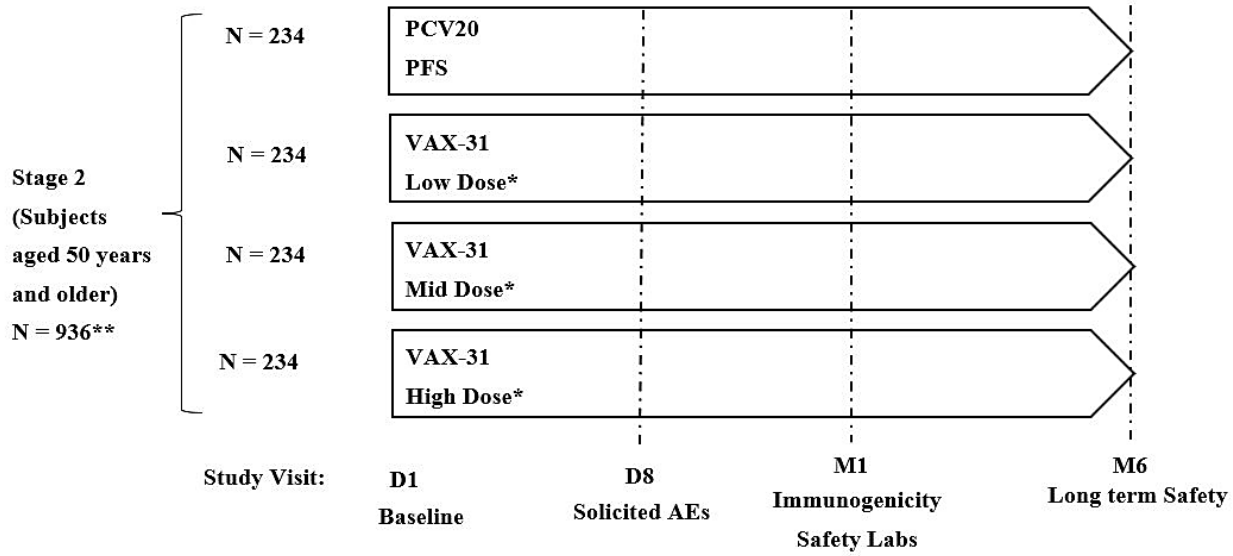


* VAX-31 doses are as follows: 1.1 mcg (Low), 2.2 mcg (Mid), and 3.3 mcg (High) for all serotypes with the exception of serotypes 1, 5, and 22F, which are 1.65 mcg, 3.3 mcg, and 4.4 mcg for the 3 dose levels, respectively.

After the safety data in the Stage 1 group is evaluated by the DMC and approved to proceed as planned, Stage 2 of the study will start enrollment and the remainder of the 800 subjects aged 50 to 64 years old (approximately 736 subjects with a minimum of 200 subjects aged 60 to 64) along with a minimum of 200 subjects aged 65 years and older will be concurrently enrolled in the study across the 4 treatment arms (Figure 2).

Solicited AE will be collected for 7 days post-vaccination and unsolicited safety information for 30 days post-vaccination, with SAE, NOCI, and MAAE collected up to 6 months post-vaccination. Females of childbearing potential will take a urine pregnancy test at screening and immediately prior to vaccination. All subjects in Stage 2 will also have a urine sample collected as well as blood samples drawn for safety labs and immunogenicity analysis (OPA and IgG) at Day 1 (predose) and Month 1 (refer to Section 6.6.3).

Figure 2 Study Schema for Stage 2



* VAX-31 doses are as follows: 1.1 mcg (Low), 2.2 mcg (Mid), and 3.3 mcg (High) for all serotypes with the exception of serotypes 1, 5, and 22F, which are 1.65 mcg, 3.3 mcg, and 4.4 mcg for the 3 dose levels, respectively.

** Minimum of 200 total subjects aged 60 to 64 years (enrolled in Stage 1 and Stage 2) and minimum of 200 subjects aged 65 years and older.

4.2 Number of Study Participants

The study population will comprise approximately 1000 healthy US adults who meet the eligibility criteria listed in Section 4.4.

Approximately 800 eligible subjects aged 50 to 64 years (with a minimum of 200 subjects aged 60 to 64 years) and a minimum of 200 subjects aged 65 years and older will be enrolled.

4.3 Estimated Study Duration

The Screening period is 30 days, the Treatment and Observation period is from Day 1 to Month 1 (30 days after vaccination), and the Follow-Up period is through Month 6 (180 days after study vaccination). The maximum possible study duration for an individual is 7 months (210 days).

4.4 Study Population

4.4.1 Inclusion Criteria

Subjects must meet all of the following criteria to be enrolled:

1) Male or female aged 50 to 64 years (inclusive) for Stage 1, or 50 years and older (inclusive) for Stage 2 at the time of randomization into the study.
2) Able and willing to complete the informed consent process.
3) Available for clinical follow-up through the last study visit at 6 months after the study vaccination.
4) In good general health as determined by medical history, vital signs, physical examination, and clinical judgment of the Investigator.
5) Willing to have blood samples collected, stored indefinitely, and used for research purposes.
6) Able to provide proof of identity to the satisfaction of the study staff completing the enrollment process.
7) Women of childbearing potential, defined as premenopausal females capable of becoming pregnant, must have a negative urine pregnancy test immediately prior to randomization and agree to use acceptable contraception, defined as: condoms, male or female, with or without a spermicide; diaphragm or cervical cap with spermicide; intrauterine device; contraceptive pills or patch, Norplant, Depo-Provera; or a female with a male partner that has previously undergone a vasectomy. Subjects must agree to consistently practice contraception if sexually active at least 7 days prior to enrollment and throughout the duration of the study.
8) Able to access and use a smartphone, tablet, computer, or other device connected to Wi-Fi or cellular network for completion of an electronic diary.

4.4.2 Exclusion Criteria

Subjects who meet any of the following criteria cannot be enrolled:

1) Previous pneumococcal disease (either confirmed or self-reported).
2) Previous receipt of a licensed or investigational pneumococcal vaccine.
3) Receipt of any investigational study product within 30 days prior to enrollment into the study, currently participating in another interventional investigational study, or having plans to receive another investigational product(s) while on study.

4) Planned or actual administration of any licensed vaccine during the period starting 30 days before enrollment into the study through Month 1.

5) Physical examination indicating any clinically significant medical condition.

6) Body Temperature >38.0°C (>100.4°F) or acute illness within 3 days prior to study vaccination (subject may be rescheduled).

7) Previous or existing diagnosis of HIV, Hepatitis B, or Hepatitis C.

8) History of severe allergic reaction with generalized urticaria, angioedema, or anaphylaxis.

9) Female who is pregnant, breastfeeding, or planning to become pregnant during study participation.

10) Bleeding disorder diagnosed by a doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions) resulting in clinically significant bruising or bleeding difficulties with IM injections or blood draws.

11) Any other chronic or clinically significant medical condition that, in the opinion of the Investigator, would jeopardize the safety or rights of the subject or confound evaluation of the study vaccine, including but not limited to diabetes mellitus type I, untreated hypertension, chronic hepatitis or clinically significant forms of drug or alcohol abuse, asthma, autoimmune disease, psychiatric disorders, heart disease, pulmonary disease, neurologic disease, cancer, or any known or suspected impairment of immune function.

12) Any medical, psychiatric, or social condition that in the judgment of the Investigator is a contraindication to protocol participation or impairs a subject's ability to give informed consent.

13) Received blood or blood product (including Immune Globulin IV) within 90 days prior to enrollment into the study.

14) Received systemic corticosteroids (except for inhaled, topical, intra-articular) for ≥14 consecutive days and has not completed treatment ≤30 days prior to enrollment into the study.

15) Receiving immunosuppressive therapy, including chemotherapeutic agents used to treat cancer or other conditions, immunostimulants, and treatments associated with organ or bone marrow transplantation, or autoimmune disease.

16) History of malignancy ≤5 years before enrollment, except for adequately treated basal cell or squamous cell skin cancer or in situ cervical cancer.

5. Study Vaccine

5.1 Investigational Vaccine (VAX-31)

VAX-31 is the Sponsor's research name for a 31-valent pneumococcal conjugate vaccine. VAX-31 comprises capsular polysaccharide antigens of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 8, 9N, 9V, 10A,11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 31, 33F, and 35B, with each of the 31 polysaccharides (1 from each serotype) individually conjugated in a site-specific manner to a proprietary non-toxic diphtheria carrier protein referred to as eCRM.

