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Protocol Title: A Phase 2, Randomized, Double-blind, Multi-dose, Dose Finding Study to Evaluate the Safety, Tolerability and Immunogenicity of AFX3772 Compared with PCVs in Healthy Infants

Protocol Number: AFX3772-003 (219651)

Compound: AFX3772 (also referred to as Pn-MAPS24v)

Brief Title: A Phase 2 Study to Evaluate the Safety, Tolerability, and Immunogenicity of Different Dose Levels of AFX3772 Vaccine in Healthy Infants

Study Phase: Phase 2

Protocol Date: 04 May 2022

Protocol Amendment 1 Date: 22 November 2022

Protocol Amendment 2 Date: 22 March 2023

Protocol Amendment 3 Date: 09 October 2023

Protocol Amendment 4 Date: 14 May 2024

Protocol Amendment 5 Date: 27 Jan 2025

Version: 1.0

Sponsor Name: GSK Biologicals SA

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Pediatric Investigational Plan	**EMEA-002641-PIP01-19**

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Approval Date: 27 Jan 2025

Sponsor Signatory:

Mariano Young, MD

Clinical Project Lead

Date

Medical Monitor's Name and Contact Information

Protocol-specific Sponsor's contact information can be found in the Investigator Study File Binder (or equivalent).

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The signature below constitutes approval of the study in full accordance with the provisions of this protocol and the attachments. I agree to conduct this study in compliance with the protocol, in-country and local regulatory requirements, applicable United States (US) Code of Federal Regulations (CFR) and ICH Good Clinical Practices (E6).

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GSK.
- To assume responsibility for the proper conduct of the study at this site.
- That I will comply with the terms of the site agreement.
- To comply with local bio-safety legislation.
- To ensure that all persons assisting me with the study are adequately informed about the GSK study intervention and other study-related duties and functions as described in the protocol.
- To supervise any individual or party to whom I have delegated study-related duties and functions conducted at the study site.
- To ensure that any individual or party to whom I have delegated study-related duties and functions conducted at the study site are qualified to perform those study-related duties and functions.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained on-site or elsewhere without the approval of GSK and the express physical or digital informed consent of the participant and/or the participant's LAR.
- To perform no biological assays on the clinical samples other than those described in the protocol or its amendment(s).
- To co-operate with representative(s) of GSK in the monitoring and data management processes of the study with respect to data entry and resolution of queries about the data.
- To have control of all essential documents and records generated under my responsibility before, during, and after the study.
- That I have been informed that certain regulatory authorities require the Sponsor to obtain and supply, as necessary, details about the investigator(s)' ownership interest in the Sponsor or the study intervention(s), and more generally about their financial ties with the Sponsor. GSK will use and disclose the information solely for the purpose of complying with regulatory requirements.

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Hence, I:

- Agree to supply GSK with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the study and for 1 year following completion of the study.
- Agree that GSK may disclose any information about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK with an updated Curriculum Vitae and all other documents required by regulatory agencies for this study.

Printed Name of Investigator

Signature of Investigator

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DOCUMENT HISTORY	
Document	**Date of Issue**
Amendment 5	27 Jan 2025
Amendment 4	14 May 2024
Amendment 3	09 October 2023
Amendment 2	22 March 2023
Amendment 1	22 November 2022
Original Protocol	04 May 2022

Overall rationale for the current Amendment:

CCI

[redacted]

Section # and title	Description of change	Brief rationale
CCI	[redacted]	[redacted]

Section # and title	Description of change	Brief rationale
CCI	[redacted]	[redacted]

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Note: PCV13 was used as the comparator in Part 1 of the study and PCV20 will be used as the comparator for Part 2 of the study. Unless specified, PCV is synonymous to PCV13 (Part 1) and PCV20 (Part 2) throughout this Protocol.

1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title	A Phase 2, Randomized, Double-blind, Multi-dose, Dose Finding Study to Evaluate the Safety, Tolerability and Immunogenicity of AFX3772 Compared with PCVs in Healthy Infants
Brief Title	A Phase 2 Study to Evaluate the Safety, Tolerability and Immunogenicity of Different Dose Levels of AFX3772 Vaccine in Healthy Infants
Rationale	The purpose of this study is to confirm the safety and tolerability of a 3+1 regimen of AFX3772 in healthy infants and to evaluate the immunogenicity of different dose levels of AFX3772 as compared with PCVs.

