STATISTICAL ANALYSIS PLAN

Title of Study:

A Phase 2 Multicenter, Randomized, Active-Controlled, Observer-Blind, Dose-Ranging Study to Evaluate the Safety, Tolerability, and Immunogenicity of a 25-Valent Pneumococcal Conjugate Vaccine in Healthy PCV-Naïve Adults

Primary Study Product: Three formulations of Pneumococcal 25-valent conjugate vaccine (IVT PCV-25):

Formulation A

Formulation B

Formulation C

Other Study Product: Pneumococcal 20-valent conjugate vaccine (conjugated to CRM_{197} carrier protein) suspension for intramuscular injection (Prevnar 20™; Pfizer).

Study Number: CVIA 105

Protocol Version: 4.0

Protocol Version Date: March 6, 2024

Study Sponsor: Inventprise, Inc, 18133 NE 68th St, D150, Redmond, Washington 98052 USA

SAP Version: 2.0

SAP Date: 11 April 2024

Authors: Lingyun Ye and Janis Breeze, Canadian Center for Vaccinology

Approved By:

CCfV Statistician

CCfV Epidemiologist

PATH Statistician

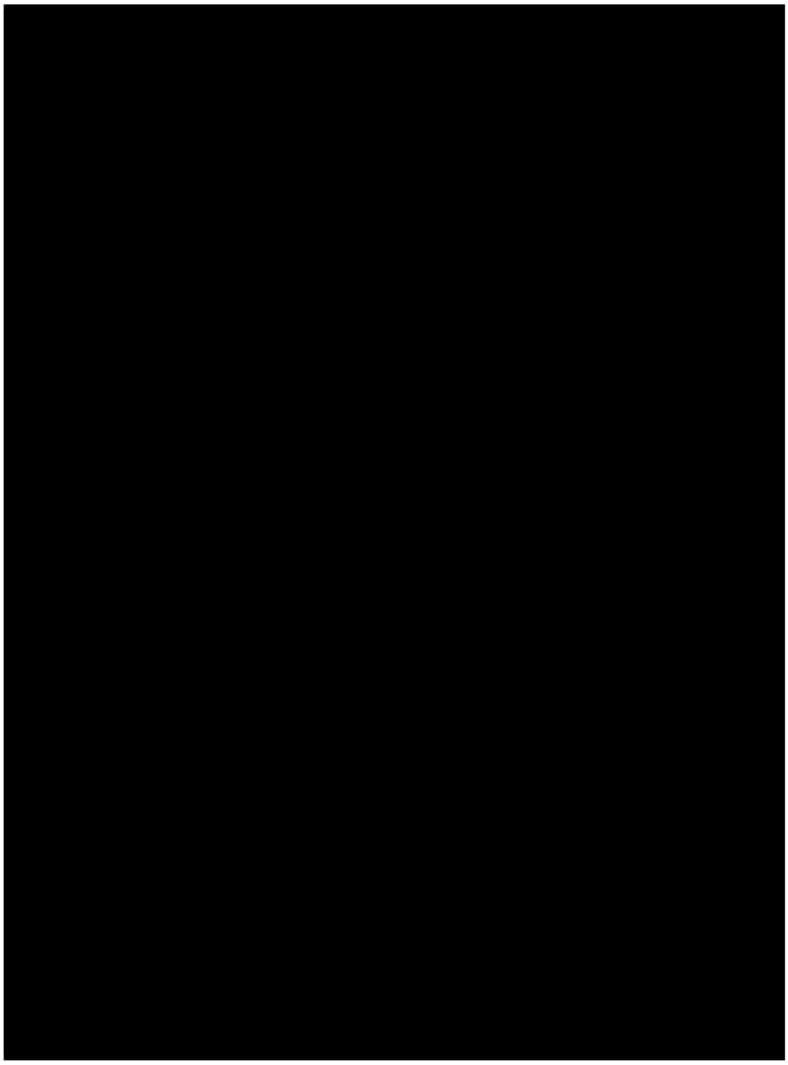

Table of Contents

List of Tables

List of Abbreviations

ABBREVIATION	DEFINITION
Ab	antibody
AE	adverse event
alum	aluminum phosphate
ALT	alanine transaminase
ANCOVA	Analysis of covariance
AST	aspartate aminotransferase
ATC	Anatomical therapeutic chemical
CCfV	Canadian Center for Vaccinology
CI	confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRM_{197}	Cross Reactive Material 197
CRO	contract research organization
DSMB	Data and Safety Monitoring Board
ECLIA	electrochemiluminescence immunoassay
ELISA	enzyme-linked immunosorbent assay
FA	full analysis
GMC	Geometric Mean Concentration
GMFR	Geometric Mean Fold Rise
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HBsAg	Hepatitis B virus surface antigen
HCV	Hepatitis C virus
Hgb	hemoglobin
Hib	*Haemophilus influenzae* type b
HIV	human immunodeficiency virus
HZ	hydrazide
ID	identification
IgG	immunoglobulin G
IP	investigational product
IPD	invasive pneumococcal disease
IVT PCV-25	25-valent candidate pneumococcal conjugate vaccine manufactured by Inventprise LLC
IWRS	Interactive Web Response System
LLOQ	lower limit of quantification
LSLV	last subject last visit
MedDRA	Medical Dictionary for Regulatory Activities
OPA	opsonophagocytic assay

