VAC-056

A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety, Tolerability, Lot-to-Lot Consistency, Immunogenicity, and Non-Interference with Concomitant Vaccinations of Serum Institute of India's 10-Valent Pneumococcal Conjugate Vaccine (PNEUMOSIL®) in Healthy Infants in The Gambia

CONFIDENTIAL

June 1, 2018

Sponsored by:
PATH Vaccine Solutions (PATH)

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION/ ACRONYM	DEFINITION
ADR	adverse drug reaction
AE	adverse event
Alum	aluminum phosphate
AMC	Advance Market Commitment
BHDSS	Basse Health and Demographic Surveillance System
BLQ	below limit of quantitation
CAPA	corrective action and preventive action
CBER	Center for Biologics Evaluation and Research
CDAP	1-cyano-4-dimethylaminopyridinium tetrafluoroborate
CFR	Code of Federal Regulations
CI	confidence interval
CRF	case report form
CRM_{197}	Cross Reactive Material 197
CRO	contract research organization
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data Safety Monitoring Board
DT	diphtheria toxoid
DTwP-HepB-Hib	Pentavalent – diphtheria, tetanus, whole-cell pertussis, hepatitis B, and *Haemophilus influenzae* type b combined vaccine
EC	ethics committee
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
EOS	End of Study
EPI	Expanded Program on Immunization
FDA	US Food and Drug Administration
FIP	Full Immunogenicity Population
FSFV	first subject first visit
Gambian	subjects enrolled from the MRC field sites in The Gambia
GCLP	Good Clinical Laboratory Practice
GCP	Good Clinical Practice
GLP	Good Laboratory Practice

GMC	Geometric Mean Concentration
GMP	Good Manufacturing Practice
GMT	geometric mean titer
GSK	GlaxoSmithKline
HbsAg	hepatitis B surface antigen
HBV	Hepatitis B 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 Harmonisation
ID	identification
IgG	immunoglobulin G
IM	intramuscular
IME	Important Medical Event
IP	investigational product
IPD	invasive pneumococcal disease
IPP	Immunogenicity Persistence Population
IPV	inactivated poliovirus vaccine
IRB	Institutional Review Board
IV	intravenous
IWC	Infant Welfare Card
LSLV	last subject last visit
MedDRA	Medical Dictionary for Regulatory Activities
MOPA	multiplexed opsonophagocytic assay
MRCG	Medical Research Council, representing Medical Research Council Unit The Gambia
NMRA	National Medicines Regulatory Authority
NRA	national regulatory authority
NTF	Note to File
OPA	opsonophagocytic assay
OPV	oral poliovirus vaccine
PATH	PATH Vaccine Solutions
PCV	pneumococcal conjugate vaccine
PE	physical examination
PFS	pre-filled syringe

PI	Principal Investigator (the term is used throughout to indicate PI or designee)
PP IMM	Per Protocol Immunogenicity Population
PSRT	Protocol Safety Review Team
RC	research clinician
RCD	reverse cumulative distribution
RDT	rapid diagnostic test for malaria
RE	reactogenicity event
REC	Research Ethics Committee (PATH)
RRF	reactogenicity record form
RV	rotavirus vaccine (Rotarix)
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SCC	Scientific Coordinating Committee
SD	standard deviation
SIDS	sudden infant death syndrome
SIIPL	Serum Institute of India Pvt. Limited
SOC	system organ class
SOP	standard operating procedure
SSP	study specific procedure
SST	Serum Separator Tube
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	treatment-emergent adverse event
TEN	toxic epidermal necrolysis
TMF	Trial Master File
TPN	total parenteral nutrition
TPP	Target Product Profile
TRS	Technical Report Series
TT	tetanus toxoid
WHO	World Health Organization
WIRB	Western Institutional Review Board

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 Harmonisation (ICH) Guidance for GCP (E6)
- World Medical Association (WMA) 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 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 ICH-GCP guidelines as outlined in the 'Statement of Compliance.'

Principal Investigator:

Signed: _____ Date: _____

Name:

Title:

Sponsor's Representative:

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Name:

Title:

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Contract Research Organization	
Immunology Laboratories	**Pneumococcal serology:**  **Diphtheria, tetanus, pertussis, and *Haemophilus influenzae* type b serology:**  **Hepatitis B, measles, and rubella serology:**  **Polio serology:** 

	[redacted] **Rotavirus serology:** [redacted] **Yellow fever serology:** [redacted]
Clinical Laboratory	[redacted]
Ethics Committees	The Gambia Government/MRC Joint Ethics Committee c/o MRC Unit, The Gambia, Fajara, PO Box 273, Banjul, The Gambia, West Africa Western Institutional Review Board 1019 39th Avenue SE Suite 120 Puyallup, WA 98374-2115, United States
The Gambia National Medicines Regulatory Agency	The Medicines Control Agency

PROTOCOL SUMMARY

TITLE	**A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety, Tolerability, Lot-to-Lot Consistency, Immunogenicity, and Non-Interference with Concomitant Vaccinations of Serum Institute of India's 10-Valent Pneumococcal Conjugate Vaccine (PNEUMOSIL®) in Healthy Infants in The Gambia**
STUDY NUMBER	VAC-056
SCC NUMBER	1517
PROJECT PHASE	Phase 3
INVESTIGATIONAL PRODUCT(S)	**Investigational Vaccine:** Pneumococcal 10-valent conjugate vaccine (PNEUMOSIL) at a dosage of 2 µg for each serotype polysaccharide, except 4 µg for 6B serotype, conjugated to a carrier protein (CRM_{197}), with adjuvant (aluminum phosphate [alum]) and preservative (thiomersal). **Active Comparator Vaccine:** Pneumococcal conjugate vaccine (Non-Typeable *Haemophilus influenzae* (NTHi) protein D, diphtheria or tetanus toxoid conjugates) adsorbed (Synflorix®; GlaxoSmithKline)
STUDY HYPOTHESES	**Primary Hypotheses:** **Immunogenicity:** • The immune responses to the 10 serotypes in PNEUMOSIL® (1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, 23F) induced by 3 different lots of PNEUMOSIL will be equivalent after a 3-dose primary series. • The immune responses to at least 7 of the 10 serotypes in PNEUMOSIL will be non-inferior to the immune responses induced by the matched serotype (for 1, 5, 6B, 7F, 9V, 14, 19F, 23F) or serotype with the lowest seroresponse rate (for 6A, 19A) in Synflorix, after a 3-dose primary series. • The immune responses induced by pentavalent, polio and rotavirus vaccines co-administered with PNEUMOSIL during a 3-dose primary series will be non-inferior to the immune responses observed when these vaccines are co-administered with Synflorix. **Safety, Tolerability:** • PNEUMOSIL administered as a 3-dose primary series, and co-administered with routine pediatric vaccines, will be safe and well tolerated. • PNEUMOSIL administered as a booster dose to primed infants at 9 months of age when co-administered with routine pediatric vaccines, will be safe and well tolerated.

	Secondary Hypotheses: **Immunogenicity:** • The immune responses induced by PNEUMOSIL for serotypes 6A and 19A will be superior to the cross-reactive responses to these serotypes induced by Synflorix after a 3-dose primary series. • PNEUMOSIL will induce a measurable booster response to each of the 10 serotypes when administered as a 4th dose at 9 months of age. • The immune responses induced by measles-rubella and yellow fever vaccines administered at 9 months of age concomitantly with a PNEUMOSIL booster dose will be non-inferior to those observed when the vaccines are co-administered with a Synflorix booster dose.
STUDY OBJECTIVES	**Primary Objectives:** **Immunogenicity:** 1. To demonstrate that the immune responses to the 10 pneumococcal serotypes in PNEUMOSIL (1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, 23F) induced by 3 different lots of PNEUMOSIL are equivalent when measured 4 weeks after a 3-dose primary series 2. To demonstrate non-inferior immune responses for at least 7 of the 10 serotypes in PNEUMOSIL in comparison to matched serotypes (for 1, 5, 6B, 7F, 9V, 14, 19F, 23F) or the lowest responder (for 6A, 19A) in Synflorix based on (a) % IgG response ≥ 0.35 µg/mL or (b) IgG GMCs measured 4 weeks after a 3-dose primary series 3. To demonstrate that the immune responses induced by routine pediatric vaccines (pentavalent, polio and rotavirus) when co-administered with a 3-dose primary series of PNEUMOSIL are non-inferior to those induced by these vaccines when co-administered with Synflorix (subset of subjects) **Safety, Tolerability:** 1. To demonstrate an acceptable safety and tolerability profile for PNEUMOSIL administered as a 3-dose primary series and booster dose, and when co-administered with routine pediatric vaccines through 4 weeks after a booster dose (subset of subjects for tolerability) **Secondary Objectives:** **Immunogenicity:** 1. To demonstrate that the immune responses to serotypes 6A and 19A in PNEUMOSIL are superior to the cross-reactive responses to these serotypes induced by Synflorix based on (a) % IgG response ≥ 0.35 µg/mL or (b) IgG GMCs measured 4 weeks after a 3-dose primary series 2. To evaluate the functional serotype-specific antibody responses induced by PNEUMOSIL in comparison to Synflorix, as measured

	by OPA at 4 weeks post 3-dose primary series (subset of subjects) 3. To evaluate the booster responses (antibody concentrations and functional responses) to PNEUMOSIL in comparison to Synflorix, from 4 weeks after a 3-dose primary series to 4 weeks after a booster dose (subsets of subjects) 4. To demonstrate that the immune responses induced by measles-rubella and yellow fever vaccines when co-administered with a booster dose of PNEUMOSIL are non-inferior to those induced by these vaccines when co-administered with a booster dose of Synflorix (subset of subjects) **Supplemental Objective:** **Immunogenicity:** 1. To evaluate the persistence of immune responses (antibody concentrations and functional responses) induced by PNEUMOSIL in comparison to Synflorix, 1 year after administration of a booster dose (subset of subjects) **Safety:** 1. To assess the safety of 3-dose primary series and booster dose of PNEUMOSIL co-administered with routine pediatric vaccines in regards to serious adverse events occurring 4 weeks after the booster dose through 12 months after the booster dose (subset of subjects)
STUDY ENDPOINTS	**Primary Endpoints:** **Immunogenicity:** **For Primary Objective 1 (lot consistency):** • Serotype-specific immunoglobulin G (IgG) geometric mean concentration (GMC) measured 4 weeks post dose 3 **For Primary Objective 2 (non-inferiority):** • Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 4 weeks post dose 3 • Serotype-specific IgG GMC measured 4 weeks post dose 3 **For Primary Objective 3 (non-interference):** • Percentage of subjects with anti-diphtheria toxoid IgG concentration ≥ 0.1 IU/mL measured 4 weeks post dose 3 • Percentage of subjects with anti-tetanus toxoid IgG concentration ≥ 0.1 IU/mL measured 4 weeks post dose 3 • Percentage of subjects with anti-Hepatitis B surface antigen (HBsAg) IgG concentration ≥ 10 mIU/mL measured 4 weeks post dose 3 • Percentage of subjects with anti-*Haemophilus influenzae* type b

	(PRP) IgG concentration ≥ 0.15 µg/mL measured 4 weeks post dose 3 • Anti-pertussis toxoid and fimbriae IgG GMCs measured 4 weeks post dose 3 • Percentage of subjects with anti-poliovirus types 1, 2 and 3 neutralizing antibody titers ≥ 1:8 measured 4 weeks post dose 3 • Percentage of subjects with anti-rotavirus IgA concentration ≥ 20 U/mL measured 4 weeks post dose 3 **Safety, Tolerability:** • Number and severity of solicited local and systemic adverse events (reactogenicity events [REs]) through Day 6 post each vaccination • Number, severity and relatedness of all AEs and serious adverse events (SAEs) during the entire study period through 4 weeks post last dose for the cohort **Secondary Endpoints:** **Immunogenicity:** **For Secondary Objective 1 (superiority):** • Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 4 weeks post dose 3 • Serotype-specific IgG GMC measured 4 weeks post dose 3 **Secondary Objective 2 (functional response):** • Percentage of subjects with OPA titer ≥ 1:8 measured 4 weeks post dose 3 • OPA geometric mean titer (GMT) measured 4 weeks post dose 3 **Secondary Objective 3 (boostability):** • Ratio of IgG GMCs measured 4 weeks post dose 4 to IgG GMCs measured 4 weeks post dose 3 • Ratio of OPA GMTs measured 4 weeks post dose 4 to OPA GMTs measured 4 weeks post dose 3 **Secondary Objective 4 (non-interference):** • Percentage of subjects with anti-measles IgG concentration ≥ 150 mIU/mL measured 4 weeks post dose 4 • Percentage of subjects with anti-yellow fever neutralizing antibody titers ≥ 1:8 measured 4 weeks post dose 4 • Percentage of subjects with anti-rubella IgG concentration ≥ 4 IU/mL measured 4 weeks post dose 4

	Supplemental Endpoints: **Immunogenicity:** **For Supplemental Objective 1 (immune persistence):** • Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 1 year post dose 4 • Serotype-specific IgG GMC measured 1 year post dose 4 • Percentage of subjects with OPA titer ≥ 1:8 measured 1 year post dose 4 • OPA geometric mean titer (GMT) measured 1 year post dose 4 **For Supplemental Objective 2 (Safety):** • Number, severity and relatedness of all serious adverse events (SAEs) 4 weeks after the booster dose through 12 months after the booster dose (subset of subjects)
STUDY DESIGN	This prospective, single center, randomized, active-controlled, double-blind, Phase 3 study in healthy Gambian PCV-naïve infants (6 to 8 weeks) will be conducted in 3 phases. In the initial priming phase, 2,250 subjects will be randomized (2:2:2:3) to receive 3 doses of either PNEUMOSIL (3 groups receiving vaccine from different lots) or Synflorix (1 group) at 6, 10, and 14 weeks of age. In the second booster phase, the first 675 randomized subjects will receive a booster dose of either PNEUMOSIL or Synflorix at 9 months of age that matches the treatment assignment for the priming phase. Standard EPI vaccinations in The Gambia will be given concomitantly with all 4 doses of the study vaccines. The third phase will include subjects from the booster phase whose parent provides additional consent to evaluate immune persistence at 1 year post booster vaccination. After parent(s) sign an informed consent form, prospective subjects will be assessed for eligibility to participate in the study, including assessment of medical history, vital signs and physical examination, and will receive the first vaccination (V1) at 6-8 weeks of age. Two subsequent primary vaccination visits will take place at 4 (+2) weeks after the prior vaccination. A follow-up visit (V4) will take place at 4 (+2) weeks after the third vaccination, during which blood will be collected for immunological assessments. This visit will serve as the end-of-study (EOS) visit for subjects not included among the first 675 subjects to be randomized (n=1,575). The first 675 subjects will continue on study and be asked to return for a booster vaccination (V5) at 9 (+1) months of age, followed 4 (+2) weeks later by a follow-up visit (V6), during which blood will be collected for immunological assessments. This visit will serve as the EOS visit for subjects whose parent does not provide consent for assessment of immune persistence 1 year after the booster vaccination.

	After the last subject in the booster cohort completes V6, the database will be closed and the study unblinded (only the Sponsor, statistical personnel and medical monitor will be unblinded) in order to analyze and report primary and secondary endpoints. Those subjects (maximum n=675) whose parent provides additional consent to participation will return for a final visit (EOS, V7) at 12 (+1) months after the booster vaccination, during which blood will be collected for immunological assessment of immune persistence.

STUDY SCHEMA

Groups	Priming Phase					Booster Phase			Immune Persistence Phase
	N	Visits*				N	Visits*		Visit*
		V1	V2	V3	V4		V5	V6	V7
		6-8 w	V1+4 (+2) w	V2+4(+2) w	V3+4(+2) w		9-10 m	V5+4(+2) w	V5+12(+1) m
PNEUMOSIL Lot 1	500	X	X	X	B	150#	X	B	B
PNEUMOSIL Lot 2	500	X	X	X	B	150#	X	B	B
PNEUMOSIL Lot 3	500	X	X	X	B	150#	X	B	B
Synflorix	750	X	X	X	B	225#	X	B	B

w = weeks; m = months

X = vaccination (+ EPI vaccines); B = blood sample for immunogenicity testing

*Age ranges indicated for V1/V5. Other vaccination/follow up visits will be at 4 weeks post prior visit + 2 week window, except for V7, which will be at 12 months post V5 + 1 month window.

The total number of subjects assessed for immune persistence at V7 will depend on number of subjects whose parent provides additional informed consent.

STUDY POPULATION	2,250 healthy, male and female PCV-naïve infants residing in The Gambia who are from 6 up to 8 weeks of age at enrollment (V1).
STUDY DURATION	All subjects will be followed for approximately 12[1] weeks after randomization (4 weeks after the third primary vaccination). A subgroup (n=675) will be followed for approximately 34[1] weeks after randomization (4 weeks after the booster dose). Those subjects in this subgroup whose parents provide consent will return for an additional blood draw at approximately 12[1] months after the booster dose (approximately 19 months after randomization). [1]Excluding any additional time due to visit windows

1. BACKGROUND AND RATIONALE

1.1. Burden of Disease

The bacterium *Streptococcus pneumoniae* kills a half million children annually before their fifth birthday, mostly in low-resource areas of the world.[1] The most common cause of childhood morbidity and mortality due to the bacterium is pneumonia, which in 2013 was estimated to be the cause of roughly 900,000 (or 15% of all) under-five deaths worldwide, making it the most deadly infectious disease of young children today.[2] 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. The highest incidence of IPD is seen at the extremes of age, in the elderly and children less than 2 years old.[3] Public health leaders agree that vaccines are the best way to address the enormous burden of pneumococcal disease, particularly in Africa and Asia, where 95% of all pneumococcal deaths occur.[4]

1.2. Pathogen and Clinical Disease

S. pneumoniae is a Gram-positive encapsulated bacterium that is commonly carried as a commensal 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.[5] Carriage is typically asymptomatic; however, it is believed to be a precondition for invasive pneumococcal infection.

The signs and symptoms of IPD depend on the type of pneumococcal infection, but may be nonspecific. The most common signs and symptoms in adults include fever, chills, sweating, aches, pain, malaise, and headache. An individual with pneumococcal pneumonia may additionally complain of cough, chest pain, shortness of breath, and rapid breathing.

1.3. Pneumococcal Epidemiology in Africa and The Gambia

Although documentation of pneumococcal disease in children is limited in low-resource countries, several studies have estimated the extent of disease in Africa generally, and in The Gambia specifically. The studies conducted prior to the introduction of pneumococcal conjugate vaccine (PCV) have estimated rates of IPD to be as much as 10-fold higher in The Gambia and other African countries than in the developed world.[6,7,8] Based on 2 studies conducted during the period 1988 through 1994, the incidence of invasive pneumococcal disease in The Gambia was estimated to be at least 500 per 100,000 in children in their first year of life, and 250 per 100,000 in children less than 5 years of age.[9] The importance of IPD in The Gambia was also highlighted in a randomized, placebo-controlled, double-blind trial of a 9-valent PCV conducted in the Upper and Central River Divisions of the country between 2000 and 2004: the incidence of IPD due to all serotypes in the placebo arm of the study was 380 per 100,000, versus 190 per 100,000 in the vaccine arm.[10] A recently published population-based surveillance study conducted in the Upper River Region of The Gambia between May 2008 and December 2014 found that, after the introduction of PCV, the incidence of IPD decreased from 253 to 113 cases per 100,000 population among children aged 2-23 months old.[11]

Though there are more than 90 serotypes of *S. pneumoniae*, a small percentage are responsible for the large majority of cases of IPD. There are important regional differences in the dominant disease-

causing serotypes (or serogroups); in particular, serotypes 1 and 5 account for a much larger percentage of IPD in the developing world. Serogroups 14, 6, 19, 18, 9, 23, and 7 are responsible for roughly 85% of IPD in the developed world, whereas the dominant serotypes causing IPD in Africa, Asia, and Latin America are 1, 2, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (Table 1).[12]

Table 1. Proportion (Percent) of Invasive Pneumococcal Disease in Children Less Than 5 Years of Age due to Serotype by Region

Region	1	2	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F
Africa	11.7	1.9	2.3	10.7	9.4	8.5	0.8	2.2	13.0	1.4	3.9	5.4	6.5
Asia	9.5	2.6	1.6	6.7	3.5	11.5	2.0	3.1	11.6	2.4	2.6	8.1	9.7
LAC	8.4	0.3	1.6	8.5	4.5	9.4	2.5	2.7	26.5	4.3	2.9	3.6	5.3

Abbreviation: LAC = Latin America and the Caribbean

1.4. Licensed Pneumococcal Conjugate Vaccines

In 1983, Pneumovax® 23, a pneumococcal polysaccharide vaccine covering 23 serotypes developed by Merck, was first approved for use in older adults and the elderly to prevent pneumococcal disease. This vaccine contains capsular polysaccharide from serotypes 1, 2, 3, 4, 5, 6b, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. While Pneumovax 23 has been shown to be effective against IPD in immunocompetent adults,[13] it is poorly immunogenic in children less than 2 years old.[14]

The first effective pneumococcal vaccine for children less than 2 years old and infants was developed based on the success of the *Haemophilus influenzae* type b (Hib) conjugate vaccine, which elicits an enhanced immune response when the polysaccharide is conjugated to a carrier protein. Prevenar®, a 7-valent pneumococcal conjugate vaccine, contains the capsular antigens from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated to Cross Reactive Material 197 (CRM_{197}, a non-toxic diphtheria toxoid protein). Developed by Wyeth (now Pfizer), Prevenar was first approved and introduced in the US for use in infants and young children in the year 2000. By the end of the decade, overall and serotype-specific IPD in the US were reduced by 45% and 94% respectively.[15] However, given the limited coverage offered by Prevenar, two second-generation PCVs, Synflorix® (GlaxoSmithKline [GSK] Biologicals) and Prevenar 13® (Wyeth, now Pfizer) were subsequently developed and approved for infants and young children, both expanding on Prevenar's 7 serotypes to offer protection against 10 and 13 serotypes, respectively. By adding serotypes 1, 5, and 7F in the case of Synflorix, and serotypes 1, 3, 5, 6A, 7F, and 19A in the case of Prevenar 13, the second-generation PCVs offer additional protection against common serotypes in Africa and Asia, most notably against serotypes 1 and 5.