Objectives, Endpoints, and Estimands

Objectives	Endpoints
Primary	
• To describe the safety and tolerability profiles of different dose levels (1**, 2 and 5 µg) of AFX3772 compared with PCV* with respect to the proportion of participants with AEs	Following any vaccination with AFX3772 or PCV*: • Solicited injection site AEs (tenderness, redness/erythema and swelling) from Day 1 through Day 7 post-vaccination • Solicited systemic AEs (irritability, fever, decrease of appetite, increased sleep, and decrease in sleep) from Day 1 through Day 7 • AEs Day 1 through Day 30 • SAEs through completion of study participation (6 months post last study vaccination)
Secondary	
• To evaluate the immunogenicity of different dose levels of AFX3772 (1**, 2 and 5 µg) compared with PCV*	• The proportion of participants with a pneumococcal serotype-specific IgG concentration ≥0.35 µg/mL or corresponding threshold at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4 • Serotype-specific IgG GMC at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4
Exploratory	
CCI [redacted]	

Objectives	Endpoints
CCI	
AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; OPA: opsonophagocytic activity; PCV13: Prevnar 13; PCV20: Prevnar 20; PD: post-dose; SAE: serious adverse event *:PCV13 [Part 1] and PCV20 [Part 2]. ** in Part 1 only	
Estimands	
Primary Estimand description: Percentage of pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age who receive at least 1 vaccination with either AFX3772, or PCV*(*PCV13 [Part 1] and PCV20 [Part 2]) and develop a solicited local or systemic AE within 7 days of each vaccination, AEs through Day 30 after each vaccination, and SAEs through completion of study participation. • A treatment policy strategy is used for assessing safety irrespective of missed vaccination or taking of prohibited medication/vaccination.	

Overall Design: This is a Phase 2, randomized, double-blind, multi-dose, dose finding study in infants approximately 2 months of age at the time of first vaccination who are naïve to licensed or investigational pneumococcal vaccines.

Brief Summary: This is a Phase 2 clinical study to support the use of AFX3772 in healthy infants for the prevention of pneumococcal disease. The purpose of this study is to determine the safety, tolerability, and immunogenicity of different dose levels of AFX3772 compared with PCV.

Up to 480 healthy infants approximately 2 months of age are planned to be enrolled and receive 4 doses of study vaccine over 8 protocol-defined visits spanning a duration of approximately 18 to 21 months. Part 1 is the dose escalation, lead-in portion of the study in which 40 infants at each dose level were randomized 3:1 in sequential cohorts of increasing doses of AFX3772 (Cohort 1: 1 µg; Cohort 2: 2 µg and Cohort 3: 5 µg) or PCV13. Enrollment in Cohorts 2 and 3 proceeded following Data Monitoring Committee (DMC) review of cumulative safety and tolerability data through Day 7 from preceding cohorts. Prior to the completion of vaccination series in Part 1, the study vaccination was halted and urgent safety measures, i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines, were implemented for participants of Part 1 and the start of recruitment in Part 2 was paused at the same time.

Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 1, Part 2 will start with target recruitment of a total of 360 healthy infants. In the dose escalation lead-in step of Part 2 (i.e., Part 2a), at each dose level, 45 infants will be randomized 2:1 into 2 cohorts of increasing dose levels of AFX3772 (Cohort 4: 2 µg and Cohort 5: 5 µg) or PCV20.

Remaining 270 infants will be enrolled in Part 2b and randomized equally to receive either AFX3772 at dose levels (2 or 5 µg) or PCV20. Enrollment in Cohorts 5 and Part 2b will proceed following Data Monitoring Committee (DMC) review of cumulative safety and tolerability data through Day 7 from the preceding cohort.

On 31 October 2024, GSK was informed of a study hold put in place by the FDA for the ongoing infant study. This study hold has been prompted by potential technical product quality concerns. As a consequence, enrollment and vaccination with investigational pneumococcal vaccine were stopped in the study.

Vaccinations are scheduled at approximately 2, 4, and 6 months of age for the primary series and 12 months of age for the booster dose. Subsequent immunogenicity assessments are scheduled at 30 days PD2, 30 days PD3, pre-dose 4 and 30 days PD4. Interim analyses of safety and immunogenicity 30 days PD3, 30 days PD4 and a final analysis of safety and immunogenicity are planned for the study.