[redacted] Each vial is filled to deliver a single 0.5 mL dose of VAX-31 vaccine for IM administration, with no preservative.VAX-31 is manufactured by Laboratoire Baccinex (Switzerland) and will be supplied at 3 strengths in single-use 2 mL glass vials. The vial is sealed with a 13 mm bromo butyl rubber stopper with FluroTec® coating on the side in contact with VAX-31 DP. The stopper is capped with a 13 mm crimped aluminum seal with a flip-off white cap for the Low dose level, a blue cap for the Mid dose level, and a natural colored cap for the High dose level.

The 3 different dose levels of VAX-31 will be identified as:

- Low, 1.1 mcg of Polysaccharide (PS)/dose per serotype
- Mid, 2.2 mcg of PS/dose per serotype
- High, 3.3 mcg of PS/dose per serotype.

Conjugate Drug Substance serotypes 1, 5, and 22F are present at 1.65 mcg for Low, 3.3 mcg for Mid, and 4.4 mcg for High dose levels.

The amount of eCRM also varies by dose level. The Low dose level contains ~30 mcg of eCRM, the Mid dose level contains ~60 mcg of eCRM, and the High dose level contains ~90 mcg of eCRM.

VAX-31 must be stored in a temperature-controlled refrigerator at 2°C to 8°C with controlled, limited access to authorized personnel only. The temperature should be monitored by checking and recording current and maximum/minimum temperature readings inside the vaccine storage unit at least once each working day and/or before dosing subjects that day.

5.1.1 Active Comparator

Prevnar 20 (PCV20), 20-valent pneumococcal conjugate vaccine, is a sterile suspension of saccharides of the capsular antigens of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F individually linked to nontoxic diphtheria CRM_{197} protein. PCV20 is manufactured by Wyeth Pharmaceuticals, LLC (subsidiary of Pfizer Inc.) and is commercially available in the US. Each 0.5 mL dose of the vaccine is formulated to contain approximately 2.2 mcg of each *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F; 4.4 mcg of the polysaccharide from serotype 6B; 51 mcg CRM_{197} carrier protein; and 100 mcg polysorbate 80, 295 mcg succinate buffer, 4.4 mg sodium chloride, and 125 mcg aluminum as aluminum phosphate adjuvant. PCV20 prefilled syringes (PFS) are to be stored at 2°C to 8°C (36°F to 46°F), as required by the manufacturer.

Refer to the PCV20 Package Insert for clinical dosage and administration, storage and handling, contraindications, warnings and precautions, and summary of clinical study results.

5.1.2 Ancillary Supplies

The following supplies are provided by the Sponsor for preparation and administration of study vaccine:

- 1 mL BD Luer-Lok™ Syringe sterile, single use polycarbonate
- 1-1/2 inch 25 Gauge Safety Hypodermic Needle Eclipse BD™ single use, sterile

5.2 Dose Preparation of Study Vaccines

A central supply depot(s) will provide study vaccines and ancillary supplies.

Unblinded site personnel will manage all aspects of the vaccine inventory; prepare the vaccine for administration; administer the vaccine and active comparator to study subjects; and store and monitor the vaccine in a secure area and maintain all accountability documentation. All study site personnel who complete subject assessments will be blinded to the vaccines being administered to study subjects. Unblinded site personnel will be trained in all aspects of vaccine management, preparation, and administration, and will be provided a Pharmacy Manual regarding such procedures.

5.2.1 VAX-31

VAX-31 should be inspected visually for particulate matter and discoloration prior to administration. The product should not be used, and the Sponsor should be notified if particulate matter or discoloration is found. VAX-31 is a suspension containing ALPO and should be

shaken vigorously (for at least 15 seconds) immediately prior to use to obtain a homogenous, white suspension in the vaccine container. The vaccine should not be used if it cannot be resuspended. All VAX-31 doses are to be withdrawn into Sponsor-provided syringes using sterile technique. No dilution or reconstitution is necessary. VAX-31 vials must be stored upright and brought to room temperature (≥10 minutes) and administered between 10 to 60 minutes, inclusive, from the time the vaccine is removed from the refrigerator. Detailed instructions on packaging, preparation, administration, and accountability are found in the VAX31-101 Pharmacy Manual.

5.2.2 PCV20

PCV20 prefilled syringes (PFS) should be inspected visually for particulate matter and discoloration prior to administration. The vaccine should not be used and the Sponsor should be notified if particulate matter or discoloration is found. PCV20 is a suspension containing ALPO and should be shaken vigorously immediately prior to use to obtain a homogenous, white suspension in the vaccine container. The vaccine should not be used if it cannot be resuspended. PCV20 will be supplied to the clinical sites as single-use PFS packaged by the manufacturer and should be stored horizontally. PCV20 should be administered by IM injection as soon as possible after being removed from refrigeration. Preparation and administration instructions are found in the PCV20 Prescribing Information (manufacturer's package insert) and should be adhered to.

5.2.3 Study Vaccine Administration

Each vaccine (VAX-31 or PCV20) is to be administered IM as a single dose in the deltoid muscle of the upper arm.

5.3 Accountability Procedures for Study Vaccines

It is the responsibility of the Investigator to supervise accurate monitoring of the receipt, storage, dispensing, and accounting of all study vaccines according to accepted industry practice. Sites must maintain and retain accurate, original site records of study vaccine inventory as well as all shipping documentation, including temperature data, invoices of shipments and records of study vaccine distribution.

Vaccine accountability information will be collected on paper source documents for tracking study vaccines (e.g., date material was received, dispensed to individual subjects, and amount used and unused on site etc.).

Each site must keep all used study vaccine cartons until the unblinded CRA has performed vaccine accountability and arranges return of unused study vaccines to the central repository

vendor. Expired vaccine should be separately quarantined until the unblinded CRA has completed accountability monitoring.

Detailed instructions on packaging, preparation, administration, and accountability are found in the VAX31-101 Pharmacy Manual.

6. Study Procedures

Source documents and source data should meet the elements of data quality and integrity (ALCOA-C: attributable, legible, contemporaneous, original, accurate, and complete).

6.1 Informed Consent

The Investigator must obtain informed consent from study subjects at the Screening visit prior to starting any study-related activities. All prospective subjects must sign and date an Institutional Review Board (IRB)-approved ICF. For further details on informed consent, refer to Section 11.3.

6.2 Screening

Screening procedures are listed in the Schedule of Events (Appendix A).

Each subject who signs an ICF will receive a sequential 3-digit identification number unique to the site (e.g., 001, 002, etc.). Upon entry of a subject into the Screening Form within the electronic data capture (EDC) system, Zelta, each subject will be automatically assigned a unique Subject identifier. This Subject ID will be in the following format:

Study Number – 2-digit site number – 3-digit identification number: **VAX31-101-01-001**

Subject numbers will also be sequential since screen-failed subjects will be entered into the EDC system.

Each site is required to record the reason(s) for screen failure for all subjects who receive a subject identification number within the EDC system. Sites may elect to use a prescreening consent form and maintain a prescreening log for those subjects contacted for the study and will provide that log to the Sponsor or designee on request.

Rescreening: A subject who meets exclusion criterion of having a current acute febrile illness at the time of scheduled enrollment may be rescreened after resolution of the acute illness. A subject who has taken any prohibited medication within the exclusionary window may be rescreened after the appropriate duration has passed. If an eligible subject is not able to be randomized and treated within 30 days of the screening period, all screening procedures must be

conducted again, including reconsenting and assigning a new subject ID number. A subject may be rescreened once only. Rescreening is not otherwise permitted.

If a subject is rescreened outside of the initial 30-day screening window, a new subject number will be assigned as they are entered into the EDC for a new Screening visit and the previous subject number will be captured in the source file of the newly assigned subject number.

6.3 Medical History

Medical history information will be collected from subjects at the Screening Visit and confirmed at the Day 1 (Baseline) Visit. Medical history will include but not be limited to demographic information, current and past medical conditions, and prior and concomitant medications taken within 30 days prior to Day 1, including history of vaccinations.