1.5. Rationale for PNEUMOSIL® Development

More than 15 years after their introduction, the most significant barriers to global access to PCVs remain their cost and complex manufacturing process. The price of the vaccine is the critical factor that determines whether PCV introduction is considered cost-effective in a low resource setting. This reality has underscored the importance of developing an affordable PCV tailored against the specific serotypes causing pneumococcal disease in the developing world.

Because of the high cost of PCV, introduction of the vaccine into low resource countries has depended on considerable external financial assistance. In 2009, Rwanda and The Gambia became the first low resource countries to introduce Prevenar, with assistance from Gavi and other international partners.[16] Since 2010 a global roll-out of Synflorix and Prevenar 13 has been underway in Gavi-eligible countries with the help of the pneumococcal Advance Market Commitment (AMC), a financing mechanism whereby donors commit funds to guarantee the price of future vaccines, creating incentives for producers and catalyzing competition to supply vaccines at long-term lower prices.[17] Fifty-four low-resource countries have now introduced PCV into their routine immunization programs with Gavi assistance.[18] In April 2011, Prevenar 13 replaced Prevenar in the Gambian EPI schedule. As these lifesaving vaccines continue to make their way into the developing world with external assistance, the rapid development of less expensive PCVs is also needed if countries in the developing world will be able to independently afford them over the long term. To this end, enhancing the participation of emerging-market manufacturers in PCV production is a critical factor in achieving a sustainable, affordable, and accessible supply of vaccine for countries with limited resources. Development of effective and more affordable PCVs is aligned with the Sustainable Development Goal of ending preventable deaths of newborns and children under 5 years of age by 2030.[19]

1.6. Introduction to PNEUMOSIL

The Serum Institute of India (SIIPL), a manufacturer of multiple WHO-prequalified vaccines, in collaboration with PATH, has been working since 2006 to develop a multivalent PCV designed to prevent pneumococcal disease and to be affordable for use in low resource countries. PNEUMOSIL*, SIIPL's 10-valent candidate pneumococcal conjugate vaccine incorporates prevalent serotypes in Africa, Asia, and Latin America (serotypes 1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, and 23F), thus offering comparable coverage to currently licensed PCVs in these settings.

In addition to selecting serotypes based on prevalence in low resource countries, SIIL has optimized 3 critical components of the manufacturing practice – carrier protein production, polysaccharide production, and conjugation efficiency – that together substantially lower the cost of manufacturing a high-quality multivalent PCV. As a result, it will be possible to provide PNEUMOSIL at a price that is significantly lower than that of the currently licensed PCVs. If it becomes available under the AMC, PNEUMOSIL would provide significant cost savings to Gavi and to the Gavi-supported countries that have introduced pneumococcal vaccines procured through the AMC. This cost savings would result not only from a price substantially below the $3.05-$3.10 per dose contributed by Gavi and Gavi-supported countries to the manufacturers of the 2 existing AMC-eligible PCVs (GlaxoSmithKline's Synflorix and Pfizer's Prevenar 13), but also from price pressure that PNEUMOSIL's AMC eligibility would place on these vaccines. Finally, availability of PNEUMOSIL would address any potential long-term supply constraints for pneumococcal vaccine.

1.7. Summary of Nonclinical Studies

PNEUMOSIL has been made in compliance with Good Manufacturing Practice (GMP), and this candidate vaccine has been tested in multiple preclinical pharmacology and Good Laboratory Practice (GLP) studies to assess immunogenicity, toxicity and local tolerance, in compliance with Schedule Y of the Drugs and Cosmetics Rules of India, ICH Harmonized Tripartite Guideline S6, and WHO recommendations.[20,21,22]

* Prior to trademark registration, SIIPL's candidate PCV was referred to as "SIILPCV10".

1.7.1. Pharmacology

A pharmacology study was conducted in New Zealand White rabbits using the GMP lot of PNEUMOSIL (#4193001) used in the Phase 1/2 clinical trial in The Gambia (VAC-017). Eight (8) animals (4 per sex) were immunized intramuscularly (IM) with a human dose-volume of PNEUMOSIL on study Day 1, 15, and 29. Serum was collected at baseline, at Day 29 (pre-dose, 2 weeks post second dose), and at Day 43 (2 weeks post the third and last dose). A second group of 8 animals was treated with Prevenar13 (human dose-volume), and served as a comparator. Serum samples were assessed individually for their humoral, serotype-specific immune response using 2 test methods: 1) a direct ELISA to quantitate serotype-specific IgG; and 2) a multiplexed opsonophagocytic assay (MOPA) to estimate the amount of functional antibodies (able to induce phagocytosis and killing) elicited in the blood of the rabbits by the vaccination. The assay results indicated that the total IgG and the functional antibody responses elicited by IM immunization with GMP lot #4193001 and measured in the blood of the animals 2 weeks post 2nd and 3rd dose were equivalent to those elicited by the Prevenar13 comparator, across all vaccine serotypes. It should also be noted that the same analyses were performed after a 3-dose schedule of immunization on multiple lots of PNEUMOSIL after 1 year of manufacture, and the IgG and OPA antibody titers achieved were comparable to the titers achieved on the lots at the time of manufacture.

1.7.2. Toxicology

A total of 7 preclinical toxicology studies of PNEUMOSIL have been conducted either in Sprague-Dawley rats or New Zealand white rabbits, 4 of which have been single-dose and 3 repeat-dose studies (Table 2).

Table 2. Summary of PNEUMOSIL Nonclinical Studies

Study No.	Animal	Route	Treatment Groups*	Doses	Sacrifice Day(s)	Recovery (Days)	Additional Assessments‡
G7628	Rat	IM	G1, G2, G3, G4, G5, G6	1	D15	14	A1, A2
G7629	Rat	SC	G1, G2, G3, G4, G5, G6	1	D15	14	A1, A2
G7630	Rabbit	IM	G1, G2, G3, G4, G5, G6	1	D15	14	A1, A2
G7631	Rabbit	SC	G1, G2, G3, G4, G5, G6	1	D15	14	A1, A2
G7557	Rat	IM	G1, G2, G3, G4, G5, G6	5	D58, D86	28	A1, A2, A3, A5
G7558	Rabbit	IM	G1, G2, G3, G4, G5, G6	5	D58, D86	28	A1, A2, A3, A5
12976	Rabbit	IM	G2, G3, G4	4	D44, D72	28	A1, A2, A3, A4, A5

Abbreviations: IM, intramuscular; SC, subcutaneous

*G1: negative control (saline), G2: vehicle control (alum), G3: Prevenar 13 (1x), G4: PNEUMOSIL (1x), G5: PNEUMOSIL (10X), G6: PNEUMOSIL (20x).

‡A1: safety labs, A2: histopathology of injection sites, A3: histopathology of select organs/tissues; A4: limited male fertility, A5: immunogenicity (IgG and OPA).

In 6 of these studies, groups of animals were administered PNEUMOSIL at doses of 1, 10, and 20 times the expected human dose; in addition, concurrent control groups were administered Prevenar 13, saline, and aluminum phosphate (alum) adjuvant. In Study 12976, groups of animals (rabbits) were administered alum, Prevenar 13, and PNEUMOSIL with or without preservative (thiomersal) at

the expected human dose. In the case of all studies, the test and control articles were administered via the intended clinical route (intramuscularly) or subcutaneously. In the repeat-dose studies, the animals were administered either 5 doses of vaccine or control at 2-week intervals, or 4 doses at 2-week intervals in the case of Study 12976, and were followed by a 28-day recovery period. Blood was collected from all animals prior to vaccination and at termination. Blood was analyzed for standard safety laboratory parameters in all studies, and for immunogenicity (IgG and OPA) against the vaccine serotypes in the repeat-dose studies. These latter analyses demonstrated a significant increase from baseline in both total IgG and functional antibody titers in the vaccinated animal sera against all the pneumococcal serotypes included in PNEUMOSIL. The immunizations with buffer and vehicle control did not give rise to any major changes in the antibody titers against any of the vaccine serotypes.

In the single-dose rat studies (G7628, G7629), visual (edema) and microscopic evidence of local inflammation was seen in all treatment groups at a similar magnitude – indicating that the alum adjuvant was most likely the cause of these changes. Recovery was noted 14 days after administration, and therefore the effects were considered transient in nature. Increases in gamma-glutamyl transpeptidase (GGT) were observed in all treatment groups in the single intramuscular (IM)-dose study (G7628) but were not seen in the single subcutaneous (SC)-dose study (G7629) or the multi-dose study (G7557). Liver enzymes (alanine transaminase [ALT]/ aspartate aminotransferase [AST]) were not altered and liver weights were normal at time of sacrifice.

In the multi-dose rat study (G7557), the albumin/globulin (A:G) ratio was decreased (as a result of an increase in globulin level) and the white blood cell count increased in the Prevenar 13 and SIILPCV13 treatment groups. These effects are commonly observed in vaccine animal testing, as they are expected pharmacological effects of stimulating the immune system. Coagulation parameters, prothrombin time (PT) and activated partial thromboplastin time (APTT), were within normal limits. Transient increases in serum fibrinogen, likely indicative of immune activation, were observed in Prevenar 13 and PNEUMOSIL treatment groups in the rat studies. Significant increases in serum creatinine were observed in all treatment groups of the multi-dose rat study (G7557), including the negative and vehicle control groups; while its cause is unclear, the consistency of the creatinine increase across groups suggests that it is unlikely to be an adverse effect of PNEUMOSIL and Prevenar 13. Microscopic examination of the kidneys at sacrifice showed that the organs were healthy.

In the rabbit, erythema was seen at the injection site in all treatment groups in the single SC-dose study (G7631) but not with IM administration. Microscopic evidence of local inflammation of a similar magnitude was seen at the injection site in all treatment groups in the 4 rabbit studies.

Subsequently it was concluded that these alterations were due to alum administration. Substantial recovery was noted at the end of the recovery period, suggesting a transient local inflammatory phenomenon. Based on assessment in the third repeat-dose study (12976), there was no effect of treatment on sperm motility and morphology.

In summary, single- and repeat-dose administration of PNEUMOSIL to rats and rabbits was well tolerated, and resulted in observed changes that were not adverse but rather a consequence of the pharmacological activity of PNEUMOSIL and the comparator Prevenar 13, or were seen across all treatment groups and not associated with the test product only.

1.8. Summary of Clinical Studies

Three clinical trials of PNEUMOSIL have been conducted:

1) A Phase 1, randomized, active-controlled, double-blind trial (PCV10-001) evaluating the safety and tolerability of PNEUMOSIL in healthy young Indian adults (n=34) has been completed. Eligible subjects were randomized 1:1 to receive a single dose of PNEUMOSIL or Pneumovax 23 and were followed through 28 days post vaccination.
2) A Phase 1/2, randomized, active-controlled, double-blind age de-escalation trial (VAC-017) evaluating the safety, tolerability and immunogenicity of PNEUMOSIL in 34 PCV-naïve adults, 112 PCV (Prevenar 13)-primed toddlers (12-15 months of age), and 200 PCV-naïve infants in The Gambia has been completed through the infant primary series. Eligible subjects were randomized 1:1 to receive a single dose of PNEUMOSIL or either Pneumovax 23 in adults or Prevenar 13 in toddlers, and a 3-dose primary series of PNEUMOSIL or Prevenar 13 (at 6, 10, and 14 weeks of age) in the infant cohort. EPI vaccines were concomitantly administered to infants, including pentavalent vaccine (diphtheria, tetanus, whole-cell pertussis, hepatitis B, and Haemophilus influenzae type b [DTwP-HepB-Hib]). Adults and toddlers were followed for 28 days post vaccination, and infants for 84 days post final vaccination. A Data Safety Monitoring Board (DSMB) granted approval to advance to the toddler and infant cohorts following the adult and toddler cohorts respectively. Following database lock and unblinded data review, the decision was made to extend the study to evaluate a matched booster dose of PNEUMOSIL or Prevenar 13 in infants.
3) A Phase 2, randomized, active-controlled, double-blind trial (PCV10-002) evaluating the safety, tolerability and immunogenicity of a 2-dose regimen of PNEUMOSIL in 114 PCV-naïve Indian toddlers (12-15 months of age) is ongoing. A DSMB formally recommended advancing to this Phase 2 'catch-up' trial in toddlers after review of results of the PCV10-001 trial post database lock. All toddlers have completed the final follow-up visit 28 days after the 2nd vaccination.

PNEUMOSIL was well tolerated in all 3 trials, and no safety concerns were identified (based only on blinded review of safety data in the case of PCV10-002). PNEUMOSIL was also shown in the VAC-017 study to be immunogenic for all 10 serotypes contained in the vaccine. A summary of safety and immunogenicity results follows. Please refer to the Investigator's Brochure (IB) for additional details on the methodology and results of the PCV10-001 and VAC-017 trials.

1.8.1. Safety

Laboratory Assessments:

In all 3 clinical studies blood samples were collected for safety hematology, clinical chemistry, and organ function tests; a coagulation panel was also evaluated in adult subjects. Laboratory assessments were only performed at baseline for infants in the VAC-017 study. There were no notable trends from baseline to post vaccination in any laboratory parameter in the adult subjects in the PCV10-001 and VAC-017 studies, or in the toddler subjects in the VAC-017 study.

Reactogenicity:

Solicited local and systemic reactogenicity were assessed daily in all subjects for the first 7 days post vaccination in all 3 clinical studies by means of a subject diary (PCV10-001 and PCV10-002) or daily home visits by field workers (VAC-017). When observed, reactogenicity after vaccination with PNEUMOSIL in all studies and age cohorts was generally mild or moderate and of limited duration.

In adults, the most common local reactogenicity event (RE) after a single dose of PNEUMOSIL was pain, reported in 70.6% and 58.8% of adults in the PCV10-001 and VAC-017 studies respectively. Only 1 Grade ≥ 2 local RE was reported in the PNEUMOSIL group in either trial (Grade 3

tenderness). Headache was the most common systemic RE after vaccination and was reported for 17.6% of adults who received PNEUMOSIL in both trials. No Grade ≥ 2 systemic RE was reported in adults who received PNEUMOSIL.

In the VAC-017 toddler cohort, the most common local RE post vaccination was tenderness (21.5% PNEUMOSIL, 21.4% Prevenar 13). A higher proportion of toddlers in the PNEUMOSIL group vs the Prevenar 13 group had Grade 1 or 2 induration/swelling at the injection site (10.7% PNEUMOSIL; 1.8% Prevenar 13). No severe (Grade ≥ 3) tenderness or other local RE was reported in toddlers in either the VAC-017 or PCV10-002 studies. Fever (axillary temperature ≥ 37.5°C) was the most common systemic RE (16.1% PNEUMOSIL, 19.6% Prevenar 13 in VAC-017). Grade 3 fever was reported in 2 toddlers who received the PNEUMOSIL booster and 1 toddler who received the Prevenar 13 booster. A higher proportion of toddlers in the PNEUMOSIL group vs the Prevenar 13 group had Grade 1 or 2 drowsiness (10.7% PNEUMOSIL; 0% Prevenar 13). Most REs were transient and resolved within 24 hours.

Table 3 presents the highest grade (Grade ≥ 1) of selected REs occurring over the first 7 days following primary vaccination of infants in the VAC-017 study.

Table 3. Highest Grade ≥ 1 of Selected Reactogenicity Events in Infants – Primary Series (VAC-017)

Reactogenicity Event Grade	Vaccination 1		Vaccination 2		Vaccination 3	
	PNEUMOSIL N = 100 n (%)	Prevenar 13 N = 100 n (%)	PNEUMOSIL N = 100 n (%)	Prevenar 13 N = 100 n (%)	PNEUMOSIL N = 100 n (%)	Prevenar 13 N = 100 n (%)
Temperature						
Grade 1: ≥ 37.5 to ≤ 38.0	29 (29.0)	34 (34.0)	11 (11.0)	13 (13.0)	15 (15.0)	16 (16.0)
Grade 2: > 38.0 to ≤ 39.0	11 (11.0)	7 (7.0)	7 (7.0)	6 (6.0)	4 (4.0)	4 (4.0)
Grade 3: > 39.0 to ≤ 40.0	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)
Irritability						
Grade 1: Crying more than usual/no effect on normal activity	33 (33.0)	29 (29.0)	35 (35.0)	30 (30.0)	33 (33.0)	37 (37.0)
Grade 2: Crying more than usual/interferes with normal activity	4 (4.0)	2 (2.0)	5 (5.0)	3 (3.0)	3 (3.0)	4 (4.0)
Grade 3: Crying that cannot be comforted/prevents normal activity	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
Drowsiness						
Grade 1: Drowsiness easily tolerated	8 (8.0)	1 (1.0)	6 (6.0)	1 (1.0)	2 (2.0)	3 (3.0)
Grade 2: Drowsiness that interferes with normal activity	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)
Decreased appetite						
Grade 1: Eating less than usual/no effect on normal activity	10 (10.0)	1 (1.0)	9 (9.0)	8 (8.0)	6 (6.0)	9 (9.0)
Grade 2: Eating less than usual/interferes on normal activity	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)	2 (2.0)
Tenderness at injection site						
Grade 1: Mild reaction to touch	15 (15.0)	12 (12.0)	18 (18.0)	26 (26.0)	21 (21.0)	19 (19.0)
Grade 2: Cries/protests on touch	4 (4.0)	5 (5.0)	2 (2.0)	1 (1.0)	0 (0.0)	2 (2.0)

Reactogenicity Event Grade	Vaccination 1		Vaccination 2		Vaccination 3	
	PNEUMOSIL N = 100 n (%)	Prevenar 13 N = 100 n (%)	PNEUMOSIL N = 100 n (%)	Prevenar 13 N = 100 n (%)	PNEUMOSIL N = 100 n (%)	Prevenar 13 N = 100 n (%)
Erythema/redness at injection site						
Grade 1: Erythema present but ≤ 2.5 cm diameter	1 (1.0)	8 (8.0)	0 (0.0)	4 (4.0)	3 (3.0)	3 (3.0)
Grade 2: Erythema > 2.5 cm diameter but < 50% surface area of the extremity segment	0 (0.0)	1 (1.0)	1 (1.0)	1 (1.0)	0 (0.0)	0 (0.0)
Induration/Swelling at Injection Site						
Grade 1: Induration/Edema present but ≤ 2.5 cm diameter	4 (4.0)	8 (8.0)	7 (7.0)	16 (16.0)	11 (11.0)	13 (13.0)
Grade 2: Induration/Edema > 2.5 cm diameter but < 50% surface area of the extremity segment	0 (0.0)	2 (2.0)	1 (1.0)	2 (2.0)	2 (2.0)	1 (1.0)

Source: VAC-017 CSR Table IS7A.

In the VAC-017 infant cohort, the most common local RE after a primary dose of study vaccine was tenderness (19-21% PNEUMOSIL, 17-27% Prevenar 13). No Grade ≥ 3 local RE was reported. A lower proportion of infants in the PNEUMOSIL group had Grade 1 or 2 erythema/redness (1.0% PNEUMOSIL; 5.0-9.0% Prevenar 13) and induration/swelling (4.0-8.0% PNEUMOSIL; 10.0-18.0% Prevenar 13) after vaccinations 1 and 2. Fever (axillary temperature ≥ 37.5°C) was the most common systemic RE after the first vaccination (40.0% PNEUMOSIL, 41% Prevenar 13). The proportion of cases of fever was lower after vaccinations 2 and 3 (18.0-20.0% PNEUMOSIL; 20% Prevenar 13). One event of Grade 3 fever was reported in both treatment groups, and 1 episode each of Grade 3 irritability and cutaneous rash in the PNEUMOSIL group. A higher proportion of infants in the PNEUMOSIL group compared with infants in the Prevenar 13 group had Grade 1 or 2 vaccine-related drowsiness (6.0% to 8.0% PNEUMOSIL; 2.0% Prevenar 13) and decreased appetite (10.0% PNEUMOSIL; 1.0% to 8.0% Prevenar 13) after vaccinations 1 and 2. Most REs were transient and resolved within 24 to 48 hours.

The highest grades (Grade ≥ 1) of selected reactogenicity events (REs) after a booster dose of study vaccine in infants are presented in Table 4. When observed, reactogenicity in infants was generally mild or moderate. Overall, there were no notable differences in RE frequency or severity between infants receiving PNEUMOSIL and infants receiving Prevenar 13. Two (4.1%) infants in the PNEUMOSIL group and 1 (2.1%) infant in the Prevenar 13 group had Grade 3 fever. All other REs were mild or moderate in severity. Fever and irritability were the most common systemic REs, both occurring in a total of 12% of subjects (fever: 12.2% PNEUMOSIL, 12.7% Prevenar 13, irritability: 10.2% PNEUMOSIL, 14.9% Prevenar 13). Tenderness at the injection site was the most common local RE (18.3% PNEUMOSIL, 23.4% Prevenar 13). Most REs were transient and resolved within 24 to 48 hours.

