VAC 040

A Phase 1/2, Randomized, Placebo-Controlled, Observer-Blind Study to Assess the Safety, Tolerability, and Immunogenicity of *Streptococcus pneumoniae* Whole Cell Vaccine, Inactivated and Adsorbed to Aluminum Hydroxide (PATH-wSP) in Healthy Kenyan Young Adults (18 to 40 years) and Toddlers (12 to 21 months)

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Sponsored by: PATH

Vaccine Solutions

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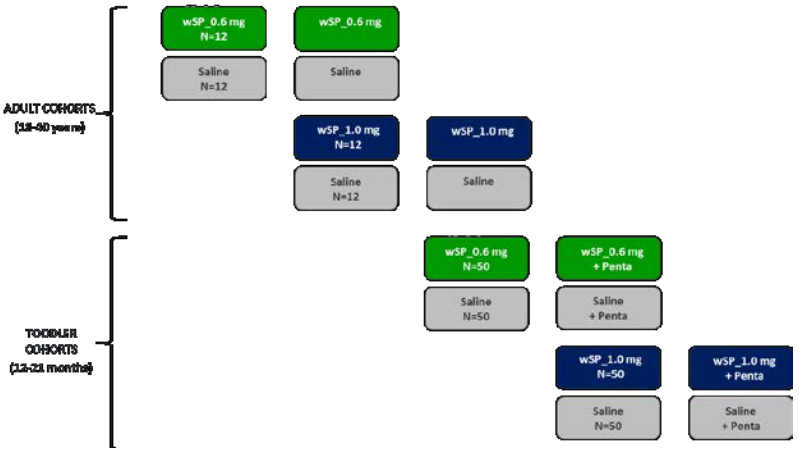
STUDY SYNOPSIS

TITLE	A Phase 1/2, Randomized, Placebo-Controlled, Observer-Blind Study to Assess the Safety, Tolerability, and Immunogenicity of *Streptococcus pneumoniae* Whole Cell Vaccine, Inactivated and Adsorbed to Aluminum Hydroxide (PATH-wSP) in Healthy Kenyan Young Adults (18 to 40 years) and Toddlers (12 to 21 months)
STUDY NUMBER	VAC 040
PROJECT PHASE	Phase 1/2
STUDY HYPOTHESES	**Primary Hypothesis**: PATH-wSP administered intramuscularly (IM) to healthy adults and toddlers will be safe and well tolerated up to a dose of 1 mg. **Secondary Hypothesis**: PATH-wSP administered IM to healthy adults and toddlers will elicit a measurable immune response.
STUDY OBJECTIVES	**Primary Objectives:** • To evaluate the safety and tolerability of 2 dose levels (0.6 mg and 1 mg) of PATH-wSP vaccine, administered in a 2-series schedule, 4 weeks apart, in healthy adults. • To evaluate the safety and tolerability of 2 dose levels (0.6 mg and 1 mg) of PATH-wSP vaccine, administered in a 2-series schedule, 8 weeks apart, in healthy toddlers when co-administered with a booster dose of licensed pentavalent vaccine (diphtheria, tetanus, whole-cell pertussis, *Haemophilus influenzae* type b, and hepatitis B combined vaccine) at the second vaccination. **Secondary Objective**: • To assess immunogenicity of PATH-wSP vaccine when given as a 2-vaccination series to adults and toddlers. **Exploratory Objectives**: • To evaluate the potential for sera from PATH-wSP-immunized subjects to afford passive protection in an animal challenge model (subset of subjects). • To identify novel antibody targets for future vaccine candidates using peripheral blood mononuclear cells (PBMCs, i.e. plasmablasts) stimulated *in vitro* (adults only).

STUDY ENDPOINTS	**Primary Endpoints** **Safety**: Safety and tolerability of PATH-wSP vaccine including co-administration of PATH-wSP and licensed pentavalent vaccine at the second vaccination to toddlers will be evaluated based on the following analyses: • Occurrence and severity of solicited local and systemic adverse events (AEs) and clinical laboratory abnormalities through 1 week post vaccination. • Occurrence, severity, and relatedness to vaccination of unsolicited AEs and serious adverse events (SAEs) from Day 0 through the last study contact. • Occurrence and severity of pneumonia cases reported from Day 0 through the last study contact. **Secondary Endpoints** **Immunogenicity**: • The Immunoglobulin G (IgG) response to pneumococcal-specific proteins (pneumolysoid [Ply] and pneumococcal surface protein A family 1 [PspA Fam 1) measured by the enzyme-linked immunosorbent assay (ELISA), and the IgG response to nine pre-selected pneumococcal proteins measured on the Meso Scale Discovery (MSD) platform, will be evaluated based on the following analyses: o IgG Geometric Mean Concentration (GMC) and Geometric Mean Fold Rise (from baseline) 4 weeks after the second vaccination. o Percentage of subjects with IgG concentration of a predefined threshold level (responders), measured 4 weeks after the second vaccination. **Exploratory Endpoints** **Immunogenicity**: • Assessment of protection of mice against intravenous (IV) S. pneumoniae challenge after passive transfer of serum obtained from a subset of subjects 4 weeks after the second vaccination. • Identification of novel antibody targets for future vaccine candidates using PBMCs (i.e. plasmablasts) stimulated in vitro (adults only).

STUDY DESIGN	This randomized, placebo-controlled, observer-blind trial is designed to sequentially evaluate PATH-wSP at two escalating doses (0.6 mg and 1 mg) in both adults and toddlers. The following cohorts will be tested: **Adult (18-40 years old) Cohorts:** • Two 0.6 mg doses of PATH-wSP (12 subjects) **or** saline (12 subjects) with a 28-day interval between doses. • Two 1 mg doses of PATH-wSP (12 subjects) **or** saline (12 subjects) with a 28-day interval between doses. **Toddler (12-21 months old) Cohorts**: • Two 0.6 mg doses of PATH-wSP (50 subjects) **or** saline (50 subjects) with a 56-day interval between doses, and pentavalent vaccine co-administered with the second dose. • Two 1 mg doses of PATH-wSP (50 subjects) **or** saline (50 subjects) with a 56-day interval between doses, and pentavalent vaccine co-administered with the second dose.  Note: All cohorts will be enrolled sequentially; each subsequent cohort will be only triggered after a favorable review of the post Dose 1 safety and reactogenicity data of the previous cohort by the Internal Safety Team (IST) or the Data Safety Monitoring Board (DSMB).

STUDY RATIONALE	Parenteral immunization with killed whole cell bacteria is one of the oldest and most successful approaches to vaccine-induced protection against several bacterial infections. A candidate pneumococcal vaccine manufactured from unencapsulated and inactivated pneumococci (*Streptococcus pneumoniae* Whole Cell Vaccine [SPWCV]), and adsorbed to aluminum hydroxide adjuvant (Alum) prior to administration, has been tested in Phase 1/2 studies in healthy US adults (VAC-002), and in healthy Kenyan adults and toddlers (VAC-010). The SPWCV and Alum used in these trials were supplied separately in a two-vial configuration; the SPWCV was manufactured at Walter Reed Army Institute of Research (WRAIR) and the Alum at Instituto Butantan. After adsorption of the Alum to SPWCV, the vaccine is referred to as PATH-wSP. A single-vial formulation of PATH-wSP, an adsorbed suspension of SPWCV and Alum, has now been manufactured by PT Bio Farma, Indonesia. The purpose of this study is to assess the safety and tolerability of this new formulation. The ultimate goal of this clinical development program is for PATH-wSP to be approved and prequalified for use in a primary infant vaccination program to protect against infections associated with *S. pneumoniae*. To achieve this aim, studies in infant populations in low-resource settings will be required that, in addition to assessment of safety and immunogenicity, will evaluate vaccine efficacy in a population at high risk for pneumococcal disease. This study (VAC-040) is required to assess the safety and tolerability of the single-vial formulation of PATH-wSP manufactured by PT Bio Farma, prior to use of this new formulation in such an infant population in a Phase 2 dose-ranging, proof-of-concept study.
STUDY POPULATION	• Approximately 48 healthy adults aged 18-40 years, inclusive, at the time of enrollment; • Approximately 200 healthy toddlers aged 12-21 months, inclusive, at the time of enrollment.
INVESTIGATIONAL PRODUCT	**PATH-wSP vaccine**: A single-vial formulation of *Streptococcus pneumoniae* Whole Cell Vaccine, inactivated and adsorbed to aluminum hydroxide. **Placebo**: Normal saline (NS).

STUDY PROCEDURES AND VISIT SCHEDULE	Each adult subject will undergo a total of 6 scheduled visits, including at least one screening visit no more than 28 days prior to vaccination (Visit 0), two vaccination visits (scheduled 28 days apart), two safety visits at 7 days post each vaccination and a final visit at 28 days post the final vaccination. Each toddler subject will undergo a total of 8 scheduled visits, including at least one screening visit no more than 28 days prior to vaccination (Visit 0), two vaccination visits (scheduled 56 days apart), and five safety visits at 7 and 28 days after the first vaccination and at 7, 28, and 56 days after the second dose. Daily reactogenicity assessments for 7 days post vaccination will be completed in all subjects by fieldworkers using a standard home visit diary. For all subjects, blood draws for safety laboratory tests and/or PATH-wSP-induced immune responses will be performed at screening (baseline safety and immunogenicity), 7 days after each study vaccination (safety only) and 28 days after the second vaccination (immunogenicity only).
STUDY DURATION	All adult subjects will be followed for approximately 56 days after randomization (28 days following the second vaccination). All toddler subjects will be followed for approximately 112 days, including 56 days after the second vaccination.

STUDY SCHEMA

Adult cohorts:

Cohorts	Vaccine	N	Visit (Study Day)					
			Visit 0 (-28 to -1)	Visit 1 (Day 0)	Visit 2 (Day 7[a])	Visit 3 (Day 28)	Visit 4 (Day 35[a])	Visit 5 (Day 56)
Adults (0.6 mg)	wSP 0.6 mg	12	BS	V	BS	V	BS	BS
	Placebo	12	BS	S	BS	S	BS	BS
Adults (1.0 mg)	wSP 1 mg	12	BS	V	BS	V	BS	BS
	Placebo	12	BS	S	BS	S	BS	BS

Toddler cohorts:

Cohorts	Vaccine	N	Visit (Study Day)							
			Visit 0 (-28 to -1)	Visit 1 (Day 0)	Visit 2 (Day 7[a])	Visit 3 (Day 28)	Visit 4 (Day 56)	Visit 5 (Day 63[a])	Visit 6 (Day 84)	Visit 7 (Day 112)
Toddlers (0.6 mg)	wSP 0.6 mg	50	BS	V	BS	Safety	V, P	BS	BS	Safety
	Placebo	50	BS	S	BS	Safety	S, P	BS	BS	Safety
Toddlers (1.0 mg)	wSP 1 mg	50	BS	V	BS	Safety	V, P	BS	BS	Safety
	Placebo	50	BS	S	BS	Safety	S, P	BS	BS	Safety

Study vaccines: V = PATH-wSP; S = saline placebo; P = pentavalent vaccine
BS = blood sample for safety and/or immunogenicity testing

[a] Solicited AEs (reactogenicity) will be collected on day of vaccination and the following 6 days via home visits by fieldworkers. The reactogenicity results will be reviewed by the investigator or a designee at Days 7 and 35 for adult subjects or at Days 7 and 63 for toddlers.

SAFETY MONITORING	The Principal Investigator (PI) will provide continuous monitoring for safety, and an Internal Safety Team (will review blinded safety data regularly throughout the trial. Initiation of the 1 mg adult cohort and the 0.6 mg toddler cohort will occur only after the IST reviews the blinded safety data for all subjects in the prior cohort through Day 7 after the first dose, and based on this review grants approval for the dose escalation and age de-escalation. A DSMB will evaluate reactogenicity and safety profile of PATH-wSP 0.6 mg vaccine in toddlers and will make a decision regarding administration of the second vaccination and initiation of the enrollment in the 1 mg toddler cohort. The composition and responsibilities of the DSMB are presented in the DSMB charter. Enrollment in the 1 mg toddler cohort will be staggered such that the IST will review blinded safety data for the first 10 subjects prior to the remainder of the cohort being enrolled. The DSMB will meet at the request of the IST or if one of the following pause rules for the investigational vaccine has been met **Rule 1**: (related death / related SAE): 1 subject experiences any vaccine-related Grade 4 AE or SAE. **Rule 2** (Grade 3 vaccine-related solicited AE): ≥ 10% of subjects in a cohort experience any vaccine-related Grade 3 solicited AE. In the case of Grade 3 fever, the episode must last longer than 24 hours. **Rule 3** (Grade 3 vaccine-related unsolicited AE): ≥ 6% of subjects in a cohort experience the same Grade 3 unsolicited AE assessed as related by the PI. **Rule 4** (Grade 3 abnormal clinical laboratory parameters): ≥ 6% of subjects in a cohort experience the same abnormal clinical laboratory parameter assessed as Grade 3 and classified as related to vaccination by the PI. Note: Assessment of pause rules in toddler cohorts will be conducted on ongoing basis starting when results for approximately 20 subjects are available. The rate of AEs and laboratory abnormalities will be computed based on the actual number of exposed subjects with available results. Severity grading and causality assessment must be confirmed by the PI.

STATISTICAL CONSIDERATIONS	Sample sizes were selected to provide adequate data to assess whether the safety and immunogenicity of PATH-wSP measured in adults and toddlers in this trial support advancing into an infant population. All vaccinated subjects are expected to provide data for safety analyses. It is estimated that with a 10% attrition rate approximately 90% of subjects will be evaluable for immunogenicity analyses. **Safety**: A sample size of 12 subjects per treatment group for the adult cohorts will provide a 90% chance of observing at least one occurrence of an AE that has an approximately 17.5% rate of occurrence. The 50 subjects per treatment group for the toddler cohorts will provide a 90% chance of observing at least one occurrence of an AE that has an approximately 4.5% rate of occurrence. If no AEs/SAEs are observed among 12 and 50 subjects receiving PATH-wSP, the upper limits of exact 2-sided 95% confidence intervals for the rate of AE occurrence will be 26.5% and 7.1%, respectively. **Immunogenicity**: In the first Phase 1 study in adults (VAC-002), the standard deviations (SD′s) of log10 of the geometric mean fold rise (GMFR) for ELISA PspA and Ply concentrations were estimated to be ≤ 0.55. Assuming SD = 0.55, then for each adult cohort, 11 evaluable subjects in each study group will provide approximately 68.5% power to find a significant difference between GMFR's if the true difference is a 4.0-fold increase in PATH-wSP recipients compared to recipients of the saline control. For the toddler cohorts, 45 evaluable subjects per study group will provide > 99% power if the true difference is a 4.0-fold increase in PATH-wSP recipients. Power for analysis of GMFR was estimated based on a 2-sample t-test and a 1-sided 0.025 significance level and calculated using PASS 12 (Number Cruncher Statistical Systems, Statistical Software, Kaysville, Utah). In VAC-002, ≥ 40% of subjects with seroresponse (≥ 2-fold-rise) were observed for PspA and Ply measured by ELISA. For each adult cohort (11 evaluable subjects in each group), the power to find a significant difference in seroresponse rates will be approximately 30.1% if the true rates are 40% in PATH-wSP recipients and 5% in recipients of the saline control. For the toddler cohorts (45 evaluable subjects in each group), the power to find a significant difference will be ≥ 88.9% if the true seroresponse rate is 40% in the PATH-wSP group and the true rate in the control group is ≤ 10%. Power for comparing seroresponse rates was estimated based on a Fisher exact test at the 1-sided 0.025 significance level, using PASS 12.

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LIST OF ACRONYMS

ACRONYM	DEFINITION
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
Alum	Aluminum Hydroxide
AST	Aspartate Aminotransferase
BCG	Bacille Calmette-Guérin Vaccine
BLQ	Below Limit of Quantitation
CAPA	Corrective Action and Preventive Action
CBC	Complete Blood Count
CBER	Center for Biologics Evaluation and Research
CFR	Code of Federal Regulations
CFU	Colony-Forming Unit
CI	Confidence interval
CRP	C-Reactive Protein
CRF	Case report form
CRO	Contract Research Organization
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
DTwPHibHep	Pentavalent Diphtheria, Tetanus, Whole-Cell Pertussis, Hepatitis B, and *Haemophilus influenzae* Type B Combined Vaccine
ERC	Ethics Review Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
EOS	End of Study
EPI	Expanded Program on Immunization
FDA	US Food and Drug Administration
FSFV	First Subject First Visit
GCLP	Good Clinical Laboratory Practice
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GLP	Good Laboratory Practice
GMC	Geometric Mean Concentration

ACRONYM	DEFINITION
GMFR	Geometric Mean Fold Rise
GMT	Geometric Mean Titer
HbsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
Hib	*Haemophilus influenzae* Type B
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ID	Identification
IgG	Immunoglobulin G
IL	Interleukin
IM	Intramuscular
IME	Important Medical Event
IND	Investigational New Drug
IP	Investigational Product
IPD	Invasive Pneumococcal Disease
IRB	Institutional Review Board
IST	Internal Safety Team
ITT	Intent to Treat
IV	Intravenous
KEMRI	Kenya Medical Research Institute
KWTRP	KEMRI-Wellcome Trust Research Programme
LLN	Lower Limit of Normal
LSHTM	London School of Hygiene and Tropical Medicine
LSLV	Last Subject Last Visit
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSD	Meso Scale Discovery
MUAC	Mid-Upper Arm Circumference
NPC	Nasopharyngeal Carriage
NRA	National Regulatory Authority
NS	Normal Saline
NSAID	Nonsteroidal Anti-Inflammatory Drug
NTF	Note to File
OPV	Oral Poliovirus Vaccine
OTC	Over the Counter

ACRONYM	DEFINITION
PASS 12	Number Cruncher Statistical Systems Statistical Software
PATH-wSP	*Streptococcus pneumoniae* Whole Cell Vaccine, Inactivated and Adsorbed to Aluminum Hydroxide
PBMC	Peripheral Blood Mononuclear Cell
PCV	Pneumococcal Conjugate Vaccine
PE	Physical Examination
PI	Principal Investigator (The term is used throughout to indicate PI or delegee.)
Ply	Pneumolysoid
PPB	Republic of Kenya Pharmacy and Poisons Board
PspA Fam 1	Pneumococcal Surface Protein A Gamily 1
PVS	PATH Vaccine Solutions
qPCR	Real-Time Polymerase Chain Reaction
RE	Reactogenicity Event
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SERU	Scientific & Ethics Review Unit
SSC	Scientific Steering Committee
SIDS	Sudden Infant Death Syndrome
SOC	System Organ Class
SOP	Standard Operating Procedure
SPWCA	*Streptococcus pneumoniae* Whole Cell Antigen
SPWCV	*Streptococcus pneumoniae* Whole Cell Vaccine
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEN	Toxic Epidermal Necrolysis
TMF	Trial Master File
ULN	Upper limit of normal
V	Visits
WBC	White Blood Cell Count
WHO	World Health Organization
WIRB	Western Institutional Review Board
WRAIR	Walter Reed Army Institute of Research

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46);
- International Conference on Harmonization (ICH) Guidance for GCP (E6);
- World Medical Association Declaration of Helsinki – Ethical Principles for Research Involving Human Subjects (Oct 2013 or subsequent amendments).

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training and ICH-GCP training.

PROTOCOL SIGNATURE PAGE

The signature below constitutes the approval of this protocol amendment and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator:

Signed: Date:

Name: …….

Title: ……

1 BACKGROUND AND INTRODUCTION

1.1 Burden of Disease

Each year the bacterium *Streptococcus pneumoniae* (pneumococcus) kills hundreds of thousands of children before their fifth birthday, mostly in low-resource areas of the world. Based on estimation methods used in the seminal Pneumococcal Global Burden of Disease Study,[1] *S. pneumoniae* was responsible for the deaths of about 826,000 children younger than 5 in the year 2000 alone. The most common cause of childhood morbidity and mortality due to *S. pneumoniae* is pneumonia, which in 2011 was estimated to be the cause of roughly 1.2 million (or 18% of all) under-five deaths worldwide, making it the most deadly infectious disease of young children today.[2,3] Although pneumonia has multiple bacterial and viral etiologies, *S. pneumoniae* is the leading cause of severe pneumonia. In addition to pneumonia, *S. pneumoniae* also causes a number of other serious invasive pneumococcal diseases (IPD), including sepsis and meningitis, which collectively result in tremendous morbidity and mortality.

1.2 Pathogen

S. pneumoniae is a gram-positive encapsulated bacterium that is recognized as normal flora in the human nasopharynx. More than 90 serotypes of the bacterium have been identified based on differences in the composition of its polysaccharide capsule, which is an essential virulence factor. Pneumococci are transmitted by direct contact with respiratory secretions from infected individuals and healthy carriers. Nearly all children harbor one or more strains, and become carriers during the first few years of life.[4] Carriage is typically asymptomatic; however, it is believed to be a precondition for invasive pneumococcal infection.[5]

1.3 Rationale for PATH-wSP Development

Public health leaders generally agree that vaccines are the best way to protect children from pneumococcal disease. Multivalent pneumococcal conjugate vaccines (PCVs) have significantly reduced serotype-specific pneumococcal disease in the industrialized world since their introduction more than 10 years ago.[6] Since 2009, a roll-out of PCVs in low-resource countries has been underway with assistance from the GAVI Alliance and other international partners. However, significant barriers to accelerated and sustainable global access to PCVs remain due to their cost and complex manufacturing process. Furthermore, PCVs do not provide broad, serotype-independent coverage against the more than 90 regionally variable serotypes of pneumococcus. As a result, these populations remain vulnerable to disease caused by non-vaccine serotypes. Also, serotype replacement after PCV introduction could offset the effectiveness and erode the value of current PCVs.

Over the long term, new vaccines are needed that can provide broad, non-serotype specific protection against pneumococcal disease in the low resource areas of the world, where 90% of global pediatric pneumococcal deaths occur. These new vaccines also need to be available at a price that even the poorest countries can sustainably afford without assistance—which would provide cost savings that could then be used for other health-related needs. To this end, PATH Vaccine Solutions (PVS) is collaborating with public- and private-sector partners to develop safe, affordable, and broadly effective vaccines against *S. pneumoniae* tailored to meet the needs of infants and children in the low resource areas of the world.[7]

Parenteral immunization with killed whole cell bacteria is one of the oldest and most successful approaches to vaccine-induced protection against several bacterial infections. Whole cell vaccines have been applied to control epidemics and have considerably diminished the burden of vaccine preventable diseases over the last century.[8,9] PVS is developing a whole cell candidate vaccine made from unencapsulated pneumococcal cells called *Streptococcus pneumoniae* Whole Cell Vaccine (SPWCV) inactivated and adsorbed to aluminum hydroxide adjuvant, or PATH-wSP. Because it elicits immunity directed to a variety of "species" antigens (i.e., antigens present in all pneumococcal serotypes), PATH-wSP should be efficacious worldwide, regardless of regional serotype prevalence, and also prevent serotype emergence and replacement. As in the case of other whole cell vaccines, the manufacturing process for PATH-wSP is simple and low cost. Successful development of PATH-wSP for protection of children from *Streptococcus pneumoniae* could solve the issues of complex manufacturing, high cost, inadequate supply, and limited serotype coverage that plague pneumococcal conjugate vaccines.