In consideration of the availability of commercial pneumococcal vaccines and ongoing investigational vaccine clinical research, the history of pneumococcal vaccination must be collected and, if the subject's medical records and/or state-mandated vaccine registry are available, the site is required to maintain this documentation as part of the eligibility review.

6.4 Physical Examination

A complete physical examination will be performed on subjects during the Screening visit. The examination should include:

- Height
- Body weight
- Vital signs
- General appearance
- Eyes-ears-nose-throat
- Head-neck
- Lungs-chest
- Heart
- Abdomen
- Musculoskeletal
- Lymph nodes
- Skin
- Extremities
- Neurological

A physical exam may be performed on subjects at additional time points if indicated by AE reporting.

6.5 Vital Signs

Vital signs collected from subjects will include blood pressure, heart rate, respiratory rate, and oral temperature. The first set of Screening vitals are to be collected for inclusion of the subject into the study. Repeat measurements on abnormal vital parameters are allowed 1 additional time

for inclusion into the study. After Day 1, abnormal vital signs can be repeated for confirmation of clinical significance. Vital signs should be taken after at least 5 minutes in supine position.

6.6 Laboratory Tests

6.6.1 Blood Volume

Blood is collected by venipuncture, in the appropriate blood container tubes according to the schedule of sample collection shown in Appendix A and Table 1. The maximum volume of blood taken from a subject is 43 mL. Phlebotomy will observe the American Red Cross limit of no more than 450 mL in any 8-week period.

Table 1 Blood Collections

Study Day	Blood Volume (mL)	Laboratory Tests	Tube Type/Volume	Minimum No. of 1 mL Cryovials
Day 1	21.5 mL	Hematology Clinical Chemistry Immunogenicity	1 EDTA 2 mL 1 SST 2.5 mL 2 SST 8.5 mL	Not applicable Not applicable 12
Month 1	21.5 mL	Hematology Clinical Chemistry Immunogenicity	1 EDTA 2 mL 1 SST 2.5 mL 2 SST 8.5 mL	Not applicable Not applicable 12

Note: All cryovials should be frozen and stored at -20°C or colder and shipped on dry ice.

Samples are obtained during the trial according to Appendix A. At each time point, blood is collected in the appropriate blood tube(s).

To minimize the impact of lost or damaged shipments, the frozen serum samples will be sent in separate consignments (on different days to minimize risk of transport delays or loss) to the central laboratory.

Further details regarding specimen collection, processing, and shipping will be provided in the Central Laboratory Manual.

6.6.2 Safety Laboratory Assessments

Biological samples will be collected for the following clinical laboratory tests and shipped to the central laboratory for analysis:

- **Hematology:** Hemoglobin, White blood cell count (absolute), Neutrophils (absolute), Eosinophils (absolute), Platelet count, Blood Film Review, Manual Differential (Panel)

- **Clinical Chemistry:** Albumin, Alkaline phosphatase, Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Bilirubin (total and direct), Blood urea nitrogen, Calcium, Carbon dioxide, Chloride, Cholesterol and triglycerides, Creatinine, Glucose (random), Gamma-glutamyl transferase (GGT), Lactate dehydrogenase, Phosphate, Potassium, Sodium, Total serum protein, Uric acid
- **Urinalysis:** Specific gravity, pH, Glucose, Protein (Total), Ketones, Bilirubin, Urobilinogen, Hemoglobin, Leucocyte esterase, Nitrite

Urine pregnancy tests will be conducted at the site using a dipstick before vaccination.

Unscheduled laboratory tests will be performed if indicated by a change in medical condition (including clinically significant abnormal values). The volume to be collected will be appropriate to the tests performed. The toxicity grading scales for clinical laboratory values (FDA, 2007) must be used when assigning severity to an out-of-range laboratory value; however, not all out of range laboratory values are considered AE and need to be evaluated according to Section 8.1.1.

Further details regarding specimen collection, processing, and shipping will be provided in the Central Laboratory Manual.

6.6.3 Immune Response Assessments

Immunogenicity samples should be collected for all subjects randomized in the study. Serum antibody responses to pneumococcal polysaccharides induced by vaccination will be measured using 2 standard assays, OPA and multiplexed Meso Scale Discovery (MSD).

The OPA assay has been developed and qualified by Nexelis, a GCP-compliant central laboratory, to analyze the functional responses to 31 polysaccharide antigens contained in VAX-31. The OPA assay has been consistently used as the method for measuring the functional capacity of pneumococcal antibodies, as antibodies to pneumococcal PS protect the host by opsonizing pneumococci (marking pneumococci for destruction) for phagocytosis (Burton, 2012).

The MSD assay has been developed and qualified by Nexelis to measure IgG responses against the 31 polysaccharide antigens contained in VAX-31. The MSD multiplex electrochemi-luminescent assay is an immunoassay platform that allows simultaneous measurement of responses against multiple antigens and has been used to evaluate responses to various PCV (Feyssaguet, 2019; Goldblatt, 2011; Marchese, 2009; Nolan, 2020).

The immune responses (as assessed by OPA and MSD assays) against the 20 polysaccharide antigens common to PCV20 and VAX-31 (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B,

18C, 19A, 19F, 22F, 23F and 33F) will be compared in the various treatment groups (VAX-31 at 3 different dose levels, and the comparator vaccine), and the 11 additional polysaccharide antigens contained in VAX-31 (2, 7C, 9N, 15A, 16F,17F, 20B, 23A, 23B, 31 and 35B) will be evaluated separately.

6.7 Pregnancy Testing and Contraception

Female subjects of childbearing potential, defined as premenopausal females capable of becoming pregnant, will undergo a urine pregnancy test at Screening and immediately prior to vaccination. The subject must have a negative pregnancy test prior to study vaccine administration. The Investigator must report any pregnancies as described in Section 8.7.1. Female subjects of childbearing potential must also use an acceptable method of contraception from prior to Day 1 through Month 6 (throughout the study). The Investigator must confirm that contraception methods were initiated prior to Day 1 (e.g., hormonal contraception) to be considered fully effective.

For reporting a pregnancy, refer to Section 8.7.1.

6.8 Randomization

Subject eligibility will be confirmed and documented by the Investigator immediately prior to randomization of each subject.

Blinded study staff will indicate on a Randomization electronic Case Report Form (eCRF) within the EDC system that they want to generate a randomization number for the subject. When they indicate yes, a randomization number will be generated from EDC. The randomization number is separate from, and does not replace, the subject identification number which is assigned at Screening. The unblinded designee will receive information from the Zelta EDC with treatment assigned for VAX-31 (1.1/1.65 mcg, 2.2/3.3 mcg, or 3.3/4.4 mcg dose) or treatment assigned PCV20 PFS. The unblinded designee may then prepare the treatment assigned for administration.

Randomization will be 1:1:1:1 for the 4 treatment groups across the sample size (1000 subjects). For Stage 1, there were no stratification factors. For Stage 2, randomization will be stratified by each of the 25 clinical sites. See Section 10.3 for further details on the sample sizes within each age group.

A subject will be considered enrolled once a randomization number has been assigned within the EDC system. The study will be conducted as an observer-blind study. No one except specific predesignated unblinded staff (at sites and CRO) will know subjects' individual treatment assignments until all subjects have completed their participation in the study and the database has been cleaned and locked.

The following safeguards will be employed to reduce the risk of inadvertent unblinding:

- Each clinical site is required to have a blinding plan that outlines its process on blinding the subject in situations such as administration of IM injection with different syringe types, transport of study vaccine to the clinic room for administration, providing initial training and reminders to the subjects on how to maintain the study blind.
- No Sponsor personnel other than the designated unblinded monitor(s), Sponsor unblinded Oversight Representative, third-party unblinded biostatistics and programming staff, and the Sponsor Medical Director, if necessary, will have access to the randomization treatment assignments. If Sponsor personnel are unblinded, they will not provide input on data for the specific subject they were unblinded to.
- Should any subject or blinded staff member become inadvertently unblinded, the Investigator will promptly (within 24 hours of the Investigator's awareness of the error) disclose the event to the Sponsor clinical study manager and medical monitor in a blinded fashion (disclosing only subject number, not treatment) so that corrective action can be initiated. The unblinding sequence of events will be documented as a protocol deviation and retained as source documents. See Section 6.15 for details on Emergency Unblinding.