Table 4 Highest Grade (≥ 1) Reactogenicity Post Booster Vaccination – Infant Booster Population (VAC-017)

Reactogenicity Event Grade	PNEUMOSIL (N = 49) n (%)	Prevenar 13 (N = 47) n (%)	Total (N = 96) n (%)
Systemic			
Temperature (°C)			
Grade 1: ≥ 37.5 to ≤ 38.0	3 (6.1)	4 (8.5)	7 (7.3)
Grade 2: > 38.0 to ≤ 39.0	1 (2.0)	1 (2.1)	2 (2.1)
Grade 3: > 39.0 to ≤ 40.0	2 (4.1)	1 (2.1)	3 (3.1)
Cutaneous rash			
Grade 1: Localized macular rash	1 (2.0)	3 (6.4)	4 (4.2)
Grade 2: Diffuse macular/maculopapular/morbilliform rash or target lesions	0 (0.0)	2 (4.3)	2 (2.1)
Irritability			
Grade 1: Crying more than usual/no effect on normal activity	5 (10.2)	7 (14.9)	12 (12.5)
Drowsiness			
Grade 1: Drowsiness easily tolerated	0 (0.0)	1 (2.1)	1 (1.0)
Grade 2: Drowsiness that interferes with normal activity	1 (2.0)	0 (0.0)	1 (1.0)
Decreased appetite			
Grade 1: Eating less than usual/no effect on normal activity	2 (4.1)	5 (10.6)	7 (7.3)
Local			
Tenderness at injection site			
Grade 1: Mild reaction to touch	8 (16.3)	11 (23.4)	19 (19.8)
Grade 2: Cries/protests on touch	1 (2.0)	0 (0.0)	1 (1.0)
Erythema/redness at injection site			
Grade 1: Erythema present but ≤ 2.5 cm diameter	1 (2.0)	1 (2.1)	2 (2.1)
Induration/Swelling at Injection Site			
Grade 1: Induration/Edema present but ≤ 2.5 cm diameter	4 (8.2)	4 (8.5)	8 (8.3)
Grade 2: Induration/Edema > 2.5 cm diameter but < 50% surface area of the extremity segment	2 (4.1)	0 (0.0)	2 (2.1)

Note: Highest toxicity grade experienced across all observations from 30 minutes through 6 days post vaccination for each subject is summarized. May include unscheduled visits.
Source: Table IBS7A.

Adverse Events:

In all 3 clinical studies subjects were monitored for AEs from enrollment to final clinic visit, and any AE was assessed by the investigator with regards to severity, relatedness, and duration. No deaths, related serious adverse events (SAEs), or AEs leading to subject withdrawal were reported following vaccination in the 3 studies. In the 2 studies that have unblinded results (PCV10-001 and VAC-017), there are no concerning trends in the frequency of any grade of treatment-emergent AEs (TEAEs), vaccine-related TEAEs, or treatment-emergent SAEs.

No adult in either the PCV10-001 or VAC-017 studies experienced a Grade >1 TEAE or serious adverse event (SAEs). The only related TEAE in the PCV10-001 study was Grade 1 postvaccination injection-site paraesthesia (1 event in both groups), and in the VAC-017 adult cohort was Grade 1 injection-site pain (1 event in both groups).

In the VAC-017 toddler cohort, there were only 2 related TEAEs were reported in the PNEUMOSIL group (Grade 1 diarrhea, Grade 2 morbilliform/papular rash) and 1 related TEAE in the Prevenar group (Grade 1 pruritus). Two (2) treatment-emergent SAEs were reported, one in each treatment group and neither deemed related to study vaccine.

In the VAC-017 infant cohort, the most common TEAEs reported in both treatment groups through 12 weeks post final primary vaccination included upper respiratory tract infection (64.0% PNEUMOSIL, 48.0% Prevenar 13), tinea infection (31.0% PNEUMOSIL; 21.0% Prevenar 13), diarrhea (29.0% PNEUMOSIL; 19.0% Prevenar 13), and conjunctivitis (27.0% PNEUMOSIL; 19.0% Prevenar 13). The most common TEAE after the booster vaccination through 4 weeks of follow up was upper respiratory tract infection (28.6% PNEUMOSIL, 12.8% Prevenar 13), dermatitis (8.2% PNEUMOSIL, 6.4% Prevenar 13), and diarrhea (4.1% PNEUMOSIL, 10.6% Prevenar 13). The differences in the frequency of these TEAEs between the treatment groups were within the expected range for this early-stage clinical study. The only vaccine-related TEAE for more than 1 infant was Grade 1 vaccination site swelling (2.0% PNEUMOSIL, 6% Prevenar 13), and the only severe TEAE reported for > 1 infant was bronchiolitis (2% PNEUMOSIL, 0% Prevenar 13).

Six (6.0%) infants in the PNEUMOSIL group and 2 (2.0%) infants in the Prevenar 13 group had a treatment-emergent SAE through 12 weeks post primary vaccination, and one (2.0%) infant in the PNEUMOSIL group had a treatment-emergent SAE after the booster vaccination through 4 weeks of follow up. There was an imbalance in serious cases of bronchiolitis (4 in the PNEUMOSIL group, 1 in the Prevenar 13 group) but no imbalance in overall TEAEs of bronchiolitis. No treatment-emergent SAE was considered to be related to study vaccine, and there was no temporal relationship to any vaccination.

1.8.2. Immunogenicity

In the VAC-017 study, serum samples were collected 28 days after vaccination in the adult and toddler cohorts, and 28 days after completion of the primary vaccination series and prior to and 28 days post booster vaccination in the infant cohort, for evaluation by ELISA to determine the IgG concentration to each of the 10 serotypes contained in PNEUMOSIL. In the case of toddlers, a random subset was selected for this analysis (n=34), and this subset of toddlers also had serotype-specific IgGs determined from sera collected at baseline. In the infant cohort, the IgG concentration was determined for each component of the co-administered pentavalent vaccine (DTwP-HepB-Hib). The functional activity of the IgG response to the 10 serotypes contained in PNEUMOSIL was also determined in randomly selected subsets of infants (n=40) and toddlers (n=34), and in all adults using

the same serum samples collected 28 days after vaccination (only after primary vaccination in the case of infants).

PNEUMOSIL was shown to be immunogenic for all 10 serotypes contained in the vaccine in all 3 age cohorts, and, in toddlers and infants, the level of responses to each serotype was comparable to the responses observed in the Prevenar 13 control group. The percentage of infants achieving an IgG concentration of 0.35 µg/mL (the reference concentration for assessment of vaccine efficacy against IPD defined by the WHO[23]) at 28 days post final primary series vaccination was substantial across all serotypes in both treatment groups, with seroresponse rates of 91% or higher achieved in all cases except for serotypes 6A (79.0%) and 6B (89.0%) in the PNEUMOSIL group (Table 5). While they were generally higher in the Prevenar 13 group, IgG GMCs were > 1 µg/mL for all 10 serotypes in both treatment groups.

Table 5. Percentage of IgG Seroresponders in the Infant Cohort – Primary Series

	PNEUMOSIL (N = 100)		Prevenar 13 (N = 100)		PNEUMOSIL vs Prevenar 13
Serotype	n (%)[a]	90% CI[b]	n (%)[a]	90% CI[b]	Difference (90% CI)[c]
IgG ELISA type 1	99 (99.0)	95.34 - 99.95	100 (100)	97.05 - 100.00	-1.0 (-5.13- 2.66)
IgG ELISA type 5	100 (100)	97.05 - 100.00	97 (97.0)	92.43 - 99.18	3.0 (-1.10- 7.95)
IgG ELISA type 6A	79 (79.0)	71.19 - 85.48	91 (91.0)	84.82 - 95.22	-12.0 (-20.94- -2.97)
IgG ELISA type 6B	89 (89.0)	82.45 - 93.71	93 (96.9)	92.12 - 99.14	-7.9 (-15.00- -1.01)
IgG ELISA type 7F	97 (97.0)	92.43 - 99.18	100 (100)	97.05 - 100.00	-3.0 (-7.95- 1.10)
IgG ELISA type 9V	94 (94.0)	88.50 - 97.36	97 (97.0)	92.43 - 99.18	-3.0 (-9.17- 2.90)
IgG ELISA type 14	98 (98.0)	93.84 - 99.64	96 (97.0)	92.35 - 99.17	1.0 (-4.00- 6.27)
IgG ELISA type 19A	92 (92.0)	86.03 - 95.96	94 (97.9)	93.59 - 99.63	-5.9 (-12.38- 0.17)
IgG ELISA type 19F	99 (99.0)	95.34 - 99.95	97 (99.0)	95.25 - 99.95	0.0 (-4.22- 4.33)
IgG ELISA type 23F	91 (91.0)	84.82 - 95.22	97 (97.0)	92.43 - 99.18	-6.0 (-12.77- 0.40)

Abbreviations: CI = confidence interval, ELISA = enzyme-linked immunosorbent assay, IgG = immunoglobulin G.

[a] Number of responders (IgG ≥ 0.35 µg/mL).

[b] Exact CIs based on Clopper-Pearson method.

c Exact CIs around treatment group differences were calculated based on Newcombe score.

Source: VAC-017 CSR Table I12.

Similarly, while the functional immune response to multiple serotypes (types 6A, 7F, 9V, and 19A) was superior for the Prevenar 13 group as measured by OPA GMTS (and as illustrated in divergent reverse cumulative distribution [RCD] curves), the percentage of infants with a functional IgG response (OPA titer ≥ 1:8) was substantial across all serotypes for both treatment groups -- and was numerically higher for the PNEUMOSIL group for more serotypes (4 vs 1) (Table 6). The small number of subjects with OPA data in all 3 cohorts limits what can be meaningfully concluded from these analyses.

Table 6. Percentage of OPA Seroresponders in the Infant Cohort – Primary Series

	PNEUMOSIL (N = 20)		Prevenar 13 (N = 20)		PNEUMOSIL vs Prevenar 13
Seroresponders on OPA Titers	n (%)[a]	90% CI[b]	n (%)[a]	90% CI[b]	Difference (90% CI)[c]
OPA - Pn 1	15 (93.8)	73.60 - 99.68	11 (84.6)	58.99 - 97.19	9.1 (-15.55 - 36.11)
OPA - Pn 5	19 (95.0)	78.39 - 99.74	19 (95.0)	78.39 - 99.74	0.0 (-18.56 - 18.56)
OPA - Pn 6A	20 (100)	86.09 - 100.00	19 (100)	85.41 - 100.00	
OPA - 6B	19 (100)	85.41 - 100.00	19 (95.0)	78.39 - 99.74	5.0 (-12.35 - 22.97)
OPA - 7F	20 (100)	86.09 - 100.00	20 (100)	86.09 - 100.00	
OPA - 9V	20 (100)	86.09 - 100.00	20 (100)	86.09 - 100.00	
OPA - 14	19 (100)	85.41 - 100.00	18 (94.7)	77.36 - 99.73	5.3 (-12.15 - 24.01)
OPA - 19A	16 (94.1)	74.99 - 99.70	20 (100)	86.09 - 100.00	-5.9 (-26.41 - 11.01)
OPA - 19F	20 (100)	86.09 - 100.00	19 (95.0)	78.39 - 99.74	5.0 (-11.64 - 22.97)
OPA - 23F	20 (100)	86.09 - 100.00	20 (100)	86.09 - 100.00	

Abbreviations: CI = confidence interval, OPA = opsonophagocytic assay.

[a] Number of responders (OPA titers ≥ 1:8).

[b] Exact CIs based on Clopper-Pearson method.

c Exact CIs around treatment group differences were calculated based on Newcombe score. Calculation of difference and CI around the difference was not possible when all subjects in both groups were responders.

Source: VAC-017 CSR Table II5.

IgG GMCs decreased substantially from 4 weeks post Vaccination 3 to just prior to the booster vaccination in both the PNEUMOSIL and Prevenar 13 groups. While GMCs were generally higher in the Prevenar 13 group at 4 weeks post Vaccination 3, pre booster vaccination GMCs were generally comparable between groups due to the more substantial reduction in IgG concentrations in the Prevenar 13 group for serotypes that were significantly higher in the Prevenar 13 group post primary series. Both PNEUMOSIL and Prevenar 13 demonstrated a substantial booster effect across serotypes, indicating that the initial 3-dose series of both vaccines effectively 'primed' infants for boosting of immune responses.

In the toddler cohort, there was a substantial booster response for all serotypes in both treatment groups. No meaningful conclusions can be drawn from the generally higher Geometric Mean Fold Rise (GMFR) response after boosting with Prevenar 13 given the small number of boosted subjects, higher baseline GMCs in the PNEUMOSIL group, and the potential immunologic advantage of boosting with the homologous vaccine (Prevenar 13) that was used for priming. Additionally, OPA GMTs were similar in toddlers boosted with Prevenar 13, and there was no divergence in RCD curves of OPA titers in favor of Prevenar 13. Results of the supplemental infant booster phase of the VAC-017 study will be more informative, given that a booster dose of PNEUMOSIL will be evaluated in PNEUMOSIL-primed infants (in comparison to a booster dose of Prevenar 13 in Prevenar 13-primed infants).

In the adult cohort, the IgG GMC and functional (OPA GMT) immune responses were generally similar 4 weeks after administration of a single dose of PNEUMOSIL or Pneumovax 23. The consistent exceptions were serotypes 6A and 6B in favor of PNEUMOSIL and serotype 1 in favor of Pneumovax 23. The small number of adults evaluated, and expected high levels of naturally acquired immunity, limit what can be meaningfully concluded from these analyses.

Based on review of seroprotection rates and GMCs to component antigens (as well as RCD curves of IgG levels), there is no evidence from this early-stage study that administration of PNEUMOSIL interferes with the immune response to any component of the pentavalent vaccine when these vaccines are given concomitantly as a 3-dose primary series to infants.

1.9. Clinical Development Plan for PNEUMOSIL

The ultimate goal of PNEUMOSIL clinical development is to achieve licensure through a WHO-recognized national regulatory authority (NRA), followed by prequalification by WHO to support product acquisition by Gavi and UNICEF for its distribution to low- and middle-resource countries. The product specifications detailed in Part C of the WHO Technical Report Series (TRS) 977 Annex 3 (2013)[23] and the associated Target Product Profile (TPP) for the Advance Market Commitment (AMC) for Pneumococcal Conjugate Vaccines (2008)[24] – which establishes additional essential criteria for the AMC for PCVs – are a critical guide for the PNEUMOSIL clinical development plan, to ensure that planned trials serve to evaluate the vaccine on the basis of the essential attributes for a PCV deemed suitable for use in Gavi-eligible countries. As was the case for the second-generation PCVs, the path to WHO prequalification and Gavi eligibility for PNEUMOSIL is to conduct a Phase 3 pivotal trial in infants to demonstrate the following: 1) vaccine efficacy based on immunologic non-inferiority to a licensed and prequalified comparator vaccine post a 3-dose primary series, 2) manufacturing quality demonstrated by post-primary lot-to-lot consistency, 3) non-interference with co-administered EPI vaccines, 4) immunologic memory as indicated by boostability, and 5) an adequate safety and tolerability profile after primary series and booster vaccination. An additional TPP requirement is that the first dose of the vaccine must be shown to be administrable at 6 weeks of life or earlier.

The current Phase 3 non-inferiority trial of PNEUMOSIL in healthy infants in The Gambia (n = 2,250) is designed to provide the data necessary to demonstrate each of these critical attributes of a licensed and prequalified PCV. Conducting such a large Phase 3 trial in infants is warranted based on the observed safety, tolerability, and immunogenicity results of VAC-017. Phase 3 trials will also be conducted in India to demonstrate the safety and immunogenicity of PNEUMOSIL administered at 6 weeks, 10 weeks and 14 weeks of age, as well as at 6 weeks, 14 weeks and 9 months of age.

1.10. Study Rationale

Given that vaccine efficacy will be demonstrated based on immunologic non-inferiority (NI), a key element in the design of this Phase 3 pivotal trial is the choice of licensed PCV comparator. After much consideration Synflorix has been selected as the active comparator for the trial. Since its initial licensure and WHO prequalification in 2009, Synflorix has been introduced in 30 countries worldwide -- including 13 Gavi-eligible countries through the AMC framework --, and has been given to more than 70 million children.[25] The safety profile of Synflorix that led to licensure and prequalification was established in clinical trials in which 63,905 doses of the vaccine were administered to 22,429 healthy children as primary vaccination, and 19,466 doses were administered as a booster dose in the second year of life.[26]

One of the key factors for selecting Synflorix as the comparator in this Phase 3 pivotal trial is that Synflorix is the only licensed and WHO-prequalified 2nd generation PCV that has been evaluated in randomized controlled trials for both efficacy and effectiveness against IPD and pneumonia in infants and children. In a large (n= 47,366) Phase 3/4, double-blind, cluster-randomized, controlled, clinical trial conducted in Finland (FinIP), effectiveness of Synflorix against culture-confirmed

vaccine-type IPD in both 3+1 (3, 4, 5 + 11 mo) and 2+1 (3, 5 + 11 mo) schedules was 100% (95% CI: 91-100; 0 vs 11 cases) and was 93.0% (95% CI: 75-99; 2 vs 14 cases) for culture-confirmed IPD irrespective of serotype.[27] Synflorix also was shown in the FinIP trial to be effective in reducing pneumonia rates: rates of hospital-diagnosed pneumonia were reduced by 26.7% (95% CI: 4.9; 43.5) and 29.3% (95% CI: 7.5; 46.3) in the 3+1 and 2+1 clusters, respectively.[25] In a large (n=23,821) Phase 3, randomized, double-blind clinical trial conducted in Argentina, Panama and Colombia (COMPAS), vaccine efficacy against culture-confirmed IPD was 66.7% (95% CI: 21.8-85.9; 7 vs 21 cases), and efficacy against likely bacterial community-acquired pneumonia was 22.0% (95% CI: 7.7, 34.2) in infants who received Synflorix in a 3+1 schedule (2, 4, 6 mo + booster at 15-18 mo).[28] Given these robust results from randomized controlled studies of Synflorix, determining that PNEUMOSIL is immunologically non-inferior to Synflorix will provide an indication of the expected efficacy of PNEUMOSIL against IPD and pneumonia.

It is important to note that Synflorix has been shown to be effective against IPD and pneumonia despite evidence that serotype-specific IgG antibody responses induced by the vaccine are generally lower than those induced by Prevenar 13 when measured 1 month after a 3-dose primary series – the timepoint specified in the TRS guidelines (Section C.2.2.3) for the primary NI analysis.[29,30] The fact that Synflorix has proven that a PCV can be highly effective while inducing somewhat lower post-primary antibody responses compared with Prevenar 13 is an important consideration in the selection of Synflorix as comparator in this Phase 3 pivotal trial.

Synflorix and PNEUMOSIL have 8 serotypes in common: serotypes 1, 5, 6B, 7F, 9V, 14, 19F, and 23F. Serotypes 6A and 19A are contained in PNEUMOSIL, but not in Synflorix. In accordance with TRS guidelines (Section C.2.2.3), non-inferiority of the immune response after primary vaccination with PNEUMOSIL will be demonstrated for any of the 8 matched serotypes if the difference in the percentage of subjects with serotype-specific IgG concentration ≥ 0.35 µg/mL (percentage responding after Synflorix vaccination minus percentage responding after PNEUMOSIL) is less than the standard[†] NI margin of 10%, or if the serotype-specific IgG GMC ratio (Synflorix GMC divided by PNEUMOSIL GMC) is less than 2 (the same standard 2-fold criterion for comparing serotype-specific IgG GMC ratios will be the basis for demonstrating equivalence of the 3 productions lots of PNEUMOSIL). Again in accordance with TRS guidance, the approach for serotypes 6A and 19A will be to assume that the response rate with Synflorix is the lowest observed rate among the 10 serotypes in Synflorix, and the GMC is the GMC of the serotype with the lowest response rate. Since the TRS guidance does not require non-inferiority to be met for each of the serotypes in the candidate vaccine, it is prespecified that the primary NI objective will be met if at least 7 of the 10 serotypes in PNEUMOSIL are shown to be non-inferior to Synflorix.

Of note, a secondary immunogenicity objective of this study is to demonstrate that the post-primary immune responses to serotypes 6A and 19A in PNEUMOSIL are superior to the responses to these serotypes induced by Synflorix, based on either the proportion with IgG antibody concentration ≥ 0.35 µg/mL or GMC ratio. Due to cross-reactivity from serotypes 6B and 19F, immune responses to 6A and 19A induced by Synflorix are not only measurable, but have been shown to provide statistically significant cross-protection in surveillance studies of vaccine effectiveness for individual serotypes against IPD (also, Synflorix is indicated for protection against serotype 19A disease in Europe, Canada and other countries).[31,32,33] Demonstrating superiority to the immune responses to these serotypes induced by Synflorix – especially superiority based on a margin of

[†] A NI margin of 10% was used in the pivotal Phase 3 trials of both Prevenar 13 and Synflorix for comparison of seroresponse rates.[29, 30]

>10% higher seroresponse rate or >2-fold GMC ratio – will indicate expected effectiveness of PNEUMOSIL against IPD due to serotypes 6A and 19A.

It is also important to emphasize that surveillance data from the Basse Health and Demographic Surveillance System (BHDSS) in the Upper River Region of The Gambia provide reassurance that this Phase 3 trial, in which infants will receive PNEUMOSIL or Synflorix, instead of Prevenar 13, can be safely conducted in the country. Because of the early and successful introduction of 7-valent Prevenar and then Prevenar 13 in the national EPI schedule, the incidence of IPD due to serotypes included in Prevenar 13, and prevalence of nasopharyngeal carriage of vaccine-type pneumococci, have decreased significantly in Gambian children since PCV introduction.[11,31] In fact, since 2013 the BHDSS has not detected a single case of IPD in children aged 2-59 months that was due to a serotype contained in Prevenar 13 but not PNEUMOSIL (serotypes 3, 4, and 18C) (G. Mackenzie, personal communication). These data, together with BHDSS surveillance data showing numerical reduction in serotype-specific IPD in adults,[11] suggest robust indirect (herd) protection against Prevenar 13 vaccine-type disease in The Gambia.