PCV	pneumococcal conjugate vaccine
PCV-13	13-valent pneumococcal conjugated vaccine (Prevnar 13®; Pfizer)
PE	physical examination
PEG	polyethylene glycol
PI	Principal Investigator (the term is used throughout to indicate PI or designee)
Plts	platelets
PSRT	Protocol Safety Review Team
PP	per protocol
PT	Preferred Term
RCD	reverse cumulative distribution
SAE	serious adverse event
SD	standard deviation
SOC	System Organ Class
SOP	standard operating procedure
T bili	total bilirubin
TEN	Toxic epidermal necrolysis
TMF	Trial Master File
ULOQ	upper limit of quantification
USA	United States of America
V	Visit
WBC	white blood cell
WHO	World Health Organization
WHO DD	WHO Drug Dictionary
WOCBP	women of childbearing potential

Revision History

Version	Date	Protocol Version	Note
1.0	17-Jan-2024	2.0	• First approved version
2.0	11-Apr-2024	4.0	• Revised to include early OPA analyses

1 Introduction

The bacterium Streptococcus pneumoniae kills approximately 300,000 children before their fifth birthday annually, mostly in low-resource areas of the world. The most common cause of childhood morbidity and mortality due to the bacterium is pneumonia, which in 2019 was estimated to be the cause of roughly 740,000 under-five deaths worldwide.

Inventprise Inc has partnered with PATH to develop a safe, effective, and low-cost third-generation pneumococcal conjugate vaccine (PCV) that will prevent invasive pneumococcal disease (IPD), pneumonia, and acute otitis media due to the remaining predominant serotypes causing disease in children, particularly those residing in Africa and other low- and middle-income regions of the world. The resulting candidate vaccine (IVT PCV-25) contains capsular polysaccharides from 25 pneumococcal serotypes: 1, 2, 3, 4, 5, 6B, 6C, 7F, 8, 9N, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 24F, 33F, and 35B.

The IVT PCV-25 vaccine was recently tested in a randomized, active-controlled, observer-blind, Phase 1 study (CVIA 096) to evaluate the safety, tolerability, and immunogenicity in adults (NCT05540028) at the Canadian Center for Vaccinology, Dalhousie University, IWK Health Centre Halifax, Nova Scotia, Canada. The single dose of IVT PCV-25 vaccine or the comparator PCV Prevnar 20™ was administered to 60 adults aged 18-40 years in a randomization ratio of 1:1. Analysis of the unblinded adult group-level serotype-specific IgG responses which indicated a lower geometric mean ratio in several of the serotype-specific IgG responses in participants who received IVT PCV-25 compared to those participants vaccinated with Prevnar 20™ and review of the Prevnar 20™ pediatric data presented to the American Committee on Immunization Practices (https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2023-02/slides-02-22/Pneumococcal-04-Watson-508.pdf; https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2023-02/slides-02-22/Pneumococcal-05-Kobayashi-508.pdf) led Inventprise, Inc (Sponsor) advised by its Scientific Review Committee to suspend the enrollment of the young child and infant cohorts originally planned in the Phase 1 trial. Inventprise, Inc. has reformulated IVT PCV-25 into three different formulations of antigen and adjuvant combinations to be tested in this new dose ranging Phase 2 trial in adults only.

The Data and Safety Monitoring Board (DSMB) reviewed the safety data after the single dose immunization in the 60-person adult cohort (NCT05540028) through day 29 and determined that the reactogenicity and safety were favorable and approved moving forward in age de-escalation prior to the Sponsor's decision to suspend the trial. No SAEs occurred, and Grade 3 AEs were determined to be unrelated to investigational product(s). The adult cohor[redacted] will continue safety follow-up through study end (day 169, estimated July 2023) for all 60 adult participants enrolled in the study, after which the study will be terminated.

This Phase 2 trial of IVT PCV-25 is designed to provide the necessary assurances of safety, reactogenicity, and immunogenicity of a single dose of three different dose-ranging formulations of IVT PCV-25 in order to proceed directly to infants for a dose-finding Phase 2 trial[redacted]

[redacted]

2 Hypothesis, Objectives and Endpoints

2.1 Study Hypotheses

IVT PCV-25 will have an acceptable tolerability and safety profile in adults. IVT PCV-25 will be immunogenic and will elicit seroresponses to the serotypes present in the candidate vaccine. All analyses in this study are considered descriptive and no statistical test of this hypothesis is planned.

2.2 Study Objectives

2.2.1 Primary Objective: Safety and Tolerability

To assess the reactogenicity, tolerability, and safety of IVT PCV-25 administered as a single-dose regimen to healthy pneumococcal vaccine-naïve young adults.

2.2.2 Secondary Objectives: Immunogenicity

To describe the immune responses elicited by a single dose of IVT PCV 25 against the 25 serotypes contained in IVT PCV-25 (1, 2, 3, 4, 5, 6B, 6C, 7F, 8, 9N, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 24F, 33F, 35B) as well as serotype 6A, alone and in comparison to the antibody responses against these serotypes induced by Prevnar 20TM in healthy adults

2.3 Study Endpoints and Estimands

2.3.1 Primary: Safety and Tolerability

Table 1: Primary Endpoints and Estimands

ENDPOINTS	ESTIMANDS

1. Local reactions (redness, swelling, and pain at the injection site) 2. Systemic events (fever, headache, fatigue, muscle pain, joint pain, and rash) 3. Unsolicited Adverse events (AEs) 4. Serious adverse events (SAEs)	**Population**: Healthy pneumococcal vaccine-naïve adult participants receiving study vaccines **Population-level summary**: 1. Number and severity of solicited local reactions within 7 days after vaccination (redness, swelling, and pain at the injection site) by group 2. Number and severity of solicited systemic AEs within 7 days after vaccination by group 3. Number and severity of unsolicited AEs within 28 days after vaccination by group 4. Number of SAEs within 6 months after vaccination by group