6.9 Study Vaccine Administration

On Day 1 before randomization, the medical history, including physical exam (if indicated by updated medical history) and vital signs, and concomitant medications will be collected, females of childbearing potential must have a negative urine pregnancy test, and information updated in the subject file to recheck subject eligibility. Immediately prior to study vaccine administration, the following biological samples will be collected: blood for hematology and chemistry, urine for urinalysis (and if applicable, pregnancy test), and serum for immunogenicity. Once these procedures are performed, study vaccine will be administered. All doses of study vaccine are 0.5 mL in volume and are administered by IM injection with a Sponsor-provided 1 mL syringe and Safety Hypodermic Needle Eclipse 1.5 inch 25 gauge as described above or PCV20 PFS, using universal precautions and sterile technique. All injections will be administered into the deltoid muscle. Injections will be administered by an unblinded staff member delegated by the Investigator.

6.10 Acute Observation

The subject will be monitored by blinded study staff for signs of an acute adverse reaction for at least 30 minutes after the injection and the blinded study staff will document any AE. Vital signs will also be obtained at least 30 minutes after the injection.

Allergic reactions to the study vaccine are possible; therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available and a medically qualified study team member trained to recognize and treat anaphylaxis must be present in the clinic during the entire post-dosing monitoring period.

6.11 Solicited Adverse Events

Solicited AE will be collected for 7 days after vaccine administration, starting on Day 1. Solicited AE for this study are local events of pain, erythema (redness) and edema (swelling) at the injection site, and systemic events of oral temperature ≥100.4ºF, fatigue, headache, muscle pain, and joint pain.

Subjects will be trained to complete an electronic diary (eDiary) via Zelta's electronic Patient Reported Outcomes (ePRO) system. The eDiary collects the primary safety endpoint data. This system is accessed via a secure URL and will be used to observe, measure, and record these solicited AE. A digital thermometer will be provided to the subject to measure oral temperature each day, and it is to be recorded daily in the diary. To record injection site local reaction, a ruler will be provided to the subject to measure the diameter of redness and swelling at the largest point of the reaction each day; it is to be recorded daily in the diary. Study staff will be provided with a Reference Guide for instructing subjects on accessing the Zelta ePRO system. Each user will be provided with login credentials and a temporary password. The Zelta ePRO system is web-based and accessed via a secure URL.

Study staff will review the signs and symptoms recorded in the diary and the action taken for the event during the subject's clinic visit. The electronic diary will serve as the primary source document and data will be automatically integrated into the subject visit Solicited AE form in the EDC platform for site staff view-only access. The Investigator will then assess all recorded solicited events for severity and relatedness. Severity will be graded according to the FDA Toxicity Grading Scale (FDA, 2007). The results of the Investigator's assessment, if different from the subject's entry, will be recorded in a separate eCRF specified for Investigators. For solicited injection site reactions, the relationship to study vaccine will be automatically recorded to be probably related, however, the Investigator will determine the relationship for each systemic solicited reaction.

Symptoms continuing beyond the solicited AE collection period (7 days following injection) will be collected and recorded as unsolicited AE in the source document. The start date of unsolicited AE will be equal to the date of onset of the solicited AE.

6.12 Unsolicited Adverse Events

Unsolicited AE (any AE not listed in the diary) will be collected for this study. Details regarding definitions, evaluation, reporting periods, and documentation are outlined in Section 8.

6.13 Prior and Concomitant Medications and Procedures

At the Screening visit, the details of prior and concomitant medications and procedures (through 30 days prior to Day 1) usage will be collected, including history of vaccinations. From Day 1 through Month 1, the details of all concomitant medications and procedures including those associated with solicited AE and unsolicited AE, not qualifying as SAE, NOCI or MAAE, will be collected.

Concomitant medications and procedures associated with an SAE, NOCI, and MAAE will be collected through the end of the study.

6.14 Prohibited Medications and Therapies

Subjects must not have received or be planning to receive:

- Licensed or investigational pneumococcal vaccine.
- Investigational agents or study product from 30 days prior to Day 1 through the duration of the study.
- Other (non-pneumococcal) licensed vaccines from 30 days prior to Day 1 through Month 1.
- Blood or blood product (including IGIV) from 90 days prior to Day 1 through the duration of the study.
- Systemic immunosuppressant therapy (e.g., chemotherapeutics) and immunostimulants.
- Immunomodulatory medications (e.g., oral corticosteroids, allergy shots) from 30 days prior to Day 1 through Month 1 or received systemic corticosteroids (except for inhaled, topical, intra-articular) exceeding physiologic replacement doses from 14 days prior to Day 1 through Month 1.

The history of all prohibited medications at any time during study participation (regardless of association with an AE) will be collected.

6.15 Emergency Unblinding

The Investigator may obtain a treatment assignment for a study subject only in the case of a medical emergency in which knowledge of the treatment is necessary for management of an AE.

The Investigator must notify the Medical Monitor (and as backup, Medical Director) no later than 24 hours following unblinding. Additional details for this unblinding process are provided in the Pharmacy Manual.

6.16 Protocol Deviations

The Investigator and delegated staff are responsible for conducting the study in accordance with the protocol. Any deviation from the protocol must be documented in the study file. Unblinded study personnel will document protocol deviations in a blinded manner for the blinded study personnel to record in the EDC. In addition, deviations must be reported to the IRB as applicable. Subject-specific deviations must be recorded in the subject's source documents. The Sponsor will review all protocol deviations on an ongoing basis and will be responsible for categorizing protocol deviations.

6.17 Dosing Errors

The Investigator or designee must report any error in the dosing of study vaccine to the site-assigned unblinded CRA and Sponsor Medical Monitor or designee within 24 hours of the Investigator's awareness of the error. Additional information regarding the dosing error may be provided as a follow-up report. A dosing error without signs or symptoms is not considered an AE but may be determined to be an important protocol deviation and investigated further.

6.18 Study Completion for Individual Subjects

An individual subject is considered to complete study participation after completion of the Month 6 visit (180 days after study vaccine administration) and completion of any required safety follow-up.

6.19 Early Termination

An individual subject is considered to undergo Early Termination if the subject stops study participation before the Month 6 visit.

An enrolled subject may voluntarily withdraw consent for further participation at any time before the Month 6 visit. The Investigator will request (but cannot require) such subjects to provide the reason(s) for withdrawal of consent and to undergo an Early Termination visit.

In addition, the Investigator, at his or her discretion, may withdraw a subject from further participation in the study. Criteria for withdrawal by the Investigator include:

- Noncompliance with the protocol
- Pregnancy
- Immediate hypersensitivity reaction associated with a study injection

- Lost to follow-up status requires documentation of at least 3 unsuccessful attempts to contact the subject. Lost to follow-up will be determined after the date of the subject's projected last visit.
- Other reason(s) which, in the opinion of the Investigator, indicate that continued participation in the study is not in the best interest of the subject.

The Investigator or designee must report any early termination for safety reasons to the Sponsor Medical Monitor or designee within 24 hours of discontinuation. Additional information regarding ongoing AE may be provided as a follow-up report.

6.20 Study Completion: Overall

The study is planned to be completed after all subjects have completed the Month 6 visit (or Early Termination, 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.

7. Study Procedures by Visit

The overall summary of procedures and assessments by visit is provided in the Schedule of Events (Appendix A). All visits are relative to the day of vaccine administration, Day 1. Acceptable time windows for the visit schedule are indicated.

7.1 Scheduled Study Visits

7.1.1 Screening (-30 days to Day 1)

The following will take place during the Screening visit, which will occur within 30 days prior to Day 1, or the visit may be combined with Day 1 (preferred):

- Informed consent
- Demographics
- Review of eligibility criteria
- Medical history
- Physical exam
- Vital signs
- Urine pregnancy test for females of childbearing potential
- Prior and concomitant medications review

Screening and Day 1 Visits are encouraged to be combined into the same day to complete 1 set of procedures.

7.1.2 Day 1

If the subject had a separate Screening visit, the following procedures will take place during the Day 1 visit and ***before*** study vaccine administration:

- Updated medical history
- Confirm Inclusion/Exclusion criteria are met
- Targeted physical examination, if indicated by updated medical history
- Vital signs
- Biological specimen collection for clinical laboratory tests:
  - Blood for hematology and chemistry
  - Urine for urinalysis
  - Serum for immunogenicity
- Urine pregnancy test for females of childbearing potential
- Prior and concomitant medications review
- Randomization

All eligible, consented, and randomized subjects will be vaccinated at the Day 1 Baseline visit.