Given the TPP requirement regarding dosage schedule ('The first dose must be shown to be administrable at 6 weeks of life or earlier'), study vaccine (PNEUMOSIL or Synflorix), and all other EPI vaccines normally administered at 2, 3, and 4 months of age in The Gambia (DTwP-HepB-Hib, OPV, RV and IPV[‡]), will be administered to infants enrolled in this Phase 3 trial at 6, 10, and 14 weeks of age. This schedule is approved by the WHO and is common in EPI programs.[22,34] Administering these infant EPI vaccines at this earlier schedule as part of the clinical trial ensures that all subjects are fully vaccinated, and avoids complications of missed vaccinations, and over-vaccination in the case of PCV. It is also required in order to evaluate whether concomitant administration of PNEUMOSIL interferes with the immune responses to these EPI vaccines. For the same reason, the booster dose of study vaccine will be administered at 9 months of age, to be aligned with the EPI schedule (see the Gambian EPI schedule in

Table 7). Seroprotection rates for DTwP-HepB-Hib and polio vaccine (type 1 and 3) after the 3-dose primary series, and for measles-rubella and yellow fever vaccines after the booster dose, will be measured in a subset of infants enrolled in the trial to demonstrate that the immune responses induced by these EPI vaccines are not inferior to those induced by these vaccines when co-administered with Synflorix. If, after completion of the trial, the immune response proves to not be comparable for an EPI vaccine when co-administered with PNEUMOSIL, infants who failed to seroconvert will be offered a booster dose of the vaccine.

Table 7. Gambian EPI Schedule for Children 0 to 18 Months Old[§]

Age at Immunization	Antigen
At birth	BCG, OPV, HepB
2 months	OPV, DTwP-HepB-Hib, PCV, RV
3 months	OPV, DTwP-HepB-Hib, PCV, RV
4 months	OPV, DTwP-HepB-Hib, PCV, IPV*

9 months	OPV, measles-rubella, yellow fever
18 months	OPV, measles-rubella, DTwP, Vitamin A

Abbreviation: BCG, Bacillus Calmette–Guérin;
*Only a single dose of IPV is given alongside the third infant priming vaccines in line with the national EPI program recommendations.

* Should any changes to the EPI schedule in the Gambia occur before or during the study the vaccines given alongside the study vaccines will be modified accordingly to reflect the current programme unless this is considered to interfere with the assessment of the study endpoints in any way.

2. HYPOTHESES, OBJECTIVES AND ENDPOINTS

2.1. Study Hypotheses

2.1.1. Primary Hypotheses:

Immunogenicity:

- The immune responses to the 10 serotypes in PNEUMOSIL (1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, 23F) induced by 3 different lots of PNEUMOSIL will be equivalent after a 3-dose primary series.
- The immune responses to at least 7 of the 10 serotypes in PNEUMOSIL will be non-inferior to the immune responses induced by the matched serotype (for 1, 5, 6B, 7F, 9V, 14, 19F, 23F) or serotype with the lowest seroresponse rate (for 6A, 19A) in Synflorix, after a 3-dose primary series.
- The immune responses induced by pentavalent, polio and rotavirus vaccines co-administered with PNEUMOSIL during a 3-dose primary series will be non-inferior to the immune responses observed when these vaccines are co-administered with Synflorix.

Safety, Tolerability:

- PNEUMOSIL administered as a 3-dose primary series, and co-administered with routine pediatric vaccines, will be safe and well tolerated.
- PNEUMOSIL administered as a booster dose to primed infants at 9 months of age will be safe and well tolerated when co-administered with routine pediatric vaccines.

2.1.2. Secondary Hypotheses:

Immunogenicity:

- The immune responses induced by PNEUMOSIL for serotypes 6A and 19A will be superior to the cross-reactive responses to these serotypes induced by Synflorix after a 3-dose primary series.
- PNEUMOSIL will induce a measurable booster response to each of the 10 serotypes when administered as a 4th dose at 9 months of age.
- The immune responses induced by measles-rubella and yellow fever vaccines administered at 9 months of age concomitantly with a PNEUMOSIL booster dose will be non-inferior to those observed when the vaccines are co-administered with a Synflorix booster dose.

2.2. Study Objectives

2.2.1. Primary Objectives:

Immunogenicity:

1. To demonstrate that the immune responses to the 10 pneumococcal serotypes in PNEUMOSIL (1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, 23F) induced by 3 different lots of PNEUMOSIL are equivalent when measured 4 weeks after a 3-dose primary series
2. To demonstrate non-inferior immune responses for at least 7 of the 10 serotypes in PNEUMOSIL in comparison to matched serotypes (for 1, 5, 6B, 7F, 9V, 14, 19F, 23F) or the lowest responder (for 6A, 19A) in Synflorix based on (a) % IgG response ≥ 0.35 μg/mL or (b) IgG GMCs measured 4 weeks after a 3-dose primary series
3. To demonstrate that the immune responses induced by routine pediatric vaccines (pentavalent, polio and rotavirus) when co-administered with a 3-dose primary series of PNEUMOSIL are non-inferior to those induced by these vaccines when co-administered with Synflorix (subset of subjects)

Safety, Tolerability:

1. To demonstrate an acceptable safety and tolerability profile for PNEUMOSIL administered as a 3-dose primary series and booster dose, and when co-administered with routine pediatric vaccines through 4 weeks after a booster dose (subset of subjects for tolerability)

2.2.2. Secondary Objectives:

Immunogenicity:

1. To demonstrate that the immune responses to serotypes 6A and 19A in PNEUMOSIL are superior to the cross-reactive responses to these serotypes induced by Synflorix based on (a) % IgG response ≥ 0.35 μg/mL or (b) IgG GMCs measured 4 weeks after a 3-dose primary series
2. To evaluate the functional serotype-specific antibody responses induced by PNEUMOSIL in comparison to Synflorix, as measured by OPA at 4 weeks post 3-dose primary series (subset of subjects)
3. To evaluate the booster responses (antibody concentrations and functional responses) to PNEUMOSIL in comparison to Synflorix, from 4 weeks after a 3-dose primary series to 4 weeks after a booster dose (subsets of subjects)
4. To demonstrate that the immune responses induced by measles-rubella and yellow fever vaccines when co-administered with a booster dose of PNEUMOSIL are non-inferior to those induced by these vaccines when co-administered with a booster dose of Synflorix (subset of subjects)

2.2.3. Supplemental Objectives:

Immunogenicity:

1. To evaluate the persistence of immune responses (antibody concentrations and functional responses) induced by PNEUMOSIL in comparison to Synflorix, 1 year after administration of a booster dose (subset of subjects)

Safety:

1. To assess the safety of a 3-dose primary series and booster dose of PNEUMOSIL co-administered with routine pediatric vaccines in regards to serious adverse events occurring 4 weeks after the booster dose through 12 months after the booster dose (subset of subjects)

2.3. Study Endpoints

2.3.1. Primary Endpoints:

Immunogenicity:

For Primary Objective 1 (lot consistency):

- Serotype-specific immunoglobulin G (IgG) geometric mean concentration (GMC) measured 4 weeks post dose 3

For Primary Objective 2 (non-inferiority):

- Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 4 weeks post dose 3
- Serotype-specific IgG GMC measured 4 weeks post dose 3

For Primary Objective 3 (non-interference):

- Percentage of subjects with anti-diphtheria toxoid IgG concentration ≥ 0.1 IU/mL measured 4 weeks post dose 3
- Percentage of subjects with anti-tetanus toxoid IgG concentration ≥ 0.1 IU/mL measured 4 weeks post dose 3
- Percentage of subjects with anti-Hepatitis B surface antigen (HBsAg) IgG concentration ≥ 10 mIU/mL measured 4 weeks post dose 3
- Percentage of subjects with anti-*Haemophilus influenzae* type b (PRP) IgG concentration ≥ 0.15 µg/mL measured 4 weeks post dose 3
- Anti-pertussis toxoid and fimbriae IgG GMCs measured 4 weeks post dose 3
- Percentage of subjects with anti-poliovirus types 1, 2 and 3 neutralizing antibody titers ≥ 1:8 measured 4 weeks post dose 3
- Percentage of subjects with anti-rotavirus IgA concentration ≥ 20 U/mL measured 4 weeks post dose 3

Safety, Tolerability:

- Number and severity of solicited local and systemic adverse events (reactogenicity events [REs]) through Day 6 post each vaccination
- Number, severity and relatedness of all AEs and serious adverse events (SAEs) during the entire study period through 4 weeks post last dose for the cohort

2.3.2. Secondary Endpoints:

Immunogenicity:

For Secondary Objective 1 (superiority):

- Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 4 weeks post dose 3
- Serotype-specific IgG GMC measured 4 weeks post dose 3

Secondary Objective 2 (functional response):

- Percentage of subjects with OPA titer ≥ 1:8 measured 4 weeks post dose 3
- OPA geometric mean titer (GMT) measured 4 weeks post dose 3

Secondary Objective 3 (boostability):

- Ratio of IgG GMCs measured 4 weeks post dose 4 to IgG GMCs measured 4 weeks post dose 3
- Ratio of OPA GMTs measured 4 weeks post dose 4 to OPA GMTs measured 4 weeks post dose 3

Secondary Objective 4 (non-interference):

- Percentage of subjects with anti-measles IgG concentration ≥ 150 mIU/mL measured 4 weeks post dose 4
- Percentage of subjects with anti-yellow fever neutralizing antibody titers ≥ 1:8 measured 4 weeks post dose 4
- Percentage of subjects with anti-rubella IgG concentration ≥ 4 IU/mL measured 4 weeks post dose 4

2.3.3. Supplemental Endpoints:

Immunogenicity:

For Supplemental Objective 1 (immune persistence):

- Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 1 year post dose 4
- Serotype-specific IgG GMC measured 1 year post dose 4
- Percentage of subjects with OPA titer ≥ 1:8 measured 1 year post dose 4
- OPA geometric mean titer (GMT) measured 1 year post dose 4

For Supplemental Objective 2 (Safety):

- Number, severity and relatedness of all serious adverse events (SAEs) 4 weeks after the booster dose through 12 months after the booster dose (subset of subjects)

3. STUDY DESIGN

This is a prospective, single center, randomized, active-controlled, double-blind, Phase 3 study in healthy Gambian PCV-naïve infants (6 to 8 weeks). The study will be conducted in 3 phases: an initial priming phase, in which all (n=2,250) eligible subjects will participate, and a second booster phase, in which only the first 675 randomized subjects will participate. In the third phase out of the 675 booster subjects, additionally consented subjects will participate to evaluate immune persistence. The study schema is presented in Table 8.

Screening: After parental informed consent is obtained, prospective subjects will be considered enrolled in the trial, and will be screened to determine eligibility. While informed consent may occur as early as 4 weeks of age, the screening window will be 6 to 8 weeks of age. Randomization will take

place only after a subject has satisfied all eligibility criteria, including confirmation of 1) no acute illness that precludes vaccination; 2) a negative rapid diagnostic test (RDT) for malaria; and 3) normal vital signs. These same criteria will need to be satisfied prior to administration of any subsequent vaccination.

Priming Phase: Subjects (n=2,250) deemed eligible to participate in the study by the Principal Investigator (PI) will be randomized in a 2:2:2:3 ratio based on a pre-established randomization scheme, to receive the first dose of either PNEUMOSIL (3 groups receiving vaccine from different lots) or Synflorix (1 group) at 6-8 weeks of age (V1). Treatment assignment will be stratified by field site. Subsequent primary vaccination visits will take place at 4 (+2) weeks after the previous vaccination.

Table 8. Study Schema

Groups	Priming Phase					Booster Phase			Immune Persistence Phase
	N	Visits*				N	Visits*		Visit*
		V1	V2	V3	V4		V5	V6	V7
		6-8 w	V1+4(+2)w	V2+4(+2)w	V3+4(+2)w		9-10 m	V5 +4(+2)w	V5+12(+1)m
PNEUMOSIL Lot 1	500	X	X	X	B	150[#]	X	B	B
PNEUMOSIL Lot 2	500	X	X	X	B	150[#]	X	B	B
PNEUMOSIL Lot 3	500	X	X	X	B	150[#]	X	B	B
Synflorix	750	X	X	X	B	225[#]	X	B	B

w = weeks; m = months

X = vaccination (+ EPI vaccines); B = blood sample for immunogenicity testing

*Age ranges indicated for V1/V5. Subsequent vaccinations/follow up visits at 4 weeks post prior visit + 2 week window except for V7, which will be at 12 months post V5 + 1 month window.

[#]The total number of subjects assessed for immune persistence at V7 will depend on number of subjects whose parent provides additional informed consent.

(V2, V3). Standard EPI vaccinations based on the Gambian EPI schedule (See Table 7) will be given concomitantly with all 3 doses of the study vaccine. A follow-up visit (V4) will take place at 4 (+2) weeks after the third vaccination visit, during which blood will be collected for immunological assessments. This visit will serve as the end-of-study (EOS) visit for subjects not included among the 675 subjects to be randomized (n=1,575).

Infants who are only enrolled in the priming phase of the study will be offered a booster dose of Prevenar 13 outside the study, at 9 to 12 months to ensure all recruited infants gain maximal long term pneumococcal protection. Prevenar 13 is used as the vaccine routinely in the EPI schedule in The Gambia.

Booster Phase: The first 675 randomized subjects will continue on study and be asked to return to clinic at 9 (+1) months of age for a booster vaccination of study vaccine that matches the original treatment assignment (V5). Standard EPI vaccinations based on the Gambian EPI schedule (measles-rubella, yellow fever vaccine, OPV) will be co-administered with the booster dose of study vaccine.

Subjects will be evaluated at a follow-up visit 4 (+2) weeks later (V6), during which a blood sample will be collected for immunological assessments. This visit will serve as the EOS visit for boosted subjects whose parent does not provide consent to continue on study for assessment of immune persistence 1 year after the booster vaccination (V7). Initial database closure and unblinding of only the Sponsor, statistical personnel and medical monitor will occur after the last subject in the booster cohort completes V6 in order to analyze and report primary and secondary endpoints. Those subjects (maximum n=675) whose parent provides additional consent to participation will return for a final visit (EOS, V7) at 12 (+1) months after the booster vaccination, during which blood will be collected for assessment of immune persistence.

Safety Monitoring: Planned safety assessments will provide the data for active monitoring of vaccine safety during conduct of the trial through 4 weeks post the final (booster) vaccination, and for the primary safety and tolerability endpoints. For subjects who are included in the third phase for immune persistence evaluation, serious adverse events will be monitored from 4 weeks post the booster vaccination to 12 months after booster vaccination.

Immediate solicited reactogenicity and vital signs will be assessed at 30 (+/- 10) minutes following vaccination in all subjects. Severity of solicited reactions will be assessed by toxicity grading scale (see Section 9.2.2). The solicited local reactions assessed will include tenderness, erythema/redness, and induration/swelling at the study vaccine injection site. The solicited systemic reactions will include cutaneous rash, fever (based on axillary temperature), irritability, drowsiness, and decreased appetite.

Half of the subjects assigned to each treatment group (n = 1,125 subjects total) will be randomly selected to be included in the "primary reactogenicity cohort." These subjects will be monitored daily at home by field workers for assessment of local and systemic reactogenicity during the 6 days after each primary series vaccination. In addition, all 675 infants who receive the booster vaccination ("booster cohort") will be monitored daily at home by field workers during the 6 days after the booster vaccination. Reactogenicity scoring will be reviewed by a research clinician (RC) prior to being entered into the electronic data capture (EDC) system.

Subjects who are enrolled in the priming phase of the study will be monitored for adverse events (AEs) at each clinic visit until the End-of-Study (EOS) visit. Subjects in the booster cohort will be monitored for AEs at each clinic visit through the follow-up visit at 4 weeks post the booster vaccination (V6). Additionally, for subjects who are included in the third phase for immune persistence evaluation, serious adverse events will be monitored from 4 weeks post the booster vaccination to 12 months after booster vaccination.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) (version 19.1 or later) and assessed by the PI with regards to severity, relatedness, and duration. Any serious adverse event (SAE) ongoing at the time of the subject's EOS visit, will be attempted to be followed until it is resolved, assessed as resolved with sequelae by the PI, or until last subject last visit (LSLV) in the trial (ie last subject completes V7). Any SAE deemed related to study vaccine that is ongoing at the time of LSLV will continue to be followed until it is resolved, assessed to be resolved with sequelae, or assessed to be stable/chronic. SAEs deemed not related to study vaccine that are unresolved at the time of LSLV will be classified as ongoing for the purposes of data lock but will continue to be followed by the investigator team or referred on if appropriate according to good practice in The Gambia.

To facilitate rigorous safety monitoring, data captured on case report forms (CRFs) at each visit will be entered into the EDC system within 3 business days from the date of the clinic visit. Home visit

data (Day 1-6 following vaccination) will be entered in the EDC system within 3 days of the Day 6 home visit.

The Protocol Safety Review Team (PSRT), including the PI, RCs, PATH Study Director, and CRO staff, will review blinded safety data and clinical trial conduct weekly throughout the trial, until the last subject completes V6. During this time, data will be aggregated for routine review by the PSRT. In addition, an independent Data Safety Monitoring Board (DSMB) will monitor conduct of the trial and vaccine safety; this will include a formal, unblinded review of all safety data accrued during the trial when approximately one-quarter of the infants in the primary reactogenicity cohort have received their first vaccination. The need for additional meetings to review unblinded safety data will be determined by the DSMB following this initial review or may be specifically requested by the PSRT.

Immunogenicity Testing: Serum samples collected 4 weeks after completion of the 3-dose primary series (V4), 4 weeks after the booster dose (V6) and 1 year after the booster dose (V7) will be analyzed by ELISA to determine the IgG concentration and seroresponse rates (≥0.35 µg/mL) to each of the 10 serotypes included in PNEUMOSIL. Comparisons for primary objectives 1 (lot-consistency) and 2 (non-inferiority), secondary objectives 1 (superiority) and 3 (boostability) and supplemental objective 1 (immune persistence) will be based on these endpoints. Serum samples collected at Visits 4, 6 and 7 will also be analyzed by OPA to determine the functional immune response to the 10 serotypes included in PNEUMOSIL. Serotype-specific OPA titers and seroresponse rates are secondary immunogenicity endpoints for objectives 2 (functional response) and 3 (boostability) and supplemental immunogenicity endpoints for objective 1 (immune persistence).

Serum collected 4 weeks after the primary series (V4) and after the booster dose (V6) will also be analyzed by ELISA and by neutralization assays (for poliovirus types 1, 2 and 3 and yellow fever) to determine immune responses to antigenic components of co-administered EPI vaccines. Seroprotection rates and GMCs calculated on the basis of these data are primary and secondary endpoints for assessment of primary objective 3 and secondary objective 4 (non-interference). Should these analyses post database lock reveal evidence of PNEUMOSIL interference to an EPI vaccine (i.e. failure to demonstrate non-inferiority to the immune response to the vaccine when co-administered with Synflorix), the PI will offer a booster dose to subjects who failed to seroconvert to the EPI vaccine.

4. STUDY POPULATION

4.1. Description of Study Population

The study population will consist of healthy, Gambian male and female, PCV-naïve infants from 6 up to 8 weeks of age, to be recruited, screened, and qualified by the site staff (under the direction of the PI) at the MRC Unit The Gambia.

Since 1947, the MRC Unit The Gambia has been conducting medical research focused on infectious diseases of significance to people of The Gambia and other African countries, with the goal of reducing the burden of illness and death in the country and throughout the developing world. The MRC Unit, The Gambia has conducted seminal vaccine trials, in particular against *H. influenzae* type b and *S. pneumoniae*, that have resulted in important benefits to The Gambia as a result of early vaccine introduction and disease surveillance. The VAC-017 study was also conducted at the MRC Unit The Gambia.

Clinical vaccine trials at the MRC are conducted at field sites based within the compounds of government urban health centers or at other facilities within the vicinity of the respective health centers if space at the

health center itself is insufficient. These field sites are within a short distance of the main MRC administrative and laboratory site in Fajara, which includes a ward and clinical unit for subject inpatient treatment, as well as clinical laboratories. The present trial will be conducted at multiple field sites.

4.2. Inclusion Criteria

Prospective subjects will only be eligible for randomization if all of the following inclusion criteria, and none of the exclusion criteria, are met at the time of screening:

- They are healthy infants. Subjects are deemed healthy if, based on medical history and clinical assessment, they are determined to be without acute or chronic, clinically significant pulmonary, cardiovascular, hepatobiliary, gastrointestinal, renal, neurological, or hematological functional abnormality or illness that requires medical therapy.
- They are between 6 and 8 weeks (ie 42 to 56 days) old, inclusive. Subjects will be eligible from the day they reach 6 weeks until the day they reach 8 weeks only.
- Subject's parent must provide voluntary written/thumb-printed informed consent for the subject to participate in the study. As local languages in The Gambia are non-written, informed consent may be obtained from an English-illiterate parent but will require an English-literate impartial witness who is also fluent in the relevant local language (and who is not an employee of MRC) to be present for consenting and to co-sign the informed consent form (ICF) to confirm that the information in the ICF has been provided in full and that the subject's parent is consenting for their infant to take part in the trial having had any questions answered to the parent's apparent satisfaction.
- Subject's parent 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 must have been born full-term, have a weight-to-height Z score of $\geq$ -2 (WHO child growth standard), and be $\geq$ 3.5 kg at randomization.

  Note: Subjects with borderline z-score or weight at initial screening may be rescreened if within the age window.
- Subject's parents must have a readily identifiable place of residence in the study area, be available for the duration of trial participation, and have a means of telephone contact.

  Note: A telephone and/or telephone credit will be provided to subjects enrolled in the trial to ensure they are always able to contact a member of the field team in the case of illness/adverse event.

4.3. Exclusion Criteria

- Use of any investigational medicinal product prior to randomization or planned use of such a product during the period of study participation.
- Previous vaccination against *S. pneumoniae*.
- History of *S. pneumoniae* infection confirmed by culture from a normally sterile site.
- History of allergic disease or history of a serious reaction to any prior vaccination or known hypersensitivity to any component of the study vaccines. This includes such reactions in older siblings and also includes all components of the EPI vaccines.
- History of anaphylactic shock.