1.4 Preclinical Studies of PATH-wSP

Preclinical toxicology and immunogenicity studies of PATH-wSP in mice and rabbits have established the proof-of-concept for vaccine efficacy and demonstrated the safety and tolerability required to support Phase 1 clinical trials. Detailed review of the preclinical program for PATH-wSP is provided in the Investigator's Brochure (IB). In two Good Laboratory Practice (GLP)-compliant toxicology studies of PATH-wSP in rabbits, doses of PATH-wSP up to 1 mg doses were well tolerated and were without evidence of adverse changes in any organ examined, including the liver. In these studies, there was minimal necrosis and increased incidence of minimal mononuclear infiltrates in the livers of some of the animals that received 1 mg of SPWCV alone (no alum). Mild to moderate increases in the hepatic-biliary markers (ALT, sorbitol dehydrogenase, and gamma-glutamyl transpeptidase [GGT]) were also detected at termination in some of the animals that received 1 mg of SPWCV alone (no alum). These did not always correlate with the microscopic findings in the livers. The liver changes were resolved by the end of the recovery period in both studies. No such changes were detected with PATH-wSP. The current acceptable tolerated dose for PATH-wSP is set at 1 mg (the highest dose tested).

Several pneumococcal challenge studies in mice have demonstrated that subcutaneous (SC) and IM injections of PATH-wSP result in significant protection against pneumococcal nasopharyngeal carriage (NPC) and IPD (specifically sepsis). In these studies PATH-wSP was shown to induce two distinct pathways involved in protective immunity: a novel T-cell mediated pathway, and an antibody-mediated response. PATH-wSP protection against mouse NPC appears to be dependent upon Th17 cells and interleukin (IL)-17A, whereas protection against pneumococcal sepsis is dependent upon antibodies.[10] If both pathways are also induced in humans, PATH-wSP has the potential to provide broad protective immunity in infants and children through both a reduction in pneumococcal NPC and direct protection against pneumococcal disease. Induction of both of these pathways of protection would confer significant herd immunity due to the dual reduction of human-to-human transmission and density of pneumococci in the nasopharynx. It is well recognized that the density of pneumococcal NPC is much higher in low resource countries, and that the age of onset is much earlier, than in other settings—undoubtedly contributing to the early and high burden of IPD in low resource countries.[11]

1.5 Clinical Studies of PATH-wSP

The first PATH-wSP candidate evaluated in clinical trials was SPWCV unadsorbed suspension which was absorbed to aluminum hydroxide adjuvant prior to administration (SPWCV+Alum). This formulation was tested in a Phase 1 trial in the US (VAC-002), in which doses of 0.1, 0.3 or 0.6 mg were given to healthy young adults in a 3-dose vaccination series at an interval of 28 days. Given the favorable safety and immunogenicity results of this study (see below), this same formulation of PATH-wSP was tested in a Phase 1/2 trial in Kenya (VAC-010), where a two-vaccination series was given to healthy young adults at dose levels of 0.6 mg and 1 mg, and to healthy toddlers (co-administered with Pentavalent and Synflorix) at dose levels of 0.3 mg and 0.6 mg. The SPWCV used in both trials was manufactured at WRAIR, and the Alum used in both trials was manufactured by Instituto Butantan.

Subsequent to the initial manufacturing of SPWCV+Alum, PVS formed a collaboration with PT Bio Farma in Bandung, Indonesia to establish PT Bio Farma as the commercial manufacturer of a single-vial formulation of PATH-wSP (as an alum-adsorbed suspension). The result of this collaboration is the vaccine candidate *Streptococcus pneumoniae* Whole Cell Vaccine, inactivated and adsorbed to aluminum hydroxide. The single-vial configuration is manufactured from the same unencapsulated strain, and inactivated and genetically modified by the same processes (See Section 4.1). The primary objective of the present study (VAC-040) is to assess the safety and tolerability of this new formulation.

1.5.1. VAC-002

The first clinical study of PATH-wSP, VAC-002, was a Phase 1, randomized, double-blind, placebo-controlled dose-escalation study in healthy US adult volunteers. The primary objective of this study was the evaluation of the safety, reactogenicity, and tolerability of PATH-wSP (SPWCV+Alum). The secondary and exploratory objectives were to examine the immunogenicity of the vaccine. Healthy adults (n=42) were randomized to receive three vaccinations, at a 28 day interval (to approximate the infant Expanded Program on Immunization [EPI] schedule for PCVs), of either 0.1, 0.3, or 0.6 mg of PATH-wSP, or saline (control). Reactogenicity events (REs), AEs, and chemistry/hematology parameters were captured through Day 84 following the first vaccination (28 days after the final vaccination). All 42 subjects received the initial dose of PATH-wSP or saline, 36 (85.7%) received at least two injections, and 35 (83.3%) received all three injections. No subject refused any vaccination, and of specific note, the two subjects who self-reported Grade 3 pain and tenderness with the first vaccination did not refuse subsequent vaccinations. The majority of subjects receiving PATH-wSP reported at least one solicited local or systemic reaction (for local reactions, 16.7% were mild, 54.8% moderate, and 4.8% severe; for systemic reactions, 28.6% were mild, 16.7% moderate, and 0% severe). Overall, local REs were common but of mild to moderate severity, with the most common reaction being pain and tenderness at the injection site (57.2% reporting mild/moderate) during the 7 days post vaccination. There were two reports of severe pain/tenderness post vaccination that were without sequelae, and subsequent vaccinations were well tolerated by these two subjects. The frequency of local REs did not appear to increase with successive vaccinations at a given dosage level or across increasing dosage levels of PATH-wSP. The pain and tenderness for two subjects extended to 8 days post vaccination (for the first vaccination only), but the remainder of subjects had only a few days of reported local symptoms that required no therapy or intervention. There were no events of necrosis or abscess formation at the site of vaccination, and no subject refused further vaccination due to any local reaction experienced. The majority of subjects experienced either no or mild systemic

REs regardless of treatment group, and there were no reports of a severe systemic RE during the 7-day period following any vaccination. Although nonspecific in nature, the total number of systemic REs was greater in PATH-wSP treatment groups than in the placebo-treated group. There was no obvious trend across successive injections or type of systemic RE, but the overall rate of systemic REs did increase with increasing dosage of PATH-wSP (Table 1). The range of systemic REs was broad, with no one event appearing dominant when considering dosage or vaccination sequence. The most frequently reported REs were mild headache and mild fatigue.

Table 1. Number (%) of Subjects With Any Solicited Local or Systemic Reactogenicity Event Within 7 Days After an Injection of PATH-wSP or Placebo*

	Treatment Group 1 (0.1 mg) N=10			**Treatment Group 2 (0.3 mg) N=10**			**Treatment Group 3 (0.6 mg) N=10**			**Placebo N=12**		
Severity	**Mild**	**Mod**	**Sev**	**Mild**	**Mod**	**Sev**	**Mild**	**Mod**	**Sev**	**Mild**	**Mod**	**Sev**
Number of Solicited Local Injection Site REs (%)												
Injection No.												
1	4 (40.0)	8 (80.0)	0	6 (60.0)	6 (60.0)	2 (20.0)	7 (70.0)	6 (60.0)	0	0	2 (16.7)	0
2	3 (33.3)	5 (55.6)	0	6 (75.0)	3 (37.5)	0	6 (66.7)	5 (55.6)	0	3 (30.0)	0	0
3	6 (66.7)	1 (11.1)	0	5 (62.5)	2 (25.0)	0	6 (66.7)	3 (33.3)	0	2 (22.2)	0	0
Number of Solicited Systemic AEs (%)												
1	1 (10.0)	0	0	3 (30.0)	0	0	6 (60.0)	1 (10.0)	0	3 (25.0)	0	0
2	0	0	0	1 (12.5)	1 (12.5)	0	2 (22.2)	2 (22.2)	0	1 (10.0)	2 (20.0)	0
3	1 (11.1)	0	0	2 (25.0)	0	0	2 (22.2)	2 (22.2)	0	1 (11.1)	0	0

Abbreviations: AE = adverse event, Mod = moderate, RE = reactogenicity event, Sev = severe

*Columns show the number of subjects with any reaction at that particular severity. For each type of reaction only the maximum severity is used. If a subject had multiple reactions at different levels of severity, they are counted in multiple columns.

The majority of solicited local or systemic REs reported by subjects during the 7-day post vaccination follow-up period were noted as first occurring in the interval from 1 to 4 days after vaccination. Table 1 summarizes by treatment group and injection number the total number of subjects with any solicited local or systemic RE, as well as the mean duration of these events. The mean duration of local REs ranged from 2 to 4 days for subjects given PATH-wSP, and showed no trend across successive injections or dosage levels. The mean duration of local REs among placebo recipients was shorter, ranging from 1 to 2 days (Table 2). The mean duration of systemic REs ranged from 1 to 3 days for subjects given PATH-wSP, which was similar to the range for placebo-treated subjects.

Table 2. Duration in Days of Solicited Local and Systemic Reactogenicity Events for VAC 002 Subjects

Vaccination		Treatment 1 (0.1 mg) N=10	Treatment 2 (0.3 mg) N=10	Treatment 3 (0.6 mg) N=10	Placebo N=12
	Solicited Local Injection Site REs				
Injection No.					
1	Subjects with reaction (N)	9	10	8	2
	Mean duration (SD)	2.8 (1.20)	3.2 (2.25)	3.6 (0.92)	1.5 (0.71)
2	Subjects with reactions (N)	7	7	8	3
	Mean duration (SD)	3.1 (1.68)	2.7 (2.56)	3.1 (1.46)	1.3 (0.58)
3	Subjects with reactions (N)	6	6	8	2
	Mean duration (SD)	2.7 (1.51)	2.2 (0.98)	3.0 (1.31)	1.0 (0.00)
	Solicited Systemic REs				
1	Subjects with reactions (N)	1	3	6	3
	Mean duration (SD)	3	2.3 (1.15)	2.3 (1.63)	1.0 (0.00)
2	Subjects with reactions (N)	0	1	4	3
	Mean duration (SD)		6	2.8 (1.71)	2.7 (2.08)
3	Subjects with reactions (N)	1	2	3	1
	Mean duration (SD)	2	1.0 (0.00)	3.3 (4.04)	1

Abbreviations: RE = reactogenicity event, SD = standard deviation

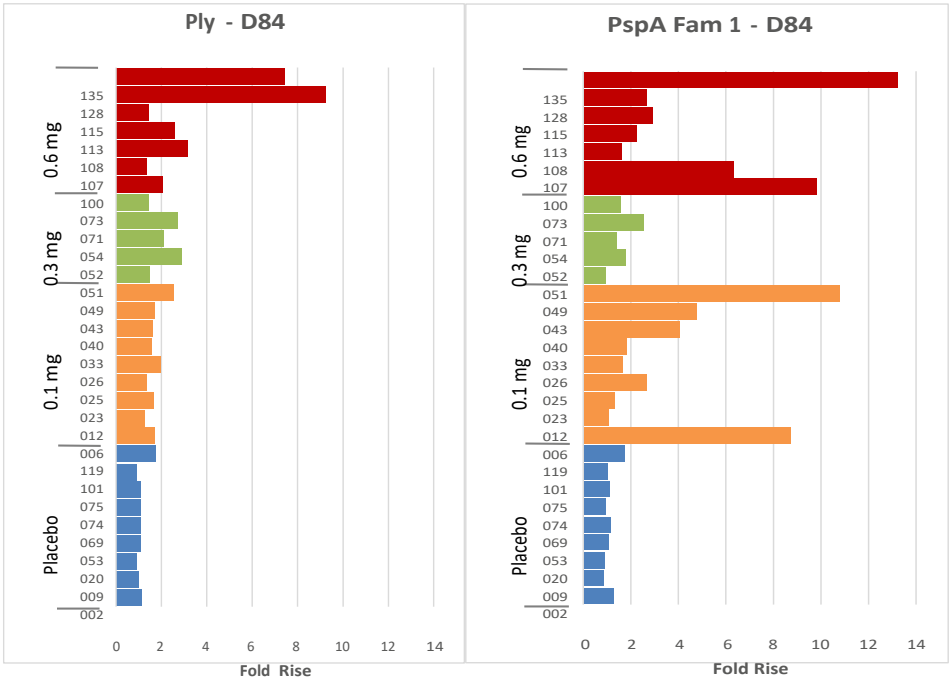
There were no vaccine-related SAEs. Only 5 subjects (all in PATH-wSP treatment groups) had AEs deemed possibly related to study vaccine, all of which were mild in severity: three cases of mild injection pain and one headache that extended beyond the 7 day post-vaccination period, and one episode of dysfunctional uterine bleeding that began three days post vaccination. In general subjects had normal clinical laboratory parameters (within the reference range of the laboratory) during the study. In the rare cases that an abnormal laboratory parameter was observed, none was attributed to vaccination, none was reported to have any sequelae, and there were no trends noted across PATH-wSP treatment groups.

The majority of immune parameters in this study were exploratory in nature. PATH-wSP was shown to elicit antibody responses to a number of specific pneumococcal proteins, was seen to stimulate IL-17A production, and demonstrated the ability to protect mice that had received serum from immunized adults and were then challenged with an IV injection of *S. pneumoniae*. The 0.6 mg dose level resulted in the most positive (and often only) response to these immune parameters, as illustrated below. Therefore, the Phase 1/2 trial of PATH-wSP in Kenya (VAC-010) includes an adult cohort that received a two-series vaccination with PATH-wSP at a 1 mg dose level, the highest level supported by preclinical toxicology results. Based on the continued favorable (but still blinded) safety results of the VAC-010 study (see below), evaluation of PATH-wSP at the 1 mg dose level is planned for both adults and toddlers in the current study (VAC-040).

Assay development work conducted using sera collected in the VAC-002 study has resulted in validation of protein-specific immunological assays that are the basis for evaluating key secondary

immunogenicity endpoints in the VAC-010 and VAC-040 studies. The proteins against which immunogenicity of PATH-wSP in the VAC-010 and VAC-040 studies will be measured are those proteins that gave rise to the highest fold increase in the immunoglobulin G (IgG) response in stimulated sera obtained at Day 84 (28 days following the final vaccination) from subjects vaccinated with PATH-wSP at the 0.6 mg dose level, when measured by a solid phase assay on the MSD platform. The IgG responses to two such proteins, Ply, and PspA Fam 1, are shown below in Figure 1. These data provide the first indication that antibody responses to biologically meaningful components of PATH-wSP are produced that have the potential to protect through a B-cell pathway.

Figure 1. IgG Response to Pneumococcal-Specific Proteins, Ply and PspA Family 1, Following a 3-Series Vaccination of Healthy US Adults with PATH-wSP



Note: Results from the MSD platform are expressed as fold increase over baseline at Day 84, for specific subjects in each VAC-002 treatment group (0.6 mg, 0.3 mg, 0.1 mg, and placebo).
Abbreviations: Ply = pneumolysoid, PspA Fam 1 = pneumococcal surface protein A family 1

In addition, a functional challenge model was evaluated in VAC-002 as an exploratory endpoint: CBA/CaHN-Btkxid/J mice (CBA/N mice) were passively immunized intraperitoneally with varying concentrations of VAC-002 sera and then challenged four hours later with 400 to 500 colony-forming units (CFU) of Type 3 pneumococci by the IV route. Serum samples from a subset of subjects in the 0.6 mg dose cohort were tested prospectively by first injecting mice with a 1:50 dilution of pre-vaccination (Day 0) or post-vaccination (Day 84) serum. Based on these results, additional dilutions of 1:10 or 1:100 were performed: donors whose serum failed to offer any protection at the 1:50 dilution were retested at the 1:10 dilution. Donors whose serum offered strong protection that could not be distinguished from the other at 1:50 were retested at a 1:100 dilution. Mice given Day 0 or Day 84 sera from two of the three placebo group subjects showed no evidence of protective immunity. The 1:50 dilution of Day 0 serum from one placebo subject was protective, since 30% (3 of 10) of the mice given this serum survived to the end of the study. However, there was no increase in median survival time or percent survival among mice given

Day 84 serum from this individual. In contrast, mice given Day 84 serum from 7 of the 9 recipients of SPWCV+Alum showed clear evidence of improved protective immunity as measured by a significantly increased median survival time compared to animals given Day 0 serum from the same individual (see Table 3). The immunity conferred by Day 84 sera from 3 of these 7 vaccinees was also manifest in an increased percent survival at the end of the study. This challenge model provides the first proof of concept that vaccination with SPWCV induces functional protective antibodies. Further investigation of this model, in addition to an antibody neutralization assay, is being performed in studies VAC-010 and VAC-040.

Table 3. Protection From IV Challenge of Pneumococcus in a Mouse Model by Passive Transfer of Serum from Subjects Vaccinated with PATH-wSP

Treatment Group	**Placebo**			**0.6 mg PATH-wSP**								
Subject No.	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Change in Median Survival Time in hours (dilution)	NS (1:50)	NS (1:50)	NS (1:50)	255* (1:50)	>336* (1:10)	92* (1:10)	137* (1:10)	89* (1:50)	>336* (1:50)	NS (1:100)	>336* (1:100)	NS (1:50)

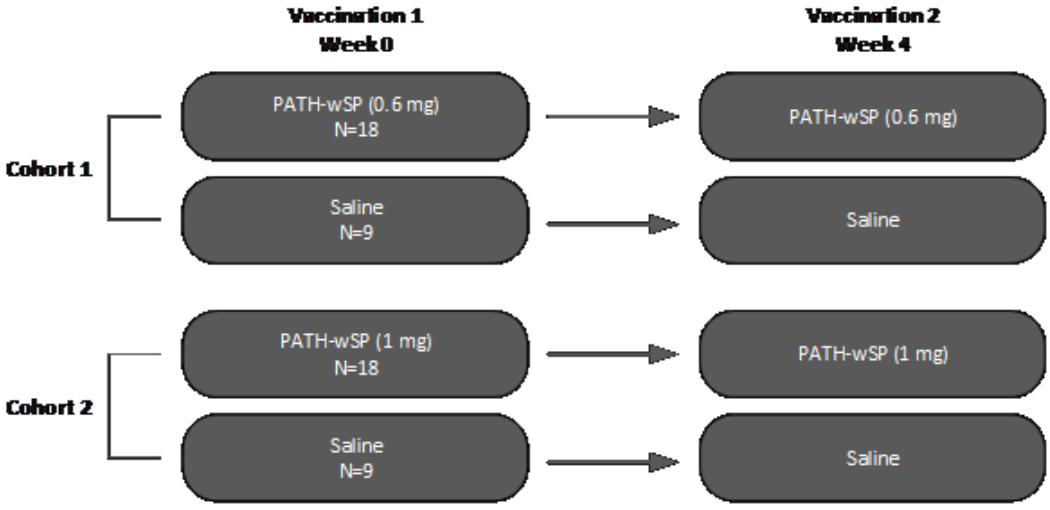
Abbreviation: NS = not significant from Day 0 (baseline).

*Significantly different from Day 0 (pre-vaccination) serum survival time ($P < 0.05$). P values for differences in survival time were calculated by a 2-tailed Wilcoxon 2-sample rank test.

1.5.2. VAC-010

Given the favorable safety, tolerability, and immunogenicity profile of PATH-wSP in the VAC-002 study, a Phase 1/2 dose escalation and age de-escalation study (VAC-010) was initiated in April 2014 at Kenya Medical Research Institute (KEMRI) in Kombewa to evaluate a two-dose regimen of PATH-wSP up to 1 mg in healthy adults, and up to 0.6 mg in healthy toddlers (Figures 2 and 3).

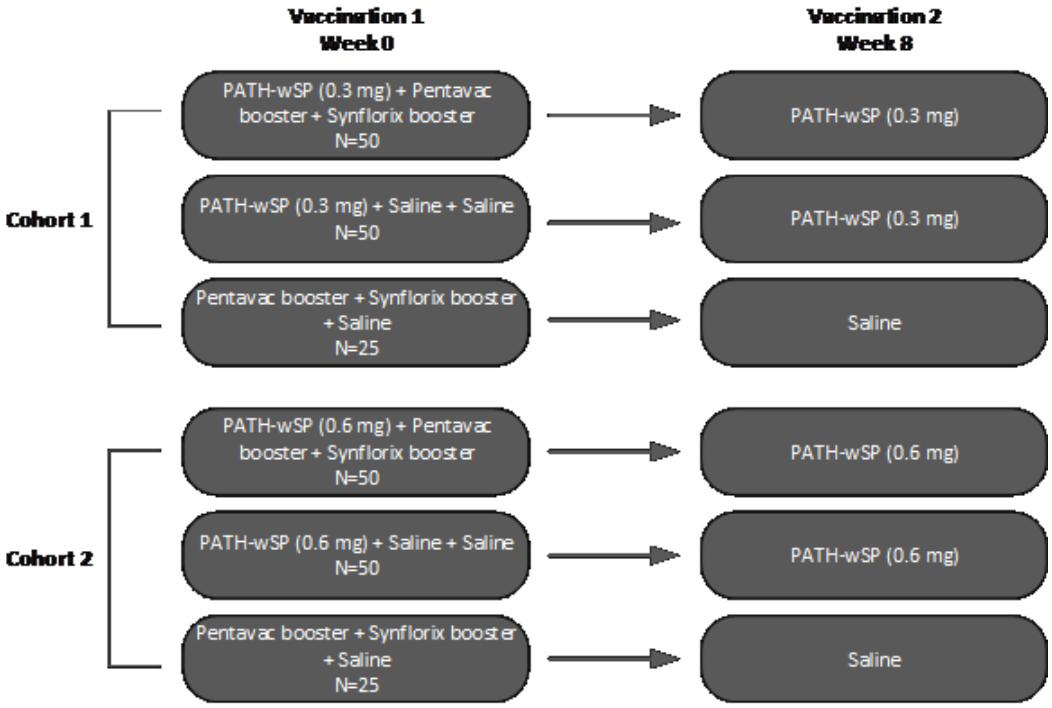
Figure 2. Adult Treatment Groups in the VAC-010 Study



The toddler phase of VAC-010 is designed to evaluate the safety, tolerability, and immunogenicity

of PATH-wSP at two dose levels (0.3 mg and 0.6 mg) administered in a two-dose regimen (with 8 weeks between vaccinations) and given with a booster dose of Pentavac and Synflorix vaccines during the first vaccination (N=50) or alone with saline placebo in lieu of the booster doses (N=50); a third treatment arm evaluates the administration of the booster doses alone during the first vaccination (N=25), with saline placebo administered as a control at both vaccination visits.

Figure 3. Toddler Treatment Groups in the VAC-010 Study



All assessed doses of PATH-wSP vaccine (0.6 mg and 1.0 mg in adults and 0.3 mg and 0.6 mg in toddlers) induce a substantial immune response against pneumolysoid and PspA protein after the first and second vaccinations. A functional immune response was elicited by the wSP vaccine as detected by the passive transfer assay and a positive trend was seen with the pneumolysin toxin neutralization assay. In toddlers, immune responses induced by the 0.6 mg dose appeared to be higher than with the 0.3 mg dose.