- Study vaccine administration, either VAX-31 or PCV20

The following procedures will take place during the visit and ***after*** study vaccine administration:

- Acute observation for at least 30 minutes after vaccine administration
- Vital signs collected at least 30 minutes after vaccine administration
- AE (solicited and unsolicited) and SAE evaluation
- Review of concomitant medications taken after vaccination
- Instructions on completion of a diary (login to new account for electronic diary), use of a ruler and thermometer

7.1.3 Day 8 (+3 days)

The following procedures will take place at this visit:

- Visual inspection of injection site location
- Review of diary data with the subject; Investigator's assessment of severity and relationship to study vaccine
- Review of concomitant medications
- AE (solicited and unsolicited) and SAE evaluation

7.1.4 Day 15 (+3 days)

This visit is a follow-up phone call for safety evaluation. The following procedures will take place at this visit:

- Review of concomitant medications
- AE (unsolicited) and SAE evaluation

7.1.5 Month 1 (30 days after Day 1, ±3 days)

The following procedures will take place during this visit:

- Review of concomitant medications
- AE (unsolicited) and SAE evaluation
- Biological specimen collection for clinical laboratory tests:
  - Blood for hematology and chemistry
  - Urine for urinalysis
  - Serum for immunogenicity

7.1.6 Months 2, 3, 4, 5, and 6 (60 to 180 days after Day 1 [±5 days])

These visits are follow-up phone calls for safety evaluation. The following procedures will take place at each visit:

- SAE, NOCI, and MAAE evaluation
- Review of concomitant medications or vaccines potentially related to SAE, NOCI, MAAE, or prohibited medications

7.2 Early Termination Visit

All subjects who discontinue study participation before the Month 6 visit will be requested to undergo an Early Termination (ET) visit.

- Review of diary data with the subject; Investigator's assessment of severity and relationship to study vaccine (if ET is within 7 days)
- Review of unsolicited AE and SAE
- Review of concomitant medications associated with new or ongoing AE and any SAE
- Biological specimen collection for clinical laboratory tests (if ET is after Day 8 and before Month 1)
  - Blood for hematology and chemistry
  - Urine for urinalysis
  - Serum for immunogenicity

7.3 Unscheduled Visits

Any study procedure, excluding study vaccination, may be conducted at an unscheduled visit as needed. Examples include repeat specimen collection and additional safety follow-up for an AE.

8. Safety

8.1 Definitions

8.1.1 Adverse Event

An AE is any untoward medical occurrence in a study subject, regardless of the suspected causal relationship with study vaccine. The definition of an AE includes:

- A new-onset symptom or disease
- An exacerbation of a pre-existing symptom or disease
- A new-onset clinical laboratory abnormality considered by the Investigator to be clinically significant
- A new-onset symptom or disease that occurs as a result of a protocol-specified procedure

The definition of an AE does ***not*** include:

- A pre-existing symptom or disease that does not worsen during the study (even if first disclosed by the subject after the start of the study)

- A medical or surgical intervention such as surgery, endoscopy, tooth extraction, or transfusion (although the condition leading to the procedure or a complication from the procedure may be an AE)
- An uncomplicated pregnancy
- A dosing error without any resulting signs or symptoms
- Any other situation where an untoward medical occurrence has not occurred (e.g., hospitalization for cosmetic elective surgery or social admissions)

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

The definition of a medically attended adverse event (MAAE) includes:

- A new onset or worsening of a condition that prompts the subject to seek unplanned medical advice at a physician's office, urgent care center, or Emergency Department

The definition of a new onset of chronic illness (NOCI) includes:

- A new onset of a disease or condition that requires ongoing medical attention and/or limits activity of daily living, e.g., heart disease, cancer, diabetes

The Investigator will attempt to establish a diagnosis based on signs, symptoms, and other clinical information. Whenever possible, the Investigator will report an AE as a diagnosis rather than 1 or more signs or symptoms. If a clinically significant laboratory abnormality meets the definition of an AE, a diagnosis or clinical signs and symptoms rather than the abnormal clinical laboratory finding should be reported if possible. If no diagnosis is known and clinical signs and symptoms are not present, but the laboratory abnormality is clinically significant by itself, then it should be reported as the AE.

8.1.2 Solicited Adverse Event

A solicited AE is a protocol-specified AE about which the Investigator or designee proactively asks the subject during a protocol-specified time period. Solicited AE for this study are local events of pain, erythema (redness), and edema (swelling) at the injection site, and systemic events of fever (oral temperature ≥100.4ºF), fatigue, headache, muscle pain, and joint pain.

8.1.3 Unsolicited Adverse Event

An unsolicited AE is an AE that is spontaneously reported by the subject or discovered by the Investigator. New onset of chronic illness(es) would be recorded as part of the collection of unsolicited AE as specified in the visit procedures. Unsolicited AE will be collected separately from solicited AE. Solicited AE occurring or persisting beyond the solicitation period or which meet SAE/NOCI/MAAE criteria are also recorded as unsolicited AE.

8.1.4 Serious Adverse Event

An SAE is an AE (either solicited or unsolicited) which meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires hospitalization or prolongs an existing hospitalization
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly or birth defect
- Important medical event that may jeopardize the patient or subject and/or may require medical or surgical intervention to prevent any of the above outcomes

The Investigator will evaluate all AE for seriousness using the above criteria.

“Life-threatening” means that in the opinion of the Investigator the subject was at immediate risk of death from the event as it occurred. It does not mean that the event might have caused death had it occurred in a more severe form.

Hospitalization for observation or for elective treatment of a pre-existing condition that did not worsen during the study is not considered an SAE.

Important medical events may be considered serious at the discretion of the Investigator.

These seriousness criteria also apply to the Study Stopping Rules in Section 8.10.

8.2 Severity Grading

The Investigator will grade all AE for severity. AE listed in the Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA, 2007) will be graded according to the criteria in the table. AE not listed in the Toxicity Grading Scale will be graded as follows:

- Mild (Grade 1): No interference with activity
- Moderate (Grade 2): Some interference with activity
- Severe (Grade 3): Significant; prevents daily activity
- Potentially Life-Threatening (Grade 4): ER visit or hospitalization

8.3 Causality Assessment

The Investigator will assess all AE, including solicited AE, for causality (relationship to study vaccine), assigning 1 of these 3 categories: Not Related, Possibly Related, or Probably Related.

An AE will be considered "Not Related" to study vaccine if **any** of the following conditions are met:

- An unreasonable temporal relationship between administration of the study vaccine and the onset of the AE (e.g., the event occurred either before or too long after administration of the study vaccine for it to be considered related).
- A causal relationship between the study vaccine and the AE is biologically implausible (e.g., injury as a passenger in an automobile accident).
- A clear alternative causality for the AE is present (e.g., typical adverse reaction to a concomitant medication).

An AE will be considered "Possibly Related" if there is a reasonable possibility that the AE may have been caused by the study vaccine.

- A single occurrence of an event that is uncommon and known to be strongly associated with vaccine exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).
- One or more occurrences of an event that is not commonly associated with vaccine exposure but is otherwise uncommon in the population exposed to the vaccine (e.g., tendon rupture).
- An aggregate analysis of specific events observed in a clinical study (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of vaccine administration) that indicates those events occur more frequently in the vaccine treatment group than in a concurrent or historical control group.

An AE will be considered "Probably Related" if there is evidence that the AE was caused by the study vaccine.

8.4 Follow-up of Adverse Events

The Investigator must follow all AE until resolution, until the condition stabilizes or is no longer clinically significant, or until no further information is likely to be obtained.

The Investigator is responsible for ensuring the conduct of any supplemental investigations considered necessary to evaluate the AE. These may include unscheduled clinical laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

In the event of a non-fatal SAE, subjects will be instructed to contact the Investigator (or designee) immediately. All subjects experiencing an SAE will be evaluated by the Investigator or designee as soon as is feasible following the report of the SAE by the subject. In the event of a fatal SAE, the Investigator must provide the Sponsor with any available post-mortem findings, including histopathology.