- Any abnormal (Grade ≥ 1) vital sign.

  Note: An abnormal vital sign, including fever (axillary temperature of ≥ 37.5°C), may be repeated to determine whether a subject is eligible for randomization. A minimum of 48 hours following a documented fever must pass before the subject can be reassessed for eligibility. The last vital sign measurement must be used as the baseline value for the study.

- Any moderate or severe (Grade ≥ 2) acute illness.

  Note: Infants with a Grade 1 acute illness may be enrolled at the discretion of the PI.

  Note: Subjects with moderate or severe acute illness may return for clinical re-assessment; if the illness has sufficiently resolved, they may still qualify for randomization.

- A positive RDT (or blood film) for malaria.

  Note: Subjects with a positive RDT may be retested post treatment. A RDT for malaria will be undertaken on the day of each vaccination to ensure a subject is not vaccinated with a concurrent malaria infection.

- History of administration of a non-study vaccine within 30 days prior to administration of study vaccine or during the course of study participation, other than EPI vaccinations, and any campaigns administered through The Gambian EPI office and Ministry of Health and Social Welfare.

  Note: BCG administered to subjects who did not receive BCG at birth must be given at least 7 days prior to study vaccine.

- Chronic administration (defined as more than 14 consecutive days) of immunosuppressant or other immune modifying drugs prior to the administration of the study vaccine, including the use of glucocorticoids. The use of topical and inhaled glucocorticoids will be permitted.

- Administration of immunoglobulins and/or any blood products or anticipation of such administration during the study period.

- History of known disturbance of coagulation or blood disorder that could cause anemia or excess bleeding (eg, thalassemia, coagulation factor deficiencies, severe anemia at birth). Any clearly documented history in a first-degree relative (eg, parent, sibling) of the same is also exclusionary.

- History of suspected primary immunodeficiency. Any clearly documented history in a first-degree relative of the same is also exclusionary.

- Subject 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.

- Evidence of a clinically significant congenital abnormality as judged by the PI.

- History of meningitis, seizures or any neurological disorder.

- Evidence by history taking alone of exposure to an HIV-positive individual through maternal fetal transmission, breast milk, or other blood-borne mechanisms.

- Subject is a direct descendant (child or grandchild) of any person employed by the Sponsor, the CRO, the PI, study site personnel, or site.

- Any medical or social condition that in the opinion of the PI may interfere with the study objectives, pose a risk to the subject, or prevent the subject from completing the study follow-up.

Note that specific exclusion criteria (eg, abnormal vital sign, acute illness, positive RDT) will be reassessed at all vaccination visits. Any subject who cannot be vaccinated due to an acute abnormality assessed at the 2nd or 3rd primary vaccination visit (V2 or V3), or at the booster vaccination visit (V5), may return once the acute issue has resolved. A minimum of 48 hours must have passed after a documented fever before a subject can be vaccinated. This safety requirement will not be deemed a protocol deviation should the visit fall outside the vaccination window; however, it will be encouraged to maintain the vaccination window whenever possible in these situations.

Note that there is no further screening once initial randomization has taken place. All subjects enrolled in the booster cohort will be eligible to continue into the cohort for immune persistence if informed consent is obtained.

5. STUDY PRODUCTS

5.1. PNEUMOSIL

5.1.1. Product Description

PNEUMOSIL consists of 10 individually fermented and purified pneumococcal polysaccharides that have been subsequently conjugated to CRM_{197}, a detoxified diphtheria toxin, using 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) conjugation chemistry.

One single 0.5mL dose of PNEUMOSIL contains 2µg of polysaccharide for serotypes 1, 5, 9V, 14, 19A, 19F, 23F, 7F and 6A, and 4µg for serotype 6B. It is formulated with aluminum phosphate (0.125mg Al^{3+} per dose) as an adjuvant in an appropriate buffer, and the multi-dose presentation used in this trial contains thiomersal (25µg per dose) as a preservative. The vaccine is a turbid white suspension.

5.1.2. Manufacturer

PNEUMOSIL is manufactured and supplied by SIIPL.

5.1.3. Presentation and Formulation

PNEUMOSIL will be supplied in a 5-dose (multi-dose) vial, in cartons containing 50 labeled vials and 1 product leaflet. Each vial label will include the following information: name of the medicinal product, composition, dose and fill volume, route of administration, lot number, manufacturing date, retest dates, storage condition, and a cautionary statement ("For Clinical Trial Use Only").

5.1.4. Storage

PNEUMOSIL is stored at between 2°C and 8°C. It must not be frozen.

5.1.5. Potential Safety Risks

As with any vaccine, severe allergic reaction is a potential rare event. Known hypersensitivity to any component of the vaccine (including diphtheria toxoid and CRM_{197}) is a contraindication to vaccination.

In the VAC-017 study, the most commonly reported solicited adverse reactions in infants (n = 100) after any of the 3 primary doses of PNEUMOSIL administered at 6, 10, and 14 weeks of age, and co-administered with DTwP-HepB-Hib vaccine, were irritability (69%), fever (53%), injection site tenderness (45%), injection site induration/swelling (21%), and decreased appetite (20%). Reported Grade 3 solicited adverse reactions in infants after any of the 3 primary doses of PNEUMOSIL were fever (1%), rash (1%) and irritability (1%).

The most commonly reported solicited adverse reactions in subjects from the infant cohort of the VAC-017 study (n = 49) after a booster dose of PNEUMOSIL administered at 10-13 months of age were injection site tenderness (18.3%), injection site induration/swelling (12.3%), fever (12.2%) and irritability (10.2%). The only reported Grade 3 solicited adverse reaction in these subjects was fever (4.1%; >39.0 – ≤40.0˚C).

The most commonly reported solicited adverse reactions in subjects from the toddler cohort (n = 56) in the VAC-017 study after a booster dose of PNEUMOSIL at 12-15 months of age were injection site tenderness (21.5%), fever (16.1%), decreased appetite (12.5%), drowsiness (10.7%), and injection site induration/swelling (10.7%). The only reported Grade 3 solicited adverse reaction in toddlers was fever (3.6%). Refer to Section 1.8.1 and to the IB for additional details on adverse reactions reported in clinical trials of PNEUMOSIL.

5.2. Synflorix

5.2.1. Product Description

Synflorix consists of 10 individually fermented and purified pneumococcal polysaccharides that have been subsequently conjugated to non-typeable *Haemophilus influenzae* protein D (serotypes 1, 4, 5, 6B, 7F, 9V, and 14), tetanus toxoid (serotype 18C), or diphtheria toxoid (serotype 19F) using CDAP conjugation chemistry.

One single 0.5 mL dose of Synflorix contains 1µg of polysaccharide for serotypes 1, 5, 6B, 7F, 9V, 14, and 23F, and 3µg of serotypes 4, 18C, and 19F. It is formulated with aluminum phosphate (0.5mg Al^{3+} per dose) as an adjuvant. The vaccine is a turbid white suspension.

5.2.2. Manufacturer

Synflorix is manufactured and supplied by GSK Biologicals.

5.2.3. Presentation and Formulation

Synflorix may be supplied in a pre-filled syringe (PFS) or in a single- or multi-dose vial. Each PFS/vial will be labeled with the following minimum information: name of the medicinal product, route of administration, expiry date, lot number, dose volume, and a cautionary statement ("For Clinical Trial Use Only").

5.2.4. Storage

Synflorix should be stored at between 2°C and 8°C. It must not be frozen.

5.2.5. Potential Safety Risks

As with any vaccine, severe allergic reaction is a potential rare event. Synflorix should not be administered to subjects with known hypersensitivity to any component of the vaccine.

The most relevant clinical data for identifying potential solicited adverse reactions in infants who receive Synflorix in the current trial are from a Phase 3 study evaluating the safety and tolerability of Synflorix administered to Filipino infants (n = 300) as a 3-dose primary series at 6, 10, and 14 weeks of age, and co-administered with DTwP-HepB-Hib vaccine.[35] The most commonly reported solicited adverse reactions after any of the 3 primary doses of Synflorix were injection site tenderness (67.2%), irritability (66.2%), fever (60.9%), injection site erythema (47.0%), drowsiness (38.8%), injection site swelling (36.4%), and decreased appetite (25.9%). Reported Grade 3 solicited adverse reactions after any of the 3 primary doses of Synflorix were injection site tenderness (9.4%), fever (6.1%; >39.0 – ≤40.0˚C), irritability (2.9%), drowsiness (1.0%), and decreased appetite (0.2%). Injection site swelling > 30mm was reported in 9.3% of infants, and injection site erythema > 30 mm was reported in 2.4%.

A Phase 3 trial evaluating the safety and tolerability of a booster dose of Synflorix administered to toddlers aged 12-18 months (n = 737), and co-administered with INFANRIX hexa® (combined diphtheria and tetanus toxoids, acellular pertussis, hepatitis B (recombinant), inactivated poliomyelitis and adsorbed conjugated *Haemophilus influenzae* type b vaccine), was conducted in Finland, France and Poland.[33] The most commonly reported solicited adverse reactions after the booster dose of Synflorix were irritability (59.6%), drowsiness (41.2%), decreased appetite (31.3%), fever (33.3%), injection site tenderness (61.5%), injection site erythema (61.4%), and injection site swelling (46.0%). Grade 3 solicited adverse reactions after the booster dose of Synflorix were injection site tenderness (6.4%), fever (3.3%; >39.0 – ≤40.0˚C), irritability (2.0%), drowsiness (0.7%), and decreased appetite (0.5%). Injection site swelling > 30mm was reported in 9.1% of toddlers, and injection site erythema > 30 mm was reported in 13.1%. Grade 4 fever (>40.0˚C) was reported in 0.1% of toddlers.

In both Phase 3 trials the incidence of solicited reactions reported after each vaccination dose was within the same range as after vaccination with the comparator, 7-valent Prevenar. Refer to the Summary of Product Characteristics for Synflorix (2014)[25] for additional information on adverse reactions that have been reported in clinical trials and post-marketing surveillance.

5.3. Vaccine Storage, Transport, and Temperature Monitoring

The temperature of study vaccines will be monitored during shipment, storage and transportation to the field sites to ensure that temperature deviations do not occur.

The temperature of all vaccine shipments will be monitored throughout transit using a continuous temperature monitoring system. Vaccines will not be used until the temperature of the vaccines throughout transit has been confirmed to be within acceptable limits.

Upon receipt at MRC Fajara, all vaccines will be stored at 2°C to 8°C in dedicated refrigerators that are safe, locked, and not accessible to unauthorized personnel – including study team personnel blinded for study conduct purposes. The refrigerators will be under continuous temperature monitoring with maintenance of 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 the main MRC site at Fajara to the field site in a cold box with continuous temperature monitoring. Any unused vaccines at the end of clinic will be returned to MRC Fajara for storage.

It is 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 vaccine(s) will be quarantined. The temperature deviation will be reported to the CRO who

will advise the unblinded investigator 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.

5.4. Dose Preparation and Administration

A limited number of appropriately trained, unblinded study personnel (herein referred to as unblinded personnel) will be responsible for preparing study vaccine doses in accordance with the randomly determined assignment, administering the study and other EPI vaccines, 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' parents, 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 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. To be prepared for the highest level of possible medical risk, vaccination will take place in a clinical setting in which there is immediate access to the medical personnel (certified in pediatric life support), equipment and medications required for emergency resuscitation.

Since both PNEUMOSIL and Synflorix are suspensions containing an alum adjuvant, study vaccine must be shaken gently immediately prior to use, in order to obtain a uniform homogenous white suspension. Inspection of each vial/PFS will occur immediately prior to use. If a vial/PFS or its contents appear unusual in any way, the vial/PFS will not be used, and procedures detailed in the Study Specific Procedures (SSP) for documentation and disposal will be followed.

Only a single dose of study vaccine (PNEUMOSIL and Synflorix) will be drawn from each vial. EPI vaccines will be administered in an unblinded manner. Unblinded nursing staff will administer vaccines based on WHO best practices.[36] Vaccination of EPI and PCV will be documented on the subject's Infant Welfare Card (IWC). A detailed account of procedures related to preparation and administration of study vaccine will be included in the SSP.

- PNEUMOSIL or Synflorix will be administered as an IM injection into the anterolateral aspect of the left thigh, using a 23G x 25mm needle.
- Injectable EPI vaccines (DTwP-HepB-Hib, IPV, measles-rubella, and yellow fever vaccines) will be administered as an IM injection into the anterolateral aspect of the right thigh.
- RV and OPV vaccines will be administered orally according to standard local procedures.

Injectable vaccines may be given at other sites if there is a good reason to do so (eg, local infection, or pre-existing swelling). In exceptional circumstances this could mean administering up to three vaccines, including study vaccine, into the same leg. Such decisions will be made on a case by case basis by the PI, and the reasons documented clearly in the clinical notes. When more than one vaccine is administered into the same leg, the leg may be temporarily marked with a pen to ensure local reactogenicity is assessed accurately. The site at which study vaccine is administered will be documented in the CRF for all subjects.

5.5. Accountability and Disposal

Following vaccination, the vaccine vials/PFS will be labeled with the screening number of the subject to which the vaccine has been administered using prepared stickers. The person who administered the vaccine and the time and date of vaccine administration will also be documented in an appropriate drug accountability log on the day of vaccination. All used vials/PFS will be stored in a dedicated space that is accessible only to the unblinded site personnel and the unblinded CRO monitor (and ultimately disposed of after completion of the study).

In case a vial/PFS of vaccine is broken or unusable, the unblinded site personnel will promptly inform the unblinded monitor and store the vial/PFS for accountability, following all safety precautions. In case a broken vial/PFS 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 designated unblinded site personnel will maintain a complete and accurate inventory of study vaccines received (including the quantity of vaccines received, date of receipt, condition at receipt, temperature noted during transit), those administered, and any broken or destroyed.

The unblinded CRO monitor will visit the site (including field sites) 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 or unused vials/PFS of 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.

6. STUDY PROCEDURES

6.1. Recruitment and Informed Consent

This will be a single-center study to be conducted at the MRC Unit The Gambia, with prospective subjects to be recruited and consented at multiple field sites.

6.1.1. Community and Individual Sensitization

Recruitment for the trial will take place at MRC clinical trial facilities (field sites) based in or close to the compounds of a number of government health centers in the peri-urban coastal region of The Gambia. The health centers provide antenatal and obstetric services as well as inpatient care. They are also the sites through which the national EPI vaccine program is delivered to infants and children.

Prior to commencing any trial-related activities a process of 'community sensitization' will take place. A series of 'kola nut'[**] meetings with the Alkalo (community leader) and other senior members of the local community including representatives of women's and mother's groups will take place. During these meetings, the PI and other members of the clinical trial team will explain the purpose of the trial, as set out in the ICF, and a chance for the attendees at the meeting to ask any questions they may have will be given. Following these meetings, information regarding the trial is disseminated throughout the

[**] Kola nuts (the seeds of *Cola nitida* and *Cola acuminata*) are given to members of the community at the end of the meeting as a sign of thanks and respect.

local community through well-established community networks. The aim here is that the community as a whole is aware of the trial and that any concerns or misunderstandings are avoided.

Information regarding the trial may also be provided to mothers attending antenatal clinics to raise awareness of the trial in advance and to give them further time to consider and discuss possible participation.

Potentially eligible infants will subsequently be identified by members of the clinical trial team in the early post-natal period at around the time that the newborn vaccines (BCG, OPV and hepatitis B) are administered and 'individual sensitization' of the parents will be undertaken. Sensitization at this age is necessary as the first set of primary EPI vaccines is recommended in The Gambia at 8 weeks of age, so recruitment at this visit is too late to catch infants in the 6 to 8 week eligibility window.

During individual sensitization, parents will be approached and, if interested, the details of the study, as outlined in the ICF, will be explained to them by the study staff. 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. It will be important to ensure that the subject's father is also aware of the study. According to the mother's preference a field worker may visit the home to provide information to the father or may provide such information by telephone. Contact details are taken from any parent who remains potentially interested following individual sensitization. The family will then be given a minimum of an overnight period to consider the information in the ICF before informed consent/enrollment can take place – ie, individual sensitization and consent cannot take place on the same day. However, in most cases the interval will be significantly longer than this given most sensitization will occur in the post-natal period and consent will not take place before an infant reaches 4 weeks of age and will generally occur at 6 week of age. Of note, neither community nor individual sensitization alter the later process of consent during which the ICF is reviewed again line by line on a one-to-one basis.

6.1.2. Initial and Continuing Informed Consent

Informed consent is the process of ensuring that study subjects' parent(s) 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 will be reviewed with the study subjects' parent(s) at designated times and as needed; this review process will be fully documented. Additionally, if any new information becomes available that, in the judgment of PATH and/or the PI, may affect parents' decision to have their infant continue in the trial, such information will be shared, and may be the basis for requiring a new consent form to be signed.

A separate informed consent procedure will be undertaken following V6 in the booster cohort only to include the additional blood draw one year after the booster vaccination (i.e. at V7) and to allow for the interim reporting of SAEs.

If interested following individual sensitization, a subject's parent(s) will be invited to one of the field sites, at which point documented informed consent can be obtained. Prior to initiation of this process at the site, parents must provide study staff with the IWC to confirm their infant's identity and age. Copies of IWCs will be retained as a part of the source documents for the trial.

A prospective subject may be as young as 4 weeks old on the day of consent. The parent providing consent must be 18 years or over on the day consent was provided. Consent from grandparents or guardians will not be accepted.

Parents who are literate in English will be provided with all the information in the ICF again by the PI or designee in English. English literacy will be confirmed in this case by asking the parent to read and explain a section of the consent form. Parents who are not literate in English (this is common in The Gambia) 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 (all members of the team are English literate). Of note, the tribe to which a parent belongs is not necessarily the same as the first language spoken. If consent is obtained in this way, an impartial witness, who is fluent in English and 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 parent. They must also confirm that the parent has had the chance to ask questions and that these have been answered to the parent's apparent satisfaction.

After understanding all aspects of the study and having all questions answered, the parent will be required to undertake an 'Assessment of Understanding' – a series of questions to check that key elements of the study have been fully understood. If understanding is confirmed (according to predefined criteria) the parent is required to sign or provide a thumb print confirming agreement to have the parent's infant participate in the study. Some parents may mark or sign the ICF rather than thumb-printing even though they are not English literate. This is acceptable according to the parent's preference. If the consent has been undertaken in a language other than English 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 providing consent for their child (eg, mother or father) 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 parent and the original ICF will be filed with other subject records by the site team.

The ICF will only be completed once at the time of enrollment and prior to screening for subjects enrolled only in the priming phase of the study (and unless new information necessitating repeat consent is required). As indicated, for subjects in the booster cohort, a second ICF will be completed at or after V6 to include an additional blood draw at 1 year post booster vaccination (V7) and to allow for the interim reporting of SAEs.

Regardless of duration on study, ongoing willingness of subjects to participate will be documented in the source documents at each visit.

7. STUDY VISITS

7.1.1. Screening (Visit 1)

Once informed consent has been documented the subject will be considered to be enrolled in the trial, and may be screened to determine study eligibility. The screening period may encompass more than 1 day to allow for resolution of an exclusionary acute illness and/or abnormal vital sign. All inclusion/exclusion criteria must be assessed from data obtained within the screening period, unless otherwise specified in the eligibility criteria. After informed consent has been obtained, the following screening procedures will be performed:

1. Screening identification (ID) number will be assigned.
2. Demographic and contact information will be obtained including address (with adequate detail for another individual to identify the residence), telephone number(s), and email (if applicable).

3. Complete medical history of relevance to study eligibility will be obtained from the subject's parent.
4. A history of medications taken that are of specific relevance to study eligibility (eg, immunosuppressant medications) will be obtained from the subject's parent. The IWC will also be reviewed for this purpose.
5. Vaccination history will be obtained from the IWC, which will represent the source document for this information.
6. Height/length and weight will be measured. The weight-for-height Z score will be calculated.
7. A PE will be performed, including vital signs (temperature, pulse rate, and respiratory rate) and assessment of the major organ systems. Any subject with a ≥ Grade 1 vital sign based on the toxicity grading tables in Appendix 1 and 2 will not be eligible for randomization (note: toxicity scores for pulse rate and > Grade 1 respiratory rate are based on severity of clinical manifestations of bradycardia/tachycardia and respiratory distress, respectively). Infants may return for repeat assessments once during the screening period to be reassessed for eligibility. The last measurement will be taken as the baseline for purposes of analysis.

The PI will use good clinical judgment in considering an infant's overall eligibility. Infants who are not eligible will be recorded as screen failures, along with the basis for this determination, on the appropriate CRF. An infant deemed a screen failure may not be rescreened. Any infant who fails screening due to an abnormal clinical finding will receive counseling from the PI, may receive initial care from the clinical trial team, and will be referred for further medical management as indicated according to normal practice in The Gambia.

7.1.2. Randomization and Vaccination Visits (Visit 1, 2, 3, and 5)

In most cases, eligibility will be determined in a single clinic visit, and no additional assessments will be needed prior to randomization and first study vaccination, when these are completed on the same day. If the screening period encompasses more than 1 day to allow for resolution of an acute illness, abnormal vital sign and / or borderline z-score or baseline weight<3.5 kg, or due to time or logistic constraints, the following procedures will need to be performed prior to randomization and first vaccination. These procedures will also be performed prior to subsequent vaccinations during the priming phase of the trial for all subjects, and prior to booster vaccination for those subjects included in the booster phase of the trial.

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 moderate or severe acute illness may be noted, and no vital sign may be ≥ Grade 1 based on toxicity score (see Appendix 1 and 2). Randomized subjects may return to clinic to receive the 2nd (V2), 3rd (V3) or booster (V5) vaccination after resolution of an acute illness (or other cause of abnormal vital signs), without this resulting in a protocol deviation. Ideally this vaccination visit will occur within the allowable visit window (+2 weeks). A minimum of 48 hours must be allowed after a documented fever (axillary temperature of ≥ 37.5°C) before a subject may receive a study vaccination.
3. If not already recorded, unsolicited AEs will be documented and graded for severity.
4. 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, based on guidelines set forth by the NRA and Investigational Review Boards (IRBs) pertaining to both reporting timelines and processing of related forms. Submission to the Sponsor will occur within a 24-hour time frame, from the time the event is first documented by the PI.