The co-administration of Synflorix and Pentavac with PATH-wSP appears to reduce immune response to wSP-associated pneumococcal proteins after the first dose; however a substantial increase in immune response was observed after the second wSP vaccination. No negative impact on immunogenicity of routine pediatric vaccines (DTPw-Hib-Hep and PCV-10) was observed after co-administration with wSP.

Reactogenicity profile of wSP concomitantly administered with routine pediatric vaccines and given alone was mild; the rates of any solicited adverse events in the wSP groups were comparable with that in the control group. The most common solicited reactions in adults were injection-site pain, fatigue and myalgia / arthralgia. The most frequently reported solicited reactions in toddlers were tenderness, decrease of appetite and irritability. Severe reactions were reported in <5% of subjects.

Overall, 15 subjects reported SAEs in the study VAC-010; one SAE (simple febrile convulsion) was assessed as vaccine-related by the investigator.

The rates of unsolicited AEs were comparable in the wSP and control groups except for pneumonia cases which were higher among toddlers who received wSP in 0.6 mg dose compared to the control group. Several pneumonia cases were reported after the first vaccination; most of them were assessed as mild-to moderate by the investigator. Two cases of pneumonia were confirmed by chest radiography and required hospital admission. These cases were reported as SAEs. All cases resolved following standard antibiotic therapy. Considering the non-equal allocation ratio in the study (100 subjects in the pooled 0.6 mg groups and 25 subjects in the control group), and the low incidence of the severe cases, the relevance of this observation is unknown.

The independent DSMB recommended, after reviewing all key safety data from study VAC-010, that a case definition for pneumonia be pre-specified in future clinical studies to standardize clinical evaluation, and that unblinded pneumonia cases be reported in an expedited manner to the DSMB.

Overall, the results of the VAC-010 study confirmed the favorable risk / benefit profile of wSP vaccine but require evaluation of the observed imbalance for pneumonia cases in further clinical studies, including the VAC-040.

1.6 Clinical Development Plan for PATH-wSP and Justification for the Study

The ultimate goal of clinical development program for PATH-wSP is to achieve vaccine licensure by a WHO-recognized national regulatory authority, followed by prequalification by WHO to support product acquisition by GAVI and UNICEF for its distribution to low- and middle-resource countries. To achieve these aims, studies in infant populations in low-resource settings will be required that, in addition to assessing safety and immunogenicity, will evaluate vaccine efficacy in a population at high risk for pneumococcal disease.

Prior to initiation of clinical development in infant population, the current study (VAC-040) will be conducted to confirm the favorable reactogenicity, safety and immunogenicity profile of a new single-vial formulation of the PATH-wSP vaccine (an alum-adsorbed suspension) manufactured by PT Bio Farma, Indonesia. A two-dose schedule at the 0.6 mg and 1 mg dose levels will be evaluated sequentially in two separate adult cohorts followed sequentially by two toddler cohorts.

2. HYPOTHESIS, OBJECTIVES, AND ENDPOINTS

2.1. Study Hypothesis

Primary Hypothesis: PATH-wSP administered IM to healthy adults and toddlers will be safe and well tolerated up to a dose of 1 mg.

Secondary Hypothesis: PATH-wSP administered IM to healthy adults and toddlers will elicit a measurable immune response.

2.2. Study Objectives

2.2.1. Primary Objectives

- To evaluate the safety and tolerability of 2 dose levels (0.6 mg and 1 mg) of PATH-wSP vaccine, administered in a 2-series schedule 4 weeks apart, in healthy adults.
- To evaluate the safety and tolerability of 2 dose levels (0.6 mg and 1 mg) of PATH-wSP vaccine, administered in a 2-series schedule 8 weeks apart, in healthy toddlers when co-administered with a booster dose of licensed pentavalent vaccine (diphtheria, tetanus, whole-cell pertussis, *Haemophilus influenzae* type b, and hepatitis B combined vaccine) at the second vaccination.

2.2.2. Secondary Objective

- To assess immunogenicity of PATH-wSP vaccine when given as a 2-vaccination series to adults and toddlers.

2.2.3. Exploratory Objectives

- To evaluate the potential for sera from PATH-wSP-immunized subjects to afford passive protection in an animal challenge model (subset of subjects).
- To identify novel antibody targets for future vaccine candidates using PBMCs (i.e. plasmablasts) stimulated *in vitro* (adults only).

2.3. Study Endpoints

2.3.1. Primary Endpoints

Safety:

Safety and tolerability of PATH-wSP vaccine including co-administration of PATH-wSP and licensed pentavalent vaccine at the second vaccination to toddlers will be evaluated based on the following analyses:

- Occurrence and severity of solicited local and systemic AEs and clinical laboratory abnormalities through 1 week post vaccination.
- Occurrence, severity, and relatedness to vaccination of unsolicited AEs and SAEs from Day 0 through the last study contact.

- Occurrence and severity of pneumonia cases reported from Day 0 through the last study contact.

2.3.2. Secondary Endpoints

Immunogenicity:

- The IgG response to pneumococcal-specific proteins (Ply and PspA Fam 1,) measured by the ELISA, and the IgG response to nine pre-selected pneumococcal proteins measured on the MSD platform, will be evaluated based on the following analyses:
  - IgG GMC and GMFR (from baseline) 4 weeks after the second vaccination.
  - Percentage of subjects with IgG concentration of a predefined threshold level (responders), measured 4 weeks after the second vaccination.

2.3.3. Exploratory Endpoints

Immunogenicity:

- Assessment of protection of mice against IV *S. pneumoniae* challenge after passive transfer of serum obtained from a subset of subjects 4 weeks after the second vaccination.
- Identification of novel antibody targets for future vaccine candidates using PBMCs (i.e. plasmablasts) stimulated *in vitro* (adults only).

3. STUDY DESIGN

This Phase 1/2, randomized, placebo-controlled, observer-blind trial is designed to sequentially evaluate PATH-wSP at two escalating doses (0.6 mg and 1 mg) in both adults and toddlers.

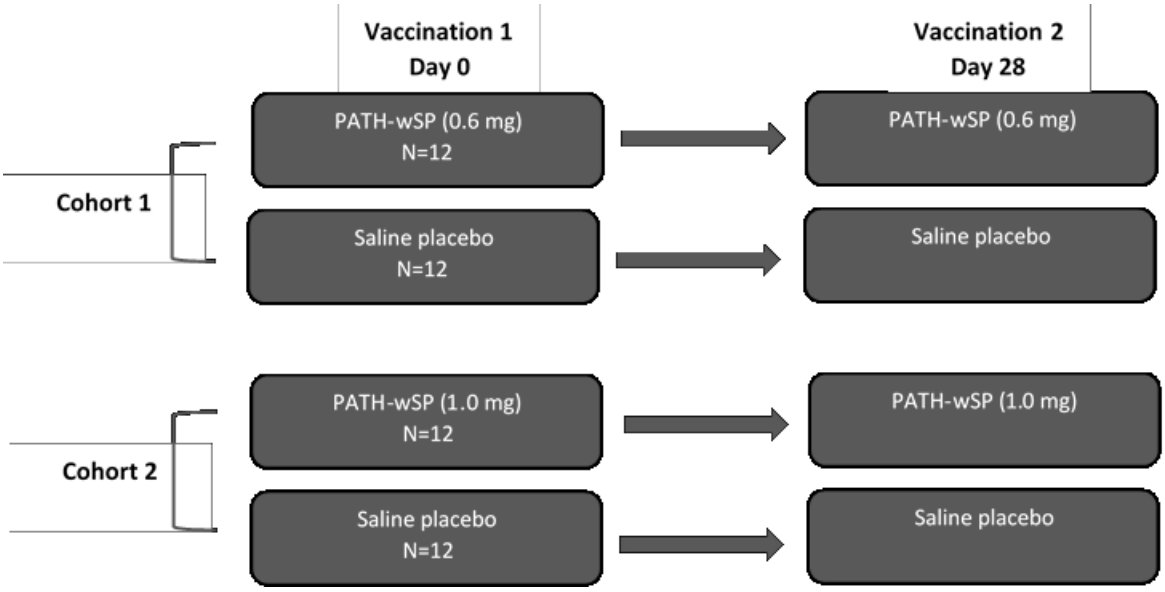
3.1. Definitions of Cohorts, Treatments, and Treatment Groups

The following 2 adult and 2 toddler cohorts will be sequentially evaluated in this study:

3.1.1. Adult Cohorts

- Cohort 1: Two 0.6 mg doses of PATH-wSP (12 subjects) **or** saline (12 subjects) with a 28-day interval between doses.
- Cohort 2: Two 1 mg doses of PATH-wSP (12 subjects) **or** saline (12 subjects) with a 28-day interval between doses.

Figure 4. Adult Cohorts



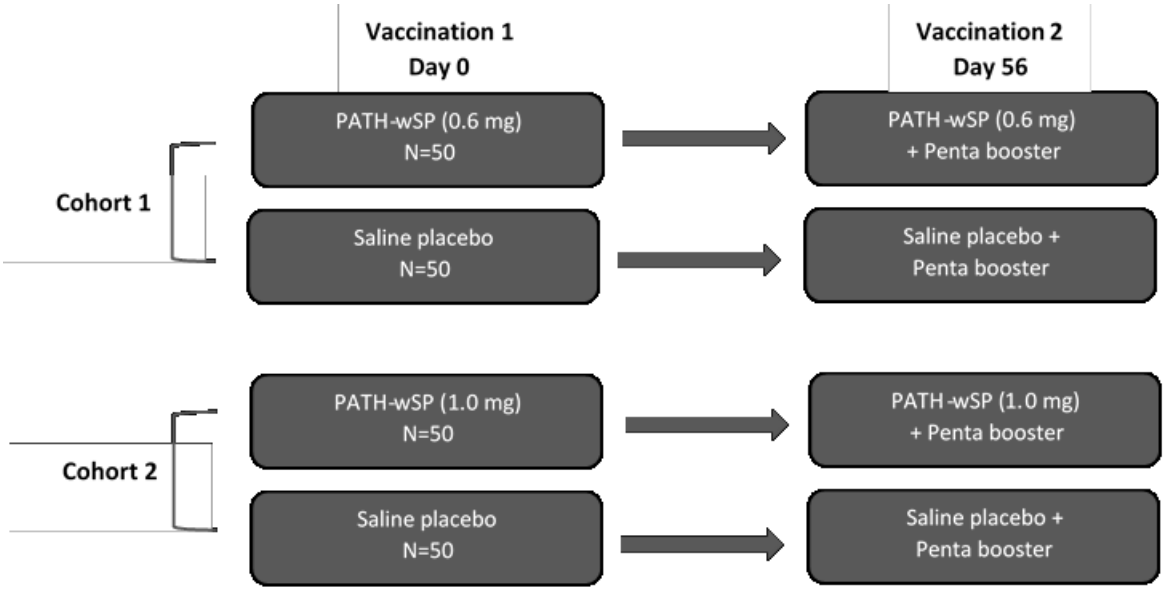
In each adult cohort, approximately 24 eligible healthy adult Kenyan subjects (18-40 years old inclusive) will be randomized in a 1:1 ratio to receive two doses of either PATH-wSP (at the 0.6 mg or 1 mg dose level for Cohort 1 and 2 respectively) or saline placebo at an interval of 28 (+7) days between injections. The use of a saline control group in both adult cohorts replicates the placebo-controlled design of the VAC-010 and VAC-002 studies, allowing for a direct comparison of REs and AEs across studies. Because some adult subjects will receive a saline control instead of a potentially efficacious vaccine (PATH-wSP), all adults will be offered prophylactic rabies vaccination at the end of the trial (given as a standard 3-dose regimen under the guidance and at the discretion of the PI), to provide all adult subjects potential health benefit from participating in the trial. Rabies is endemic in Kenya: 1623 cases of suspected rabid dog bites were handled in 2009 in one Kenyan district hospital alone.[12]

3.1.2. Toddler Cohorts

Cohort 1: Two 0.6 mg doses of PATH-wSP (50 subjects) **or** saline (50 subjects) with a 56-day interval between doses, and pentavalent vaccine co-administered with the second dose.

Cohort 2: Two 1 mg doses of PATH-wSP (50 subjects) **or** saline (50 subjects) with a 56-day interval between doses, and pentavalent vaccine co-administered with the second dose.

Figure 5. Toddler Cohorts



In each toddler cohort, approximately 100 eligible healthy Kenyan toddlers (12-21 months old inclusive) will be randomized in a 1:1 ratio to receive two doses of either PATH-wSP (at the 0.6 mg or 1 mg dose level for Cohort 1 and 2 respectively) or saline placebo at an interval of 56 (+7) days between injections.

A booster dose of a licensed pentavalent diphtheria, tetanus, whole cell pertussis, *Haemophilus influenzae* type b, and hepatitis B vaccine will also be administered at the second vaccination visit to toddler subjects in both treatment arms of both cohorts. Currently the Kenyan EPI program does not include a pentavalent booster during the second year of life. Given that boosters of Hib, diphtheria, pertussis, and tetanus vaccines have been shown to provide increased protection and improved immune responses, and to be safe during the second year of life,[13,14] the pentavalent booster provides a potential health benefit to the toddlers enrolled in this trial.

3.2. Overview of Enrollment and Cohort Advancement

The sequential enrollment of study cohorts allows the IST to review blinded safety data from individual cohorts prior to granting approval for dose escalation or age de-escalation. The 1 mg adult cohort will be initiated only after the IST reviews the blinded safety data for all subjects in the 0.6 mg adult cohort through Day 7, and based on this review grants approval for the dose escalation. Similarly, the 0.6 mg toddler cohort will be initiated only after the IST reviews the blinded safety data for all subjects in the 1 mg adult cohort through Day 7, and based on this review grants approval for the age de-escalation.

Finally, the 1 mg toddler cohort will be initiated only after the DSMB reviews the safety data in the 0.6 mg toddler cohort through 6 weeks after the first vaccination for each subject, including reactogenicity results and distribution of pneumonia cases, and based on this review grants approval for the dose escalation. In addition, enrollment in the 1 mg toddler cohort will be staggered such that the IST will review safety data through Day 7 for the first 10 subjects prior to approving enrollment of the remainder of the cohort. Additional details on the IST and the DSMB are provided in Section 10.1.

3.3. Overview of Study Procedures

Each adult subject will undergo a total of 6 scheduled visits (V), including at least one screening visit (V0) no more than 28 days prior to randomization and first vaccination (V1, Day[D]0); two vaccination visits (V1 and V3) scheduled 28 (+7) days apart; two safety visits (V2 and V4) occurring 7 (+3) days post vaccination visits; and a final visit (V5) occurring 28 (+14) days after the final vaccination.

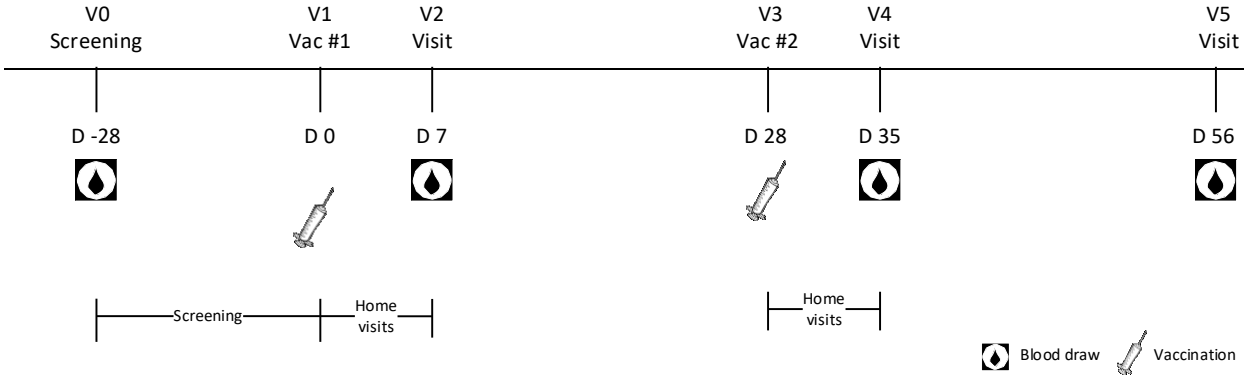
Each toddler subject will undergo a total of 8 scheduled visits, including at least one screening visit no more than 28 days prior to vaccination (V0), two vaccination visits (V1 and V4, scheduled 56 days apart), two safety visits at 7 and 28 days after the first vaccination (V2 and V3, respectively) and three safety visits at 7, 28 and 56 days after the second dose (V5, V6 and V7, respectively).

Toddlers will receive two vaccinations at the second vaccination visit (V4), whereas adults will only receive one (see Figures 6 and 7).

Daily reactogenicity assessments for 7 days post vaccination will be completed in all subjects by fieldworkers using a standard home visit diary.

For all subjects, blood draws for safety laboratory tests and/or PATH-wSP-induced immune responses will be performed at screening (baseline safety and immunogenicity), 7 days after each study vaccination (safety only) and 28 days after the second vaccination (immunogenicity only).

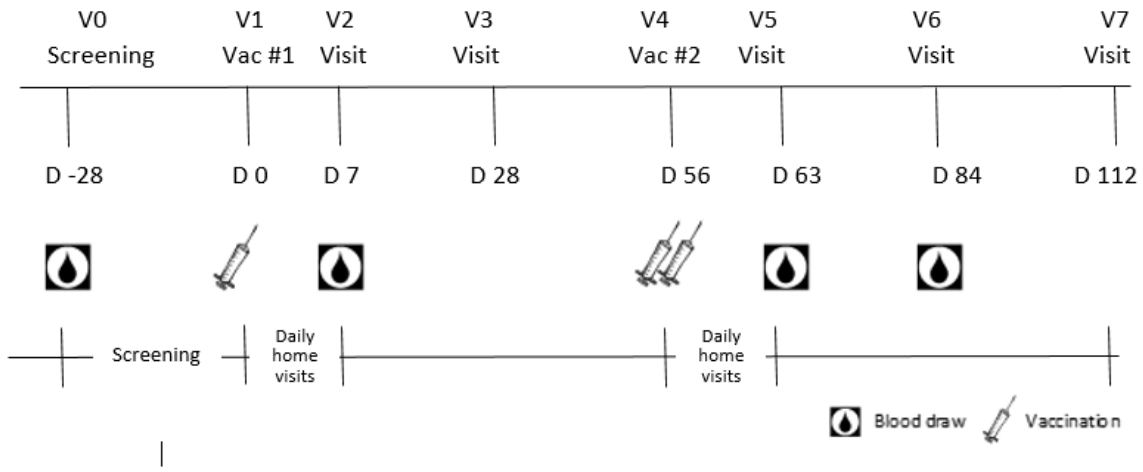
Figure 6. Study Schematic for Adult Cohorts



Abbreviations: D = day; V = visit

Notes: Screening must occur not more than 28 days before the Day 0 (V1) vaccination visit. Home visits by fieldworkers occur on Days 1 through 6 and Days 29 to 34 following vaccinations.

Figure 7. Study Schematic for Toddler Cohorts



Abbreviations: D = day; V = visit
Notes: Screening must occur not more than 28 days before the Day 0 (V1) vaccination visit. Home visits by fieldworkers occur on Days 1 through 6 and Days 57 to 62 following vaccinations.

Screening: Subjects in all cohorts must complete screening procedures within 28 days of signing an informed consent form (i.e., 'enrollment'). Screening procedures include medical history, targeted physical examination (PE), laboratory assessment, and confirmation of eligibility for randomization based on cohort-specific inclusion/exclusion criteria. The screening visit may encompass more than one day in order for a subject to meet study eligibility criteria. During screening up to two laboratory or vital sign measurements per parameter may be taken to determine whether a subject is eligible for randomization. The last laboratory measurement or vital sign must be used as the baseline value for the study. If the screening period exceeds 28 days, ongoing willingness to participate in the study must be confirmed verbally from the subject/subject's parent or legal guardian and documented in the case report form (CRF) and all screening procedures repeated. On the day of randomization (Day 0, V1), a urine pregnancy test will be performed on all adult females, a finger prick rapid antigen test for malaria, and a targeted history and PE will be performed to exclude acute illness on all subjects before eligibility is confirmed by the PI and subjects randomized (See Table 6 for the complete schedule of events).

Randomization: Eligible subjects will be randomized to 1 of 2 treatment groups (based on cohort) using a 1:1 randomization ratio. Randomization will take place only after a subject 1) has satisfied all eligibility criteria specific to the qualifying age cohort; 2) is without acute infection/illness that precludes vaccination; 3) has a negative malaria rapid diagnostic test; 4) has normal vital signs and safety laboratory values; and 5) has a negative urine pregnancy test in the case of adult female subjects. Randomization will occur at V1, the day of vaccination. The PI, clinic staff, the Sponsor, and the contract research organization (CRO) will remain blinded to subject treatment assignment until after database lock, unless unblinding is warranted due to subject safety concerns or for related SAEs (see Section 6.7). Site personnel responsible for preparation and administration of study vaccine and the CRO personnel who are monitoring procedures related to vaccine preparation and administration will remain unblinded and will not participate in other activities of the clinical trial. All monitoring reports from the unblinded monitor will be written such that the blind will be maintained during trial conduct.

Safety Monitoring: After each vaccination subjects will be monitored for solicited reactogenicity, which is evaluated for severity by toxicity grading scale (see Section 9.2.2.); systemic solicited reactogenicity will also be evaluated for relatedness to the vaccination.

- The solicited local reactions to be assessed for **adults** will include pain, induration/swelling, and erythema/redness at the injection site, and the solicited systemic reactions will include cutaneous rash, headache, fever (based on axillary temperature), fatigue/malaise, arthralgia, and myalgia.
- For **toddlers** the solicited local reactions will include tenderness, erythema/redness, and induration/swelling at the injection site, and the solicited systemic reactions will include cutaneous rash, fever (based on axillary temperature), irritability, drowsiness, and decreased appetite.

For each study vaccination, solicited reactogenicity will be assessed 1) at approximately 60 minutes following vaccination, 2) daily by fieldworkers during days 1 to 6 after each study vaccination, and 3) Day 7 post each vaccination, i.e. in clinic at Visit 2 (Day 7 [+3]; all subjects), and Visit 4 (Day 35 [+3]; adults only) or Visit 5 (Day 63 [+3]; toddlers only).

During 6 calendar days after a subject receives study vaccine, fieldworkers will visit the subject at home daily to assess and record solicited reactogenicity, and determine whether the subject needs to be seen by the PI for any medical condition or issue (unscheduled visit). **Any Grade 3 or greater reactogenicity assessed by a fieldworker will result in immediate clinic contact, and the subject will be seen in the clinic within 24 hours** once a fieldworker becomes aware **of the event.** All reactogenicity scoring will be reviewed by the PI and (in the case of systemic events) graded for relatedness to vaccination on the first scheduled clinic visit after vaccination, prior to being entered into the electronic-CRF (eCRF).

All subjects or parents / legal guardians will be instructed to contact the site as soon as possible if he / she feel unwell or has adverse events or a medical condition that required medical attention or of concern. In addition, parents / legal guardians will be instructed to contact site staff in case of any respiratory symptoms in child that persist for more than 3 days.