2.3.2 Secondary: Immunogenicity

Table 2: Secondary Endpoints and Estimands

ENDPOINTS	ESTIMANDS
1. Serotype-specific immunoglobulin G (IgG) antibody responses 2. Serotype-specific functional antibody responses measured by Opsonophagocytic Assay (OPA)	**Population**: Healthy pneumococcal vaccine-naïve adult participants receiving study vaccines, complying with the key protocol criteria, and providing evaluable sample at baseline and 28 days after vaccination **Population-level summary:** 1. Geometric mean concentrations (GMC) of serotype-specific IgG at each time point (Day 1 and Day 29) by group 2. Geometric mean fold rise (GMFR) in serotype-specific IgG GMCs from baseline to Day 29 after vaccination by group 3. Percentage of participants achieving a ≥4-fold IgG rise from baseline to Day 29 after vaccination by group 4. Ratio of GMCs between IVT PCV-25 and Prevnar 20 groups 28 days after vaccination 5. Ratio of IgG GMFR between IVT PCV-25 and Prevnar 20 groups 6. Geometric mean titers (GMTs) of serotype-specific OPA antibodies at each time point (Day 1 and Day 29) by group

	7. Geometric mean fold rise (GMFRs) in serotype-specific OPA GMTs from baseline to Day 29 after vaccination by group 8. Ratio of GMTs between IVT PCV-25 and Prevnar 20 groups 28 days after vaccination 9. Ratio of OPA GMFR between IVT PCV-25 and Prevnar 20 groups

3 Study Design

In this prospective, multicenter, randomized, active-controlled, observer-blind Phase 2 study, 220 adult (age 18-49 years) eligible participants will be randomized to four groups as shown below (Table 3). The four groups will be conducted concurrently and randomized in a 2:3:4:2 ratio.

Each adult will undergo a total of 4 clinic visits (V), including at least one screening visit (V0) not more than 28 days prior to Day 1 (V1), a vaccination visit on Day 1 (V1), follow-up clinic visits at 7 (+3) and 28 (+14) days post-vaccination (V2 and V3, respectively) as shown in Table 8 in Appendices. Re-screening is permissible if the window period exceeds 28 days. Daily local and systemic reactogenicity will be monitored during the 7 days after vaccination (Day 1 and following 6 days) by memory aid. Blood will be collected for hematological and biochemical laboratory tests at screening (V0) and Day 8 (V2). Blood will be collected at baseline (V1) and 28 days post-vaccination (V3) to assess serotype-specific IgG antibody and OPA functional antibody responses in all participants. There will be a final follow-up call on Day 169 (V4).

The study visit schedule is presented in Table 8 in the Appendices.

4 Statistical Considerations

4.1 Overview

This is a multicenter, randomized, active-controlled, observer-blind Phase 2 trial in 220 healthy adults (age 18-49 years) residing in Canada. Eligible participants will be randomized to four groups as shown below (Table 3) in a 2:3:4:2 ratio.

No formal statistical hypothesis tests between treatment groups are planned in this study. All analyses for safety and immunogenicity data will be descriptive. When appropriate, continuous data will be summarized using descriptive statistics such as number of subjects with non-missing values, mean, standard deviation (SD), median, minimum, and maximum. Categorical data will be presented using the percentage of subjects in each category. A two-sided 95% CI will be provided for estimates, as appropriate. Individual subject data will be provided in listings. All statistical analyses will be performed using SAS® software Version 9.4 or later. Subject-wise data listings will be provided.

4.2 Randomization

An interactive web response system (IWRS) will be used to randomize participants to the four study groups in a ratio of 2:3:4:2, as shown in shown in Table 3. A block randomization scheme will be employed to ensure that the randomization ratio is maintained among the treatment groups at each site.

Table 3: Randomization Scheme

Group	n	[redacted]	[redacted]
[redacted]			
Group D	40	Prevnar 20™ comparator	Prevnar 20
[redacted]			

4.3 Sample Size

A total of 220 eligible adult participants will be randomized. The sample size for the study is not based on any statistical hypothesis testing. The sample sizes for each group were selected and weighted toward the higher antigen and adjuvant dose combinations to ensure that the safety assessment in each group at the interim analysis was adequate to advance to an infant Phase 2 trial and would reflect the probability for similar dose ranges to be included in a study in infants. With 40, 60, and 80 adults vaccinated per group, the probabilities of observing at least one unsolicited AE, solicited local AE, or solicited systemic AE when the true event rates are 0.5%, 1%, and 5%, respectively, are presented in Table 4. For example, if the true AE rate is 5%, with 60 participants in a treatment group, there is a 95% probability of observing at least one AE.

Table 4: Probability of Observing at Least One Unsolicited AE, Solicited Local AE, or Solicited Systemic AE by Assumed True Event Rates

Sample Size Per Group	Assumed True Event Rate		
	0.5%	**1.0%**	**5.0%**
N = 40	0.182	0.331	0.871
N = 60	0.260	0.453	0.954
N = 80	0.330	0.552	0.983

The expected precision of GMC ratio between the IVT PCV-25 and Prevnar 20 groups at the first (interim) analysis is presented in Table 5 below.