5. Negative malaria parasitemia will be confirmed by RDT/blood film for malaria (generally a capillary sample will be used for this purpose).
6. Targeted PE will be performed, to confirm absence of exclusionary acute illness or abnormality of the extremities (skin and lymph nodes) targeted for vaccination.
7. After assessment of items 1-6 above any basis for determining that an infant is a screen failure, or for withholding re-vaccination in the case of subjects returning for the 2nd (V2), 3rd (V3) or booster (V5) vaccination, will be documented. Any subject who is not randomized will be referred back to their normal clinic for routine EPI vaccination.

The PI must approve the randomization of the subject, based on confirmation that the subject meets all eligibility criteria. Randomization will occur at Visit 1 using a predefined randomization scheme, with allocations occurring in a 2:2:2:3 ratio to PNEUMOSIL (3 groups receiving vaccine from different lots) or Synflorix (1 group) and with stratification by field site. A subject's allocation will be selected in numeric order from a set of sealed randomization envelopes. The assignment will be associated with a unique randomization ID. 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. 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 eligibility has been confirmed and randomization has taken place -- or it has been determined that a subject returning for a vaccination at Visits 2, 3 or 5 may be re-vaccinated --, unblinded study personnel will perform the following procedures in an area of the clinic that is not readily accessible to blinded study personnel:

1. Subject randomization ID will be recorded.
2. The assigned study vaccine will be administered and documented on the CRF as to timing and location of administration (see Section 5.4 for details).
3. The EPI vaccines due at the given vaccination visit will be administered and documented on the IWC and CRF (see Section 5.4 for details). It will also be noted on the IWC that a pneumococcal conjugate vaccine has been given.
4. All subjects will be provided with a study ID card documenting their screening ID, the fact that they are enrolled in the clinical trial, and that they have received a study vaccine. It will also include telephone contact details for study personnel, and state that, should the subject become unwell, a member of the clinical trial team should be contacted immediately. This card will be attached to the IWC.
5. All subjects will also be provided with a photo ID card confirming their randomization in the study.

After vaccination is complete, the subject will be observed by blinded study personnel for the remainder of that visit and for all subsequent non-vaccination-related trial conduct. Immediately following vaccination the following will occur:

1. Subjects will be monitored for vital signs and solicited reactogenicity with recording of all these

events at 30 (+/-10) minutes post vaccination. See Appendices 1 and 2 for appropriate severity grading scales.

2. Parents of subjects in the primary reactogenicity cohort or booster cohort will be reminded about daily home visits for the subsequent 6 days after vaccination, and place of residence and phone contact details will be reconfirmed. Note: inclusion in the primary reactogenicity cohort will be determined at the time of randomization.
3. The date of the subsequent clinic visit will be established.

Note: Study vaccination **must** occur on the day of randomization. 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.

Home Visits – Primary Reactogenicity and Booster Cohorts (Days 1 through 6 Post Vaccination)

Half of the subjects assigned to each treatment group (n = 1,125 subjects total) will be randomly selected as part of the randomization scheme to be included in the "primary reactogenicity cohort." These subjects will be monitored daily at home by field workers for assessment of local and systemic reactogenicity during the 6 days after each primary series vaccination. In addition, all 675 infants who receive the booster vaccination ("booster cohort") will be monitored daily at home by field workers during the 6 days after the booster vaccination.

Field workers will be provided with a reactogenicity record form (RRF) for recording of reactogenicity events. The RRF will be retained as part of the source documents in the subject's file. The grading scales to be used for assessment of severity of local and systemic reactogenicity events are listed in Appendix 1 (Section 16.1). **Any Grade 3 or higher reactogenicity assessed by a field worker will result in immediate clinic contact, and the subject will be seen in the clinic within 24 hours of the event.** Field workers will contact the site to assist with scheduling if subjects are noted to be experiencing any medical condition (ie, solicited or unsolicited AE) that needs to be evaluated by the PI at an unscheduled clinic visit.

Independent of a clinic visit, a Research Clinician (RC) will review the reactogenicity scoring with the field worker at the end of the 6-day assessment period, and sign off to confirm that this review has occurred. Following this review, an anonymized copy of the RRF will be submitted for data entry. If the condition of the RRF is such that data entry could be difficult, the source RRF could be transcribed to a new RRF for copying and submission. In this case the reason for this will be explained in the clinical notes. If more than 1 measurement of a particular parameter is taken and recorded, the value corresponding to the greatest magnitude of the RE will be used as the basis for categorizing and recording the event on the CRF during the given period of assessment. Any local or systemic reactogenicity or other AE ongoing at the day 6 home visit will prompt a day 7 follow-up visit in clinic. If a solicited AE is ongoing on day 7 post vaccination, or occurs after 7 days post vaccination, the event will be recorded on the AE CRF and continued to be followed as per AE monitoring requirements.

7.1.3. Post-Vaccination Visits (Visit 4, 6, 7 and Unscheduled Visits)

All subjects will be seen in clinic at Visit 4 (4 weeks post completion of the 3-dose primary series), and subjects in the booster cohort will be seen in clinic at Visit 6 (4 weeks post booster vaccination). The following procedures will be completed at these visits, as well as at any required unscheduled visit before Visit 6:

1. Screening ID, address and telephone number(s) will be confirmed.
2. Unsolicited AEs will be recorded, including assessment of relatedness to vaccination and severity grade.
3. The occurrence of any SAE will be documented – inclusive of location, duration, severity, relatedness and clinical summary – and will result in notification as set forth by the NRA and IRBs pertaining to both reporting timelines and processing of related forms. Submission to the Sponsor will occur within a 24-hour time frame, from the time the event is first documented by the PI.
    a. Follow-up will be attempted on any 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 last subject exits the study (LSLV). Any SAE deemed related to study vaccine that is ongoing at the time of LSLV will continue to be followed until it is resolved, assessed to be resolved with sequelae, or assessed to be stable/chronic. SAEs deemed not related to study vaccine that are unresolved at the time of LSLV will be classified as ongoing.
4. Concomitant medications will be recorded.
5. Vital signs will be measured, recorded, and graded. See Appendix 1 and 2 for severity grading of abnormal vital signs.
6. Targeted PE will be performed, including local examination of the vaccination site and for any clinically significant finding.
7. Blood sample for immunologic testing will be obtained by venipuncture (Visit 4 and 6).
8. Any follow-up visits will be scheduled.
9. Exit from the study will occur after Visit 4 (following completion of the final disposition CRF) for any subject not participating in the booster phase of the study or after Visit 6 for subjects in the booster cohort.

Parents of subjects in the booster cohort will be asked during Visit 6 whether they are interested in continuing in the immune persistence phase (i.e. they will be individually sensitized to the immune persistence phase at V6). At the same visit or at a subsequent visit, those that are interested will then provide informed consent for the immune persistence phase based on the new informed consent document. The procedures for informed consent will be the same as for the initial consent for the main trial.

The following procedures will be completed at Visit 7 for subjects from the booster cohort whose parent provides prior informed consent:

1. Screening ID, and other identifiers (e.g. mother's name/father's name) will be confirmed.
2. Any unrecorded SAE will be captured and reported
3. Record any specific medications (e.g. immunosuppressives), vaccines (e.g. additional pneumococcal vaccines) or other treatments (e.g. blood products) which could influence immune persistence and which may be accounted for in the final analysis subsets
4. Blood sample for immunologic testing will be obtained by venipuncture.
5. Exit from the immune persistence phase of the study.

Between V6 and V7 the study team will continue to provide medical care at the trial site, in line with good medical practice in The Gambia, for those participants enrolled in the immune persistence phase. The ongoing provision of clinical care in this way is essential to ensure any SAE occurring in these participants are captured real-time. In addition, for consistency purposes, the study team will generally offer the routine EPI vaccines and Vitamin A due within the schedule in The Gambia at around 18 months of age (DTwP, Measles and Rubella, OPV). However, if a participant was away from the trial site at this point they would be encouraged to attend a nearby clinic rather than delaying these vaccines. Data regarding these activities will be captured on clinical notes and other appropriate source documents by the clinical trial team but are not otherwise part of the clinical trial dataset. The administration of the routine EPI vaccines will be documented in the IWC of the infant.

The occurrence of any SAE will be documented and reported in the immune persistence cohort as for the primary and booster cohorts between V1 and V4 or V6 respectively. The procedures will be set out in the safety management plan for the immune persistence phase.

Follow-up will be attempted on any 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, assessed to be stable and the outcome unlikely to change (chronic) by PI or until the last subject exits the immune persistence phase (LSLV). Any SAE deemed related to study vaccine that is ongoing at the time of LSLV will continue to be followed until it is resolved, assessed to be resolved with sequelae, or assessed to be stable/chronic. SAEs deemed not related to study vaccine that are unresolved at the time of LSLV will be classified as ongoing.

Evaluations to be performed at each study visit are shown in Table 9 as follows:

Table 9. Study Visits

Step No.	Evaluation	V1*	V2	V3	V4	V5[#]	V6[#]	V7[#]
		6-8 weeks	V1+4 (+2)	V2+4 (+2)	V3+4 (+2)	9-10 mos	V5+4 (+2)	V5+12 (+1)
1	Signing of ICF and confirmation of ongoing informed consent (+)	✓	+	+	+	+	+✓[$]	+
2	Assign screening ID and confirm (+)	✓	+	+	+	+	+	+
3	Demographics	✓						
4	Record contact information – address and telephone number(s) – and confirm (+)	✓	+	+	+	+	+	+
5	Full medical history (including concomitant medications) and vaccination history.	✓	~	~	~	~	~	
6	Vital signs and PE (targeted after screening)*	✓^✓	✓^✓	✓^✓	✓	✓^✓	✓	
7	Blood sample for immunogenicity testing				✓		✓	✓
8	Rapid malaria diagnostic test*	✓	✓	✓		✓		
9	Eligibility check*	✓	✓	✓		✓		
10	Assign randomization ID	✓						
11	Study vaccination	✓	✓	✓		✓		
12	EPI vaccination	✓	✓	✓		✓		
13	Record local/systemic solicited reactions	✓	✓	✓		✓		
14	Record adverse events (including SAE)*	✓	✓	✓	✓	✓	✓	✓ (only SAEs)
15	Record concomitant medications*	✓	✓	✓	✓	✓	✓	✓[%]
16	Schedule/confirm next visit	✓	✓	✓	✓[#]	✓	✓	
17	Exit study				✓[‡]		✓[‡]	✓

Age ranges indicated for V1/V5. Other vaccination/follow up visits to be scheduled at 4 weeks post prior visit + 2 week window, or at 12 months post booster dose + 1 month window in the case of V7.

* If screening extends beyond 1 clinic visit assessments (*) need to be repeated on the day of randomization/1st vaccination.

[#] Visits 5 and 6 will only be completed by subjects in the booster cohort. Visit 7 will only be completed by subjects in the booster cohort whose parent provides additional consent for this assessment.

(~) Confirmation of medical history

^ Evaluations will be conducted twice – before and after vaccination

[‡] V4 is the EOS visit for subjects who do not participate in the booster phase, and V6 is the EOS visit for subjects in the booster phase whose parent does not additionally consent for assessment at V7.

[$] Informed consent for subjects in the booster cohort to continue on study in order to be assessed at V7 will be obtained at or after V6.

[%]- if relevant to immune persistence objectives

7.1.4. Interim Contacts and Visits

Interim unscheduled contacts and visits (eg, unscheduled visits) in between regularly scheduled follow-up visits may occur at any time at the request of the subject's parent or as deemed necessary by the PI. Up to V6, all unscheduled interim contacts and visits will be captured in the subject's study records and on applicable CRFs.

In the immune persistence phase, data for unscheduled contacts and visits will be captured in clinical notes and other source documents but not on CRF. SAEs during this period will continue to be documented in the internet-based SAE database and on the AE CRF; this will be described in the safety management plan.

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

Subjects' parents may refuse procedures on behalf of subjects at any time during enrollment in the study and can withdraw consent at any time. The PI may also, at his discretion, withdraw the subject from participating in the study at any time if he considers it in the best interest of the subject, with clear documentation as to the reason.

Minor protocol deviations (eg, a missed visit window for a follow up visit, 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 on a protocol deviation CRF and in the clinical notes. 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, or if the visit will be deemed a "Missed Visit." If a subject has exceeded the visit window for a vaccination visit, the PI, in consultation with the Sponsor, will have the discretion to determine whether the subject ought to be withdrawn from further study vaccinations and only be offered the routine EPI vaccines. For major protocol violations (eg, a subject 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 PSRT 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:

- The subjects' parent requests that the subject be withdrawn.
- The PI determines that the subject is unable to comply with the protocol.
- The subject is lost to follow-up.

  Note: A subject will be considered lost to follow-up only after telephonic attempts to contact the subject's parent have failed, and a visit to the home to attempt a contact has occurred and the subject still cannot be located.
- The Sponsor decides to suspend or discontinue development of PNEUMOSIL.

If a subject is withdrawn for a major protocol violation prior to V6, the subject may continue to be followed for safety monitoring if he/she has received a study vaccination.

In the event of a subject's withdrawal or early termination prior to V6, the following activities will be attempted to be performed and information recorded in the database:

1. Contact information will be reviewed and updated.
2. Results from prior visits will be reviewed and any outstanding data queries completed.
3. 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).
4. The reason for withdrawal or early termination will be documented.
5. PE will be performed if possible.
6. New AEs since the last visit will be documented.

7. All previously documented AEs and SAEs will be updated in regards to classification (ongoing, resolved, etc.).
8. Concomitant medications since last visit will be documented.
9. The subject's final study visit will be documented and the final disposition CRF completed. Efforts made to complete the final disposition CRF in the event that the subject's parent cannot or is unwilling to be contacted will be documented.

In the event of a subject's withdrawal or early termination after V6, the following activities will be attempted to be performed and information recorded in the database:

1. Contact information will be reviewed and updated.
2. Any previously recorded SAEs will be updated if necessary with regard to outcome and unrecorded SAEs will be captured
3. The final disposition CRF will be completed. The date of withdrawal will be the date when the last contact with the subject occurred (telephone or face-to-face)

In the case of early subject withdrawal or early termination, samples already collected will be retained for appropriate immunogenicity measurements unless the parent 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.

7.3. Concomitant Medications and Treatments

Up to and including V6, 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 based on good medical practice in The Gambia. To enable the PI to directly assess potential AEs, subjects' parents will be encouraged to obtain initial medical care for subjects at the field site during their enrollment in the trial except in the event of an emergency situation in which another health facility is more readily accessible. Any necessary medical care will follow what is considered to represent good medical practice in The Gambia, and access to such medical therapies will be made available to all subjects during enrollment in the trial. Essential treatments and medications will be provided by the trial team within the scope of their clinical expertise, although referral to government facilities will be appropriate in some cases (eg surgical conditions or trauma).

That said, certain medications will not be allowed according to the protocol (unless for clinical need which will always take priority); if a subject uses the following medications, the PSRT will determine whether to discontinue the subject from the study or from receiving further vaccinations:

- Use of any investigational drug or vaccine other than the study vaccines.
- Administration of a vaccine not part of the EPI, or not administered as part of a national campaign.
- Chronic administration (defined as more than 14 days) of immunosuppressant or other immune modifying agents during the vaccine period. For corticosteroids, this means prednisone or equivalent >10 mg per day; topical and inhaled steroids are allowed.
- Administration of immunoglobulins or any blood products during the study period.

Data regarding concomitant medications (other than those detailed in SAE reports) will not be routinely captured after V6. However, parents will be questioned at V7 regarding the administration of certain medications not permitted per protocol (listed above). Reported use of these specific

medications will be documented on the CRF, if possible, and will be taken into consideration regarding the participant's inclusion in the relevant analysis population.

7.4. 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 first database closure and study unblinding. All CRO personnel, with the exception of the unblinded monitor, clinical supplies manager, an administrator and statistician for the DSMB will remain blinded to the treatment assignments until this time. After the last subject completes V6, the study will be unblinded (only the Sponsor, statistical personnel and medical monitor will be unblinded) following closure of all data, at which time primary and secondary endpoints will be analyzed. Study unblinding will be conducted and documented in accordance with SOP. Personnel who had remained blind would be unblinded only at the end of the study (post V7). The randomization scheme will be appended to the integrated clinical study report (CSR) for reporting of these endpoints, as well as to the CSR addendum reporting the supplemental immunogenicity and safety endpoints.

During conduct of the study through completion of Visit 6 by the last subject in the booster cohort, a limited number of unblinded site personnel will be responsible for preparing and administering study vaccines, performing drug accountability, and maintaining the security of the treatment assignments. The unblinded site personnel will not be involved in the safety assessment of the subjects, or in any other aspect of the study. All other site personnel, including those who perform the clinical evaluations (such as but not limited to assessment of medical history, vital signs assessment, and PE), will be blinded with respect to the identity of the vaccine administered to the subjects.

The CRO will assign blinded monitor(s) to visit the site (including all field sites) 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 monitor(s) 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 PSRT. The CRO will also assign an unblinded study monitor, who will visit the site (including all field sites) 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 PSRT 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.

7.5. Unblinding Procedure

In the event of a medical emergency, the PI 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.

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.

Training surrounding such unblinding will be done during the site initiation visit. Documentation of the unblinding event (including the rationale and requestor) will be recorded and duly entered into the EDC system. Every effort will be made not to unblind the subject unless it is considered necessary for the welfare of the subject. Prior to unblinding, 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 will remain in the study and continue with protocol-defined study visits, but not receive further study vaccines. The decision to unblind will be communicated to the Gambian Government/MRC joint EC 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.

8. LABORATORY EVALUATIONS

Blood samples will be collected from subjects for immunogenicity testing.

8.1. Sample Collection, Distribution, and Storage

Blood samples collected for the immunogenicity endpoints will be separated into aliquots by the MRC research laboratories as per study SSP and stored at -70°C or lower in the MRC biobank before being shipped to the central immunology laboratories (see Section 8.3). Continuous temperature monitoring and backup generators will be in place to ensure proper sample storage. Any blood samples obtained for clinical evaluations will be transported to the MRC clinical laboratory for testing, although for efficiency a blood film for malaria parasites may be undertaken in the government laboratory at the health center when deemed necessary.

Volumes of blood required at different time points for immunogenicity testing are shown in Table 4.

Table 4. Total Blood Volume Required

Immunogenicity Test:	Visit 4	Visit 6	Visit 7	Total
Study Vaccine: ELISA IgG for 10 serotypes in PNEUMOSIL	3.0 mL	3.0 mL	3.0 mL	**9.0 mL**
OPA for 10 serotypes in PNEUMOSIL				
EPI Vaccines: ELISA IgG for components of DTwP-HepB-Hib	2.0 mL	-	-	**2.0 mL**
Neutralization assay for poliovirus 1, 2 and 3		-	-	
ELISA IgA for rotavirus		-	-	
ELISA IgG for measles and rubella	-	2.0 mL	-	**2.0 mL**
Neutralization assay for yellow fever	-		-	
Total Blood Volume	**5.0 mL**[1]	**5.0 mL**	**3.0 mL**	**13.0 mL**[2]

[1]Total volume for infants in priming phase only.
[2]Total volume for infants assessed for immune persistence at V7. Infants in the booster cohort whose parent does not consent to this assessment will require a total blood volume of 10.0 mL.

8.2. Clinical Laboratory Assays

Clinical laboratory tests obtained at the discretion of the PI and RCs will be performed at the Clinical Laboratories Services, MRC in Fajara. The Clinical Laboratories Services subscribes to proficiency testing programs, and operates based on the principles of Good Clinical Laboratory Practice (GCLP) and ISO15189.

Additional investigations may also be undertaken on subjects for research and/or diagnostic purposes in order to more fully characterize particular AEs (eg, to identify respiratory viruses in the nasopharynx, or investigate the cause of focal chest findings on PE with a chest x-ray). The circumstances in which such additional investigations may be undertaken, beyond those required as part of routine clinical care, will be specified in study specific procedures for the trial.

8.3. Immunological Assays

The following immunological assays are to be undertaken:

- ELISA IgG: The IgG concentration to each of the 10 serotypes contained in PNEUMOSIL will be measured by ELISA in serum samples collected at Visit 4 (all subjects), 6 (booster cohort) and 7 (immune persistence cohort). The IgG concentration to components of the co-administered pentavalent (DTwP-HepB-Hib) vaccine will also be determined in serum samples collected at Visit 4, and to measles and rubella antigens in serum samples collected at Visit 6 (subset of subjects for each assay). The IgA concentration to co-administered rotavirus vaccine will be measured by ELISA in serum samples collected at Visit 4 (subset of subjects). The ELISAs for the PCV vaccines will be performed at the [redacted], using the WHO reference PCV

ELISA protocol. The ELISAs for pentavalent and measles-rubella vaccines will be performed by [redacted]. The ELISA for rotavirus vaccine will be performed by the [redacted].

- OPA: The functional activity of the IgG response to the 10 serotypes contained in PNEUMOSIL will be determined in serum samples collected at Visit 4, 6 and 7 (subsets of subjects). This activity will be determined using the 4-fold multiplexed opsonophagocytic assay (MOPA) developed at the University of Alabama at Birmingham, also to be performed at [redacted].
- Neutralization assays: The immune responses to co-administered poliovirus and yellow fever vaccines will be determined in serum samples collected at Visit 4 and 6, respectively, by neutralization assays for poliovirus types 1, 2 and 3 and yellow fever (subset of subjects in both cases). The assay for poliovirus types 1, 2 and 3 will be performed by [redacted], and for yellow fever by [redacted].