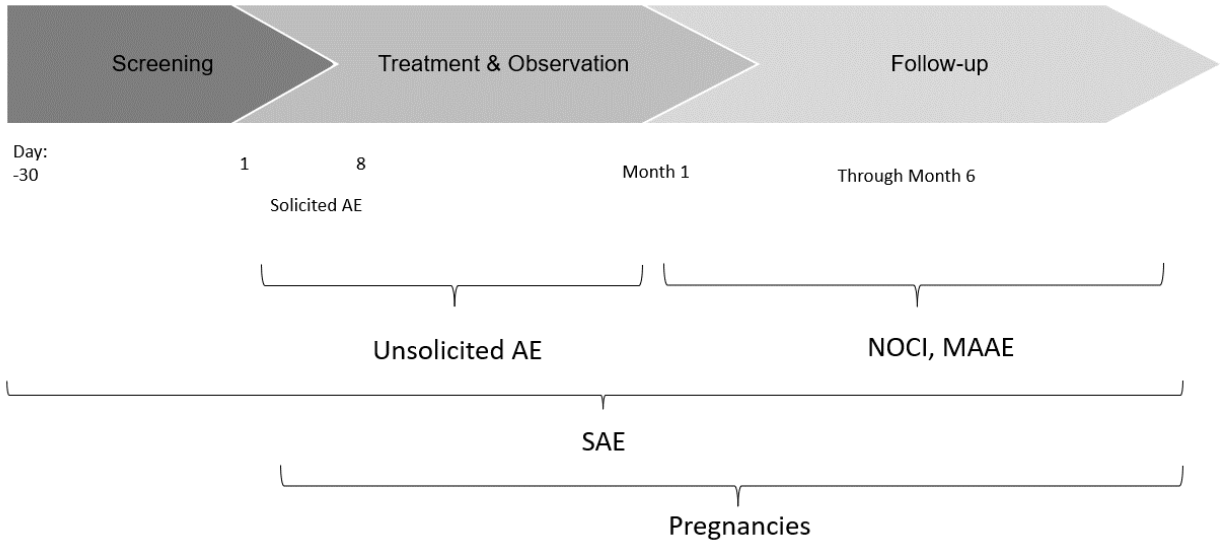
Additionally, the Sponsor may request that the Investigator perform or arrange for the conduct of supplemental investigations for 1 or more AE.

8.5 Reporting of Adverse Events

8.5.1 Reporting Periods

The reporting period for solicited AE begins immediately after study vaccine administration and continues for 7 days after the injection. The reporting period for unsolicited AE is immediately after study vaccine administration on Day 1, continuing through Month 1. AE that correspond to solicited AE terms but occur outside of (or continue past) the solicited AE collection periods are also collected through the unsolicited AE reporting periods. The reporting period for SAE begins at the time of informed consent and continues for the duration of study participation. A summary of the reporting periods is presented in Figure 3.

Figure 3 Safety Events Reporting Periods



8.5.2 Documentation

The Investigator or designee will document all AE in the subject's source documents and solicited AE in the source document file within 24 hours of awareness. All AE should include:

- Event term
- Start and stop date
- Severity
- Seriousness (Yes/No) and if Yes, which seriousness criteria were met
- Relationship to study vaccine
- Action taken in response to the AE
- Action taken with study vaccine

8.6 SAE Reporting

The Investigator or designee must report all SAE to the contract research organization (CRO) Drug Safety Group within 24 hours of becoming aware of the event, using the SAE Report Form. The Investigator or designee must also enter SAE into the source document.

The CRO will provide safety support for SAE report processing. Email transmission of the CRO SAE Report Form is the preferred method to transmit this information to the CRO Drug Safety group. If initial notification is provided via telephone, this does not replace the need for the

Investigator to complete and sign the CRO SAE Report Form within the designated reporting time frame.

Email to the CRO Drug Safety Group: VAX31-101-Safety@vaxcyte.com

The SAE Report Form should be completed as thoroughly as possible and be signed by the Investigator or designee before reporting to the CRO. The SAE Report Form must include an assessment of causality and should include a preliminary diagnosis if possible. All SAE assessed as "Not Related" must include an alternate causality.

To avoid delays in initial reporting, additional information regarding the SAE may be provided as a follow-up report. The Investigator may also modify the diagnosis, seriousness, and/or causality assessment based on this information.

The CRO will notify the Investigator of SAE that meet criteria for expedited reporting to regulatory authorities. The Investigator is responsible for notifying the applicable IRB of these events and adhering to any other applicable local reporting requirements.

The Sponsor will report AE to FDA in accordance with 21 CFR 312.32. Specifically, the Sponsor will report unexpected fatal or life-threatening suspected adverse reactions no later than 7 days after initial receipt of the information, and other serious and unexpected suspected adverse reactions (SUSAR) no later than 15 calendar days after determining that the information qualifies for expedited reporting. The Sponsor's Medical Director may be unblinded to a specific subject for SUSAR reporting.

Determination of which SAE are SUSAR, as defined in 21 CFR 312.32:

- **Suspected adverse reaction** means any AE for which there is a reasonable possibility that the drug caused the AE.
- **Unexpected adverse reaction** means an AE that is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

All SUSAR will be reported to FDA. SUSAR will be provided to all participating Investigators by the CRO.

For further information regarding SAE reporting, please refer to the VAX31-101 Safety Management Plan.

8.7 Other Events Requiring Immediate Reporting

8.7.1 Pregnancy

The Investigator or designee must report all pregnancies to the Vaxcyte Clinical Safety Team within 24 hours of becoming aware of the pregnancy, using the Pregnancy Report Form. All pregnancies will be followed to outcome. Additional information regarding the pregnancy may be provided as a follow-up report.

An uncomplicated pregnancy is not considered an AE. Complications of pregnancy may qualify as AE or SAE and would therefore be documented and reported as specified above.

8.8 Medical Monitor

The Medical Monitor will be the first point of contact regarding safety and eligibility questions and will assess AE and SAE that trigger the stopping rules and address all other safety related questions from the sites.

[redacted]

8.9 Data Monitoring Committee

The DMC will comprise 2 or more persons (independent Subject Matter Experts) and an unblinded statistician to compile the data and present to the members. The DMC will independently review the 7 days post-vaccination unblinded safety data for the Stage 1 subjects aged 50 to 64 years before advancing the study into Stage 2, enrolling the remaining adult population aged 50 to 64 years and 65 years and older. Stopping rules will be observed during the active study vaccine administration periods (see Section 8.10) and the DMC will conduct ad hoc reviews if the study is paused, triggered by one of the Stopping Rules, in order to identify any emerging safety concerns.

The DMC will refer to the DMC Charter for procedures.

8.10 Study Stopping Rules

The rules governing the stopping of the study vaccine administration at any time during the study are defined by the Stage 1 and Stage 2 segments of the clinical study. 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. The DMC may recommend resumption of enrollment after stopping if the DMC determines it is safe to proceed

with no modifications or with modifications to the protocol plans. Vaxcyte must approve the recommendation to resume enrollment.

Stage 1:

- 1 SAE assessed as at least possibly related to study vaccine
- 1 subject with a Grade 4 (potentially life-threatening) assessed as at least possibly related to study vaccine
- 2 subjects with the same or similar Grade 3 (severe) AE assessed as at least possibly related to study vaccine
- Any concern from the Investigator or study team that warrants a study halt

Stage 2:

- 1 SAE assessed as at least possibly related to study vaccine
- 1 subject with a Grade 4 (potentially life threatening) AE assessed as at least possibly related to study vaccine
- 2 subjects with the same or similar Grade 3 (severe) unsolicited AE assessed as at least possibly related to study vaccine other than solicited events
- Thirteen (13) subjects with the same or similar Grade 3 (severe) solicited AE
- Any concern from the Investigator or study team that warrants a study halt

The appropriate regulatory authority will be informed in writing when either of the following occurs:

- Study dosing is stopped.
- DMC has decided to resume or discontinue study activities.

Enrollment may be resumed following review of available safety data by the Sponsor.

Vaxcyte's clinical safety team will refer to the VAX31-101 Safety Medical Monitoring Plan for detailed procedures on stopping rule reporting and safety oversight for the study.

9. Data Handling

9.1 Source Documentation

Source documents and source data will meet elements of data quality and integrity (ALCOA-C: attributable, legible, contemporaneous, original, accurate, and complete).