Table 5: Expected Precision of GMC Ratio by Assumed Standard Deviation (SD) of Log_{10} Antibody Concentration and GMC Ratio with Different Sample Sizes

Assumed SD of log_{10} Antibody Concentration in IVT PCV-25 group	Assumed SD of log_{10} Antibody Concentration in Prevnar 20	Assumed GMC Ratio (IVT PCV-25 vs. Prevnar 20)	Expected 95% CI for GMC Ratio		
			N_1:N_2 = 20:20	N_1:N_2 = 30:20	N_1:N_2 = 40:20
0.3	0.20	0.5	0.34-0.75	0.35-0.71	0.36-0.69
		0.75	0.50-1.12	0.53-1.06	0.55-1.03
		1.0	0.67-1.49	0.71-1.41	0.73-1.37
	0.25	0.5	0.32-0.77	0.34-0.73	0.35-0.72
		0.75	0.49-1.15	0.51-1.10	0.52-1.07
		1.0	0.65-1.54	0.68-1.47	0.70-1.43
	0.30	0.5	0.31-0.80	0.33-0.77	0.33-0.75
		0.75	0.47-1.20	0.49-1.15	0.50-1.12
		1.0	0.63-1.60	0.65-1.53	0.67-1.50
0.4	0.30	0.5	0.29-0.87	0.31-0.81	0.32-0.78
		0.75	0.43-1.30	0.46-1.22	0.48-1.18
		1.0	0.58-1.74	0.62-1.63	0.64-1.57
	0.35g	0.5	0.28-0.90	0.30-0.85	0.31-0.82
		0.75	0.42-1.35	0.44-1.27	0.46-1.23
		1.0	0.56-1.80	0.59-1.69	0.61-1.64
	0.40	0.5	0.27-0.93	0.28-0.88	0.29-0.86
		0.75	0.40-1.40	0.42-1.32	0.44-1.29
		1.0	0.54-1.87	0.57-1.76	0.58-1.72
0.5	0.40	0.5	0.25-1.01	0.27-0.94	0.28-0.90
		0.75	0.37-1.52	0.40-1.40	0.42-1.35
		1.0	0.49-2.03	0.53-1.87	0.56-1.79
	0.45	0.5	0.24-1.05	0.26-0.97	0.27-0.94
		0.75	0.36-1.58	0.38-1.46	0.40-1.41
		1.0	0.48-2.10	0.51-1.95	0.53-1.88
	0.50	0.5	0.23-1.09	0.25-1.02	0.25-0.98
		0.75	0.34-1.64	0.37-1.53	0.38-1.47
		1.0	0.46-2.18	0.49-2.03	0.51-1.96
0.6	0.50	0.5	0.21-1.18	0.23-1.08	0.24-1.03
		0.75	0.32-1.78	0.35-1.62	0.37-1.54
		1.0	0.42-2.37	0.46-2.15	0.49-2.05
	0.55	0.5	0.20-1.23	0.22-1.12	0.23-1.07
		0.75	0.31-1.84	0.33-1.68	0.35-1.61
		1.0	0.41-2.45	0.45-2.25	0.47-2.15
	0.60	0.5	0.20-1.27	0.21-1.17	0.22-1.12
		0.75	0.29-1.91	0.32-1.76	0.33-1.69
		1.0	0.39-2.55	0.43-2.34	0.44-2.25

Note: CI = confidence interval; N1 is the sample size in the IVT PCV-25 group; N2 is the sample size in the Prevnar

20 group. An attrition rate of 10% was used in calculating the expected 95% CIs.

If the true SD of $\log_{10}$ antibody concentrations for a specific serotype is 0.5 in the IVT PCV-25 group and 0.45 in the Prevnar 20 group and the true GMC ratio between the two groups is 0.75, the expected two-sided 95% CI for the GMC ratio between the two groups is (0.36, 1.58) with 18 evaluable participants per group, (0.38, 1.46) with 27 evaluable participants in the IVT PCV-25 group and 18 evaluable participants in the Prevnar group, and (0.40, 1.41) with 36 evaluable participants in the IVT PCV-25 group and 18 evaluable participants in the Prevnar group.

4.4 Study Cohorts

4.4.1 Enrolled Population

All subjects who provide written informed consent, regardless of the subject's screening, randomization, and treatment status in the study.

4.4.2 Exposed Population

All subjects in the enrolled population who were randomized and received a vaccination dose (i.e., were accrued).

4.4.3 Safety Analysis Population

All subjects in the exposed population for whom any safety data is available. All safety analyses will be performed using this population. The denominators for different safety endpoints may vary according to the number of subjects with available data for the specific endpoint. For instance, the solicited local and systemic adverse event endpoint will be based only on those who have the corresponding CRF data regardless of other safety data. Treatment groups for safety analysis will be assigned according to the actual treatment received.

4.4.4 Full Analysis Population

This population will include all subjects in the exposed population for whom any post-study vaccine administration immunogenicity results are available. If it differs from the per protocol population, an immunogenicity analysis will also be performed using this population as a sensitivity analysis to support the robustness of the final results. The analysis based on this population will serve as supportive results for all secondary immunogenicity objectives. Subjects in the full analysis (FA) population will be analyzed as randomized, i.e., according to the treatment group to which subjects were assigned.

4.4.5 Per Protocol Population

This population will include all subjects in the FA population who were correctly vaccinated per randomization and have no protocol deviations that are determined to potentially interfere with the immunogenicity assessment of study vaccine.