At each study visit or unscheduled study contact, all toddler subjects with respiratory symptoms (cough or difficulty of breathing) will be assessed by the investigator or a medically qualified designee. The assessment will include physical exams and collection of relevant symptoms and signs. In addition, a nasopharyngeal swab will be taken in all subjects with cough and/or difficulty of breathing and respiratory rate of > 40 per minute (suspected pneumonia). A rapid malaria test will be performed in subjects with body temperature ≥ 37.5 C or history of fever in the last 24 hours.

Assessment of toddlers with suspected severe pneumonia (defined as respiratory rate of ≥ 50, or presence of lower chest in drawing or an oxygen saturation < 90% or presence of other danger signs, such as grunting, central cyanosis, convulsion, vomiting everything or unable to feed) will include chest radiography. Reading of the chest radiograms for clinical diagnostic and treatment purposes will be done according to the routine practice by the local radiologist, physician and/or pediatrician. Digital x-ray images from suspected pneumonia cases will be sent to PATH for further classification. A nasopharyngeal swab and samples for blood culture and C-reactive protein (CRP) measurement will be collected from all children with suspected severe pneumonia within a maximum of 12 hours after a chest radiography or within 12 hours after hospitalization and preferably before the first administration of any antibiotic.

All subjects will be monitored for unsolicited AEs at each clinic visit until the End-of-Study (EOS) visit, with events coded using the Medical Dictionary for Regulatory Activities (MedDRA) (version 15.1 or later) and assessed by the PI with regards to severity, relatedness, and duration.

Safety laboratory parameters will be monitored at screening (V0) and 7 days after each study vaccination. Laboratory values that are outside the normal laboratory range will be assessed by the PI with regard to clinical significance, relatedness, and severity. Any abnormal laboratory value from post-vaccination assessments with toxicity score $\geq$ Grade 2 must be repeated within 7 days (and may be repeated more than once). Any laboratory value that is outside the normal range should be assessed on the basis of toxicity score, change from baseline, and clinical assessment of the subject, to determine if an AE has occurred. Any laboratory value with a toxicity score $\geq$ Grade 3 (after confirmation by repeat testing within a brief window) must be attributable to an existing AE or be classified as an AE based on the laboratory abnormality. Additional laboratory assessments may be obtained at the discretion of the PI based on clinical assessment. A listing of all safety labs and schedules can be found in Section 7.2.

Any AE/SAE ongoing at the time of the subject's EOS visit will be attempted to be followed up until resolved, or assessed to be resolved with sequelae by the PI, until last subject last visit (LSLV) in the trial. If any AE/SAE remains unresolved at the time of LSLV, it will be classified as ongoing in the database for data management purposes.

To facilitate rigorous safety monitoring, data captured at each visit will be entered into the electronic data capture (EDC) system within 3 business days from the date of the clinic visit, including all reactogenicity measurements collected during home visits. For ancillary data that may be obtained after a visit (e.g., laboratory test results, inpatient records) the goal of data entry will be to maintain data integrity and timely safety assessments. The IST will monitor the safety of study subjects during their active enrollment in the study. Study pause rules will be in place for specific events $\geq$ Grade 3 (see Section 10.3) that would trigger convening of the DSMB. The IST or DSMB will grant approval to advance through the cohorts as detailed in Section 3.2.

Immunogenicity Testing: During screening (V0), all subjects will have blood drawn to be stored for later evaluation of baseline immunogenicity. Only those subjects who are randomized will have baseline immunogenicity assays performed. Blood will be obtained for post-vaccination immunogenicity testing at 28 (+14) days after the final vaccination (Visit 5 in adults or Visit 6 in toddlers). Immunogenicity will be assessed by ELISA and MSD assay to determine IgG response to pneumococcal-specific proteins as well as by passive transfer on a subset of subjects.

In addition, consenting adult subjects will have blood drawn at Visit 4 (7 [+3] days after the final vaccination) to collect PBMCs for novel pneumococcal antigen screening. This is an exploratory endpoint for discovering novel antigens that may provide alternatives for future pneumococcal vaccine development. Due to the blood volumes needed for PBMCs, this is an option for adult subjects only, who must agree to the blood draw separately on the informed consent. Opting out of this blood draw will not affect enrollment of subjects into the trial.

4. STUDY PRODUCTS

4.1. Product Description

PATH-wSP is made from whole, unencapsulated and autolysin-deficient pneumococcal cells that are inactivated and adsorbed to aluminum hydroxide adjuvant. *Streptococcus pneumoniae* whole cell antigen (SPWCA) bulk is manufactured from strain RM200 RX1E PdT ΔlytA and is inactivated with beta-propiolactone. The gene for pneumolysin, a 471-amino-acid toxin produced by virtually all clinical isolates of *S. pneumoniae* that has both cytolytic and complement activation properties, has been knocked out and replaced with the gene for pneumolysoid, a derivative with three-point mutations known to abolish the virulent properties of the toxin. PATH-wSP is an opalescent suspension.

The saline placebo to be used will consist of normal saline (0.9% sodium chloride, preservative-free).

The licensed pentavalent vaccine that will be used in this trial (Pentavalent vaccine, Serum Institute of India Limited) is a homogeneous liquid containing purified diphtheria and tetanus toxoids, inactivated whooping cough (pertussis) organisms, highly purified, noninfectious particles of Hepatitis B surface antigen (HBsAg), and highly purified, non-infectious *Haemophilus influenzae* type b (Hib) capsular polysaccharide chemically conjugated to a protein (tetanus toxoid).

4.2. Products and Manufacturers

The single-vial formulation of PATH-wSP being evaluated in this study is manufactured by PT Bio Farma in Bandung, Indonesia.

4.3. Presentation and Formulation

PATH-wSP is aseptically formulated with saline (0.9% sodium chloride), aluminum hydroxide (1.2 mg Al^{+3}/mL) and thimerosal (0.01%) at one of the clinical target concentrations of antigen (1.2 mg/mL or 2 mg/mL). PATH-wSP is provided as a 5-dose configuration (2.5 mL in 5 mL glass vials). Two different dose levels of PATH-wSP will be evaluated in this trial: 0.6 mg and 1 mg. The label for each vial of PATH-wSP will contain information that identifies the manufacturer, dose level, retest date, lot number, storage conditions required, and a cautionary statement ("For Clinical Trial Use Only").

4.4. Stability and Storage

PATH-wSP and Pentavalent vaccine (Diphtheria, Tetanus, Pertussis, Hepatitis B and Haemophilus influenzae type B conjugate vaccine) are stored at between 2°C and 8°C. It must not be frozen. The normal saline (sodium chloride, 0.9% for injection) to be used as placebo will be stored at room temperature. The temperature of study vaccines will be monitored during shipment, storage and transportation using a continuous temperature monitoring system to ensure that temperature deviations do not occur. Vaccines will not be used until the temperature of the vaccines throughout transit and storage has been confirmed to be within acceptable limits.

Upon receipt at the clinical site, KEMRI-Wellcome Trust Research Programme in Kilifi, Kenya (KWTRP Kilifi), PATH-wSP will be stored at 2°C to 8°C in a dedicated refrigerator that is safe, locked, and not accessible to unauthorized personnel—including study team personnel blinded for study conduct purposes. The refrigerator will be under continuous temperature monitoring (documented on daily temperature logs), and connected to a power source with a reliable back-up

system. Vaccine needed for a particular day will be transported from KWTRP Kilifi to the field site in a cold box with continuous temperature monitoring. Any unused vaccines at the end of clinic will be returned for storage provided the vials have remained within their defined temperature range.

It will be the responsibility of designated unblinded site personnel to ensure that vaccine has not been exposed to temperatures outside the allowed range during transport or storage at the facility prior to being dispensed for vaccination. Should there be a deviation outside the allowed temperature range, the affected vaccines will be quarantined. The temperature deviation will be reported to the CRO, who will advise the unblinded team of the action to be taken based on the magnitude and duration of the temperature deviation. All drug accountability procedures, including cold chain monitoring will be documented and are the responsibility of the unblinded study personnel.

The lot of PATH-sWP to be used in this study was evaluated in a formal stability program per International Conference for ICH guidelines.

4.5. Preparation and Administration

A single dose of study vaccines (PATH-wSP and placebo) is 0.5 mL. It should only be administered as an IM injection. The vaccine should not be given IV or SC.

4.5.1. Dose Preparation and Administration

A limited number of appropriately trained, unblinded study personnel will be responsible for preparing study vaccine doses (i.e., drawing up vaccine from a multi-dose vial, and masking and labeling the syringe) in accordance with the randomly determined assignment, vaccine administration and handling all drug accountability procedures. The number of unblinded personnel will remain limited, and these personnel will not participate in the other aspects of the clinical trial, to help ensure the integrity of the blind at the site. The unblinded personnel will not reveal subjects' randomization assignments to the subjects, subjects' parents / legal guardians, or staff associated with the Sponsor, CRO, or site.

Unblinded personnel will retrieve a subject's randomization assignment after being informed by the PI that a subject is eligible for randomization. They will prepare the study vaccine based on the subject's randomization assignment (see Section 6.2.3) in a setting distinct from the clinic staff, and then the unblinded study nurse will administer study vaccine to a subject in a separate clinic setting. For all cohorts, vaccination will take place in a clinic setting in which there is immediate access to the medical personnel (certified in adult and pediatric life support), and emergency resuscitation equipment and medications, appropriate to the level of expected medical risk to the subjects being vaccinated. Emergency evacuation to a hospital will also be available.

Multiple doses of PATH-wSP and pentavalent may be drawn up from a single vial during the same vaccination day as long as the cold chain requirements are maintained for that vaccine. PATH-wSP and saline will be drawn up into identical syringes, and the barrels masked, to maintain the blind at the time of administration.

For all cohorts, unblinded nursing staff will administer study vaccine based on WHO best practices (WHO 2010).

- For adult subjects, PATH-wSP or saline placebo will be administered as an IM injection into the mid-deltoid muscle of the subject's non-dominant arm. If the non-dominant arm is not acceptable based on clinical assessment (e.g., due to local infection) the dominant arm may be used and the reason for the change documented. Alternate locations are allowed if following WHO immunization guidelines, but are not encouraged.
- For toddler subjects, PATH-wSP or saline placebo will be administered as an IM injection into the anterolateral aspect of the right thigh. If the right thigh is not acceptable based on clinical assessment, the left thigh will be the alternative injection site and the reason for the change documented. At the second vaccination visit (V4), the pentavalent vaccine will be administered into the anterolateral aspect of the left thigh. If either thigh is not acceptable for vaccination based on clinical assessment, the other thigh would be used for both vaccinations and the reason for the change noted. The study vaccine would be administered into the proximal part of the thigh and the pentavalent vaccine into the more distal part of the thigh with a minimum of 2 cm between the two injection sites.
- The site at which all vaccines have been administered will be documented in the CRF, to ensure accurate assessment of local reactogenicity—both immediately after vaccinations and during daily home visits by fieldworkers for six days following the vaccination day. In the case of toddlers, local reactogenicity of both the investigational vaccine and pentavalent booster will be documented.

A detailed account of procedures related to preparation and administration of study vaccine and the pentavalent booster will be included in the Manual of Procedures (MOP).

The blinded clinic staff will assume further management of the subject in the trial immediately after vaccination is completed.

4.5.2. Accountability and Disposal

All vaccinations will be documented in a drug accountability log on the day of vaccination, and used vials of study vaccine stored in a dedicated space that is accessible only to the unblinded site personnel and the unblinded CRO monitor.

The unblinded site personnel will maintain a complete and accurate inventory of study vaccine received (including the quantity of vaccine received, date of receipt, condition at receipt, temperature noted during transit), those administered, and any broken or destroyed. A log identifying each vial will also be included with any shipment of study product. All records related to receipt, storage, and disposal of study vaccine will be accessible only to the unblinded site personnel and the unblinded CRO study monitor during the conduct of the trial.

In case a vial of vaccine is broken or unusable, the unblinded site personnel will immediately inform the unblinded monitor and store the vial for accountability, following all safety precautions. In case a broken vial cannot be stored safely for accountability, appropriate discard and documentation will be followed after consultation with the unblinded monitor. Study product prepared but not administered to subjects, and all unused study product will likewise be documented per drug accountability processes and discarded after the study is completed or terminated after notification by the CRO study drug monitor.

The unblinded CRO monitor will visit the site periodically throughout the trial to review and verify vaccine accountability records, as well as to ensure compliance with all trial procedures by the unblinded site personnel. After final drug accountability is completed by the unblinded CRO

monitor, any used vials and syringes or unused study vaccine will be destroyed at the site under the supervision of the unblinded site personnel. Due to the need to maintain blinding, no drug accountability records will be sent to the Sponsor or included in the trial master file (TMF) until after database lock.

5. STUDY POPULATION

5.1. Clinical Trial Site

This study is a single-site clinical trial, to be performed at the KEMRI-Wellcome Trust Research Programme in Kilifi, Kenya (KWTRP Kilifi). The study population will be recruited, screened and qualified by the site staff, under the direction of the PI.

5.2. Study Population

The study population will consist of healthy Kenyan adults (18-40 years old) and toddlers (12-21 months old) residing in the vicinity of the study site, who have provided consent for participation—or, in the case of toddlers, whose parents or legal guardians have provided consent—after being fully informed about the study.

5.3. Eligibility

Following informed consent ('enrollment'), the subject will be assessed for study eligibility by undergoing a PE, providing a detailed medical history (including medication use), and having blood drawn for screening laboratory analyses. Subjects who meet all eligibility criteria for their age cohort, including demonstrating normal vital signs and laboratory test results based on the local normal ranges, will be eligible for randomization. Baseline clinical laboratory tests and vital signs may be repeated only once to determine whether a subject is eligible for randomization. The last laboratory / vital measurement must be used as the baseline value for the study. The PI will use good clinical judgment in considering a subject's overall eligibility.

5.4. Inclusion Criteria

All subjects must satisfy ALL the following criteria at Day 0 (prior to randomization) based on their age cohort:

- They are healthy adults who are 18 to 40 years old, or toddlers who are 12 to 21 months old on the day of randomization (Day 0), inclusive of the age ranges.

  Adults will be eligible from the day they reach 18 years of age until the day **before** they reach 41 years of age.

  Toddlers will be eligible from the day they reach 12 months of age until the day **before** they reach 22 months.

  Note: 'Healthy' adults and toddlers are those who are without acute or chronic, clinically significant pulmonary, cardiovascular, hepatobiliary, gastrointestinal, renal, neurological, or hematological functional abnormality or illness that requires medical therapy, as determined by medical history or clinical assessment before being randomized.

- Subjects (or parent / legal guardian in the case of toddlers) must provide voluntary written/thumb-printed informed consent for the subject to participate in the study.

- Subject (or parent / legal guardian in the case of toddlers) must be able to comprehend and comply with study requirements and procedures and must be willing and able to return for all scheduled follow-up visits.
- Subjects (or parent / legal guardian in the case of toddlers) must have a readily identifiable place of residence to ensure home visits can be undertaken reliably and must also have a consistent means of telephone contact for the duration of trial participation or make use of the study's local contact person (fieldworker) whom subjects can approach to communicate with the study staff by telephone, as necessary.
- Subjects must be resident in the study area with no plans to travel outside the study area during the period of study participation.
- Subjects must be willing to not consume (ingest) herbal or other local traditional medications within 28 days of randomization and during the course of the study. Subject's parents / legal guardians must be willing to not administer such medications to their toddler, and subject's mother / legal guardian must be willing to not consume herbal medication that may transmit to her toddler through breast milk.

Adults only

- Adult female subjects must have a negative serum pregnancy test at screening and urine pregnancy test prior to each vaccination. They will be advised through the informed consent process to avoid becoming pregnant for the duration of the study, and must agree to employ an effective form of birth control for the duration of the study. Adult female subjects with documented sterilization via tubal ligation or hysterectomy may be eligible for the study without required use of birth control.

  Note: Effective forms of birth control are the following: credible history of continuous abstinence from heterosexual activity, hormonal contraceptives (oral, injectable, implant, patch, and ring), double-barrier contraceptives (condom or diaphragm, either with spermicide) and intrauterine device. When using contraceptives, subjects must have been using their current contraceptive for the past two months to be eligible.

Toddlers only

- Toddlers must have been born full-term, and have a mid-upper arm circumference (MUAC) > 11.5 cm at the time of enrollment.
- Toddlers must have completed their Kenyan infant EPI schedule (Table 4) through 9 months, with the following minimum requirements: 1 birth dose of BCG, 3 doses of DTwPHibHep, 3 doses of oral polio vaccine (OPV) (birth dose is not required), 3 doses of PCV, 2 doses of rotavirus vaccine, and 1 dose of measles vaccine. If additional vaccines are introduced into the routine EPI schedule in Kenya or are given in campaigns during the period of the study, receipt of these vaccines would not be required for a toddler to be eligible for randomization.

Table 4. Kenyan EPI Schedule for Infants and Children

Vaccine	Age at Immunization
Bacille Calmette-Guérin vaccine (BCG)	Birth
Diphtheria and tetanus toxoid with whole cell pertussis, *Haemophilus influenzae* Type b vaccine, and Hepatitis B vaccine (DTwPHibHep)	6, 10, 14 weeks
Rotavirus Vaccine	6. 10 weeks
Measles	9, 18 months
Oral polio vaccine (OPV)	Birth, 6, 10, 14 weeks
Pneumococcal conjugate vaccine (PCV)	6, 10, 14 weeks
Vitamin A supplementation	6-11, 12-59 months
Yellow fever vaccine (YF)	9 months, *part of country (Kilifi County not included)*

Source: WHO Immunization Profile – Kenya

5.5. Exclusion Criteria

A subject who meets any of the following criteria at screening will not be eligible for randomization but may be reconsidered based on resolution of exclusion criteria at a later date if reevaluation falls within the screening period:

- Use of any investigational or nonregistered drug within 90 days of randomization or planned during the course of study participation.
- Use of any of the potentially hepatotoxic drugs listed in Appendix 9 within 28 days of randomization or during the course of study participation. This list will be provided to all subjects / subjects' parents or legal guardians at screening, who will be instructed to present this to any prescribing party such as doctors, pharmacists or any health facility where they may present for medical attention during enrollment in the study, and notify study staff immediately in the event that use of any listed drug becomes necessary.
- Administration of any vaccine within 14 days prior to enrollment in the study or planned use of any non-study vaccine 7 days prior to and 14 days after any study vaccination.
- Chronic, clinically significant pulmonary, cardiovascular, hepatobiliary, gastrointestinal, renal, neurological, or hematological functional abnormality or major congenital defects or illness that requires medical therapy, based on medical history or clinical assessment.
- History of anaphylactic shock.
- History of a serious reaction to any prior vaccination or known hypersensitivity to any component of the study vaccines.
- History of immunosuppression or immunodeficiency, inclusive of human immunodeficiency virus (HIV) infection by medical history (including that of an enrolled toddler's mother or legal guardian) or by HIV testing at screening. Toddler subjects will be excluded if their mother or legal guardian is known to be HIV positive from testing during the perinatal or post-delivery period.

Note: In toddlers, HIV seropositivity will result in a subject being excluded from the trial even though this may reflect maternal antibody transfer rather than HIV infection in the toddler. In all cases these individuals will be referred for follow-up care that is consistent with the normal care provided in Kenya under these circumstances.

- Evidence of active hepatitis infection (B or C) by serologic testing at screening.
- Any screening laboratory test (chemistry or hematology) or vital sign measurement with toxicity grade ≥ 1. A subject may repeat each abnormal laboratory assessment or vital sign once during the screening period for a subject to remain eligible for randomization.
- Acute illness (moderate or severe) and/or fever (axillary temperature ≥ 37.5°C).

  Note: Subjects with an acute illness/fever may return once for a repeat screening visit within the 28-day screening period and still qualify for randomization if the acute illness has resolved. A minimum of 72 hours following a documented fever must pass before the subject can be re-screened and vaccinated.
- Positive test for malaria (blood film) at screening that remains positive post treatment when retested prior to vaccination.

  Note: Subjects may be treated for malaria and retested during the screening period. A rapid antigen test for malaria will be undertaken on the day of each vaccination to ensure a subject with a concurrent malaria infection is not vaccinated.
- Disorders that require chronic administration (defined as more than 14 consecutive days) of immunosuppressants or other immune-modifying drugs within the past 6 months prior to the administration of the study vaccine. An immunosuppressant dose of glucocorticoid will be defined as a systemic dose > 10 mg of prednisone per day for adults or ≥ 0.5 mg/kg of prednisolone per day or equivalent in toddlers. The use of topical glucocorticoids will be permitted.
- Administration of immunoglobulins and/or any blood products within the 6 months preceding enrollment in the study, or anticipation of such administration during the study period.
- Known disturbance of coagulation or other blood disorder (e.g., thalassemia, sickle cell disease, thrombocytopenia, disorders of the lymphocytes, and severe anemia at birth) in adult subject or in self/first-degree relative of toddler subject; or receipt of anticoagulants in the past three weeks (aspirin as needed and nonsteroidal anti-inflammatory drugs are acceptable).
- History of meningitis, seizures or any neurological disorder (all participants) or major psychiatric disorder (adults).
- Any medical or social condition that in the opinion of the investigator will interfere with the study objectives or pose a risk to the study subject or may prevent the subject from completing the study follow-up.
- An employee (or first-degree relative of employee) of the Sponsor, the CRO, or any investigator or site personnel.

Adults only

- Female subjects who are pregnant or breast-feeding.
- Suspicion or recent history (within the past year) of alcohol or substance abuse.

Toddlers only

- Prior vaccination with a pentavalent booster (following the primary series).
- Family history of suspected primary immunodeficiency in first-degree relative.
- Had a sibling die of likely sudden infant death syndrome (SIDS) or die suddenly and without apparent other cause or preceding illness in the first year of life.
- Has evidence of a clinically significant congenital abnormality as judged by the PI.
- Evidence of fetal alcohol syndrome or maternal history of alcohol abuse during pregnancy.

Note that specific exclusion criteria (vital signs and clinical examination, history of acute illness, blood test for malaria, urinary pregnancy test for fertile women) will be reassessed at the time of V1 and prior to confirming final eligibility and proceeding to randomization to ensure only those subjects appropriate for vaccination are included in the study. These assessments will be repeated at the final (2nd) vaccination visit for all cohorts. Any subject who cannot be vaccinated on a given day (e.g., due to fever, abnormal vital signs, or acute illness) may return once the acute issue has resolved. A minimum of 72 hours must have passed after a documented fever before a subject can be vaccinated. In the case of the randomization visit, the return to clinic needs to fall within the 28-day screening period; otherwise, ongoing willingness to participate in the study must be confirmed verbally from the subject/subject's parent or legal guardian and documented in the CRF, and all screening procedures repeated.

6. STUDY PROCEDURES

6.1. Overview of the Recruitment Plan

This will be a single-center study to be conducted at the KEMRI-Wellcome Trust Research Programme in Kilifi, Kenya.