The Investigator must maintain source documentation of all study conduct data and observations relevant to the study. This source documentation includes but is not limited to ICF, original medical records, progress notes from the Investigator and study staff, laboratory reports, eDiary for solicited AE, and documentation of study vaccine accountability.

The Investigator will maintain all study documentation, including copies of ICF, source documents, and documentation of study vaccine accountability, for either 2 years following FDA or other regulatory approval of VAX-31 or 2 years after clinical development of VAX-31 is discontinued, unless a longer period is required by applicable law or regulation. The Investigator will destroy study documentation only on instruction by the Sponsor and must notify the Sponsor on completion of such destruction. Subject identity information will be maintained for 15 years unless a longer period is required by applicable law or regulation.

These source data will be verified by the Clinical Research Associate (CRA) and the location of the source data documented before the start of the study. Remote monitoring of original source records may be performed, but on-site monitoring would occur for review of any paper source documents and their transcription into the system, etc.

9.2 Data Monitoring

The Sponsor or designee will monitor completed source documents at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The Investigator must make source documentation accessible to the Sponsor or designee as needed to verify the information in the source documents. The Investigator agrees to cooperate with the Sponsor or designee to ensure that any problems detected during data monitoring are resolved.

9.3 Electronic Patient Reported Outcome Data

This study will employ electronic transfers of external eDiary data generated from the subjects recording of solicited AE and actions taken for 7 days post-vaccination within a validated system that is 21 CFR Part 11 compliant. The eDiary is integrated into the EDC (Zelta) system and subject-reported outcome data are available as soon as the subject submits data. The eDiary is considered source documentation and should be limited to the subject's recording. The Investigator is responsible for the adequacy and accuracy of data associated with severity and relationship to study vaccine, and this is recorded on a separate source document from the eDiary.

9.4 Audit Compliance

The Investigator must permit the Sponsor and/or designee, regulatory agencies, and/or the IRB direct access to facilities and study documentation for the purpose of auditing study conduct. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are needed for the evaluation of the study.

9.5 Laboratory Data

This study will employ electronic transfers of external laboratory data generated from clinical specimens collected by the Investigator. The Investigator is responsible for the adequacy and accuracy of these specimens, and for determining clinical significance for all out-of-range values.

10. Statistical Analysis

This Phase 1/2 study is designed to achieve both safety and immunogenicity objectives.

The primary objectives are to evaluate the safety of a single injection of VAX-31 at 3 dose levels and to compare the safety of VAX-31 to that of PCV20 administered to 4 groups:

- Subjects aged 50 to 59 years receiving PCV20
- Subjects aged 50 years and older receiving PCV20
- Subjects aged 60 years and older receiving PCV20
- Subjects aged 65 years and older receiving PCV20

The secondary immunogenicity objectives are to assess the induction of OPA and MSD antibody responses by VAX-31 at 3 dose levels compared to the 20 PCV20 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F), as measured at 30 days after the injection (Month 1) and 11 non-PCV20 serotypes (2, 7C, 9N, 15A, 16F,17F, 20B, 23A, 23B, 31 and 35B), as compared to the standard regulatory criteria for efficacy (e.g., lower bound of the 2-sided 95% CI for the geometric mean ratio GMR for a given serotype greater than 0.5 for noninferiority of shared serotypes and the lower bound of the 2-sided 95% CI for the geometric mean ratio GMR for a given serotype greater than 2.0 for superiority of the novel serotypes), measured at 30 days after the injection (Month 1) for subjects aged 50 to 59 years, 50 years and older, 60 years and older, and 65 years and older.

A statistical analysis plan (SAP) will be generated and approved prior to the interim analysis and treatment group unblinding for the primary safety and secondary immunogenicity endpoints through Month 1 visits. Individual treatment unblinding will occur after the data are cleaned

through the last Month 6 visits and final database lock. A single completed Clinical Study Report (CSR) will summarize safety and immunogenicity findings through Month 6 for all 1000 subjects.

10.1 Sample Size Calculation

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.

10.2 Treatment Period

The Treatment period begins at the time of study vaccine administration and extends through the Month 1 visit. The Follow-up period spans the time following the Month 1 visit through Month 6 (180 days after study vaccine administration).

10.3 Treatment Groups

Subjects will be randomized into 4 treatment groups:

- 250 subjects will receive VAX-31 Low Dose
- 250 subjects will receive VAX-31 Mid Dose
- 250 subjects will receive VAX-31 High Dose
- 250 subjects will receive PCV20

For Stage 1, there were no stratification factors. For Stage 2, randomization will be stratified by clinical site.

10.4 Populations for Analysis

Randomized Population: All screened subjects who have informed consent, provide demographic and other baseline measurements, are randomized, and are assigned a study subject

ID (number). Each subject will be analyzed as randomized (i.e., according to the vaccine regimen to which the subject was randomized).

Exposed Population: All subjects who receive 1 study vaccination.

Safety Population: All subjects in the Exposed Population who provide safety assessment data. This generally includes any subject in the Exposed Population that is not lost to follow-up at Day 1, as they will be at risk for reporting an SAE. Each subject will be analyzed as treated (i.e., according to the vaccine regimen a subject received, rather than the vaccine regimen to which the subject may have been randomized).

Immunogenicity Evaluable Population: For this Phase 1/2 study, the immunogenicity population will be analyzed as treated (i.e., according to the vaccine regimen a subject receives, which may be different from the vaccine regimen to which the subject is randomized in the case of treatment errors).

The Immunogenicity Evaluable Population (IEP) includes all subjects in the Exposed Population who:

- Have no major protocol deviation that would impact immunogenicity assessment or other reason to be excluded as defined prior to unblinding or analysis.
- Have not received a prohibited medication or vaccine.
- Provide 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 study vaccine administration
  - Month 1: 30 days after vaccination ±5 days, inclusive

10.5 Demographic and Baseline Characteristics Analysis

The demographic and baseline characteristics will be summarized descriptively according to age group, treatment group, and overall. Age, height, weight, and body mass index at enrollment will be summarized by reporting the mean, standard deviation, median, minimum, and maximum. The frequencies and percentages of subjects by sex, race, and ethnicity will be presented.

Demographic data will be tabulated for the Randomized, Immunogenicity Evaluable, and Safety Populations.

10.6 Safety Analysis

All safety analyses will be based on the Safety Population.

10.6.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations will be summarized by age group and treatment group for the Randomized Population.

10.6.2 Solicited Adverse Events

With the exception of redness and swelling, all solicited AE (local and systemic) will be summarized according to severity grading scales defined in Section 8.2, from "mild" to "potentially life-threatening."

Solicited AE will be recorded daily until 7 days post-injection within an eDiary. The analyses of solicited AE (any event, after any injection, and after each injection) will be performed by maximum severity and by treatment group. In addition, solicited AE ongoing after the solicitation period (7 days post-injection) will also be recorded as unsolicited AE.

Frequencies and percentages of subjects experiencing each solicited AE will be presented by maximum severity. Summary tables showing the occurrence of any local or systemic solicited AE overall and at each time point will also be presented.

The severity of redness and swelling recorded as diameters (cm) will be summarized according to categories based on the largest diameter linear measurement when the local reaction is present:

- Grade 0/absent: 0–2.4 cm.
- Grade 1/mild: >2.5–5.0 cm.
- Grade 2/moderate: >5.1–10.0 cm.
- Grade 3/severe: >10.0 cm.

The following summaries of subjects will be performed:

- Solicited events by day post-injection, for each event and for any event.
- Time of first onset of solicited AE, after injection, for each event and any event.
- Solicited AE by maximum event severity, after injection, for each event and for any event.
- Duration of solicited AE, after injection, for each event and maximum duration of any event.
- Solicited AE, occurrence of at least 1 event by category (local, systemic), after injection.

For each of the time points or time intervals presented in the summaries, only subjects with at least 1 observation (i.e., any non-missing values but excluding "Not done/unknown") for the solicited AE will be summarized.

10.6.3 Unsolicited Adverse Events

All the unsolicited AE occurring during the study will be recorded, regardless of their assessment of relatedness by the Investigator.

The original verbatim terms used by Investigators to identify AE will be mapped to preferred terms using the MedDRA dictionary. The unsolicited AE will then be grouped by MedDRA preferred terms into frequency tables according to system organ class (SOC). All reported AE, as well as AE judged by the Investigator as at least possibly related to study vaccine, will be summarized by treatment group, according to SOC and preferred term within SOC. When an unsolicited AE occurs more than once for a subject, the maximum severity reported and strongest reported relationship to the treatment group will be counted.