The per protocol (PP) population will serve as the primary analysis population for the (secondary) immunogenicity endpoints. The population will be adapted by time point to include subjects' data up to

the time of disqualifying protocol deviation or discontinuation. The criteria for exclusion of subjects from the PP population will include the following:

- Missed vaccinations
- Significant non-compliance with visit windows
- Any eligibility criteria not met
- Receipt of a non-study vaccine
- Receipt of immunosuppressants or immune modulators
- Receipt of study vaccine not stored as per manufacturers approved storage condition
- Incomplete vaccine dose administration
- Serological results unavailability
- Incorrect randomization
- Dosed with incorrect study product

This list is not exhaustive, as unexpected deviations may arise requiring unique consideration and will be reviewed by a blinded adjudication committee prior to the interim analysis.

4.5 Blinding

This is an observer-blind clinical trial where unblinded study personnel will be responsible for preparing study vaccine doses in accordance with the randomly determined assignment, administering the study vaccines, and handling all vaccine accountability procedures. Unblinded personnel will not participate in the other aspects of the clinical trial after vaccine administration, to help ensure the integrity of the blind at the site. The unblinded personnel will not reveal participants' randomization assignments to the participants, or staff associated with the Sponsor, PATH, CRO, or site.

Emergency unblinding decisions are expected to be rare and could be justified only when that information is needed for the future clinical management of that participant. The decision of emergency unblinding must be approved by the principal investigator (PI) and the sponsor after sufficient evaluation. If a subject's treatment assignment is unblinded, the subject will remain in the study and continue with protocol-defined study visits and procedures, unless there is another reason for subject discontinuation. The safety data collected after the unblinding will be reported separately. The decision to unblind will be communicated to all regulatory bodies as required. At the end of the study, documentation of all unblinded subjects (and the rationale for unblinding) will be incorporated into the trial master file (TMF).

For the first, interim analysis, an independent CRO statistician and programmer team (whose responsibilities are otherwise limited to supporting the DSMB) will be unblinded to conduct the analyses. Study unblinding will be conducted and documented in accordance with relevant CRO SOPs. The independent CRO statistician and programmer team will provide the unblinded group-level safety report directly to the Sponsor, its consultants, PATH, the Bill & Melinda Gates Foundation, and the site investigators. The independent CRO statistician and programmer team will provide the unblinded group-level and individual-level (participant ID masked) serotype-specific IgG responses to the Sponsor, its consultants, PATH, and the Bill & Melinda Gates Foundation. All other personnel will continue to remain blinded to individual treatment assignments until all subjects complete Visit 4 and the database is locked.

The second analysis will include both group-level and individual-level (with participant ID-masked) serotype-specific OPA results. Unblinded group-level second analysis will be shared with the Sponsor and its consultants, PATH, the Investigators, and representatives from the Bill & Melinda Gates Foundation. The individual-level, participant ID-masked results will be shared with the Sponsor and its consultants, PATH, and representatives from the Bill & Melinda Gates Foundation as they become available.

4.6 Estimands and Analytical Methodology

4.6.1 Estimands

The estimands corresponding to each primary and secondary objective are described in Table 1 and Table 2 in Section 2.3.

For the primary safety estimand, receipt of concomitant medications will be handled with treatment policy strategy, which means that participants who take concomitant medications will be included in the safety analysis. The estimates will be calculated based on the safety analysis population.

For the estimand to evaluate the immunogenicity objective, receipt of immune-modifying medications/vaccines/treatments will be handled according to principal stratum strategy, which means participants who receive immune-modifying medications/vaccines/treatments will not be included in the primary immunogenicity analysis. The corresponding estimates will be computed based on both PP and Full Analysis populations, to examine robustness of the estimates. Immunogenicity results that are below the lower limit of quantification (LLOQ) will be set to half of the LLOQ (LLOQ/2) in the analysis. Immunogenicity results above the upper limit of quantification (ULOQ) will be set to the ULOQ in the analysis.

4.6.2 Descriptive Methodology

When appropriate, continuous data will be summarized using descriptive statistics such as number of participants with non-missing values, mean, standard deviation (SD), median, minimum, and maximum. Categorical data will be presented using the percentage of participants in each category. A two-sided 95% CI will be provided for estimates, as appropriate. Individual participant data will be provided in listings.

4.6.3 Analysis Sequence

Upon completion of the Day 29 Visit 3 from all 220 participants, the CRO will prepare an unblinded safety report for review and approval by the DSMB to continue the study as planned.

There will be three analyses. The first analysis (interim) includes the dataset of the safety from all participants having completed the Day 29 visit, and the serotype-specific IgG responses from approximately 50% or more of the participants having completed the Day 29 visit, in order to provide the Sponsor an initial indication of the comparative immunogenicity among the three different IVT PCV-25 formulations and to compare to the immune responses elicited in participants vaccinated with Prevnar 20™.

The Sponsor and its consultants, PATH, and representatives from the Bill & Melinda Gates Foundation will review both group-level and individual-level (with participant ID masked) serotype-specific IgG antibody responses (baseline and Day 29) from the interim analysis, and the group-level unblinded safety data to ensure that both the safety profile and the immune responses to IVT PCV-25 are sufficient to proceed to planning for a Phase 2 study in infants. The Investigators will be provided the group-level safety and immunogenicity results. All site and CRO personnel will remain blinded to individual treatment assignment until end of study.