6.2. Informed Consent, Screening and Study Visits

6.2.1. Initial and Continuing Informed Consent

Informed consent is the process of ensuring that study subjects, or (in the case of the toddler phase of the study) subjects' parents / legal guardians, fully understand the purpose of the study and what will and may happen during participation in the research study and what the risks are. The informed consent process continues throughout the study. Key study concepts are reviewed with the study subjects (if adults) or study subjects' parents / legal guardians (if toddlers) at designated times and as needed; this review process must be fully documented. Additionally, if any new information becomes available that, in the judgment of PATH and/or the PI, may affect subjects' or their parents' / legal guardians' decision for them to continue in the trial, such information will be shared, and may be the basis for requiring a new consent form to be signed.

Prior to performing any study-related screening procedures on the subject, voluntary written or thumb-printed informed consent will be obtained. The parent / legal guardian providing consent must be 18 years or over on the day consent was provided.

Prior to initiating the study, the PI will facilitate forums to present study information, consult and discuss key issues and concerns with members of the community from which the study participants will be recruited. This will be done through the established system of community engagement at KWTRP Kilifi. Through this system, community members and leaders, healthcare workers, researchers and administrative officials will engage with the study team throughout the study period in order to identify and address any concerns. The implications of any issues identified will be carefully considered and, if necessary, action taken to safeguard the ethical integrity and scientific validity of the study.

During initial 'individual sensitization,' potential subjects / subjects' parents or legal guardians will be approached and, if interested, the details of the study, as outlined in the approved ICF, will be explained to them by the study staff. This ICF will be provided to potential subjects/subjects' parents or legal guardians in the language in which they are most literate. The ICF will be translated into both Kiswahili and Giriama, and the translations certified by qualified translators. The ICF will also be read to potential subjects / subjects' parents or legal guardians in their native language upon request. Having had a chance to ask initial questions, they will then be given a copy of the ICF and encouraged to discuss the study with other close family members. For the toddler cohorts it will be important to ensure that the subject's father or legal guardian is also aware of the study. According to the mother's or legal guardian's preference a fieldworker may visit the home to provide information to the father / legal guardian or may provide such information by telephone (if the father / legal guardian did not participate in the community sensitization). Following community sensitization, the subject/family will be given the individual information to consider in the ICF before enrollment can take place (i.e., community sensitization and individual consent cannot take place on the same day).

If interested, potential subjects / subjects' parents or legal guardians will be invited to the clinical trial site so documented informed consent can be obtained. Potential subjects / subjects' parents or legal guardians who are not literate will have all the information in the ICF explained to them verbally in their local language by a member of the study team who is documented to be fluent in the language in question. If consent is obtained in this way, an impartial witness, also fluent in the local language, must be present throughout the process of informed consent and is required to attest that all the information in the ICF has been given to the potential subject / subject's parent (or legal guardian). They must also confirm that the potential subject / subject's parent (or legal guardian) has had the chance to ask questions and that these have been answered to the potential subject's / subjects' parents' (or legal guardians') apparent satisfaction.

After understanding all aspects of the study and having all questions answered, the subject or parent / legal guardian will be required to undertake an 'Assessment of Understanding' – a series of questions to check key elements of the trial have been fully understood. If understanding is confirmed (according to predefined criteria) the subject / parent (or legal guardian) is required to sign or provide a thumb print confirming agreement to participate (or agreement to the toddler participating). Some subjects / parents (or legal guardians) may opt to mark or sign the ICF rather than thumb-printing even though they are not literate. This is acceptable according to subjects' / parents' (or legal guardians') preference. If the consent is given verbally the impartial witness must also sign and date the ICF to confirm the information has been given (as above). The language of consent and the relationship of the person giving consent for the subject (e.g., mother or father or legal guardian) will also be documented on the ICF. The PI or designee who has taken consent will also sign and date the form.

A copy of the ICF will be provided to the subject/parent (or legal guardian) and the original ICF will be filed with other subject records by the site team. Every effort will be made to get consent from both parents / legal guardians of toddler participants, though one parent / legal guardian may sign the consent form.

The ICF will only be completed once at the time of enrollment and prior to screening (unless new information necessitating repeat consent is required). However, ongoing willingness of subjects to participate will be documented in the source documents at each visit.

6.2.2. Screening – Day -28 to Day -1

Once informed consent has been documented the subject will be considered to be enrolled in the trial. The following procedures will be completed during screening to determine study eligibility. These procedures may occur over multiple screening visits, between 28 days prior to and on Day 0, the day of first vaccination. Additional screening visits may be scheduled for any follow-up as needed, but will not be required. All inclusion/exclusion criteria must be assessed from data obtained within that period, unless otherwise specified in the eligibility criteria. After study information has been provided, all study questions addressed, understanding of the study verified, and the appropriate informed consent has been obtained, the following screening procedures will be performed:

- Screening identification (ID) number will be assigned.
- Demographic and contact information will be obtained including address (with adequate detail for another individual to identify the residence), telephone numbers, and email (if applicable).
- Complete medical history will be obtained from the subject/subject's parent.
- A history of medications taken of specific relevance to study eligibility (e.g., hepatotoxic drugs, immunosuppressive medications, recent antibiotics) will be obtained.
- Vaccination history; for toddlers, the vaccination history will be obtained from the maternal-child health booklet or other records, which will represent the source document for this information.
- Height / length and weight will be measured for adults and toddlers. Mid-upper arm circumference (MUAC) will also be measured for toddlers. Of note, any subject with a MUAC $\leq$ 11.5 cm will not be eligible for randomization.
- A PE will be performed, including vital signs (temperature, pulse rate, and respiratory rate, and for adults only, sitting blood pressure) and assessment of the major organ systems. Of note, any subject with an elevated temperature or other abnormal vital sign (i.e. vital sign with toxicity score $\geq$ Grade 1) will not be eligible for randomization (see Appendices 2-5). Individuals may return for repeat assessments once during the screening period to reassess for eligibility. The last measurement will be taken as the baseline for purposes of analysis.
- A blood sample will be obtained for screening laboratory and immunological testing. See Section 7, Laboratory Evaluations, for testing and blood volume details. This will include a serum pregnancy test for women.
- If the subject is provisionally eligible for randomization into the study based on these assessments, the randomization and vaccination visit will be scheduled (Visit 1).
- Subjects (or parents / legal guardians in the case of toddlers) will be provided with a list of

prohibited drugs (Appendix 9) due to their potential hepatotoxicity. Subjects / subjects' parents (or legal guardians) will be instructed to present this to any prescribing party such as doctors, pharmacists or any health facility where they may present for medical attention during enrollment in the study, and, notify study staff immediately in the event that use of any listed drug becomes necessary prior to randomization or during the course of study participation.

- Any subject who fails screening due to abnormal (and clinically significant) laboratory test results or other clinical findings, will receive counseling from the clinician and be referred for further medical management as indicated according to normal practice in Kenya.

6.2.3. Study Visits

Randomization and Vaccination Visits

Randomization will occur at Visit 1 (Day 0) using a predefined randomization scheme which will be available to the unblinded vaccination personnel only after the PI / designee has determined that the subject is eligible for randomization based on a review of the screening information against the inclusion and exclusion criteria for the relevant cohort. Special attention will be given to the screening laboratory assessments to assure that subjects with lab values ≥ Grade 1 are not randomized. In addition, prior to final determination that the subject is eligible for randomization (and vaccination) the following will occur and be recorded:

1) Ongoing willingness to participate in the study will be documented.
2) Interval medical and medication history will be obtained and eligibility confirmed based on review of inclusion / exclusion criteria.

   No acute illness may be noted, and no vital sign may have a toxicity score ≥ Grade 1 (see Appendices 2-5). Note: Any subject with an elevated temperature, evidence of acute illness or other abnormal vital sign as noted above may return for randomization/vaccination at a subsequent time if the subject still qualifies within the 28-day screening period, and has not had repeat vital signs already performed on a separate occasion during the screening period. Subjects may also return after resolution of an acute illness (or other cause of abnormal vital signs) to receive the final (2^{nd}) vaccination. Ideally this vaccination visit will occur within the allowable visit window [+7 days]. A minimum of 72 hours must be allowed after a documented fever (axillary temperature of ≥ 37.5°C) before a subject receives a vaccination.
3) Any unsolicited AEs will be documented and graded for severity. The presence of a new AE may be reason for excluding a subject from the study or for delaying the randomization or vaccination.
4) The occurrence of any SAE will be documented—inclusive of location, duration, severity, relatedness, and clinical summary—and will result in notification as outlined in Section 9.2.8. Submission to the Sponsor will occur within a 24-hour time frame, from the time the event is first documented.
5) Negative malaria parasitemia will be confirmed by rapid diagnostic test for malaria (finger prick to obtain blood for testing).
6) Targeted PE will be performed, to confirm absence of acute illness or abnormality of the extremities (skin and lymph nodes) targeted for vaccination.
7) A negative urine pregnancy test will be confirmed for all women.

8) When subjects return for the final (2nd) vaccination, Items 1 through 7 will be performed prior to vaccination, and any basis for withholding re-vaccination will be documented.

The PI (or designated physician) must approve eligibility of the subject for randomization. Prior to the initiation of the study the randomization assignments for this study will be provided to site staff as sets of specially designed opaque, tamper-evident sealed randomization envelopes, with randomization ID on the outside and treatment assignment on the inside. Unblinded study personnel will retrieve the randomization assignment at V1 by opening the next sequentially numbered envelope for the given age/dose cohort, which will be associated with a unique randomization ID. Failure to use the next sequentially numbered envelope or opening an envelope prematurely will be a protocol violation. Following assignment, the unblinded study personnel will maintain a list documenting the vaccine assigned and administered to given randomization IDs in a secure location that is not accessible to blinded study personnel. While the subject will be referred to by screening ID for the remainder of the study, the randomization ID will be required on select CRFs.

Once randomization has taken place, the following procedures will be performed:

- Unblinded nursing staff will administer the assigned study vaccine and document the timing and location of administration on the CRF (see Section 4.5.1. for details).
- At the second vaccination visit toddlers will also be administered licensed pentavalent vaccine, which will be documented on the CRF, including the location of administration (see Section 4.5.1. for details).
- All subjects will be provided with a card documenting the subject's randomization ID, recording the fact that the subject is enrolled in the clinical trial, that they have received a study vaccine, and providing telephone contact details for study personnel. In the case of toddlers this card will be attached to the maternal-child health booklet. The card will also state that, should the subject become unwell, a member of the clinical trial team should be contacted immediately.

Immediately following vaccination the following will be conducted by blinded site personnel:

- Subjects will be monitored for vital signs, solicited reactogenicity and any unsolicited AEs with recording of all these events at least 60 minutes post vaccination. See Appendices 1-5 for appropriate severity grading scales.
- Subjects (or parents / legal guardians in the case of toddlers) will be reminded about daily home visits during 6 calendar days following the study vaccination, and place of residence and phone contact details will be reconfirmed.
- Subject (or parents / legal guardians in case of toddlers) will be reminded of the clinic visit on day 7 following study vaccination.
- Subjects (or parents / legal guardians) will be instructed to contact the site as soon as possible if he / she feel unwell or has adverse event(s) or a medical condition that required medical attention or of concern. In addition, parents/legal guardians will be instructed to contact site staff in case of any respiratory symptoms in child that persist for more than 3 days.
- The date of the subsequent clinic visit will be established. Study vaccination **must** occur on the day of randomization.

Replacement

Subjects who are discontinued from the study after vaccination will not be replaced. However, if a subject is discontinued after randomization but prior to vaccination, he or she will be replaced using a new randomization assignment for the replaced subject.

*Note: Adults who qualify for randomization but are not enrolled due to the study being full will be offered rabies vaccination under the guidance and at the discretion of the PI. Toddlers who quality for randomization but are not enrolled due to the study being full will be offered a booster dose of licensed pentavalent vaccine.

Home Visits – (Days 1 through 6 Post Vaccination)

Fieldworkers will conduct daily home visits during 6 calendar days after each study vaccination to evaluate the presence and severity of local and systemic solicited AEs in each subject. Fieldworkers will be provided with a standard contact script and a standard home visit diary for recording purposes. The diary will be retained as part of the source documents in the subject's file. See Appendices 1-3 for systemic AE severity grading and for severity grading of local injection site reactions. **Any Grade 3 reactogenicity assessed by a fieldworker will result in immediate clinic contact, and the subject will be seen in the clinic within 24 hours of the event.** A presence of any unsolicited AEs will be also assessed during these visits. Fieldworkers will contact the site to assist with scheduling if subjects are noted to be experiencing any medical condition (i.e., solicited or unsolicited AE) that needs to be evaluated by the PI or a designee at an unscheduled clinic visit.

Post-Vaccination Safety Assessments

All subjects will be seen in clinic and the following steps will be conducted by blinded site personnel:

- Screening ID, address and telephone numbers will be confirmed (**All Visits**).
- Solicited local and systemic AEs through 7 days post vaccination will be reviewed and graded for relatedness by the investigator or a designee and recorded on the specific reactogenicity CRF (Note: only systemic AEs are assessed for relatedness). If more than one measurement of a particular parameter is taken and recorded, the value corresponding to the greatest magnitude of the AE will be used as the basis for categorizing and recording the event on the eCRF during the given period of assessment (**Visit 2 and Visit 4 (adults) or Visit 5 (toddlers)**).

If a solicited AE extends beyond 7 days post vaccination, or if onset of a solicited AE is after 7 days post vaccination, the event will be recorded on the AE CRF and continued to be followed as per AE requirements.

- If not already recorded, the occurrence of any SAE will be documented—inclusive of location, duration, severity, relatedness, and clinical summary—and will result in notification as outlined in Section 9.2.8. Submission to the Sponsor will occur within a 24-hour time frame from the time the event is first documented (**All Visits**).
- If not already recorded, investigator-queried unsolicited AEs will be recorded, including assessment of relatedness to vaccination and severity grade (**All Visits**).

- Follow-up will be attempted on any AE / SAE that is ongoing at the time of a subject's last visit, until the event is resolved, assessed to be resolved with sequelae by the PI, or until the LSLV.
- If a subject experiences an SAE that is classified as ongoing at the time of the subject's final visit, the subject will continue to be monitored for 6 months after this date to obtain updated safety data for annual reporting requirements. However, the database will be locked for purposes of analysis following LSLV regardless of the ongoing nature of any SAE. After six months the subject will continue to be monitored by the PI or referred for further investigations and care locally, according to the judgment of the PI.

- Concomitant medications will be recorded (**All Visits**).
- Vital signs will be measured, recorded, and graded (including assessment for relatedness to vaccination). See Appendices 2-5 for severity grading of abnormal vital signs (**All Visits**).
- Targeted PE will be performed, including local examination of the vaccination site and for any clinically significant finding (**All Visits**).
- Blood sample for clinical laboratory testing will be obtained at the 7-day post vaccination visits (**Visit 2 and Visit 4 in adults or Visit 5 in toddlers**). Assessment for clinical significance and severity grading will be performed upon receipt of laboratory results. See Section 7 and the Laboratory Manual for testing and blood sampling/volume details. See Appendices 6-8 for severity grading of abnormal laboratory results. Blood sample for immunologic testing will be obtained at **Visit 5 (adults) or Visit 6 (toddlers**). In a subset of adult subjects who provide consent for additional immunological testing, additional blood sample will be collected at day 7 after the second vaccination. (**Visit 4**).
- A urine pregnancy test will be performed on all women prior to the second vaccination (**Visit 3**) and at the final visit (**Visit 5**).
- Any follow-up visits will be scheduled (**All Visits**).
- Study termination will occur following completion of the EOS eCRF page (**Visit 5 in adults or Visit 7 in toddlers**).

Evaluations to be performed at each study visit are shown in Table 5 (adults) and Table 6 (toddlers) as follows:

Table 5. Study Visits (adult cohorts)

Evaluation	V0	V1	V2	V3	V4	V5
	D-28 to D-1	**D0**	**D7 (+3)**	**D28 (+7)**	**D35 (+3)**	**D56 (+14)**
Signing of ICF (enrollment) and confirmation of ongoing consent (+)	✓	+	+	+	+	+
Assign screening ID	✓					
Demographics	✓					
Contact information: Record and Confirm (+)	✓	+	+	+	+	+
Full medical history, including medication and vaccination history	✓	~	~	~	~	~
Recording adverse events (including SAE)	✓	✓	✓	✓	✓	✓
Record concomitant medications	✓	✓	✓	✓	✓	✓
Vital signs and targeted PE	✓	✓^✓	✓	✓^✓	✓	✓
Blood Sample Viral serology	✓					
Hematology panel	✓		✓		✓	
Clinical Chemistry panel	✓		✓		✓	
Immunogenicity	✓				✓[a]	✓
Rapid malaria diagnostic test (prior to vaccination)	✓	✓		✓		
Serum pregnancy test for adult female subjects	✓					
Urine pregnancy test for adult female subjects		✓		✓		✓
Eligibility check	✓	✓		✓		
Randomization ID: Assign and Confirm (+)		✓	+	+	+	+
Vaccination(s)		✓		✓		
Recording local/systemic solicited AEs		✓	✓	✓	✓	
Provide list of hepatotoxic drugs	✓					
Schedule / confirm next visit	✓	✓	✓	✓	✓	
Exit study						✓

(~) Confirmation of medical history
^Evaluations will be conducted twice – before and after vaccination.
[a]PBMC collection in consenting adults only (see Table 7).

Viral serology: human immunodeficiency virus (HIV) rapid antibody test, hepatitis B surface antigen (HbsAg), hepatitis C virus (HCV) serology
Hematology: complete blood count (CBC) including white blood cell (WBC) count, hemoglobin, and platelet count
Clinical Chemistry panel: albumin, total and direct bilirubin, alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, creatinine
Refer to Section 7 for Tests and Blood Volume requirements.

Table 6. Study Visits (toddler cohorts)

Evaluation	V0	V1	V2	V3	V4	V5	V6	V7
	D-28 to D-1	**D0**	**D7 (+3)**	**D28 (+7)**	**D56 (+7)**	**D63 (+3)**	**D84 (+7)**	**D112 (+14)**
Signing of ICF (enrollment) and confirmation of ongoing consent (+)	✓	+	+	+	+	+	+	+
Assign screening ID	✓							
Demographics	✓							
Contact information: Record and Confirm (+)	✓	+	+	+	+	+	+	+
Full medical history, including medication and vaccination history	✓	~	~	~	~	~	~	~
Recording adverse events (including SAE)	✓	✓	✓	✓	✓	✓	✓	✓
Record concomitant medications	✓	✓	✓	✓	✓	✓	✓	✓
Vital signs and targeted PE	✓	✓^✓	✓	✓	✓^✓	✓	✓	✓
Blood Sample Viral serology	✓							
Hematology panel	✓		✓			✓		
Clinical Chemistry panel	✓		✓			✓		
Immunogenicity	✓						✓	
Rapid malaria diagnostic test (prior to vaccination)	✓	✓			✓			
Eligibility check	✓	✓			✓			
Randomization ID: Assign and Confirm (+)		✓	+	+	+	+	+	+
Vaccination(s)		✓			✓[a]			
Recording local/systemic solicited AEs		✓	✓		✓	✓		
Provide list of hepatotoxic drugs	✓							
Schedule / confirm next visit	✓	✓	✓	✓	✓	✓	✓	
Exit study								✓

(~) Confirmation of medical history

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

[a]Toddlers will receive two vaccinations at this visit: PATH-wSP or placebo + pentavalent.

Viral serology: human immunodeficiency virus (HIV) rapid antibody test, hepatitis B surface antigen (HbsAg), hepatitis C virus (HCV) serology

Hematology: complete blood count (CBC) including white blood cell (WBC) count, hemoglobin, and platelet count

Clinical Chemistry panel: albumin, total and direct bilirubin, alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, creatinine

Refer to Section 7 for Tests and Blood Volume requirements

6.3. Refusing of Procedures, Missed Visits, Withdrawal, and Early Termination

See Section 10.4 for a discussion of refusal of procedures, reasons for withdrawal, and early termination. If a subject fails to come to clinic for a study visit, extensive follow-up will be undertaken to locate and recall him / her. If the subject still fails to present to clinic within the allowed window for the visit, then he or she may still be permitted to complete the visit and related procedures at a suitable later date on a case-by-case basis. The PI will use discretion regarding the window allowed. However, if the subject has exceeded the visit window to the extent that he or she is due for the next visit (e.g., did not return for Visit 2 and now is due for Visit 3) then that original visit will be deemed a "Missed Visit" and the subject assigned to the subsequent visit to maintain the visit schedule. A Note to File (NTF) reporting the protocol deviation would be logged in such a case. In case the delay is too great, that visit may also be deemed a "Missed Visit" and the subject permitted to continue in the study. All efforts will be made to ensure that he or she attends the next clinic visit on time as per the original schedule date. Note: the exception to this practice will be if a subject has not completed the final vaccination visit (V3) and has exceeded the visit window; in such a case, the PI (in consultation with the Sponsor) will have the discretion to determine the appropriate visit schedule, or whether the subject ought to be withdrawn from further study vaccination.

If the subject decides to withdraw from the study, all efforts will be made to complete and report study observations as thoroughly as possible until the date of withdrawal. A reason for study withdrawal / termination will be assigned in each case.

In the event of a subject's withdrawal or early termination, the following activities will be attempted to be performed and information recorded in the database:

- Contact information will be reviewed and updated.
- Results from prior visits will be reviewed and any outstanding data queries completed.
- Date of withdrawal will be recorded. The date of withdrawal will be designated as the date when the last contact with the subject occurred (telephone or face-to-face).
- The reason for withdrawal or early termination will be documented if available.
- PE will be performed if possible, including urine pregnancy test for all adult females
- New AEs since the last visit will be documented.
- All previously documented AE and SAE will be updated in regards to classification (ongoing, resolved, etc.).
- Concomitant medications since last visit will be documented.
- The subject's final study visit will be documented and the EOS eCRF page completed. Efforts made to complete the EOS eCRF in the event the subject cannot, or is unwilling to be contacted, will be documented.
- Safety laboratory testing will be performed if possible, if the subject's withdrawal occurs prior to scheduled testing at a 7-day post-vaccination visit (V2 or 4). The subject/parent (or legal guardian) should specifically be encouraged to allow these safety bloods to be obtained to ensure safety is not compromised. The reason for the safety blood should be explained although the ultimate decision to consent to these samples remains with the subject/parent (or legal guardian).
- In the case of early subject withdrawal or early termination, samples already collected will be retained for appropriate immunogenicity measurements unless the subject / parent (or

legal guardian) asks that these samples not be tested or be destroyed. If immunogenicity testing has already been carried out the data will be retained within the final analysis set irrespectively.

A subject will be considered lost to follow-up only after every effort, including telephonic attempts to contact the subject have failed, and a visit to the home to attempt a contact has occurred and the subject still cannot be located.

A subject / subject's parent (or legal guardian) may alternatively actively withdraw consent for the trial.

6.4. Interim Contacts and Visits

Interim contacts and visits (e.g., unscheduled visits) in between regularly scheduled follow-up visits may occur at any time at the subject's request or as deemed necessary by the PI. Similarly, unscheduled laboratory assessments may be performed at the PI's discretion. All interim contacts and visits will be documented in the subject's study records and on applicable CRFs.