Only TEAE will be summarized as defined in Section 8.1.1. The selection of unsolicited AE and their assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

Unsolicited AE will be summarized by alphabetic SOC and preferred term as follows:

- Any unsolicited TEAE
- Possibly or probably related unsolicited TEAE
- SAE
- Possibly or probably related SAE
- NOCI
- MAAE
- Unsolicited TEAE leading to withdrawal
- Any AE leading to death

Listings of all TEAE will be provided by subject.

10.6.4 Combined Solicited and Unsolicited Adverse Events

Solicited AE continuing beyond 7 days after an injection will be coded by MedDRA and combined with the unsolicited AE. An overall summary of subjects with all combined solicited and unsolicited AE, by SOC and preferred term, will be provided as well.

10.6.5 Analysis of Other Safety Data

The frequencies and percentages of concomitant medications will be tabulated by age group, treatment group, and overall. Medications will be coded using the WHODrug dictionary.

10.7 Immunogenicity Analysis

10.7.1 Geometric Mean Titer, Geometric Mean Concentration, and Geometric Mean Titer Fold Rise

Immunogenicity analyses will be performed by age group (50 to 59, 60 years and older, 65 years and older) and overall (50 years and older). Analyses will be performed for both OPA titers (log_{10}) and IgG titers (mcg/mL). All immunogenicity analyses will be based on the IEP and Exposed Population.

The Geometric Mean Titer (GMT) and Geometric Mean Concentration (GMC) will be analyzed via linear models. The primary model is an analysis of variance (ANOVA), with logarithmically-transformed titers (log_{10}) as the dependent variable and treatment group and study site as the fixed effects in the model. The results included in the main tables of the report will be based on this basic ANOVA model. As a secondary analysis, in order to remove the effect of baseline concentration on the GMT, an analysis of covariance (ANCOVA) will also be performed. The ANCOVA includes treatment group and study site as the fixed effects and log_{10} baseline titer as the 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.

The least squares means and their 95% confidence intervals (CI) calculated based on the ANOVA and ANCOVA will be back transformed and reported as the group GMT and GMC values (adjusted for the mean baseline in the case of the ANCOVA). GMR will also be calculated from the ANCOVA and ANOVA models.

Comparisons between relevant groups will be based on the estimated adjusted GMT measured at Month 1 for 31 serotypes in VAX-31 (of those 20 in PCV20), and mean square error calculated from the basic ANOVA model using contrast statements. The analysis of GMFR relative to Day 1 will also be computed using similar models.

The main comparison of interest will be the 3 VAX-31 dose level groups vs the PCV20 (20 serotypes) group. However, the 3 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.

10.7.2 Threshold Analyses

For the 11 non-PCV20 serotypes that are in VAX-31, the % of subjects with a ≥4-fold increase in OPA titer will be evaluated using a logistic regression model, with an indicator variable for achieving a ≥4-fold increase in OPA titer as the dependent variable, and treatment group and study site as fixed effects and $\log_{10}$ baseline titer as a covariate. The difference in proportions achieving a ≥4-fold increase in OPA titer in each of the 3 VAX-31 dose levels group vs the PCV20 and the corresponding 95% CI will be calculated. If the lower bound of the 95% CI is >0.1, the VAX-31 dose will be deemed statistically superior to PCV20.

The percentage of subjects achieving specified thresholds (e.g., GMFR ≥4 in serotype-specific OPA and IgG, achieving OPA titer of at least serotype-specific LLOQ of the assay), and associated Wilson (score) 95% CI, will be calculated for the Month 1 visit data for each treatment group. A reverse cumulative distribution curve will be provided. Differences between pair-wise treatment groups may be determined via Fisher's exact tests.

10.8 Defined Safety Evaluation

A defined safety evaluation will be conducted for the Stage 1 cohort of 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 DMC concludes that no safety concerns exist, the study will resume enrollment of the remaining subjects aged 50 to 64 years (approximately 736 subjects) as well as all subjects 65 years and older.

10.9 Interim Analysis

There will be a safety and immunogenicity interim analysis based on the data collected through Month 1. The results will be reported by treatment group preserving the double-blind status on the subject level.

For further information, please refer to the VAX31-101 Statistical Analysis Plan (SAP).

11. Additional Information

11.1 Ethical Conduct of the Study

The study will be performed in accordance with the protocol and consistent with ICH GCP Guidelines and applicable local regulatory requirements and laws.

11.2 IRB Oversight

The study (protocol, informed consent form, recruiting materials, and any documents seen by the subject) will be reviewed and approved by an IRB appropriate to each study site. Subjects will

not be recruited, consented, screened, or enrolled until the IRB has approved the required documentation. In addition, the IRB will review amendments to the protocol before their implementation.

The Investigator will retain all correspondence with the IRB in the trial master file and forward copies of all IRB approvals to the Sponsor.

11.3 Informed Consent

The Sponsor or designee will provide a master ICF template to each site for development of a site-specific ICF. At the Investigator's discretion, the site may develop a separate ICF for prescreening as described in Section 6.2.

All site-specific ICF must be approved by the Sponsor or designee and the IRB and must be in compliance with ICH GCP, local regulatory requirements, and legal requirements. The Sponsor or designee will advise the site of required changes to the master ICF template during the course of the study.

The Investigator will ensure that each potential study subject is fully informed about the nature and objectives of the study and possible risks associated with participation. Before informed consent is obtained, the Investigator, or a qualified person designated by the Investigator, will provide the potential study subject with ample time and opportunity to inquire about the details of the study, and will answer all relevant questions to the potential study subject's satisfaction. The potential study subject will then decide whether or not to participate in the study. The Investigator, or a qualified person designated by the Investigator, will obtain written informed consent from each study subject before any study-specific activity is performed.

The Investigator will retain the original and any amended signed and dated ICF at the study site and provide a copy to each study subject.

11.4 Subject Confidentiality

The Investigator will ensure that each subject's anonymity is maintained. In all documents submitted to the Sponsor and/or its designee, subjects can be identified by subject ID number and initials. Documents not intended for submission to the Sponsor and/or its designee (e.g., signed ICF) must be maintained by the Investigator securely and must be in compliance with all federal laws and regulations and ICH GCP Guidelines.

11.5 Compensation for Injury

The Sponsor will adhere to local regulations and guidelines regarding clinical study compensation to subjects whose health is adversely affected by taking part in the study. The applicable policy for compensation for injury will be described in the master ICF template.

11.6 Clinicaltrials.gov

For purposes of reporting to clinicaltrials.gov, the Sponsor is the responsible party and will provide information regarding this study in accordance with applicable regulations.

11.7 Public Disclosure and Publication Policy

All publication rights are delineated in the Clinical Study Agreement with the Investigator(s).

11.8 Amendments

The protocol may be amended only by the Sponsor.

The IRB must generally be informed of all amendments prior to implementation. In addition, the Investigator must obtain IRB approval for any amendments likely to affect the safety of study subjects prior to implementation.

The Sponsor may implement an amendment prior to IRB notification or approval only to eliminate an apparent, immediate hazard to study subjects. In that event, the Sponsor will notify the IRB in writing within 7 calendar days after the implementation.

Amendments, including descriptions and rationales, will be documented in this section of the protocol.

12. Appendix A: Schedule of Events

Study Event	Screening Visit Screening[a] (-30 days)	Visit 1 Day 1[a]	Visit 2 Day 8 (+3 days)	Visit 3 Day 15 – Phone (+3 days)	Visit 4 Month 1 (±3 days)	Visit 5 to Visit 9 Months 2, 3, 4, 5, 6 –Phone (±5 days)	Early Termination
Informed Consent	X						Refer to Section 7.2 for Procedures
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 minutes)		X					
Issue eDiary instructions, Ruler, Thermometer; Conduct Training/Retraining		X					
Review eDiary Data			X				
AE Evaluation (Solicited and/or Unsolicited)		X	X	X	X	X[d]	
Clinical Labs							
Hematology, Chemistry, Urinalysis		X[b]			X		
Urine Pregnancy	X[b]	X[b]					
Serum for Immunogenicity		X[b]			X		

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.

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