The second analysis will be conducted once serotype-specific OPA data from the baseline and Day 29 visits from all 220 participants become available and will include both group-level and individual-level (with participant ID-masked) serotype-specific OPA results. The unblinded group-level second analysis will be shared with the Sponsor and its consultants, PATH, the Investigators, and representatives from the Bill & Melinda Gates Foundation. The individual-level, participant ID-masked results will be shared with the Sponsor and its consultants, PATH, and representatives from the Bill & Melinda Gates Foundation as they become available.

The third and final analysis will include all late safety events from all participants through Day 169 (end of study) with the complete IgG and OPA immunogenicity results from all participants. This will occur after all safety data through Visit 4 from all 220 participants have been collected following the adult LSLV (Visit 4). The database will be cleaned and locked, and a final analysis of all data will be performed.

4.6.4 Analysis of Demographic and Baseline Characteristics

Demographics and baseline characteristics, including age, height, weight, sex, race, and ethnicity will be summarized for both the exposed and per protocol populations by treatment group.

For the exposed population, the number and percentage of participants with medical history will be tabulated by MedDRA System Organ Class (SOC), Preferred Term (PT) and treatment group. Using the WHO DD, the number and percentage of participants with concomitant medications will be tabulated by anatomical therapeutic chemical (ATC) classification, preferred drug name and treatment group.

Summaries of participant disposition will be prepared for all participants, including the number and percent enrolled, screened, randomized, and administered study vaccine, as well as a Consolidated Standards of Reporting Trials (CONSORT) diagram describing study participation and discontinuation. The reasons for screen failures and discontinuations will be summarized and listed.

A summary and listing of visit attendance will be prepared, in addition to a summary and listing of study vaccine administration and sample collection/availability for each sample.

4.6.5 Analysis of Primary Objective

All safety analyses will be conducted in the safety analysis population, according to the treatment received. Frequencies and percentages of participants with any solicited AE, solicited local AE, solicited systemic AE, unsolicited AE, clinical safety laboratory abnormality, SAE will be tabulated along with an exact two-sided 95% CI computed via the Clopper-Pearson method. The denominators for percentages

will be the number of participants with the data available for analysis for the given endpoint. Participant-level data listing will be provided.

Solicited local and systemic AEs: All solicited AEs reported during 7 days post vaccination will be summarized according to defined severity grading scales. Frequencies and percentages of participants experiencing each solicited AE will be presented by treatment group and severity.

Hematological and biochemical measurements: For clinical safety laboratory data collected at screening and 7 days post-vaccination, individual Hgb, WBC count, Plts, AST, ALT, and creatinine values will be graded according to Table 10 and summarized by toxicity grading and study group. In addition, summaries of changes from baseline will be presented. Clinically significant laboratory abnormalities will be considered an AE and relatedness will be reported.

Unsolicited AEs: All unsolicited AEs with onset occurring during the first 28 days post vaccination will be assessed for severity and relatedness to study product by the PI. Frequencies and percentages of participants with unsolicited AEs will be summarized by the SOC, PT, and treatment group. Similar summaries will be provided for severity and relatedness of the unsolicited AEs.

SAEs: All SAEs through Visit 4 (Day 169) will be recorded and summarized. Frequencies and percentages of participants with SAEs and relatedness to study vaccine of the events will be summarized by SOC, PT, and treatment group.

When an AE occurs more than once for a participant, the participant will be only counted once for the corresponding PT according to the maximum severity of the events. A summary table will be prepared for unsolicited AEs comprised of the following categories:

- Unsolicited AEs
- Related unsolicited AEs
- SAEs
- Related SAEs
- SAEs leading to death
- Unsolicited AEs leading to participant discontinuation

All reported AEs that start after vaccination will be tabulated. If a given disease/condition is already reported as ongoing at the first visit on the medical history form, it will be counted and tabulated as an AE only if it worsens after vaccination with the study vaccines.

Onset day, duration, and outcome of unsolicited AEs will be summarized by treatment group. For SAEs, seriousness criteria will be summarized as well.

4.6.6 Analysis of Secondary Objective

The analysis of immunogenicity will be performed on the PP population as the primary analysis and the full analysis population as a secondary analysis.

Immunogenicity data will be descriptively analyzed. Serotype-specific IgG antibody responses will be measured at baseline (V1), and 28 days after study vaccination (V3).

- GMC with two-sided 95% CIs
- GMFR from baseline with two-sided 95% CI
- Percentage of participants achieving a ≥4-fold IgG rise from baseline to Day 29 after vaccination by group with two-sided Clopper-Pearson 95% CI

Serotype-specific functional antibody responses will be measured by OPA and summarized at baseline (V1) and 28 days after study vaccination (V3),

- GMTs with two-sided 95% CIs,
- GMFR from baseline with 95% CI

GMCs and GMTs will be calculated for each treatment group along with their two-sided 95% CI, by exponentiating the corresponding log-transformed mean and their two-sided 95% confidence limits.

The ratio of the GMC/GMT between IVT PCV-25 formulations [redacted] to Prevnar 20™ and corresponding two-sided 95% CI will be provided. The log-transformed antibody responses will be used to construct a mean difference between the treatment groups and the comparator vaccine, Prevnar 20™ and its two-sided 95% CI using the two-sample t-test method. The mean difference and corresponding 95% CI will be back-transformed to obtain the GMC/GMT ratio between the two groups and corresponding 95% CI.