6.5. Concomitant Medications and Treatments

All concomitant medications, therapies and procedures will be recorded in source documents during each clinic visit of the study, as outlined above. Subjects may receive all medications and procedures deemed necessary to provide adequate health care. In fact, subjects and parents / legal guardians will be encouraged to obtain all medical care for the subject at the clinical trial site during their enrollment in the trial to enable the PI to directly assess potential AEs. Any necessary medical care will follow standard treatment practice at the site, and access to appropriate medical therapies will be made available to all subjects during enrollment in the trial. Medications (e.g., paracetamol, antibiotics, zinc, oral rehydration solutions) will be provided by the site team to ensure adequate health care is available.

That said, certain medications will not be allowed; if a subject uses the following medications, the IST will determine whether to discontinue the subject from the study or from receiving further vaccinations or to exclude subject's data from per-protocol analysis:

- Use of any investigational drug or vaccine other than the study vaccines.
- Administration of a forbidden vaccine.
- Use of any potentially hepatotoxic drug listed in Appendix 9 (Subjects / subjects' parents or legal guardians will be instructed to present this to any prescribing party such as doctors, pharmacists or any health facility where they may present for medical attention during enrollment in the study, and notify study staff immediately in the event that use of any listed drug becomes necessary prior to randomization or during the course of study participation).
- Chronic administration (defined as more than 14 days) of immunosuppressants or other immune modifying agents during the vaccine period. For corticosteroids, this means prednisone or equivalent > 10 mg per day for adults or ≥ 0.5 mg/kg per day for toddlers; topical and inhaled steroids are allowed.
- Administration of immunoglobulins or any blood products during the study period.

6.6. Blinded and Unblinded Study Personnel

With the exception of the designated unblinded site personnel described below, all study site personnel, including the PI and the Sponsor, will remain blinded to subjects' treatment assignments until after database lock has occurred. All CRO personnel, with the exception of the unblinded monitor, and an administrator for the DSMB, will also remain blinded to the treatment assignments until after database lock. At the end of the study, the randomization scheme will be appended to the integrated clinical study report (CSR), and the site will also be notified of the randomization assignments of subjects.

A limited number of unblinded site personnel will be responsible for preparation and administration of study vaccines, performing drug accountability, and maintaining the security of the treatment assignments. The unblinded site personnel will not be involved in the safety and reactogenicity assessment of the subjects, or in any other aspect of the study. All other site personnel, including those who perform clinical evaluations (such as but not limited to assessment of medical history, PE, and evaluation of laboratory test results), will be blinded with respect to the identity of the vaccine administered to the subjects. Unblinded staff at the CRO consist of the unblinded monitor, randomization statistician and independent statistician who prepares unblinded reports for the DSMB. These individuals will not have a further role in the study while the study is still blinded. Following database lock, the Sponsor, CRO and PI will be unblinded for data analysis purposes.

The CRO will assign blinded monitors to visit the site during the study period, in order to assess and verify activities of the blinded study personnel, review appropriate documentation, and provide a report to the CRO and Sponsor of ongoing activities and issues requiring resolution. The blinded monitors will be responsible for all aspects of the clinical trial related to subjects, the blinded site staff, and regulatory and audit readiness. Monitoring can occur both at the site and remotely with standard reports and escalation as needed to the PI or IST. The CRO will also assign an unblinded study monitor, who will visit the site during the study period to assess and verify activities of the unblinded site personnel, review appropriate documentation, and provide a report to the CRO and Sponsor of ongoing activities and issues requiring resolution. The unblinded study monitor will be responsible for review of treatment assignments, vaccine storage and accountability, and dosing-related matters. The unblinded monitor will be responsible for escalating issues to the PI or IST in a blinded manner. Any unblinding of additional project team personnel required to resolve issues will be clearly documented in the TMF. Of note, all reports to blinded personnel by the unblinded CRO monitor will be constructed in order to maintain the blind during the trial. No report that would break the blind will be released into the TMF until after database lock.

6.7. Unblinding Procedure

In the event of a medical emergency, the PI or designee may require that the blind be broken for the subject experiencing the emergency when knowledge of the subject's treatment assignment may influence the subject's clinical care. Unblinding is also required by the US Food and Drug Administration (FDA) for any unexpected and related SAEs that occur during the trial.

An identical set of sealed randomization envelopes will be available at the site for this purpose, and should unblinding be necessary the PI will access these envelopes and obtain the envelope corresponding to the randomization ID of the subject in question. The blinded monitor will routinely review the integrity of these envelopes as part of interim monitoring.

Details and documentation surrounding such unblinding will be described in the relevant operations manual and staff will be trained on during the site initiation visit. Documentation of the unblinding event (including the rationale and requestor) will be recorded and duly entered into the EDC. In the case of a medical emergency, every effort will be made not to unblind the subject unless it is considered necessary for the welfare of the subject. Prior to unblinding for a medical emergency, the PI must attempt (to the extent possible, without jeopardizing the subject's health) to contact the Sponsor (or designee) to discuss the decision to break the blind. The PI will be expected to provide a rationale for the necessity of unblinding based on the expectation that knowledge of the subject's treatment assignment will have a meaningful impact on the subject's medical care in the short term. If a subject's treatment assignment is unblinded, the subject may remain in the study and continue with protocol-defined follow-up evaluations, but not receive further study vaccines. The decision to unblind will be communicated to the KEMRI Scientific and Ethics Review Unit (SERU) and all other 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 TMF.

7. LABORATORY EVALUATIONS

Blood samples will be collected from subjects for both safety and immunogenicity testing.

7.1. Blood Sample Collection, Distribution, and Storage

Samples to evaluate vaccine safety will be obtained and processed at the clinical trial site and transported to the KWTRP Kilifi clinical laboratory for testing. Research specimens collected for immunogenicity will be separated into aliquots by the KWTRP Kilifi research laboratories as per study standard operating procedure (SOP) and stored at -70°C in freezers before being shipped to immunology laboratories in Kisumu, Kenya, the UK, and US (see Section 7.4.). Continuous temperature monitoring and backup generators will be in place to ensure proper sample storage. The PBMCs will be separated and processed per study SOP and then stored in liquid nitrogen.

Volumes of blood required for the different categories of assays at different time points are shown in Table 7 and Table 8.

These volumes are for the expected standard laboratory testing; retesting may require additional blood draws. Provision for additional blood draws (for the purposes of safety assessments) will be included in the ICF.

In toddlers with suspected pneumonia additional assessments may be performed, including collection of blood sample for blood culture and CRP measurement and collection of nasopharyngeal swab for respiratory pathogens detection.

Table 7. Total Blood Volume Required (Adults)

Lab Panels Tests	**Visit 0**	**Visit 2**	**Visit 4**	**Visit 5**	**Total**
Hematology: CBC including WBC count, hemoglobin, and platelet count	0.5 mL	0.5 mL	0.5 mL	-	**1.5 mL**
Viral Serology: HIV rapid antibody test, HBsAg, HCV serology	5 mL	-	-	-	**15 mL**
Blood Chemistry: albumin, total and direct bilirubin, AST/SGOT, ALT/SGPT, GGT, alkaline phosphatase, creatinine		5 mL	5 mL	-	
Serum Pregnancy Test		-	-	-	
Immunogenicity Assays: IgG level by ELISA and MSD platform, passive transfer[a]	2 mL	-	-	5 mL	**7 mL**
Novel Antigen Screening: PBMCs for *in vitro* stimulation	-	-	60 mL[b]	-	**60 mL[b]**
Total Blood Volume	**7.5 mL**	**5.5 mL**	**5.5 mL or 65.5 mL[b]**	**5.0 mL**	**23.5mL or 83.5 mL[b]**

[a]Will be assessed only in a subset of subjects.
[b]This quantity of blood will be collected if the subject consents to the additional blood draw at Visit 4 to be used for novel pneumococcal antigen screening.

Table 8. Total Blood Volume Required (Toddlers)

Lab Panels Tests	**Visit 0**	**Visit 2**	**Visit 5**	**Visit 6**	**Total**
Hematology: CBC including WBC count, hemoglobin, and platelet count	0.5 mL	0.5 mL	0.5 mL	-	**1.5 mL**
Viral Serology: HIV rapid antibody test, HbsAg, HCV serology	5 mL	-	-	-	**15 mL**
Blood Chemistry: albumin, total and direct bilirubin, AST/SGOT, ALT/SGPT, GGT, alkaline phosphatase, creatinine		5 mL	5 mL	-	
Immunogenicity Assays: IgG level by ELISA and MSD platform, passive transfer[a]	2 mL	-	-	5 mL	**7 mL**
Total Blood Volume	**7.5 mL**	**5.5 mL**	**5.5 mL**	**5.0 mL**	**23.5 mL**

[a]Will be assessed only in a subset of subjects.

7.2. Safety Clinical Laboratory Assays

Protocol-mandated screening and clinical safety laboratory tests will be performed at the Clinical Laboratories Services, KWRTP in Kilifi. The Clinical Laboratory Services subscribes to proficiency testing programs and operates based on the principles of Good Clinical Laboratory Practice (GCLP). Institutional normal reference ranges will be provided in the Laboratory Manual, and any adjustments to the toxicity tables due to discrepancies with local reference ranges will also be documented in the Laboratory Manual. Laboratory results will be reviewed promptly by the PI.

HIV testing will be undertaken only following pre-test counseling of the subject / subject's parent or legal guardian as to the implications of the test result. Post-test counseling will also be undertaken, and on the basis of a positive result the subject and subject's parents / legal guardians will be referred on for HIV care according to normal local practice in Kenya.

If clinically significant abnormalities are identified during screening, subjects will be referred for further medical management. If identified during the study, subjects may be asked to return to the study clinic for further evaluation, including clinical evaluation and repeat laboratory testing as warranted.

7.3. Nasopharyngeal Swabs Collection and Storage

Nasopharyngeal swabs will be collected from subjects with suspected pneumonia cases (defined as cough or difficulty of breathing and respiratory rate ≥ 40 per minute) during the entire study period. Swabs will be taken by staff trained in the WHO endorsed procedure of NP swab collection, transported according to site-specific procedures and processed in the KEMRI Wellcome Trust laboratory in Kilifi, Kenya.

Following processing, the sample will be divided into multiple aliquots, with a single aliquot being stored at KEMRI Wellcome Trust Lab in Kilifi. Real-time polymerase chain reaction (qPCR) will be performed at qualified laboratories to evaluate presence and density of the viral and bacterial respiratory pathogens. Positive samples (e.g. SPn) may be further processed with a confirmatory culture step.

7.4. Immunogenicity Assays

The following immunological assays are to be undertaken:

- ELISA IgG: this testing will be performed at Charles River Laboratories, Canada (or other qualified and certified laboratory), to determine the IgG responses to *S. pneumoniae*-specific proteins (Ply and PspA Fam 1).
- Meso Scale Discovery (MSD) platform: this testing (based on an electrochemiluminescence detection assay) will be performed at Institute of Child Health, University College London, U.K., to also determine the IgG responses to *S. pneumoniae*-specific proteins.
- Passive transfer: this testing will be performed at University of Alabama in Birmingham, U.S. Mice will be challenged IV with virulent *S. pneumoniae* after passive transfer of paired pre-vaccination and post- vaccination sera to test whether passively transferred antibody will protect the animals against morbidity and mortality.
- Novel protein screening: this testing will be performed at Atreca, U.S., to discover novel antigens that may provide alternatives for future pneumococcal vaccine development. PBMC preparation for this testing will take place at KWTRP Kilifi.

If adequate blood volumes are not obtained to perform all assays, the order of assay completion (and retesting) will be the following: blood chemistry, hematology, MSD platform, ELISA IgG and passive transfer.

At the completion of all the testing at the laboratories, the samples will be either destroyed or stored at an appropriate place in a designated freezer at KWTRP Kilifi or at a PATH-designated facility. PATH will be responsible for the oversight of sample storage and destruction. As part of the consent process, subjects / subjects' parents or legal guardians will be asked whether they consent to any remaining samples being used for other, ethically approved research which could be of benefit to the people of Kenya. Any such future testing of samples would require consent and approval of PATH. All study results will be shared with contributing laboratories at the conclusion of the study.

7.5. Assay Qualification, Standardization, and Validation

All assays employed to determine primary safety endpoints have been properly validated and will be run with adequate controls. All assays employed to determine secondary and exploratory endpoints have been standardized, have associated SOPs, and are run with adequate controls.

7.6. Biohazard Containment

As transmission of blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and processing of blood, and shipping and handling of all specimens for this study. The laboratory SOPs will ensure appropriate coverage of the needs for this trial. All biological specimens will be transported using packaging mandated by the site and CRO SOPs, and aligned with other applicable regulations. All dangerous goods materials, including diagnostic specimens and infectious substances, will be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

8. DATA EVALUATION: CRITERIA FOR EVALUATION OF OBJECTIVES

The planned statistical analyses for this study are outlined below. A detailed statistical analysis plan (SAP) for preparation of the final study report will be created and made final prior to database lock and unblinding for each age group. All statistical analyses will be performed using SAS® software Version 9.4 or later.

Medical history and AEs will be coded using MedDRA dictionary Version 15.1 or higher. The frequency count and percentage of subjects will be summarized according to the coded terms of system organ class and preferred term. Subject-wise data listings will be provided.

8.1. Estimated Sample Size and Power Calculation

Sample sizes were selected to provide adequate data to assess whether the safety and immunogenicity of PATH-wSP measured in adults and toddlers in this trial support advancing into an infant population. All vaccinated subjects are expected to provide data for safety analyses. It is estimated that with a 10% attrition rate approximately 90% of subjects will be evaluable for immunogenicity analyses.

Safety: A sample size of 12 subjects per treatment group for the adult cohorts will provide a 90% chance of observing at least one occurrence of an AE that has an approximately 17.5% rate of occurrence. The 50 subjects per treatment group for toddler cohorts will provide 90% chance of observing at least one occurrence of an AE that has an approximately 4.5% rate of occurrence. If no AEs/SAEs are observed among 12 and 50 subjects receiving PATH-wSP, the upper limits of exact 2-sided 95% confidence intervals for the rate of AE/SAE occurrence will be 26.5% and 7.1%, respectively.

Immunogenicity: In the first Phase 1 study in adults (VAC-002), the standard deviations (SD's) of log10 of the GMFR for ELISA PspA and Ply concentrations were estimated to be ≤ 0.55. Assuming SD = 0.55, then for each adult cohort, 11 evaluable subjects in each study group will provide approximately 68.5% power to find a significant difference between GMFR's if the true difference is a 4.0-fold increase in PATH-wSP recipients compared to recipients of the saline control. For the toddler cohorts, 45 evaluable subjects per study group will provide > 99% power if the true difference is a 4.0-fold increase in PATH-wSP recipients. Power for analysis of GMFR was estimated based on a 2-sample t-test and a 1-sided 0.025 significance level and calculated using PASS 12 (Number Cruncher Statistical Systems, Kaysville, Utah).

Table 9. Power Calculations for GMFR

Sample Size				
PATH-wSP	PATH-wSP or Control	Power, %*	Fold Difference to Detect in GMFR	Standard Deviation in Log Scale
11	11	23.0	2.0-fold	0.55
11	11	68.5	4.0-fold	
11	11	95.5	8.0-fold	
45	45	32.4	1.5-fold	
45	45	72.8	2.0-fold	
45	45	99.9	4.0-fold	

* Based on two-sample t-test with pooled variance estimate, using PASS 12.

In the previous Phase 1 study in adults (VAC-002), ≥ 40% of subjects with seroresponse (≥ 2-fold-rise) were observed for PspA and Ply measured by ELISA. For each adult cohort (11 evaluable subjects in each group), the power to find a significant difference in seroresponse rates will be approximately 30.1% if the true rates are 40% in PATH-wSP recipients and 5% in recipients of the saline control. For the toddler cohorts (45 evaluable subjects in each group), the power to find a significant difference will be ≥ 88.9% if the true seroresponse rate is 40% in the PATH-wSP group and the true rate in the control group is ≤ 10%. Power for comparing seroresponse rates was estimated based on a Fisher exact test at the 1-sided 0.025 significance level, using PASS 12.

Table 10. Power Calculations for Seroresponse/Seroconversion Rate

Sample Size					
PATH-wSP	Saline Control	Power* (%)	Proportion in PATH-wSP Group (%)	Control Group (%)	Rate Difference
11	11	30.1	40	5	35
11	11	51.3	50	5	45
11	11	71.5	60	5	55
45	45	98.4	40	5	35
45	45	88.9	40	10	30
45	45	69.5	40	15	25
45	45	46.4	40	20	20

* Based on one-sided Fisher exact test, using PASS 12.

8.2. Study Populations to Be Evaluated

The ITT Population includes all randomized subjects. Treatment group will be assigned according to the initial randomization, regardless of whether subjects receive any investigational product or receive an investigational product different from that to which they were randomized. Unless specified otherwise, this population will be used for summaries of subject disposition.

The Safety Population includes all subjects who receive any study vaccine and have post-vaccination safety data available. Treatment groups for safety analysis will be assigned according to the actual treatment received at Dose 1.

The Immunogenicity Per-Protocol Population includes all subjects who receive investigational product and had post-dose immunogenicity measurement(s) with no major protocol violations that are determined to potentially interfere with immune response to the study vaccine. Treatment groups for immunogenicity analysis will be assigned according to the actual treatment received at Dose 1.

The criteria for exclusion of subjects from the Immunogenicity Per-Protocol Population will be established before breaking the blind and will be based on the blinded review of protocol violations.

8.3. Conduct of the Analyses

A single database lock for the both age groups, adults and toddlers, is planned for this study. Data will be provided in data listings sorted by treatment group and subject screening ID. All tabular summaries will be presented by treatment group. Categorical data will be summarized by the number and percentage of subjects falling within each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation or error, median, minimum, and maximum. Confidence intervals (CIs) will be 2-sided at $\alpha = 0.05$ and the statistical significance will be evaluated at 2-sided $\alpha=0.05$, unless otherwise stated. Details of endpoint analyses will be described in the SAP.

Analysis of safety data as specified in the DSMB charter or as requested by DSMB will be performed by an independent statistician. The results of this analyses will be kept as a part of blinded DSMB documentation. No other interim analyses are planned for the study.

8.3.1. Handling of Dropouts or Missing Data

Generally no imputation will be made for missing values in safety and immunogenicity analyses, except for the following:

> All immunogenicity values below the limit of quantification (BLQ) that are reported as such will be assigned a value of ½ of the lower limit of quantification.

Any additional imputation for missing values will be documented in the SAP.

8.4. Statistical Methods

8.4.1. Safety Endpoints

The following safety and reactogenicity endpoints will be evaluated to address the primary objectives of the study when 2 doses of PATH-wSP vaccine are given alone in healthy young adults or given alone at the first vaccination or concomitantly with pentavalent booster at the second vaccination visit in healthy toddlers:

- Occurrence and severity of solicited local and systemic AEs (reactogenicity) and clinical laboratory abnormalities through 1 week post vaccination.
- Occurrence, severity, and relatedness to vaccination of unsolicited AE and SAEs through 4 weeks (in adults) or 8 weeks (in toddlers) after each study vaccination and from Day 0 through the last study contact.

Only treatment-emergent unsolicited AEs (i.e., onset on or after first vaccination) will be included in the analysis. Unsolicited AEs and SAEs will be summarized by system organ class and preferred term using the MedDRA dictionary. AEs and SAEs will also be summarized by severity and relatedness to vaccine.

Generally, safety evaluations will be descriptive in nature, and observed differences will be evaluated for medical relevance. For both age groups, all of the safety tabular summaries will be provided for each treatment group and respective control.

For reactogenicity, 2-sided 95% exact confidence intervals (CIs) for each of the proportions will be provided. Also 2-sided exact 95% CIs and *P*-values for the proportion differences between each PATH-wSP dose level and the respective control will be computed using the unconditional exact method proposed by Newcombe.

Adult Cohorts

After each vaccination, the safety data will be summarized for the PATH-wSP 0.6 mg dose group and the saline control (N=12 in each group), and the PATH-wSP 1 mg dose group, and the saline control (N=12 in each group).

Toddler Cohorts

After each vaccination, the safety data will be summarized for the PATH-wSP 0.6 mg dose group and the respective control (N=50 in each group), and for the PATH-wSP 1 mg dose group and the control (N=50 for each group).

8.4.2. Immunogenicity Endpoints

The following endpoints will be evaluated to address the secondary objective of measuring immune responses of a 2-series schedule of 0.6 mg and 1 mg PATH-wSP dose levels in both adults and toddlers:

Secondary Endpoints:

- The Immunoglobulin G (IgG) response to pneumococcal-specific proteins (Plyand PspA Fam 1) measured by ELISA, and the IgG response to nine pre- selected pneumococcal proteins measured on the MSD platform, will be evaluated based on the following analyses:
    - GMC and GMFR (from baseline) 4 weeks after the second vaccination.
    - Percentage of subjects with IgG concentration of a predefined threshold level (responders), measured 4 weeks after the second vaccination.

Exploratory Endpoints:

- Assessment of protection of mice against IV *S. pneumoniae* challenge after passive transfer of serum obtained from a subset of subjects 4 weeks after the second vaccination.
- Identification of novel antibody targets for future vaccine candidates using PBMCs (i.e. plasmablasts) stimulated *in vitro* (adults only).

For all cohorts, comparisons will be made between each PATH-wSP dose group and its respective saline control.

For GMC/GMT/GMFR, GM will be calculated as:

$$GMT = \text{antilog (mean } [\log_{10} x])$$

Where x is the assay result or fold rise (post dose vaccination / baseline).

GMTs / GMCs will be summarized by treatment group and by visit with corresponding 2-sided 95% CIs based t-distribution to provide population estimates. Treatment group differences will be based on the ratio of treatment group GMFRs (active PATH-wSP vs. saline) assuming log-normal distribution. The 95% CI around the mean ratio will be calculated on the log scale using t-distribution for the mean difference between the 2 treatment groups, then exponentiated to obtain the treatment group GMFR ratio and corresponding CI in the original scale. If the values deviate from log normal distribution, alternative i.e. nonparametric methods may be used.

For seroresponse/seroconversion rates, 2-sided 95% exact CIs for each of the proportions will be provided. Two-sided exact 95% CIs for the proportion differences between the treatment groups will be computed using the unconditional exact method proposed by Newcombe or another appropriate method.[15]

8.4.3. Efficacy Endpoints

NA

8.4.4. Multiple Comparisons/Multiplicity

No multiplicity adjustment is planned for this early phase study.

For the analysis of the safety data, the statistical comparisons will be carried out for reactogenicity events using a 2-sided 5% Type I error rate without an adjustment for multiple comparisons. The primary purpose of statistical comparisons is to screen out potential AEs that need further clinical evaluation.

Due to its hypothesis generating nature, all statistical comparisons for immunogenicity endpoints, all testing and estimations will be carried out using a 2-sided 5% Type I error rate without an adjustment for multiple comparisons.

It is acknowledged that performing multiple unadjusted comparisons will lead to inflated Type I error (i.e., inflated false statistical significance). This will be taken into account when interpreting results.