If there are limitations to blood volumes, appropriate subsets and priorities for immune testing will be established with the immunology laboratories to ensure measurements will be unbiased and be representative of the entire cohort. After the completion of immune testing, all remaining samples at the central laboratories will be destroyed.

All study results will be shared with contributing laboratories at the conclusion of the study. In addition, subjects' parents will be provided an overall summary of the findings of this study through a community meeting held at each recruitment site.

8.4. Assay Qualification, Standardization, and Validation

The ELISA IgG and MOPA assays that will be used to measure the magnitude and the functional activity of the polysaccharide antibody responses – constituting primary and secondary endpoints of the trial – are standardly used in the field to measure immunogenicity as a surrogate marker for efficacy of PCVs, and were validated at the [redacted]. A detailed description of these 2 standard assays can be found at: [redacted].

The ELISAs that will be used for pentavalent vaccine and for rotavirus vaccine and the neutralization (plaque reduction) assay for yellow fever have all been validated following ICH guidelines as specified in Q2 (R1) "Validation of Analytical Procedures: Text and Methodology." The ELISAs that will be used for measles and rubella are commercially available kits (Enzygnost® anti-measles and anti-rubella virus IgG; Siemens, Munich, Germany). The neutralization assay for poliovirus will be undertaken at a [redacted]

8.5. 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.

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 period through Visit 6. In addition, for subjects in the immune persistence cohort, the PI is responsible for also detecting and documenting events meeting the criteria and definition of a SAE from Visit 6 through Visit 7.

9.1. Collection of Safety Events

AEs will be systematically collected at all clinic visits through Visit 6 and for subjects enrolled only in the primary phase of the study, through Visit 4. For subjects in the immune persistence cohort, SAEs will also be collected from Visit 6 through Visit 7. Reactogenicity events will be assessed in all subjects immediately (30 minutes) after vaccination and for subjects included in the primary reactogenicity cohort and/or booster cohort, at the daily home visits for the first 6 days following each vaccination.

In addition, subjects' parents will be instructed to contact the PI immediately should the subject manifest any signs or symptoms. Subjects' parents will be provided with contact details for the field site team and will be provided with telephone credit for this purpose. Site staff will be available 24 hours a day by telephone and in person for emergency needs and during clinic hours to assess subjects for the duration of the trial (FSFV to 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 (PNEUMOSIL or Synflorix). 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 for a particular cohort 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, ie, 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.
- Reactogenicity events include local and systemic reactions noted immediately post vaccination, or during follow-up visits through 1 week after vaccination by field workers and confirmed by the PI.
  - Any solicited AE that is ongoing on day 7 post vaccination, or occurs after 7 days post

vaccination will be entered as an unsolicited AE and followed appropriately at subsequent visits until resolved or EOS visit.

9.2.2. Severity (Intensity) of Adverse Event

The severity of all solicited AEs will be graded from Mild (Grade 1) to Potentially Life Threatening (Grade 4), based on the criteria given in Appendix 1 (Section 16.1). 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 for solicited AEs in the Appendix have been derived from the *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events* (Version 2.0, November 2014), from the US National Institutes of Health.

All other unsolicited AEs will be classified as an AE and graded based on the AE severity scale in Table 5 below.

Table 5. 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.
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE.

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

9.2.3. Causal Relationship of an Adverse Event

A suspected adverse drug reaction (ADR) means any AE for which there is a reasonable possibility that the study 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 CRFs and on the SAE form (in case of SAEs).

The likelihood of the relationship of the AE to study vaccine is to be recorded as follows:

- 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.

  Note: solicited reactogenicity events will not be judged for relatedness.

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

An AE or suspected ADR is considered "unexpected" if it is not listed in the Investigator's Brochure (IB) for PNEUMOSIL or if it is not listed at the severity that has been observed. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation. If the classification of an unexpected AE/ADR is serious and is thought to be related to the study vaccine, then it is classified as a suspected unexpected serious adverse reaction (SUSAR).

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.

***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 (eg, government health center, MRC clinical services department, Edward Francis Small Teaching Hospital) 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.

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

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 enrolled only in the primary phase of the study, and through Visit 6 for each study subject enrolled in the booster cohort. For subjects in the immune persistence cohort, SAEs will also be collected from Visit 6 through Visit 7. In this case study staff will continue to see infants under follow-up between V6 and V7 with any illnesses but will document their assessment in the clinical notes only. AEs that are not serious nor considered IMEs will not be formally reported as study-specific data.

The PI must completely and promptly record each AE in the source documentation and on the AE CRF, 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 (ie, 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. For the purposes of data capture they will be closed at the point the subject is deemed ineligible.

Reporting of AEs will follow the regulatory guidelines of the National Medicines Regulatory Authority (NMRA), the local EC in The Gambia, WIRB and the PATH Research Ethics Committee (REC) in the US, in regards to requirements, processes and forms.

9.2.8. Serious Adverse Event Reporting

If an AE is classified as serious or an IME, 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. This will initiate evaluation by the PSRT through V6 and any additional reporting requirements for the duration of the study. In addition, the SAE submission will follow the regulatory guidelines of the NMRA, the local EC in The Gambia, and WIRB in the US, in regards to requirements, processes and forms. It is the responsibility of the Sponsor to ensure that the manufacturer (SIIPL) is notified of SAEs. Any SAE deemed related to study vaccine that is ongoing at the time of LSLV will continue to be followed until it is resolved, assessed to be resolved with sequelae, or assessed to be stable/chronic. SAEs deemed not related to study vaccine that are unresolved at the time of LSLV will be classified as ongoing.

9.3. Unanticipated Problems

All unanticipated problems will be reported in the continuing review report submitted to the NMRA, the local EC, REC and WIRB 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.

10. SAFETY MONITORING

The PI will be responsible for continuous monitoring of all study subjects' safety through to their final EOS visit. In case of urgent need, subjects' parents will have the means to get in contact with field site staff at any time (24 hours per day) – and the site will have the means to transport subjects to clinic (or another appropriate clinical setting) or will provide fares for this purpose – to allow for expeditious clinical evaluation of, 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. Protocol Safety Review Team

Safety will be monitored routinely throughout the study by the PSRT, until the last subject of the booster cohort completes Visit 6. The PSRT will include the PI and clinical trial coordinators from the MRC, the PATH Study Director and Clinical Operations Lead, and CRO staff (including the Clinical Project Manager, and data management personnel). The Study Director will serve as the PSRT Chairman. The PSRT will review blinded safety data and clinical trial conduct weekly

throughout the trial until the last subject of the booster cohort completes Visit 6 and any individual case safety concerns thereafter as deemed necessary (in this case the PSRT is unblinded except for site staff). Blinded safety reports will be prepared routinely by the CRO for the PSRT that will include at a minimum the following:

- Accrual data and subject status data with regard to completion of/discontinuation from the study, sorted by field site.
- Visit windows expected, deviations, and completions, sorted by field site.
- Summary of reactogenicity data by vaccination number (#1, #2, #3, boost), classified by severity.
- AEs sorted by MedDRA term, severity, and relatedness to study vaccine.
- 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, sorted by field site.
- 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 PSRT, 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.

The PSRT has the authority to implement a study pause based on review of safety findings, or if alerted to unexpected clinical findings by the PI. If the PSRT 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.

If a study pause is initiated, randomized subjects will continue with their scheduled follow-up visits (V4 and V6), but not vaccination visits. In that case, the visit will be on hold during the pause; when the study is resumed, the subject will still be considered to be within their vaccination window, and all future visits adjusted based on the date of resumed vaccination. Such a pause in the study would not constitute a protocol deviation in regards to subject visit windows, and the pause would be taken into consideration for restarting the assigned vaccination visit schedule. A NTF will be written to explain such an occurrence. If at any time a decision is made to permanently discontinue further vaccinations, the PI will notify The Gambia Government/MRC Joint EC and NMRA, and the Sponsor will notify WIRB expeditiously. In this case, those subjects already enrolled in the study who have received a vaccine in either phase of the study will complete the 4-week safety follow-up period. Such safety follow-up could be extended by the PSRT based, if necessary, on the advice of the DSMB if judged to be appropriate according to the reasons for study discontinuation.

10.2. Data Safety Monitoring Board

The DSMB will provide external monitoring of vaccine safety and clinical trial conduct. It will be comprised of independent experts in vaccines, infectious diseases, pediatrics and biostatistics. The DSMB will conduct a formal, unblinded review of all safety data accrued during the trial when approximately one-quarter of the infants in the primary reactogenicity cohort have received their first

vaccination. The need for additional meetings to review unblinded safety data will be determined by the DSMB following this initial meeting. The PSRT may also request additional guidance based on occurrence of certain events.

During meetings the DSMB will not only review all accrued safety data, including all solicited and unsolicited AEs, but also the quality of data generated and any protocol violations and significant protocol deviations at the field site level, with specific attention to inclusion/exclusion criteria, AE/SAE documentation, unblinding, and EDC completion. The DSMB may also take into consideration factors external to the study such as scientific or therapeutic developments that may have an impact on subject safety or the ethical conduct of the study.

DSMB reviews will indicate whether or not safety concerns were identified, and whether the trial should continue without change, be modified, or be terminated. The Sponsor will carefully consider the DSMB recommendations. If the Sponsor does not agree with these recommendations, a meeting will be held between the Sponsor, PI and DSMB to reach consensus on the appropriate action(s) to take in regard to the trial. However, if attempts to reach consensus fail, the Sponsor's opinion will prevail. In such situation, the Sponsor will inform the regulatory authorities, The Gambia Government/MRC Joint ethics committee and WIRB of the DSMB findings, the Sponsor's perspective, and any changes to the trial.

10.3. 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 site team/PI.

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 subject(s) who violates the protocol be discontinued from the study.[37]

When appropriate, 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's study monitors will visit the site at regular intervals (including field sites) as per the monitoring plan and perform pre-agreed source data verification of the data recorded in the paper CRF 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 dates and windows at each field site 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 AEs will be coded using MedDRA dictionary version 19.1 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 pertaining to subject visits through completion of Visit 6 by the last subject in the booster cohort, the database will be declared to be accurate and will be closed for statistical analysis of primary and secondary endpoints. A final database lock will occur after completion of data coding and resolution

of all the queries in the database following completion of Visit 7 by the last subject of the booster cohort (LSLV).

11.1. Case Report Form Development and Completion

Based on the final protocol of the study, a comprehensive set of paper CRFs will be prepared to capture all the relevant data required for analysis and reporting. This study will utilize 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 paper CRFs or EDC system respectively.

All study data will be collected by the clinical study staff using designated source documents, wherever applicable, and will be entered in the appropriate CRFs and EDC system in an anonymized form. In most cases, the CRF will be the source document. 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. CRFs will be reviewed by the clinical team who are responsible for ensuring that they are accurate and complete.

The data management activities will be performed as per the CRO's SOPs. The appropriately trained site personnel will ensure double data entry of the study data recorded on the CRFs into the EDC system. 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 3 business days from subject clinic visit or last home visit. The study monitor plan will include assessments of data entry timeliness.

The study site will maintain the source documents for each study subject. The source documents and other supporting documents will be kept in a secure location. Source documentation will be available for review by the study monitor to ensure that the collected data are consistent with the CRFs.

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. The final database will be locked and transferred to the Sponsor for long-term storage.

11.2.2. Data Storage and Archival

The PI will maintain an Investigator Site File, which will be used to file the IB, protocol, drug accountability records, correspondence with the EC/IRB, Sponsor, 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 2 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 2 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 MRC 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 the [redacted] until it is determined whether the samples are to be retained or destroyed under the direction of PATH. During the informed consent procedures, 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' parents 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.

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 MRC Unit The Gambia on the publication of the complete Phase 3 study outlined in this protocol in a timely fashion. Primary publication of the trial results will be shared between the MRC and PATH. Other individuals having input into the study justifying authorship from MRC Unit, The Gambia, from collaborators and from PATH will similarly be included in publication(s). Additional publications resulting from the analysis of the study data will be agreed between PATH and the MRC 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. STATISTICAL DESIGN AND ANALYSIS

The planned statistical analyses for sizing and assessing 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 first database closure and unblinding of only the Sponsor, statistical personnel and medical monitor for primary and secondary endpoint analyses.

AEs will be coded using the MedDRA Version 19.1 or later. The frequency count and percentage of subjects will be summarized according to the coded terms of system organ class (SOC) and preferred term (PT). Data listings will show AEs by subject.

12.1. Study Populations

All randomized subjects are expected to provide data for safety analyses. We estimate that data for at least 90% of randomized subjects will be available for immunogenicity analyses, with 10% loss of data because of withdrawal, loss to follow-up, problems with specimens, etc.

The Enrolled Population includes all screened subjects who provide informed consent, regardless of whether the subject is randomized to receive a study treatment. This population will be used to account fully for subject disposition, starting with the informed consent. The enrolled population will not be analyzed as such but will be available in the clinical database.

The Safety Population includes all subjects who were randomized, received a study vaccination, and provided at least some post-vaccination safety data. Treatment groups for safety analyses will be assigned according to the actual treatment received at Visit 1. This population will serve as the primary analysis population for demographics and study disposition as well as safety and is the basic population for all analyses except immunogenicity.

The Full Immunogenicity Population (FIP) includes subjects in the enrolled population who were randomized, received a study vaccination, and have post-vaccination immunogenicity measurement(s). Analysis will be according to the treatment received by each subject, even if different from that to which the subject was randomized. The analysis based on this population will serve as supportive results for the immunogenicity objectives if warranted as defined in the SAP.

The Per Protocol Immunogenicity Population (PP_IMM) includes all subjects in FIP who received all study vaccines and have post-dose immunogenicity measurement(s) with no major protocol violations that were determined to potentially interfere with immune response to the study vaccine. This population will serve as the primary analysis population for the immunogenicity primary and secondary objectives.

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

The Immunogenicity Persistence Population (IPP) is a subset of PP_IMM consisting of subjects in the booster cohort who provide evaluable data at Visit 7, excluding subjects with protocol violations that could affect this analysis (e.g. use of prohibited medication or treatment).

12.2. Conduct of the Analyses

Analysis of primary and secondary endpoints as well as supporting analysis (e.g. baseline, study disposition) will be conducted following initial study unblinding of the Sponsor, statistical personnel and medical monitor. Analysis of supplementary endpoints will be conducted following closure of the Visit 7 data. Data listings will be sorted by treatment group and subject identification number. 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. Testing for superiority will be done at the two-sided 0.05 level. Details of endpoint analyses will be described in the SAP.

No formal interim analyses are planned for this study.

12.2.1. Handling of Dropouts or Missing Data

Generally, no imputation will be made for missing values in safety and immunogenicity analyses, except that immunogenicity values below the limit of quantitation (BLQ) that are reported as < BLQ will be assigned a value of ½ the limit of quantification. Any additional imputation for missing values will be documented in the SAP.

12.3. Statistical Methods

12.3.1. Immunogenicity Analysis

Comparisons for primary immunogenicity objectives will be based on the serotype-specific concentrations of IgG antibody 4 weeks after the primary vaccination series, measured by ELISA. Comparisons for secondary objectives will be based on the serotype-specific IgG concentration and OPA titer 4 weeks after the 3-dose primary series and 4 weeks after the booster dose. Comparisons for the supplemental objective will be based on the serotype-specific IgG concentration and OPA titer 1 year after the booster dose.

Response to study vaccination is defined as concentration ≥ 0.35 μg/mL for IgG and titer ≥ 1:8 for OPA.

For each of the 10 serotypes in PNEUMOSIL, the distributions of IgG concentration and OPA titer will be displayed in tabular form (eg, number of observations, number of responders, percentage responding, geometric mean and its 95% confidence interval (CI)) and graphically by reverse cumulative distribution (RCD) curves. These curves will allow visual comparison of percentiles (eg, median, 25^{th} and 75^{th} percentiles) for each serotype in PNEUMOSIL. Summaries will include percentage of responders, geometric mean concentration (GMC) or geometric mean titer (GMT), and ratio of GMCs or GMTs for PNEUMOSIL to those for Synflorix.

Primary objective 1 is to show equivalence of 3 lots of PNEUMOSIL 4 weeks after the primary vaccination series. For each serotype in PNEUMOSIL and each pair of lots, the two-sided 95% confidence interval (CI) for the ratio of the geometric mean concentration (GMC) of IgG antibody in one lot to the GMC in the other lot will be calculated, assuming a normal distribution for $\log_{10}$ (concentration). The 3 lots will be considered equivalent if, for each serotype, all 3 of the CIs for GMC ratio lie within the interval (0.5, 2). If equivalence of lots is shown, data for the three lots will be combined for all further immunogenicity analyses.

Primary objective 2 is to show non-inferiority of the IgG antibody response after PNEUMOSIL vaccination to the response after Synflorix, for at least 7 of the 10 serotypes in PNEUMOSIL 4 weeks after the primary series. NI comparisons will be based on a CI for the difference in proportions (calculated by a likelihood score method) or a CI for a ratio of GMCs (calculated by exponentiating the limits of a CI for the difference in means of $\log_{10}$ (concentration), which will be calculated assuming a normal distribution for $\log_{10}$ (concentration)).[38] NI will be established for each serotype separately based on evaluation of two NI criteria: for each serotype, NI will be shown if a two-sided 97.5% CI for the absolute difference in proportions responding (proportion responding after Synflorix vaccination minus proportion responding after PNEUMOSIL) has upper limit < 0.10, or if a two-sided 97.5% CI for the GMC ratio (Synflorix GMC divided by PNEUMOSIL GMC) has upper limit

< 2. For serotypes 6A and 19A, the response rate for Synflorix will be assumed to be the lowest observed rate among the 8 serotypes in common with PNEUMOSIL, and the GMC will be assumed to be the GMC of the serotype with the lowest response rate.

Primary objective 3 is to show NI of responses, 4 weeks after the primary series, to pentavalent, polio and rotavirus vaccines when co-administered with PNEUMOSIL to responses when co-administered with Synflorix. Except for pertussis, for each antigen in the pentavalent, polio or rotavirus vaccines NI will be shown if a two-sided 95% CI for the difference in response proportions (proportion with Synflorix co-administration minus proportion with PNEUMOSIL co-administration) has upper limit < 0.10; for pertussis, NI will be demonstrated if the two-sided 95% CIs for the GMC ratios of the response to both pertussis toxoid and fimbriae (GMC with Synflorix co-administration to the GMC with PNEUMOSIL co-administration) has upper limit < 2. Response is defined for the various antigens as follows: anti-diphtheria toxoid ≥ 0.1 IU/mL; anti-tetanus toxoid ≥ 0.1 IU/mL; anti-HBs concentration ≥ 10 mIU/mL; anti-PRP concentration ≥ 0.15 µg/mL; anti-polio types 1, 2 and 3 titer ≥ 1:8 anti-rotavirus concentration ≥ 20U/mL.

Secondary objective 1 is to demonstrate that the immune responses to serotypes 6A and 19A in PNEUMOSIL recipients 4 weeks after the 3-dose primary series are superior to the responses to these serotypes induced by Synflorix, either for the proportion of IgG antibody concentrations ≥ 0.35 µg/mL or for GMC. For each of the two serotypes, proportions with IgG concentration ≥ 0.35 µg/mL will be compared using a z-test for proportions and GMCs will be compared by a two-sample t-test on the difference between means of $\log_{10}$ (antibody). Both tests will be done at the two-sided 2.5% significance level to adjust for the two superiority tests.

Secondary objective 2 is to evaluate, in a subset of subjects (250 PNEUMOSIL recipients and 250 Synflorix recipients), the serotype-specific functional antibody responses, measured by OPA, to PNEUMOSIL in comparison with Synflorix for each of the 10 serotypes in PNEUMOSIL, when measured 4 weeks after a 3-dose primary series. The percentage of subjects with OPA ≥ 1:8 and OPA GMT will be reported.

Secondary objective 3 is to evaluate, in a subset of subjects, serotype-specific booster responses (antibody concentrations and functional responses) to PNEUMOSIL in comparison to Synflorix, from 4 weeks after a 3-dose primary series to 4 weeks after a booster dose. The comparisons will be based on the ratio of IgG GMC post-booster to the IgG GMC post-primary series, and on a similar ratio of OPA GMT. The vaccines will be compared using the ratios of these ratios for the two vaccines (ie, the Synflorix ratio divided by the PNEUMOSIL ratio), and the corresponding 95% CIs. The number of subjects with evaluable data for this comparison is planned to be approximately 600 (400 PNEUMOSIL recipients and 200 Synflorix recipients) for the analysis of IgG GMC ratios, and 200 (100 PNEUMOSIL recipients and 100 Synflorix recipients) for the analysis of OPA GMT ratios.

Secondary objective 4 is to demonstrate non-inferior immune responses to routine pediatric vaccines co-administered with the booster dose (measles, rubella, yellow fever). The analysis will consist of comparison by z-test at the two-sided 5% level of proportions responding, using a NI margin of 10%. It is planned to have data on approximately 600 subjects (400 PNEUMOSIL recipients and 200 Synflorix recipients) for these comparisons.

Supplemental objective 1 is to evaluate, in subsets of subjects, the persistence of serotype-specific immune responses (antibody concentrations and functional responses) to PNEUMOSIL in comparison to Synflorix for each of the 10 serotypes in PNEUMOSIL, when measured 1 year after a booster dose. To evaluate the persistence of serotype-specific antibody concentrations, treatment-group-specific IgG GMCs and percentage of responders (IgG concentration ≥ 0.35 µg/mL) will be

reported. PNEUMOSIL will be compared to Synflorix by the ratio of IgG GMCs (and its 95% CI) and the difference in percentage of responders (and the 95% CI around the difference). The number of subjects with evaluable data for these analyses is planned to be approximately 600 (400 PNEUMOSIL recipients and 200 Synflorix recipients). The same approach will be used to evaluate the persistence of serotype-specific functional responses based on treatment-group GMTs and percentage of responders (OPA titer ≥ 1:8). The number of subjects with evaluable data for these analyses is planned to be 100 (50 PNEUMOSIL recipients and 50 Synflorix recipients). Analyses will be supported by summary descriptive tables, by treatment group, RCD curves and graphs of GMCs and 95% confidence intervals across time.