An adjusted GMC/GMT ratio between the treatment groups will be also provided along with its two-sided 95% CI. The log-transformed antibody responses will be used to compute a mean difference between each of the IVT PCV-25 groups and the Prevnar 20 group, along with corresponding two-sided 95% CIs, using analysis of covariance (ANCOVA) with log-transformed baseline antibody responses as a covariate. Other variables, such as age and sex, will be evaluated for inclusion in the ANCOVA model as well, using stepwise selection method. The mean difference and its 95% CI will be exponentiated to obtain the adjusted GMC/GMT ratio between the two groups and corresponding 95% CI.

GMFRs and corresponding two-sided 95% CIs will be computed for each treatment group. The mean difference in the log-transformed antibody responses between 28 days post vaccination and baseline and corresponding two-sided 95% CI will be calculated using the paired t-test method and then back-transformed to obtain GMFR and its 95% CI. The two-sample t-test method will be used to obtain the two-sided 95% CI of the ratio of GMFRs between the study groups. The distribution of concentrations/titers will be summarized using reverse cumulative distribution (RCD) curves at applicable time points. Forest plots will be created to visualize the IgG and OPA GMRs at 28 days post-vaccination. The distribution of antibody concentrations/titers at baseline and 28 days post-vaccination, as well as their fold-rises at 28 days post-vaccination, will be summarized using box plots.

4.7 Multiplicity

Due to the descriptive and exploratory nature of the analyses, no adjustment for multiplicity will be performed.

4.8 Handling of Participant Discontinuations and Missing Data

Missing immunogenicity data will not be imputed and will be analyzed as if missing completely at random. Missing reactogenicity data will not be imputed. No other missing safety information will be imputed. Over the study period, the frequency and percentage of participants who discontinue from the study will be provided by treatment group. All participants who discontinue post-randomization will be further described regarding their time to and their reasons for discontinuation. For participants who discontinue from the study, their data collected before discontinuation will be analyzed under the analysis populations as applicable. If missing data rate for immunogenicity measures is higher than 10% or any patterns are observed in the missing data, sensitivity analyses will be performed to evaluate the robustness of the analysis results.

4.9 Conventions for Presentation

The conventions for presentation in the analysis displays are shown in Table 6.

Table 6: Conventions for Presentation

Convention	Description
Decimals for summary statistics	General rule: Relative to number of decimals in original data, use 1 more decimal for mean, median, and percentiles, 2 more decimals for standard deviation/error, and same number for minimum, maximum, and range. Do not exceed 4 decimals. Some laboratory parameters or other data may require judicious deviation from this rule
Format for percentages	Display percentages with 1 decimal. When the percentage is low, the number of decimals could be increased appropriately.

The conventions for calculation and tabulation are shown in Table 7.

Table 7: Conventions for Calculation and Tabulation

Convention	Description
Age Calculation	Age is calculated as the difference between the subject's date of informed consent and the date of birth with one decimal place. If only the year of birth is reported, age is calculated as the difference between year of informed consent and year of birth.
Duration Calculation	Duration of an event is calculated as end date – start date + 1. Duration of an ongoing event or event with unknown start date is considered missing.
Percentage calculation	Percentages are calculated as 100*numerator/denominator. Rounding is not necessary because rounding is handled by the display format. Denominator is total number of subjects with non-missing data unless specified otherwise in a particular analysis display specification.

5 Appendices

5.1 Schedule of Events

Table 8: Study Visits

VISIT (study day)	V0 (D0)	V1 (D1)	V2 (D8)	V3 (D29)	V4 (D169)*
Allowed window in days	***-28 to 0***	***1***	***V1+7 (+3)***	***V1+28 (+7)***	***V1+168 (+14)***
Assign participant ID	✓				
Demographics[A]	✓				
Eligibility check		✓			
Medical history[B]	✓	✓	✓	✓	✓
Concomitant medications	✓[C]	✓	✓	✓	✓
Eligibility check	✓				
Vital signs[D]	✓	✓^✓	✓	✓	
Complete physical exam[E]	✓				
Targeted physical exam[F]		✓	✓	✓	
Clinical chemistry[G]	✓		✓		
Hematology[H]	✓		✓		
Viral serology tests[I]	✓				
Pregnancy test[J] (serum or urine)	✓	✓		✓	
Randomization		✓			
Administer study vaccine[K]		✓			
Observation/solicited AEs		✓	✓		
Unsolicited AEs		✓	✓	✓	
SAE		✓	✓	✓	✓
Blood for immune testing		✓		✓	
Provide memory aid		✓			
Review memory aid			✓	✓	
Exit study					✓

*Telephone call "visit"

^= Evaluations will be conducted twice – before and after vaccination

[A]Contact information, including home address, telephone number(s), and email address

[B]Complete medical history of relevance to study eligibility including vaccination history. V1-V4 to collect changes in medical condition during interval period between visits not captured on memory aid

[C]History of medication use in the past 28 days, and of medications taken that are of specific relevance to study eligibility (e.g., immunosuppressive medications)

[D]Temperature, pulse rate, respiratory rate, blood pressure

[E]Complete physical exam may be performed at either V0 or V1. Height and weight will be measured; a physical examination (PE) will be performed in all participants and include assessment of the major organ systems

[F]Targeted PE will be conducted only in the event of new symptom, sign or new AE; Injection site assessed at Visit 2
[G] Serum creatinine, ALT, AST
[H]WBC count, Hgb, Plts
[I]HIV 1/2 Ab, HBsAg, HCV Ab
[J]Women of childbearing potential (WOCBP) only. Serum pregnancy testing is preferred at V0 (D0) and urine pregnancy testing is acceptable. Urine will be collected at V1 (D1) and V3 (D29) for pregnancy testing.
[K]Date and time documented