9. SAFETY ASSESSMENT AND REPORTING

The PI is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as provided in this protocol for the duration of the study.

9.1. Collection of Safety Events

AEs will be systematically collected at all clinic visits and through the home visits undertaken daily for 6 days following the day of each vaccination visit.

All subjects or parents / legal guardians will be instructed to contact the site as soon as possible if he / she feel unwell or has adverse events or a medical condition that required medical attention or of concern. In addition, parents / legal guardians will be instructed to contact site staff in case of any respiratory symptoms in child that persist for more than 3 days.

The subject / subject's parent or legal guardian will be provided with contact details of the site team. Site staff will be available 24 hours a day by telephone for emergency needs and during

clinic hours to assess subjects throughout the clinical period for each cohort (First Subject Last Visit (FSLV) to Last Subject Last Visit (LSLV).

9.2. Definitions

9.2.1. Adverse Event or Medical Event

- An AE is any untoward, undesired, or unplanned event in the form of signs, symptoms, disease, or laboratory or psychological/physiologic observations occurring in a subject enrolled in the clinical trial. This includes all subjects from whom consent has been obtained whether or not they have yet been randomized and received a study vaccine. The event does not need to be causally related to trial participation or receipt of a study vaccine. An AE is temporally related to participation in the study and will be documented as to whether or not it is considered to be related to vaccine. An AE includes, but is not limited to, the following:
    - An intercurrent illness or injury during the course of the study.
    - Any clinically significant worsening of a preexisting condition.
- A protocol-related AE is one that occurs from the time of enrollment until the EOS visit that is not considered to be related to receipt of the study vaccine, but is considered by the PI or the medical monitor (Sponsor or designee) to be related to the research conditions, i.e., related to the fact that a subject is participating in the study. For example, a protocol-related AE may be an untoward event occurring during blood sampling or other protocol-specified activity.
- A treatment-emergent AE is defined as an event that is not present prior to administration of the study medication, or, if present prior to the administration of the study medication, increases in intensity after administration of the study medication during the course of the study.
- Solicited AEs include local and systemic reactions noted during the follow-up visits through 1 week after vaccination by fieldworkers and reviewed by the PI or a designee.
    - Any solicited AE that extends beyond 7 days after vaccination, or occurs after this date, will be entered as an unsolicited AE and followed appropriately at subsequent visits until resolved or LSLV.
- In toddler cohorts, the detailed data about all AEs that meet definition of suspected pneumonia (defined as cough and/or difficulty of breathing and respiratory rate of > 40 per minute) will be collected and expedited to IST or/and DSMB review.

9.2.2. Severity (Intensity) of Adverse Event

The severity of all abnormal vital signs, solicited AEs and abnormal laboratory values will be graded from Mild (Grade 1) to Potentially Life Threatening (Grade 4), based on the criteria given in Appendices 1-8. All AEs leading to death are Grade 5 events. Adverse events are graded based on the worst severity grade during the illness/symptoms. The grading scales in the Appendices have been derived from two toxicity tables: the *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events* (2014) from the US National Institutes of Health, and the *Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* (September 2007) from the US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research.

The grading scales for abnormal vital signs and abnormal laboratory values in Appendices 4-8 are guidelines that may be modified based on institutional normal reference ranges. These ranges will be provided by the KWTRP Kilifi Central Laboratory, and any adjustments to the toxicity tables due to these reference ranges will be documented in the laboratory manual or as a NTF. Only those values that are outside the reference ranges will be graded and assessed for clinical significance. Laboratory values which are Grade 3 or greater must be entered as AEs, classified as to relatedness to vaccination and followed until resolution or LSLV.

All other unsolicited AEs and laboratory parameters not listed in the Appendices will be classified as an AE and graded based on the AE severity scale in Table 11 below.

Table 11 Severity Grading

Grade	Description
0	No AE (or within normal limits).
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE.

Source: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf

9.2.3. Causal Relationship of an Adverse Event

A suspected ADR means any AE for which there is a reasonable possibility that the vaccine caused the AE. A reasonable possibility means there is evidence to suggest a causal relationship between the vaccine and the AE. All cases judged by either the PI or the Sponsor as having a reasonable suspected causal relationship to the study vaccine will qualify as ADRs. Medical judgment will be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, confounding factors such as concomitant medication, concomitant diseases, and relevant history. Assessment of causal relationship will be recorded on the eCRFs and on the SAE form (in case of SAEs).

- Related: There is a reasonable causal relationship between the vaccine administered and the AE.
- Not Related: There is no reasonable causal relationship between the vaccine administered and the AE.

9.2.4. Assessment of Outcome of Adverse Event

The outcome of the AE will be assessed and recorded as per the following categories:

- Ongoing.
- Recovered/resolved.
- Recovered/resolved with sequelae.
- Fatal.
- Unknown.

9.2.5. Unexpected Adverse Event / Drug Reaction

All SAEs will be evaluated by the Sponsor or designee for "expectedness." An unexpected AE is one that is not listed in the current Summary of Product Characteristics or the Investigator's Brochure or an event that is by nature more specific or more severe than a listed event.

An investigator safety report is prepared for a SAE that is both related to the investigational vaccine and unexpected. The purpose of the report is to fulfil specific regulatory and GCP requirements, regarding the product under investigation.

9.2.6. Serious Adverse Event

An SAE is a specific AE that:

- Results in death.
- Is life-threatening.*
- Requires inpatient hospitalization or prolongation of an existing hospitalization.**
- Results in a persistent or significant disability or incapacity.***
- Results in a congenital anomaly or birth defect.

Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

***Life-threatening** refers to immediate risk of death as the event occurred per the reporter. A life-threatening event does not include an event that, had it occurred in a more severe form, might have caused death but, as it actually occurred, did not create an immediate risk of death. For example, hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though hepatitis of a more severe nature can be fatal. Similarly, an allergic reaction resulting in angioedema of the face would not be life-threatening, even though angioedema of the larynx, allergic bronchospasm, or anaphylaxis can be fatal.

****Hospitalization** is an admission to a health facility in the situation where there is an AE. A period of observation at a clinical trial site or government health facility is not considered to represent hospitalization for the purposes of SAE reporting. Hospitalization or prolongation of a hospitalization constitutes a criterion for an AE to be serious; however, it is not in itself considered an SAE. In absence of an AE, a hospitalization or prolongation of a hospitalization should not be reported as an SAE by the PI on a SAE form. Such situations include, but are not limited to, the following:

- A hospitalization for a preexisting condition that has not worsened.
- Hospitalization for social reasons.

*****Disability** is defined as a substantial disruption in a person's ability to conduct normal life functions. If there is any doubt about whether the information constitutes an SAE, the information is treated as an SAE.

9.2.7. Adverse Event Recording and Reporting

Recording and reporting of all AEs will occur from signing of the ICF (enrollment) through the EOS visit for each study subject. The PI must completely and promptly record each AE in the source documentation and in the AE section of the eCRF, regardless of relationship to the vaccine administered/procedure as determined by the PI. The PI will attempt, if possible, to establish a diagnosis based on the signs and symptoms. When a diagnosis for the reported signs or symptoms is known, the PI will report the diagnosis as the AE, not the signs and symptoms. Adverse events will be classified by MedDRA term and by severity/intensity, relatedness, and outcome.

Enrolled subjects who subsequently screen fail (i.e., who never underwent randomization) will have any AEs recorded from enrollment until the time they are determined to be ineligible for randomization. These AEs will be listed in separate appendices from those subjects randomized and vaccinated.

Any AE that is ongoing when the subject's EOS visit is due will continue to be followed-up until an outcome or resolution has occurred, or until LSLV (i.e., EOS will be delayed). Should an AE remain unresolved at LSLV, that AE will be classified as ongoing at the time of database lock.

Reporting of AEs will follow the regulatory guidelines of the FDA, Republic of Kenya Pharmacy and Poisons Board (PPB), KEMRI SERU, London School of Hygiene and Tropical Medicine (LSHTM) Research Ethics Committee (REC), Western Institutional Review Board (WIRB) and PATH REC, in regards to requirements, processes and forms.

9.2.8. Serious Adverse Event Reporting

If an AE is classified as serious, an SAE form will be completed and submitted within 24 hours of the PI becoming aware of the SAE, including information on the location, severity, relatedness, and clinical summary of the event to the Sponsor to initiate the IST evaluation and any additional reporting requirements. In addition, the SAE submission will follow the regulatory guidelines of the FDA, PPB, KEMRI SERU, LSHTM REC, WIRB, and PATH REC in regards to requirements, processes and forms.

At this early stage in this vaccine development program, all ongoing SAEs at the time of the LSLV will be classified as ongoing for database lock purposes but will be followed for a period of 6 months or until stabilized (recovered / resolved with or without sequelae), whichever occurs first.

9.3. Unanticipated Problems

All unanticipated problems will be reported in the continuing review report submitted to the FDA, PPB, KEMRI SERU, LSHTM REC, WIRB, and PATH REC per reporting requirements of each regulatory body. All serious unanticipated problems involving risk to participants or others will be promptly (within 48 hours) reported by telephone, by email, or by facsimile to the Sponsor. Follow-up reports will be submitted as soon as additional information becomes available.

9.4. Medication Errors

A medication error is any preventable event that may cause or lead to inappropriate investigational use or subject harm while the investigational product (IP) is in control of the healthcare professional, subject, or consumer. Examples of medication error that will require reporting to the Sponsor include the following:

- Administration of unassigned treatment.
- Administration of expired investigational material.
- Injection by the wrong route.

All AEs and SAEs will be handled as specified in this protocol whether or not they are associated with a medication error.

9.5. Exposure *In Utero*

Pregnancy during a clinical trial is required to be reported for female subjects exposed to the IP or a product not approved for use during pregnancy.

In the event of a pregnancy during the study, a pregnancy report form needs to be submitted to the Sponsor within 24 hours from the time the PI has knowledge of the pregnancy. Pregnancy during a clinical trial itself is not regarded as an AE / SAE unless one of the following applies:

- There is a suspicion that study vaccine administered may have interfered with the effectiveness of a contraceptive medication.
- The pregnancy is either ectopic or a molar pregnancy.
- The concerned pregnancy results in one of the following outcomes:
    - Miscarriage/Late spontaneous abortion.
    - Stillbirth.
    - Congenital Malformation / Anomaly.
    - Neonatal death (within 28 days from birth).

In the above cases the PI is required to record the outcome as an AE / SAE and the reporting timelines are subject to the normal AE/SAE requirements.

If a subject becomes pregnant during the trial she will be followed for safety and will remain in the study until LSLV is completed. She will not receive further vaccinations. At the conclusion of the study, should the pregnancy still be ongoing, the subject would continue to receive care and be followed through birthing and assessment of the child at the time of delivery. If the subject terminates from the study prior to the pregnancy outcome, the site should make every effort to remain in contact with the subject in order to ascertain the pregnancy outcome.

10. SAFETY MONITORING

The PI will be responsible for continuous monitoring of all study subjects' safety. In case of urgent need, subjects will have the means to get in contact with the site at any time (24 hours per day)—and the site will have the means to transport subjects to clinic or will provide fares for this purpose—to allow for expeditious clinical evaluation and provision of medical care to subjects. The PI will also be available by cell phone 24-hours per day for medical emergencies.

10.1. Internal Safety Team

Safety will be monitored routinely throughout the study by the IST, which will include the PI, trial physician and clinical trial coordinator from KWTRP Kilifi, the PATH Medical Officer and Clinical Research Officer, and CRO staff (the Medical Safety Monitor, Clinical Project Manager, and data management personnel). The PATH Medical Officer will serve as the IST Chairman. The IST will meet weekly throughout the period of active vaccination. Blinded safety reports will be prepared routinely by the CRO for the IST that will include at a minimum the following:

- Accrual data and subject status data with regard to completion of/discontinuation from the study.
- Visit windows expected, deviations, and completions.
- Summary of reactogenicity data and clinical laboratory values (classified by severity and cohort).
- AEs sorted by MedDRA term, severity, relation to study vaccine, and cohort.
- Any new or updated AEs that have occurred in the interval from the previous report.
- Data management summaries and status of missing data, missing CRFs and manual queries.
- Quality review of any site findings by blinded or unblinded monitors that are critical to the integrity of the study. These findings will be provided in a manner that maintains the blind.
- All SAEs will be provided to the IST, with history and subsequent follow-up information as pertains to the SAE, within the first 24 hours following site awareness of the SAE (as per other SAE notification rules).
- Site-specific performance issues with source data verification, inclusion/exclusion criteria, documentation practices and audit readiness.
- Additional reports as required by ongoing conduct of the trial.

Except for the 1 mg toddler cohort, the IST will review blinded safety data from individual study cohorts prior to granting approval for dose escalation or age de-escalation. The 1 mg adult cohort will be initiated only after the IST reviews the blinded safety data for all subjects in the 0.6 mg adult cohort through Day 7, and based on this review grants approval for the dose escalation. Similarly, the 0.6 mg toddler cohort will be initiated only after the IST reviews the blinded safety data for all subjects in the 1 mg adult cohort through Day 7, and based on this review grants approval for the age de-escalation.

The 1 mg toddler cohort will be initiated by DSMB only after review of reactogenicity and safety data, collected in each subject of the 0.6 mg toddler cohort up to 6 weeks after the first vaccination In addition, enrollment in the 1 mg toddler cohort will be staggered such that the IST will review safety data through Day 7 for the first 10 subjects prior to approving enrollment of the remainder of the cohort. The IST has the authority to pause or alter the rate of subject enrollment to ensure the safety oversight is appropriate for the nature of this age de-escalation

study.

The IST will review safety data to determine whether conditions of a pause rule (see Section 10.3) have been met, requiring a pause in the study and convening of the DSMB (see Section 10.2). If safety data indicate that a pause rule has been met, the IST must be notified and be provided with the relevant safety data—including clinical assessment by the PI—within 24 hours. The IST clinical evaluators (the PATH Medical Officer, PI, and CRO Medical Safety Monitor) must subsequently determine within 24 hours whether to implement a study pause immediately, convene the IST emergently to verify that a pause rule has been met, or to implement a study pause due to other safety concerns.

The CRO data management team is responsible for coordinating the IST notifications and providing the needed data for review. In addition, the PI may alert the IST to unexpected clinical or laboratory findings during the study and request additional IST review. The IST, PI or PATH Medical Officer may also seek additional guidance from the DSMB or from independent medical experts as dictated by the occurrence of certain events.

If the IST elects to implement a study pause, the study team will pause the study for randomization and vaccination purposes, until the DSMB approves lifting the pause. Should a study pause be initiated, subjects already enrolled will continue with their scheduled visits except in the case of a visit associated with vaccination. In that case, the visit will be on hold during the pause; when the study is resumed, the visits windows may be adjusted based on the date of resumed vaccination. A NTF will be written to explain such an occurrence. If at any time a decision is made to permanently discontinue further vaccinations, the Sponsor will notify the FDA and PPB, and the PI will notify the KEMRI SERU, LSHTM REC, and WIRB expeditiously. In this case, those subjects already enrolled in the study who have completed the last vaccination will complete the safety follow-up period.

10.2. Data Safety Monitoring Board

The DSMB is composed of independent experts in vaccines, infectious diseases and pediatrics. The unblinded statistician attends the closed session but is not considered a member of the DSMB. The DSMB will meet for review of the 0.6 mg toddler cohort data and at the request of the IST, or if one of the study pause rules is met, to review all relevant unblinded safety data from the trial to determine whether or not safety concerns were identified, and whether the trial should continue without change, be modified, or be terminated. The study will continue as planned unless the DSMB notifies the Sponsor of the need to alter the protocol, pause the study for further analyses, or advises halting the trial. Notification of DSMB findings will be sent to the appropriate regulatory authorities after each convening of the DMSB.

10.3. Pause Rules

The DSMB will be convened if it is established that any of the following pause rules as related to administration of the (blinded) study vaccine has been met during the conduct of the trial:

- **Rule 1** (related death / related SAE): 1 subject experiences any vaccine-related Grade 4 AE or SAE.
- **Rule 2** (Grade 3 vaccine-related solicited AE): ≥ 10% of subjects in a cohort experience any vaccine-related Grade 3 solicited AE. In the case of Grade 3 fever, the episode must last longer than 24 hours.
- **Rule 3** (Grade 3 vaccine-related unsolicited AE): ≥ 6% of subjects in a cohort experience the same Grade 3 unsolicited AE assessed as related by the PI.

- **Rule 4** (Grade 3 abnormal clinical laboratory parameters): ≥ 6% of subjects in a cohort experience the same abnormal clinical laboratory parameter assessed as Grade 3 and classified as related to vaccination by the PI.

Note: Assessment of pause rules in toddler cohorts will be conducted on ongoing basis starting when results for approximately 20 subjects are available. The rate of AEs and laboratory abnormalities will be computed based on the actual number of exposed subjects with available results. Severity grading and causality assessment must be confirmed by the PI.

10.4. Refusing of Procedures, Missed Visits, Withdrawal, and Early Termination

Subjects may refuse procedures at any time in the study and can withdraw consent at any time. The PI may also, at their discretion, withdraw the subject from participating in the study at any time if they consider it in the best interest of the subject, with clear documentation as to the reason. Minor protocol deviations (e.g., post-vaccination safety assessment out of window, but the subject is seen for the visit within a reasonable time frame) do not constitute grounds for withdrawal of the subject per se, though these will be clearly documented with a NTF. For major protocol violations (e.g., a toddler receives a non-trial investigational medical product) a notification to the appropriate regulatory authorities may be required, and the subject may be withdrawn from the study. Such decisions will be made by the IST on a case-by-case basis. However subjects will be withdrawn from the study if any of the following events occur after informed consent has been given:

- Ineligibility criteria are met or incorrect enrollment of the subject occurs.
- The subject develops a vaccine-related SAE.
- The subject requests to be withdrawn.
- The PI determines that the subject is unable to comply with the protocol.
- The subject is lost to follow-up (as defined in Section 6.3).
- The Sponsor decides to suspend or discontinue development of PATH-wSP or this study. In

the case that ineligibility criteria are met or incorrect enrollment occurs, the subject may need to continue to be followed if they have received vaccination.

See Section 6.3 for a discussion of procedures for subjects who withdraw or terminate early from the study.

See Section 5.5 for criteria for deferral of the second vaccination.

10.5. Protocol Deviation and Protocol Violation

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or site SOP requirements. The noncompliance may be either on the part of the subject or the PI / site team. The PI / site team should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study subjects. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the PI / site team should consult with the Sponsor or designee (and SERU, as required) to determine the appropriate course of action.

A protocol violation is a significant departure from processes or procedures required by the protocol. Violations often result in data that are not deemed evaluable for a per-protocol analysis, and may require that the subjects who violates the protocol be discontinued from the study.[16]

Corrective actions and preventive actions (CAPAs) will be developed by the site to address protocol violations and deviations, and will be implemented promptly. These practices will be consistent with ICH E6 Guidelines.

11. DATA MANAGEMENT

The Sponsor-designated CRO will draft the Data Management Plan (DMP), which will provide full details of procedures for data handling, including specification of all edit-checks to be performed by the EDC system. The DMP will be reviewed and approved by the Sponsor.

The CRO's study monitors will visit the study site at regular intervals as per the monitoring plan and perform pre-agreed source data verification of the data recorded in the EDC system against the source documents available at the site. In addition, missing data forms and fields will be queried by daily electronic edit checks or through manual edits of the data by the data management team. Study monitors will closely evaluate pre-screening data, inclusion/exclusion criteria, informed consents, data entry timeliness, and visit window capture to ensure integrity of the study is maintained.

Any data discrepancies generated by the system will be flagged in the EDC system for the PI to provide a satisfactory resolution within the EDC system. The data management team will review all the data discrepancy responses by the site to ensure the correctness of data. The medical history events and the AEs will be coded using MedDRA dictionary version 15.1 or later and the concomitant medications will be coded using standard nomenclature. After completion of data coding and resolution of all the queries in the database, the database will be declared to be complete and accurate and will be locked for final statistical analysis of primary and secondary endpoints.

11.1. Case Report Form Development and Completion

Based on the final protocol of the study, a comprehensive set of CRFs will be prepared to capture all the relevant data required for analysis and reporting. This study will utilize eCRFs through an EDC system such that the entire study data can be maintained in a secure electronic system. No written or electronic data recorded prior to the study will be included in the eCRFs.

All study data will be collected by the clinical study staff using designated source documents and will be entered in the appropriate eCRFs by the PI or in an anonymized form. The study database will identify study subjects only by unique study identification numbers through screening (screening ID) and randomization assignments (randomization ID) and will not contain any identifying information such as name, address or personal contact information, or any other regional/state/national identification number. The data management activities will be performed as per the CRO's SOPs. The appropriately trained site personnel will ensure that the study data recorded in the EDC system are verifiable with the source documents available at the site. To ensure that data are entered in a timely fashion so as to monitor safety of the study, it is expected that the site will maintain data entry with a minimal expectation of three business days from subject clinic visit. Data that is not obtained on the day of the study visit (laboratory analyses, hospital records, etc.) will be entered in as timely a manner as possible. The study monitor plan will include assessments of data entry timeliness.

The study site will maintain the source documents for each study subject. Source documentation will be available for review by the study monitor to ensure that the collected

data are consistent with the eCRFs. eCRFs and laboratory reports will be reviewed by the site clinical team, who are responsible for ensuring that they are accurate and complete as well as to determine if any laboratory abnormalities are clinically significant for reporting purposes. The source documents and other supporting documents will be kept in a secure location.

11.2. Record Archival

11.2.1. Archiving Data at Study Site

The study site will maintain appropriate medical and research records for this trial, in compliance with ICH GCP, regulatory, sponsoring organization, and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of the Sponsor and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. After completion of data coding and resolution of all the queries in the database, the database will be declared to be complete and accurate and will be locked for final statistical analysis and made available to the Sponsor for long-term storage in a master file.

11.2.2. Data Storage and Archival

The study monitor will provide the PI with an Investigator Site File, which will be used to file the IB, protocol, drug accountability records, correspondence with the KEMRI SERU, Sponsor, and CRO, and other study-related documents. The PI will maintain, and store securely, complete, accurate, and current study records throughout the study.

As required by ICH GCP guidelines, the PI will keep essential documents until at least two years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least two years have elapsed since the formal discontinuation of clinical development of the IP. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. The documents will be archived either in the KWTRP Kilifi Archive or at any other secure location as agreed upon with the Sponsor. It is the responsibility of the Sponsor to inform the PI/institution as to when these documents no longer need to be retained.

Following completion of the study, serum samples will be stored at an appropriate place in a designated freezer at KWTRP Kilifi or at a PATH-designated facility until it is determined whether the samples are to be retained or destroyed under the direction of PATH. During the informed consent procedure, additional consent for the use of any serum remaining at the end of the trial for other ethically approved research will be sought from subjects/subjects' parents or legal guardians by the PI. Any such use must be with the consent/approval of PATH. When such additional consent has not been obtained the PI will destroy remaining serum samples based on the Sponsor's instructions (with proper audit documentation, reconciliation, and certification).