12.3.2. Safety Analysis

Safety and tolerability of study vaccines will be evaluated using the following endpoints:

- Number and severity of solicited local and systemic adverse events (reactogenicity events [REs]) through Day 6 after each vaccination
- Number, severity and relatedness of all AEs and serious adverse events (SAEs) during the entire study period through 4 weeks post dose 4
- Number, severity and relatedness of all serious adverse events (SAEs) from 4 weeks post dose 4 through 12 months post dose 4
- Number, severity and relatedness of all AEs and SAEs during the 2 week period post each vaccination

Unsolicited AEs and SAEs will be summarized by SOC and PT using the MedDRA dictionary. Adverse events and SAEs will also be summarized by severity and relationship to vaccine.

Generally, safety evaluations will be descriptive in nature, and observed differences will be evaluated for medical relevance. Tabular summaries of safety data will be provided for each treatment group.

Occurrence of local and systemic reactions (REs) within 6 days after vaccination, as well as AEs during the entire study period through 4 weeks post dose 4 (Visit 6), will be reported for both PNEUMOSIL and Synflorix. Proportions of severe reactions and classes of AEs of particular interest will be compared.

For RE's, treatment groups will be compared on the distribution of highest reactogenicity grades (0,1,2,3,4) (a) post any vaccination and (b) post each vaccination using the Cochran-Mantel-Haenszel test stratifying on field site. The modified ridit method will implemented to take advantage of the ordinality of grades. To avoid sparse data, categories may be pooled (e.g. 0, 1, 2+) based on the statistician's blinded review of distributions. The analysis will be supported by appropriate descriptive tables.

12.3.3. Multiple Comparisons/Multiplicity

No adjustment of significance levels (α) is planned for immunogenicity comparisons in this study, except in testing for NI when NI can be established either by a comparison of proportions of responders or by a GMC ratio, or in superiority testing when superiority can be established based on either a comparison of proportions or a GMC ratio. In that case the NI testing will be done with $\alpha = 0.0125$ (ie, using the upper limit of a two-sided 97.5% CI).

For analysis of safety data, statistical comparisons will be done for solicited AEs as described above with no adjustment for multiple comparisons, since the primary purpose of these statistical comparisons is to screen out potential AEs that need further clinical evaluation, and we therefore

don't want to miss an important association. It is acknowledged that the overall type I error rate for these comparisons will be greater than the nominal two-sided 0.05.

12.4. Sample Size and Power Calculations

The study is designed to have at least 90% power to meet all 3 primary objectives. The sample size was chosen in an iterative, trial-and-error fashion to give the desired power. We plan to enroll (i.e., assign a treatment by a randomization process) 2250 subjects: 1500 subjects to receive PNEUMOSIL (500 in each of 3 lots) and 750 to receive Synflorix. The sample size for the primary non-interference study will be lower: 675 total (450 recipients of PNEUMOSIL and 225 recipients of Synflorix). Power calculations were done using PASS 13 (Number Cruncher Statistical Systems, Kaysville, Utah).

Primary objective 1: Lot-to-lot comparisons

Assuming a 10% loss of data from withdrawals, loss to follow-up, etc., the numbers of subjects with analyzable data on immune response will be approximately 450 per lot of PNEUMOSIL and 675 for Synflorix. With these numbers, as shown below the power will be ~94% for showing equivalence of lots for all serotypes, under somewhat conservative assumptions about variability of antibody levels and differences between lots. The standard deviations (SDs) of $\log_{10}$(antibody concentration) assumed for the power calculations were chosen by the following procedure. The observed SDs for PNEUMOSIL serotypes from study VAC-017 were arranged in descending order. For each of the 5 pairs of ranked SDs, beginning with the largest SD, the SD for power calculations was assumed to be the higher of the 2 SDs in the pair. Thus for 5 of the serotypes, the assumed SD was the same as observed in VAC-017, and for the other 5 the assumed SD was higher than the observed SD in VAC-017. We estimated the overall power by assuming the values for different serotypes were independent, so that the power to show equivalence for multiple serotypes is estimated by multiplying the powers for the individual serotypes. For each serotype there are 3 possible comparisons of two lots; for the two serotypes with the highest SDs, we assumed a true ratio of GMCs of 1.4 for 2 of the comparisons of lots, which implies a ratio of 1 for the 3rd comparison. (This is a slightly more conservative assumption – ie, gives a lower power – than assuming equal spacing on the multiplicative scale of the GMCs of the three lots). For the other serotypes, we assumed a ratio of 1.3 for 2 of the comparisons and 1 for the 3rd comparison. The assumed values of the SDs by serotype and the resulting power estimates are given in Table 6 below.

Table 6. Power of lot-to-lot comparisons for 3 lots to show equivalence of IgG GMCs after the 3-dose primary series, for sample sizes of 450 per lot and equivalence margins of 0.5 and 2 for the GMC ratio

Serotype	SD of $\log_{10}$ (concentration), VAC-017	Assumed SD for power calculations	Assumed true GMC ratio*	Power**
6A	0.56	0.56	1.4	0.9856
14	0.55	0.56	1.4	0.9856
6B	0.53	0.53	1.3	0.9996
19A	0.45	0.53	1.3	0.9996
23F	0.43	0.43	1.3	1.0000
19F	0.38	0.43	1.3	1.0000
7F	0.38	0.38	1.3	1.0000
9V	0.37	0.38	1.3	1.0000
1	0.36	0.36	1.3	1.0000
5	0.34	0.36	1.3	1.0000

*Ratio for 2 of the 3 between-lot comparisons; ratio is 1 for the 3rd comparison.

**Power for the 2 comparisons with the indicated ratio; power for the 3rd is 1.0000.

The power to show equivalence of lots for all serotypes is approximately 94.2%, which is calculated by taking the product of the power estimates in the above table and then calculating the square of that product.

Primary objective 2: Comparison of responses to PNEUMOSIL and Synflorix

Assuming 1350 subjects who received PNEUMOSIL and 675 recipients of Synflorix are included in the analysis, the power will be approximately 99.8% to show NI of PNEUMOSIL to Synflorix for at least 7 of 10 serotypes in PNEUMOSIL, under the assumption that the true underlying proportions responding with IgG antibody ≥ 0.35 µg/mL after the 3-dose primary series are 0.02 lower after PNEUMOSIL vaccination than after receipt of Synflorix for each serotype. This assumption is made to introduce some conservativeness into the calculations. For each serotype, NI will be shown if either a two-sided 97.5% CI for the absolute difference in response proportions (proportion of responders with Synflorix minus proportion with PNEUMOSIL) has upper limit < 0.10, or a two-sided 97.5% CI for the GMC ratio (Synflorix GMC divided by PNEUMOSIL GMC) has upper limit < 2. For serotypes 6A and 19A, the response rate with Synflorix will be assumed to be the lowest observed rate among the 8 serotypes in common with PNEUMOSIL, and the GMC will be assumed to be the GMC of the serotype with the lowest response rate. Table 7 shows the assumed proportions and resulting power for each serotype.

Table 7. Power of comparisons between PNEUMOSIL and Synflorix to show non-inferiority of proportion of subjects with IgG antibody concentration ≥ 0.35 µg/mL after the 3-dose primary series, for sample sizes of 1350 for PNEUMOSIL and 675 for Synflorix and NI inferiority margin of 0.10 for the difference between the proportion responding for PNEUMOSIL and the proportion responding for Synflorix

Serotype	Assumed true proportion responding after Synflorix*	Assumed true proportion responding with PNEUMOSIL	Power
6A	0.89	0.87	0.9993
14	0.98	0.96	1.0000
6B	0.89	0.87	0.9993
19A	0.89	0.87	0.9993
23F	0.91	0.89	0.9999
19F	0.92	0.90	1.0000
7F	0.97	0.95	1.0000
9V	0.94	0.92	1.0000
1	0.99	0.97	1.0000
5	0.999	0.979	1.0000

*Assuming response proportions are as for PNEUMOSIL in VAC-017 for the 8 common serotypes and the lowest of these observed proportions for 6A and 19A.

The approximate power for meeting primary objective 2, obtained by multiplying the 7 highest powers in Table 7, is 99.8%.

Primary objective 3: Non-interference with responses to EPI vaccines

For each EPI vaccine antibody tested (except antibodies to pertussis antigens), NI will be shown if a two-sided 95% CI for the absolute difference in response proportions (proportion of responders with Synflorix minus proportion with PNEUMOSIL) has upper limit < 0.10. For pertussis, NI is defined as a two-sided 95% CI for the GMC ratio (GMC with Synflorix co-administration divided by GMC with PNEUMOSILco-administration) with upper limit < 2 for each of two separate antigens (pertussis toxoid and fimbriae). Based on prior studies, we assume response proportions of at least 96% for each antibody level tested (other than anti-pertussis antibodies) and a standard deviation of 0.82 for $\log_{10}$ (anti-pertussis antibodies). We assume there is no interference, ie, the underlying response probabilities are equal for co-administration with PNEUMOSIL and with Synflorix. For 450 recipients of PNEUMOSIL and 225 recipients of Synflorix, each comparison will have > 99% power, and the power will also be > 93% to show non-interference of PNEUMOSIL with routine vaccinations for all the comparisons simultaneously, ie, to show NI of PNEUMOSIL to Synflorix for all responses to co-administered pentavalent, polio and rotavirus vaccines that are tested.

The overall power to show lot-to-lot consistency, NI of PNEUMOSIL to Synflorix for responses to antigens in PNEUMOSIL, and non-interference of PNEUMOSIL with responses to pentavalent, polio

and rotavirus vaccines, is obtained by multiplying the powers of the study to meet primary objectives 1, 2, and 3 – approximately 93%.

Secondary objective 1: Superiority of responses to 6A and 19A

We assume the true GMCs and proportions of responders 4 weeks after the 3-dose primary series are as in VAC-017 for PNEUMOSIL and as in the COMPAS study for Synflorix cross-reactivity; in the COMPAS study the seroresponse rate was 64.4% for 6A and 61.1% for 19A, and the GMC was 0.32 (95% CI 0.27-0.37) for 6A and 0.29 (95% CI 0.25-0.33) for 19A.[27] For tests at the two-sided 0.025 significance level on data from 1350 recipients of PNEUMOSIL and 675 recipients of Synflorix, the power of the study is virtually 100% to show that the GMC for PNEUMOSIL is significantly higher than the GMC for Synflorix for both serotype 6A and serotype 19A. The power is > 99% to show that the GMC ratio (GMC for PNEUMOSIL divided by GMC for Synflorix) is greater than 2 for both serotypes. The power is virtually 100% to show the response rates for both serotypes are significantly higher for PNEUMOSIL. For showing the response rate after PNEUMOSIL vaccination is higher by at least 0.10 than the response rate after Synflorix vaccination, the power is approximately 69% for serotype 6A and 100% for serotype 19A.

Safety

The sample size for evaluation of solicited AEs (local and systemic reactions to vaccine, i.e., REs) will be approximately 1125. For 750 recipients of PNEUMOSIL and 375 recipients of Synflorix, the power to find a significant increase in the rate of severe local or systemic reactions after PNEUMOSIL vaccination compared to Synflorix, using a z-test at the one-sided 2.5% significance level, will be about 84% if the rate of severe reactions or adverse events is 5% after Synflorix vaccination and 10% after PNEUMOSIL vaccination. The power will be approximately 99% if the respective rates are 10% and 20%.

In a sample size of 1500 infants vaccinated with PNEUMOSIL, the probability of observing at least one occurrence of an unsolicited adverse event that occurs with frequency 1% will be virtually 100%. For an event that occurs with frequency 0.1%, the probability will be ~78%. If there are no events of a specific adverse event in 1500 PNEUMOSIL recipients, the upper limit of a two-sided 95% CI for the probability of the event's occurrence will be 0.25%.

13. 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 7.4 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 site (including field sites) 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 to ensure that 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 CRFs.

The PI and the monitor will cooperate to ensure that any problems detected in the course of these monitoring visits, including EDC 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 for all subjects, 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.

13.1. Independent Auditing

PATH representatives may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs of the 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 CRFs with the source data and evaluate the study site for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements.

13.2. Regulatory Agency Auditing

The PI must be aware that representatives from regulatory authorities may wish to inspect the CRFs 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 his qualification as PI in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance in responding to regulatory audits or correspondence.

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

This study will be conducted according to GCP as well in accordance with Gambian 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, and 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 immediately informed (within 24 hours of the Sponsor becoming aware) of significant new adverse effects or risks with respect to the study vaccine. The Sponsor will ensure that they will be immediately informed (within 24 hours of SIIPL becoming aware) of significant new adverse events in the Phase 3 trial in India or of any other safety concerns which could influence decisions regarding informed consent, enrollment and vaccination in this trial.

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.

15. ETHICAL CONSIDERATIONS AND INFORMED CONSENT

The study will be performed in accordance with SSPs and study plans generated and agreed between the Sponsor-designated CRO and the PI. The CRO has the responsibility for ensuring the site has the appropriate SSPs to perform the study. These SSPs have been developed in accordance with ICH Guidelines for GCP (1996), Directive 2001/20/EC, and GCPs for Clinical Research in The Gambia, 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 EC/IRB listed in this protocol and national authorities under Gambian law.

15.1. Institutional Review Board/Ethics Review Committee

The PI at the study site will be responsible for obtaining approval from the MRC Scientific Coordinating Committee (SCC) and The Gambia Government/MRC Joint EC for the conduct of the study. The PI will submit the final protocol, IB, proposed ICF, any proposed advertising material, and all other relevant study-related information in writing for The Gambia Government/MRC Joint EC review and written approval, according to guidelines. The Sponsor will ensure approval to undertake the study is obtained from WIRB. The PI will obtain import authorization and clinical trials authorization from the NMRA in The Gambia. Recruitment and enrollment of subjects will not take place until all approvals from regulatory authorities involved in this trial are received. The PI will notify the EC/IRB of SAEs, protocol amendments, and protocol violations and deviations according to the EC/IRB requirements.

15.2. 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's parent. Only one parent (usually the mother) will provide written consent but in general both parents should agree to a subject's participation. If either parent specifically states that they do not want their infant to participate, the infant will not be enrolled. Consent will only be obtained from birth parents. Consent will not be obtained from guardians in this study. Once informed consent has been obtained the subject will be considered to be enrolled. The method of explanation to the subject's parent or impartial witness, and obtaining of parental 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 6.1.2 for a detailed explanation of the informed consent process. Of note, as consent in many cases will be obtained through verbal translation of the ICF from English into the local language, the ICF will be concise, appropriate for use in this context, and consistent with the requirements of ICH-GCP. Individuals at The MRC Unit have longstanding expertise in this area. The local languages are not written therefore translation and back-translation has been proven to be unreliable/ineffective. The approach taken has been approved by The Gambia Government/MRC Joint Ethics Committee.

15.3. Research Involving Children

Before undertaking research involving children, the PI must ensure that the research has the goal of bettering the health of children. PNEUMOSIL contains 10 pneumococcal serotypes chosen specifically because of their prevalence in low-resource countries such as The Gambia. This Phase 3 study aims to provide the data necessary for licensure and WHO prequalification of PNEUMOSIL, which is being developed as an affordable and effective alternative PCV for children in low- and middle-resource countries. Because of the early successful introduction of PCV into the national EPI program, and continued high coverage rates for Prevenar 13, rates of vaccine-type IPD are low in The Gambia. This fact, together with the substantial immune response to all 10 serotypes in PNEUMOSIL seen in infants in the Phase 1/2 trial (VAC-017), and the demonstrated efficacy and effectiveness of Synflorix against IPD and pneumonia in randomized controlled trials, provides assurance that it is safe for infants to be vaccinated with a 3-dose primary series of PNEUMOSIL or Synflorix instead of Prevenar 13 in this clinical trial.

15.4. Insurance and Indemnity

Subjects will be insured against injury caused by the study according to legal requirements. The parent will be informed about the insurance and the responsibilities on their part. In the event that a subject suffers injury or death directly attributable to participation in this study, appropriate treatment and/or compensation will be provided by and/or paid to the subject by the Sponsor in accordance with applicable national laws and/or guidelines.

15.5. Risk/Benefit

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

As with any vaccine, severe allergic reaction is a potential rare event. In the VAC-017 study, conducted in The Gambia, PNEUMOSIL was well-tolerated in all age cohorts, and no meaningful safety signals were identified in any cohort, including 100 infants who received a 3-dose primary series of PNEUMOSIL (at 6, 10, and 14 weeks of age) co-administered with EPI vaccines, including DTwP-HepB-Hib vaccine. When observed, reactogenicity was primarily mild or moderate and of short duration. There were no related SAEs or severe TEAEs reported during the study and no meaningful trends in SAEs, vaccine-related TEAEs, or overall TEAEs. As was the case in the VAC-017 trial, additional risk mitigation in the planned Phase 3 trial will be provided by clinical monitoring and access to clinical evaluation and management.

Potential health benefits include the clinical assessments and physical examinations by a study clinician outlined in the protocol which may identify illnesses or other medical issues, thus allowing for their prompt treatment. Medical issues will also be investigated and managed by the study team according to good clinical practice in The Gambia and within the limits of the regular practice of the MRC clinical services department and associated laboratory, radiology and pharmacy facilities. Certain issues beyond this (including in particular, but not limited to, any surgical issues) would instead be referred to the appropriate government health facility for management, in which case transport and other small costs would generally be covered by the study team (note that these limitations do not apply to study-related injury, which are covered by clinical trial insurance).

Infants who participate in the booster phase (n = 675) will receive a booster dose of PNEUMOSIL, which is expected to provide additional protection against pneumococcal disease by boosting immunity. A booster dose of PCV is not currently offered by the Gambian EPI program. The overall

aim of the program is to license an affordable pneumococcal conjugate vaccine targeting those pneumococcal serotypes most prevalent in The Gambia and other low- and middle-income countries; parents (and in the future infants) may feel benefit from knowing that they have been a part of this endeavor.

15.6. 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 may 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.

15.7. Reimbursement

Pending EC approval, parents of subjects will be compensated for travel to study visits. In addition, appropriate food during a visit and services to allow phone contact for follow-up purposes will be provided. The study ICF will explain this. Parents of study subjects will not be charged for vaccines, research clinic visits, research-related examinations, or research-related laboratory tests or health care in line with good practice in The Gambia while on follow-up in the study.

15.8. Storage of Specimens

15.8.1. Use of Specimens during the Study

Each blood 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 during the primary and secondary analyses. After the blind is broken, the laboratories performing the assays will continue to be blinded for the supplemental analysis. Stored study research samples will be labeled by screening ID. All stored research samples will be logged into a secure database that tracks 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 SSP for specifics on sample labeling, transport, tracking and logging. Samples may be stored at several different central laboratories in order

to complete the analyses required to meet study primary and secondary analyses. After the completion of immune testing, all remaining samples at the central laboratories [redacted].

15.8.2. Future Use of Stored Specimens

Some blood samples will be retained at the [redacted] in case testing needs to be repeated. When these samples are no longer needed for the purposes of the study, they will be kept or destroyed, depending on whether subjects' parents consented to any remaining samples being used for other, ethically approved research [redacted]. Samples that will remain at the [redacted] will have the same label as was used in the current study. Their use will be governed by a repository plan that is mutually agreeable to PATH and MRC. The samples will be used in accordance with what is stated in the study consent form and with review by relevant ethics committees in accordance with laws of [redacted] policies. No genetic testing will be done on the samples.

16. APPENDICES

16.1. Appendix 1: Solicited Local and Systemic Reactions Toxicity Grading Table

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Tenderness[a]	Minor reaction to touch	Cries / protests on touch	Cries when limb is moved / spontaneously painful	Hospitalization
Erythema/Redness[b]	Erythema present but ≤ 2.5 cm diameter	Erythema >2.5cm diameter but < 50% surface area of the extremity segment (e.g., upper arm/thigh)	Erythema involving ≥ 50% surface area of the extremity segment (e.g., upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper) of tissue OR Hospitalization
Induration/Swelling[b]	Induration OR Edema present but ≤ 2.5 cm diameter	Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (e.g., upper arm/thigh)	Induration OR Edema involving > 50% surface area of the extremity segment (e.g., upper arm/thigh) or Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper) of tissue OR Hospitalization
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[a]	Crying more than usual / no effect on normal activity	Crying more than usual / interferes with normal activity	Crying that cannot be comforted / prevents normal activity	Hospitalization
Drowsiness	Drowsiness easily tolerated	Drowsiness that interferes with normal activity	Drowsiness that prevents normal activity	Hospitalization

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Decreased Appetite[a]	Eating less than usual / no effect on normal activity	Eating less than usual / interferes with normal activity	Not eating at all	Hospitalization
Cutaneous Rash	Localized macular rash (not directly associated with the injection site – ie not a local reaction at the site of injection)	Diffuse macular; maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal Necrolysis (TEN)

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

[a] Standard pediatric reactogenicity scales used in PCV studies.

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

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

16.2. Appendix 2: Vital Signs Toxicity Grading Table

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Respiratory distress[b]	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[c]	Asymptomatic, intervention not indicated	Symptomatic, non-urgent medical intervention indicated	Severe, medically significant, medical intervention indicated	Life-threatening consequences; urgent intervention indicated
Sinus tachycardia[c]	Asymptomatic, intervention not indicated	Symptomatic; non-urgent medical intervention indicated	Severe, medically significant, medical intervention indicated	Life-threatening consequences; urgent intervention indicated

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

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

[c] Derived from Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, Published May 28, 2009 (v4.03: June 14, 2010).

16.3. Appendix 3: References

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