5.2 Pause Rules

The DSMB will be convened if any of the following study pause rules are met during the conduct of the trial:

- Rule 1: 1 or more participant experiences any vaccine-related Grade 4 AE or any vaccine-related SAE.
- Rule 2: 1 or more participant experiences Grade 3 or greater local reaction classified as related to vaccination by the PI: ulceration, necrosis, or sterile abscess at the injection site requiring drainage or surgical intervention.
- Rule 3: ≥ 5 participants in the same group inclusive of participants from Groups A, B, C, and D that experience the same Grade 3 (or greater) AE or laboratory abnormality attributed (related) to study vaccine. In the case of fever and pain at the injection site, the episode must last longer than 48 hours, and, in the case of fever, be confirmed by the PI without evidence of other medical causes.

5.3 Solicited Local and Systemic Reactions Toxicity Grading Tables

Table 9: Solicited Reactions Toxicity Grading Scales

Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain at injection site (tenderness)	Does not interfere with activity / Mild discomfort to touch	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity / Discomfort with movement	Any use of narcotic pain reliever or prevents daily activity / Significant discomfort at rest	Emergency room (ER) visit or hospitalization
Redness at injection site[a]	2.5 – 5 cm	5.1 – 10 cm	>10 cm	N/A
Swelling at injection site[a]	2.5 – 5 cm	5.1 – 10 cm	>10 cm	N/A

Ulceration, Secondary Infection, Sterile Abscess, Drainage and/or Necrosis	N/A	N/A	Presence of any reaction and confirmed by site investigator	N/A
Fever (oral temp)	38.0 – 38.4°C	38.5 – 38.9°C	39.0 – 40°C	>40°C
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours OR some interference with activity	Significant; any use of narcotic pain reliever OR prevent daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevent daily activity	ER visit or hospitalization
Reaction	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Myalgia	No interference with activity	Some interference with activity	Significant; prevent daily activity	ER visit or hospitalization
Arthralgia	No interference with activity	Some interference with activity	Significant; prevent daily activity	ER visit or hospitalization
Rash	Localized macular rash (not a local reaction at the site of injection)	Diffuse macular, maculopapular, or morbilliform rash OR target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bulbous lesions OR Stevens-Johnson syndrome OR ulceration of mucous membrane involving two or more distinct mucosal sites OR toxic epidermal necrolysis (TEN)

[a] Redness and swelling should be measured at greatest surface diameter in millimeters using a ruler.

5.4 Serum and Hematology Toxicity

Table 10: Serum and Hematology Toxicity Grading

Serum	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Creatinine (mg/dL)	1.1 – 1.3 x ULN	>1.3 – 1.8 x ULN OR increase of >0.3 above baseline	>1.8 – <3.5 x ULN OR increase of 1.5 – <2.0 x above baseline	≥3 .5 x ULN OR increase of >2.0 x above baseline
Liver function tests – AST, ALT increased	1.25 – <2.5 x ULN	2.5 – <5.0 x ULN	5.0 – <10.0 x ULN	≥10.0 x ULN
Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin [Female] (g/dL)	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin [Male] (g/dL)	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
OR decrease Hemoglobin, change from baseline value (g/dL)	1.0 – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC, increased (cell/mm^3)	10,800 – 15,000	15,001 – 20,000	20,0001 – 25,000	> 25,0000
WBC, decreased (cell/mm^3)	2,500 – 3,400	1,500 – 2,499	1,000 – 1,499	< 1,000
Platelets, decreased (cell/mm^3)	100,000 – <125,000	50,000 – <100,000	25,000 – < 50,000	< 25,000

Abbreviation: ULN = upper limit of normal range

Note: the laboratory values provided in this table serve as guidelines and are dependent upon institutional normal parameters.

Note: the severity grading scales used in this study are derived in part from *Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*, U.S. Department of Health and Human Services, FDA, CBER, September 2007, and the *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events* (Version 2.1, July 2017), US National Institutes of Health.

5.5 Vital Signs Toxicity

Table 11: Vital Signs Toxicity Grading

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)

Fever – Temp (^{0}C)	38.0 – 38.4°C	38.5 – 38.9°C	39.0 – 40°C	> 40°C
Tachycardia – beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization
Bradycardia –beats per Minute	50 – 54	45 – 49	< 45	ER visit or hospitalization
Hypertension (systolic) – mm Hg	141 – 150	150 – 165	> 165	ER visit or hospitalization
Hypertension (diastolic) – mm Hg	91 – 99	100 – 105	> 105	ER visit or hospitalization
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization
Respiratory Rate – breaths per minute	21 – 23	24 – 27	> 27	ER visit or hospitalization

[a] Participant should be at rest for all vital sign measurements.

5.6 Deviations from Protocol

- The protocol included an ad hoc analysis that combined immunogenicity data from the Prevnar 20TM Phase 1 (NCT05540028) trial with data from this study. It was decided to remove this analysis as it was not necessary and the results, based on pooled ELISA and ECLIA data, would be difficult to interpret.
- The protocol specified that missing dates for the timing of AEs would be imputed. However, no imputation will be performed. In this study with only a single dose administered, every AE will occur after the first dose.
- The threshold for missing data to trigger sensitivity analyses was reduced from 20% to 10%, to be consistent with the earlier Prevnar 20TM Phase 1 trial.