No data will be destroyed without the agreement of the Sponsor. The applicable records include source documents, site registration documents and reports, correspondence, ICFs, and notations of all contacts with the subject. The Sponsor will inform the PI in writing of the need for record retention and will notify the PI in writing when the trial-related records are no longer needed. Subjects' medical records and other original data will be archived in accordance with the local regulations or facilities of the investigational site.

11.3. Posting of Information on Clinicaltrials.gov

Study information from this protocol will be posted on Clinicaltrials.gov prior to initiation of the study. Informed consent documents will contain the FDA-required language concerning the posting of this study.

11.4. Confidentiality

Documented evidence that the PI is aware and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement.

All information provided by PATH and all data and information generated by the site as part of the study (other than a subject's medical records) will be kept confidential by the PI and other site staff. This information and data will not be used by the PI or other site personnel for any purpose other than conducting the study.

11.5. Publication

PATH will work with the PI and other relevant personnel at KWTRP Kilifi on the publication of the complete Phase 1/2 study outlined in this protocol. Primary publication of the trial results will be shared between KWTRP Kilifi and PATH. Other individuals having input into the study justifying authorship from KWTRP Kilifi, from collaborators and from PATH will similarly be included in publications. Additional publications resulting from the analysis of the study data will be agreed between PATH and KWTRP Kilifi on a case-by-case basis but will generally include authors from both organizations. PATH will be acknowledged in all publications as the Sponsor of the trial.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement, is executed between PATH and the study site, that contract's publication provisions shall apply rather than this statement.

12. STUDY MONITORING

Sponsor monitoring responsibilities will be provided by the CRO. A site initiation visit will be conducted prior to beginning the study, and monitoring will be conducted at initiation, during, and at closeout of the study by the study monitor or designee. See Section 6.6 for a discussion of the roles of blinded and unblinded study monitors in this study.

During the course of the study, the monitor will visit the clinical site at intervals to verify compliance to the protocol; completeness, accuracy, and consistency of the data and study product accountability; adherence to protocol and regulatory obligations and ensure the conduct of the research follows GCP. The monitor should have access to subject medical records, study product accountability and other study-related records needed to verify the entries on the eCRFs.

The PI and the monitor will cooperate to ensure that any problems detected in the course of these monitoring visits, including eCRF completion and query resolution, are resolved in a predefined time frame to be agreed in the Clinical Monitoring Plan.

To ensure the quality of clinical data across all subjects at the site, a clinical data management review will be performed on subject data received by the CRO. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or site notifications will be sent to the site for resolution as soon as possible and within the time frame described in the Clinical Monitoring Plan; all queries must be resolved prior to database lock.

Essential documents must be filed in the site study file on an ongoing basis and be available for review by the Sponsor's contracted site monitor. Monitoring visits will be performed according to the Clinical Monitoring Plan.

12.1. Independent Auditing

PATH representatives may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs at the clinical site and the CRO, and that data are correct and complete. The PI will permit auditors (employees of the Sponsor or an external company designated by the Sponsor) to verify source data validation of the regularly monitored clinical study. The auditors will compare the entries in the eCRFs with the source data and evaluate the study site for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements.

12.2. Regulatory Agency Auditing

The PI must be aware that representatives from regulatory authorities may wish to inspect the eCRFs and associated study records. The PI will notify the Sponsor within 24 hours following contact by a regulatory agency. The PI will make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The PI will provide the Sponsor with copies of all correspondence that may affect the review of the current study or their qualification as PI in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance in responding to regulatory audits or correspondence. The Sponsor has the authority to also request an audit or review by an independent third party. The PI will be notified with appropriate preparatory time to ensure the audit can be conducted appropriately.

13. OBLIGATIONS AND ROLES OF THE SPONSOR, PI AND STUDY PERSONNEL

This study will be conducted according to GCP as well in accordance with all US federal regulations regarding the protection of human subjects in research including US 21 CFR Part 50 and US 21 CFR Part 312, as well as in accordance with Kenyan PPB regulations.

The Sponsor will assure the trial is conducted in compliance with the protocol, GCP, and regulatory authority requirements. The Sponsor will provide the PI with the funding and information needed to conduct the trial properly, ensuring proper monitoring of trial activities, ensuring that the trial is conducted in accordance with the general investigational plan, and protocol contained in the submissions to the regulatory authorities. The Sponsor will ensure that the PI and regulatory authorities are promptly informed of significant new adverse effects or risks with respect to the study vaccine.

The PI agrees to perform the research in strict accordance with this protocol, the ICH GCP (E6), as well as in conformity with applicable US or local regulations regarding the conduct of clinical studies (see Statement of Compliance).

In addition, the PI will follow local and institutional requirements including, but not limited to, investigational vaccines, clinical research, informed consent and ethics regulations. The Sponsor will provide notification to the PI of protocol and amendment approvals by regulatory authorities when applicable. Any modifications to the research protocol, the ICF, and/or change in PI will be submitted for review and approval to regulatory authorities per their guidelines. The PI may deviate from the protocol without prior approval only when the deviation is necessary to eliminate an apparent immediate hazard to the study subject.

While the PI may delegate study duties to appropriate study personnel, the PI is ultimately responsible for the conduct of all aspects of the study.

14. ETHICAL CONSIDERATIONS AND INFORMED CONSENT

The study will be performed in accordance with SOPs generated and agreed between the Sponsor-designated CRO and the PI. The CRO has the responsibility for ensuring the site has the appropriate SOPs to perform the study and the authority to defer to the site SOP rather than the CRO SOP when appropriate. These SOPs have been developed in accordance with ICH Guidelines for GCP (1996), Directive 2001/20/EC, and GCPs for Clinical Research in Kenya, which are consistent with the Ethical Guidelines outlined in the Declaration of Helsinki (2013), thus ensuring protection of the subjects. The study will commence only after receipt of a favorable opinion from the KEMRI SERU and national authorities under Kenyan law, as well as from the LSHTM REC. Also the study protocol will be submitted to FDA prior to study initiation.

14.1. Institutional Review Board/Ethics Review Committee

The PI at the study site will be responsible for obtaining approval from the KEMRI scientific and ethics review unit (SERU) and the KEMRI SERU for the conduct of the study, as well as approval from the LSHTM REC. The Sponsor will ensure approval to undertake the study is obtained from WIRB. The Sponsor will submit the protocol under the existing IND at the FDA. The PI will obtain import authorization and clinical trials authorization from the PPB in Kenya. The first subject will be enrolled only after all approvals from regulatory authorities involved in this trial are received. The PI will notify KEMRI SERU of SAEs, protocol amendments, and protocol violations and deviations according to the KEMRI SERU requirements.

14.2. Informed Consent Process

Prior to any study-related screening procedures being performed on the subject, written or thumb-printed informed consent will be obtained from each subject or subject's parents / legal guardians (as the case may be). Every effort will be made to get consent from both parents or legal guardians of toddler participants, though one parent or legal guardian may sign the consent form. If either parent / legal guardian specifically states that they do not want the child to participate, the child will not be enrolled. Once informed consent has been obtained the subject will be considered to be enrolled. The method of explanation to the subject/impartial witness or subject's parent / legal guardian (as the case may be) and obtaining their consent will comply with the ICH GCP Guidelines and the ethical principles in the amended Declaration of Helsinki (2013), whichever represents the greater protection for the individual. The PI will obtain and document the informed consent process in accordance with the requirements for source documentation in PATH-sponsored clinical trials. See Section 6.2.1 for a detailed explanation of the informed consent process.

14.3. Research Involving Children

Before undertaking research involving children, the PI must ensure that the research has the goal of bettering the health of children. As discussed in Section 1, an investigational vaccine such as PATH-wSP that is based on killed whole cell bacteria has the potential to protect children against pneumococcal disease caused by all pneumococcal serotypes. In addition, a booster dose of licensed pentavalent vaccine will be provided to all toddlers eligible for randomization. Such a booster is not currently offered by the Kenyan EPI program and is likely to enhance the protection of individual subjects against the five pathogens included in the vaccine. Lastly, the PI will provide acute medical care (in line with routine care provided in Kenya) to subjects through the EOS visit.

14.4. Permission of a Parent or Legal Guardian for Participation of Minor

The PI must obtain the permission of at least one parent / legal guardian in accordance with local laws or established procedures, and mothers / legal guardians are encouraged to discuss the participation of their child with their husbands before consenting.

14.5. Risk/Benefit

No benefits can be guaranteed to subjects for their participation in this research study.

This is the first clinical trial evaluating the safety and tolerability of a single-vial formulation of PATH-wSP manufactured by PT Bio Farma, and the first time that a 1 mg dose of PATH-wSP will be given to a child. In a Phase 1 trial in healthy U.S. adults (VAC 002) and in a Phase 1/2 trial in healthy Kenyan adults and toddlers (VAC 010), PATH-wSP has been well-tolerated, with reactogenicity, when it has occurred, predominantly of mild severity. The most common solicited reactions in adults were injection-site pain, fatigue and myalgia / arthralgia. The most frequently reported solicited reactions in toddlers were tenderness, decrease of appetite and irritability. Severe reactions were reported in < 5% of subjects.

The rates of unsolicited AEs were comparable in the wSP and control groups except for pneumonia cases which were higher among toddlers who received wSP in 0.6 mg dose compared to the control group. Several pneumonia cases were reported after the first vaccination; most of them were assessed as mild-to moderate by the investigator. Two cases of pneumonia were confirmed by chest radiography and required hospitalization. These cases were reported as SAEs. All cases resolved following standard antibiotic therapy. Considering the non-equal randomization in the study (100 subjects in the pooled 0.6 mg groups and 25 subjects in the control group), and the low incidence of the severe cases, the relevance of this observation is unknown. Additional information on these findings is provided in the latest version of the IB.

To date, only one related SAE has occurred across reported clinical studies: a toddler subject who received the PATH-wSP (0.6 mg) vaccine in study VAC 010 experienced a simple febrile seizure the day after the first vaccination. The SAE resolved with no sequelae. Please refer to the IB for additional details on this event.

As with any vaccine, severe allergic reaction is a potential rare event.

Potential health benefits to participation in this study include standard screening laboratory assessments, complete physical examination and treatment for any medical illness while a subject is enrolled in the study. All adult subjects will be offered prophylactic rabies vaccination at the end of the trial (given as a standard 3-dose regimen under the guidance and at the discretion of the PI). A booster dose of licensed pentavalent vaccine, which is currently not offered by the Kenyan EPI program, will be provided to toddlers eligible for randomization.

Additional risk mitigation will be provided by clinical monitoring and access to clinical evaluation and management.

14.6. Subject Confidentiality

Every effort will be made to protect subject privacy and confidentiality. Personal identifiers will not be included in any study reports. All study records will be kept confidential to the extent provided by national and local laws. Medical records containing identifying information will be made available for review when the study is monitored by the Sponsor or an authorized

regulatory agency. Direct access may include examining, analyzing, verifying, and reproducing any records and reports that are important in the evaluation of the study.

All study-related information will be stored securely at the study site. All subject information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, and other reports will be identified only by a unique trial-related subject identification code (screening / randomization ID) to maintain subject confidentiality. Laboratory reports will include the name and date of birth of the subject to minimize the risk of errors in the busy clinical laboratories. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link subject ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Subjects' study information will not be released without their written permission, except as necessary for monitoring, or as required/permitted by law/regulatory authorities.

14.7. Reimbursement

Subjects/parents (or legal guardians) of subjects will be compensated for costs associated with travel to study visits (depending on the distance travelled), and appropriate meals during study visits will be provided. Subjects/parents (or legal guardians) will also receive a standardized compensation for loss of earnings incurred due to scheduled visits that last for more than three hours. The study will provide a local contact (fieldworker) whom the subjects can approach to communicate with the study staff by telephone. The study ICF will state the plan for reimbursement. Subjects/parents (or legal guardians) of study subjects will not be charged for study vaccines, research clinic visits, research-related examinations, or research-related laboratory tests.

14.8. Storage of Specimens

Each sample drawn for a subject will be uniquely labeled at the subject level to allow the site, the laboratories performing the assays, and the Sponsor to remain blinded to treatment assignment until the blind is broken. Stored study research samples will be labeled by randomization ID. All stored research samples will be logged into a secure database which is a repository for total samples collected and used. The transport of samples to any laboratory outside of the clinical site will be traceable and logged at the time of transit (at the package level) and receipt (at the sample level) and temperature monitored when appropriate to ensure sample integrity. Any deviations identified during transport that might affect the integrity of the sample analysis will be reported to the data management system for logging. Refer to the Laboratory Manual for specifics on sample labeling, transport, tracking and logging. Samples may be stored at several different repositories and laboratories in order to complete the analyses required to meet study primary, secondary, and exploratory analyses. Of note, these samples are not being utilized for DNA banking or DNA analysis.

15. APPENDICES

15.1. APPENDIX 1: Solicited Local Reactions Toxicity Grading Table (Adults)

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Present but does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room (ER) visit or hospitalization
Erythema / Redness at injection site	25 – 50 mm	51 – 100 mm	>100 mm	Necrosis or exfoliative dermatitis
Induration/ Swelling at injection site	25 – 50 mm and does not interfere with activity	51 – 100 mm or interferes with activity	>100 mm or prevents daily activity	Necrosis

The intensity grading scores are based on FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007.

- For erythema/redness, and induration/swelling, longest diameter should be noted in millimeters by using a ruler.
- Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

15.2. APPENDIX 2: Solicited Systemic Reactions Toxicity Grading Table (Adults)

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever/Temperature (axillary)	≥ 37.5 °C (99.5 °F) to ≤ 38.0 °C (100.4 °F)	> 38.0 °C (100.4 °F) to ≤ 39.0 °C (102.2 °F)	> 39.0 °C (102.2 °F) to ≤ 40.0 °C (104.0 °F)	> 40.0 °C (104.0 °F)
Headache	No interference with daily activity	Some interference with daily activity	Prevents daily activity	Hospitalization
Fatigue/ Malaise	No interference with daily activity	Some interference with daily activity	Prevents daily activity	Hospitalization
Myalgia/ Muscle aches all over the body	No interference with daily activity	Some interference with daily activity	Prevents daily activity	Hospitalization
Arthralgia / Aching in several joints	No interference with daily activity	Some interference with daily activity	Prevents daily activity	Hospitalization
Cutaneous Rash	Localized area of the skin (1 arm or leg only)	Moderate area of the skin (2 or more body regions without whole body involvement)	Most of the skin	Hospitalization

The severity grading scores are based on FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007.

15.3. APPENDIX 3: Solicited Local and Systemic Reactions Toxicity Grading Table (Toddlers)

Adverse Event	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Gra
Fever/Temperature (axillary)	≥ 37.5 °C (99.5 °F) to ≤ 38.0 °C (100.4 °F)	> 38.0 °C (100.4 °F) to ≤ 39.0 °C (102.2 °F)	> 39.0 °C (102.2 °F) to ≤ 40.0 °C (104.0 °F)	> 40.0 °C (104.0 °F)
Irritability	Requires more cuddling and he / she is less playful than usual	More difficult to settle	Unable to console	Hospitalization
Drowsiness	Shows as increased drowsiness	Sleeps through feeds	Sleeps most of the time and it's hard to arouse him/her	Hospitalization
Loss of Appetite	Eating less than normal for 1 to 2 feeds	Missing 1 or 2 feeds	Missing more than 2 feeds	Hospitalization
Cutaneous Rash	Localized area of the skin (1 extremity only)	Moderate area of the skin (2 or more body regions without whole body involvement)	Most of the skin	Hospitalization
Tenderness at injection site	Minor reaction to touch	Cries/protests on touch	Cries when injected limb is moved	Hospitalization
Erythema / Redness at injection site[a]	>0 to ≤20 mm	>20 to ≤50 mm	>50 mm	Hospitalization
Induration/ Swelling at injection site[a]	>0 to ≤20 mm	>20 to ≤50 mm	>50 mm	Hospitalization

Note: The preferred route for recording temperature in this study will be axillary.

[a]Record erythema/redness and induration/swelling at greatest surface diameter in millimeters using a ruler.

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15.4. APPENDIX 4: Vital Signs Toxicity Grading Table (Adults)*

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threateni (Grade 4)
Tachycardia, beats per minute	101 – 115	116 – 130	> 130	Hospitalization for arrhythmia
Bradycardia, beats per minute[b]	50 – 54	45 – 49	< 45	Hospitalization for arrhythmia
Hypertension (systolic), mm Hg	141 – 150	151 – 155	> 155	Hospitalization for malignant hypertension
Hypertension (diastolic), mm Hg	91 – 95	96 – 100	> 100	Hospitalization for malignant hypertension.
Hypotension (systolic), mm Hg	85 – 89	80 – 84	< 80	Hospitalization for hypotensive shock
Respiratory Rate, breaths per minute	17 – 20	21 – 25	> 25	Intubation

NOTE: The above table is derived 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, Food and Drug Administration, Center for Biologics Evaluation and Research, September 2007.

[a] Subject should be at rest for all vital sign measurements.
[b]When resting heart rate is between 60 to 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

*The values provided in this table are guidelines that are dependent upon institutional normal parameters. Institutional normal reference ranges will be provided by KWTRP Kilifi, and this guidance adjusted accordingly. Only those measurements that are outside the locally defined normal reference ranges for vital signs will be toxicity graded.

15.5. APPENDIX 5: Vital Signs Toxicity Grading Table (Toddlers)

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Respiratory Distress	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90– 95%	Dyspnea at rest causing inability to perform usual social and functional activities OR Pulse oximetry < 90%	Hospitalization
Sinus bradycardia#	Asymptomatic, intervention not indicated	Symptomatic, non-urgent medical intervention indicated	Severe, medically significant, medical intervention indicated	Life-threatening consequences; urgent intervention indicated
Sinus tachycardia#	Asymptomatic, intervention not indicated	Symptomatic; non-urgent medical intervention indicated	Severe, medically significant, medical intervention indicated	Life-threatening consequences; urgent intervention indicated

NOTE: The above table is derived from the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Version 2.0, November 2014) and (#) the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, Published May 28, 2009 (v4.03: June 14, 2010).

15.6. APPENDIX 6: Serum Chemistry Toxicity Grading Table (Adults and Toddlers)*

Serum	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Creatinine	1.1 – 1.3 x ULN	> 1.3 – 1.8 x ULN OR Increase of > 0.3 mg/dL 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
Albumin - hypoalbuminemia g/dL	3.0 - < LLN	≥ 2.0 – < 3.0	< 2.0	NA
Liver Function Tests (ALT or AST or gamma-glutamyl transpeptidase)	1.25 – < 2.5 x ULN	2.5 – < 5.0 x ULN	5.0 – < 10.0 x ULN	≥ 10.0 x ULN
Alkaline Phosphatase	1.25 – < 2.5 x ULN	2.5 – < 5.0 x ULN	5.0 – < 10.0 x ULN	≥ 10.0 x ULN
Bilirubin (Total)	1.1 – < 1.6 x ULN	1.6 – < 2.6 x ULN	2.6 – < 5.0 x ULN	≥ 5.0 x ULN

Abbreviation: ULN = upper limit of normal range; LLN = lower limit of normal range

NOTE: The above table is based on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Version 2.0, November 2014).

*The laboratory values provided in these tables are guidelines that are dependent upon institutional normal parameters. Institutional normal reference ranges will be provided by KWTRP Kilifi, and this guidance adjusted accordingly. Only those measurements that are outside the normal reference ranges for the clinical laboratory at KWTRP Kilifi will be toxicity graded. Any abnormal laboratory measurement that does not have an associated toxicity grade but is deemed clinically significant by the investigator will be classified as an AE using the AE severity scale in Table 11 (Section 9.2.2.).

**The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4).

15.7. APPENDIX 7: Hematology Toxicity Grading Table (Adults and Toddlers)*

Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin change from baseline value - g/dL	Any decrease – 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	20,001 – 25, 000	> 25,000
WBC Decreased - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Platelets Decreased[a] – cell/mm^3	100,000 – < 124,999	50,000 – < 100,000	25,000 – < 50,000	< 25,000

NOTE: The above table is derived 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, Food and Drug Administration, Center for Biologics Evaluation and Research, September 2007.

[a]Derived from Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Version 2.0, November 2014).

15.8. APPENDIX 8: Hepatotoxic Drugs

This appendix lists commonly used over-the-counter (OTC) and prescription drugs with recognized hepatotoxic potential. Use of paracetamol, nonsteroidal anti-inflammatory drugs (NSAIDs) and vitamin A will not be prohibited, but monitored closely in regards to appropriate dosing. At screening (Visit 0) this list will be provided to subjects/subjects' parents (or legal guardians), who will be instructed to present this to any prescribing party such as doctors, pharmacists or any health facility where they may present for medical attention during enrollment in the study, and, notify study staff immediately in the event that use of any listed drug becomes necessary.

Section A. OTC and prescription drugs that may be in use by individuals with medical conditions not necessarily disqualifying for this study

- allopurinol (brand name: Zyloprim)
- chlorzoxazone (brand names: Paraflex, Parafon Forte)
- nicotinic acid or niacin, long-acting
- leukotriene synthase inhibitors
    - zafirlukast (brand name: Accolate)
    - zileuton (brand name: Zyflo)
- anabolic steroids

Antihypertensives

- captopril (brand name: Capoten)
- enalapril (brand name: Vasotec)
- alpha methyldopa (brand name: Aldomet)
- hydralazine (brand name: Apresoline)

Antibiotics/Antimicrobials

- amoxicillin/clavulanate (brand name: Augmentin)
- fluconazole (brand name: Diflucan)
- ketoconazole (brand name: Nizoral)
- nitrofurantoin (Macrodantin)
- sulfonamides/sulfa antibiotics
    - e.g., sulfamethoxazole with trimethoprim (brand names: Septra, Bactrim)
- tetracycline(s)
- erythromycin(s) or macrolides
    - telithromycin (brand name: Ketek)
- clindamycin (brand name: Cleocin)

Antidepressants

- bupropion (brand names: Wellbutrin, Zyban)
- fluoxetine (brand names: Prozac, Sarafem)
- sertraline (brand name: Zoloft)
- tricyclic antidepressants
    - amitriptyline (brand name: Elavil)
    - nortriptyline (brand names: Pamelor, Aventyl)
    - imipramine (brand name: Tofranil)
    - doxepin (brand names: Sinequan, Adapin)

Section B. Drugs with hepatotoxic potential that may be in use by individuals with medical conditions likely to be disqualifying for this study

- amiodarone (brand name: Cordarone)
- carbamazapine (brand name: Tegretol)
- phenytoin (brand name: Dilantin)
- valproate (brand name: Depakene)
- chlorpromazine (brand name: Thorazine)
- clopidogrel (brand name: Plavix)
- azathioprine (brand name: Imuran)
- 6-mercaptopurine (brand name: Purinethol)
- isoniazid (also "INH" – brand name: Nydrazid)
- methotrexate (brand names: Rheumatrex, Trexall)
- rifampin (brand names: Rifadin, Rimactane)
- tolcapone (brand name: Tasmar)
- quinidine (brand name: Quinidex)
- ticlopidine (brand name: Ticlid)
- troglitazone (brand name: Rezulin)
- antiretrovirals for HIV therapy

15.9. APPENDIX 9: REFERENCES

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