Number of Participants: Up to 480 healthy infants are planned to be enrolled in this study with a subset of 120 initially enrolled (Part 1) followed by 360 participants (Part 2). Accounting for an 85% evaluable rate, such a sample size predicts approximately 102 evaluable participants will be available for analysis in each treatment arm in Part 2.

Note: *Enrolled* means a participant's legally acceptable representative (LAR)'s agreement to participate in a clinical study following completion of the informed consent process. Potential participants who are pre-screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled, unless otherwise specified by the protocol.

Intervention Groups and Duration: For Part 1 participants, the Sponsor estimates the study will require approximately 10 months from the time the first participant's LAR signs the informed consent until the last participant's last study-related telephone call or visit, and for Part 2 participants, the study duration will be approximately 23 months.

For the purposes of analysis and reporting, the overall study ends when the last data available are received by the Sponsor.

Data Monitoring Committee: Yes

Internal Data Review Committee: Yes

Safety Review Team: Yes

Executive Oversight Committee: No

Steering Committee: No

Clinical Adjudication Committee: No

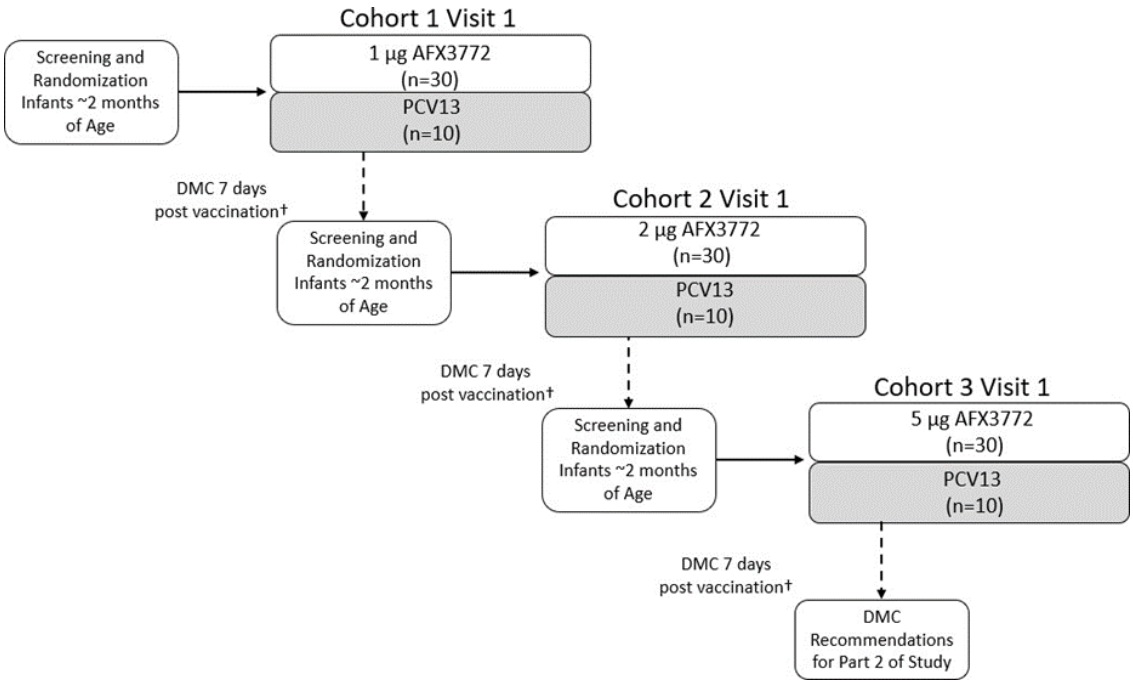
An independent DMC has been appointed for this study. This external group of experts will conduct periodic reviews of blinded and, if they request it, unblinded safety and tolerability data for each cohort. The DMC provides recommendations to ensure participant safety and ethical integrity throughout the study. The decision to progress to a higher AFX3772 dose level will be determined by the Sponsor, based on the safety and tolerability data available at the time and the DMC's recommendation. In addition, the

DMC will review all available data at 6-month intervals to ensure the benefit/risk profile continues to support the continued dosing of AFX3772 in infants. If the DMC does not recommend moving all AFX3772 dose levels into Part 2, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to the next step.

The DMC will consist of external independent physician(s) and a statistician based on expertise in vaccine clinical studies and other considerations, such as the disease area and age of the participants (e.g., infants). A DMC charter will detail the roles, responsibilities, and procedures for safety reviews.

1.2. Schema

Figure 1 Study Schema Part 1 - Dose Escalation



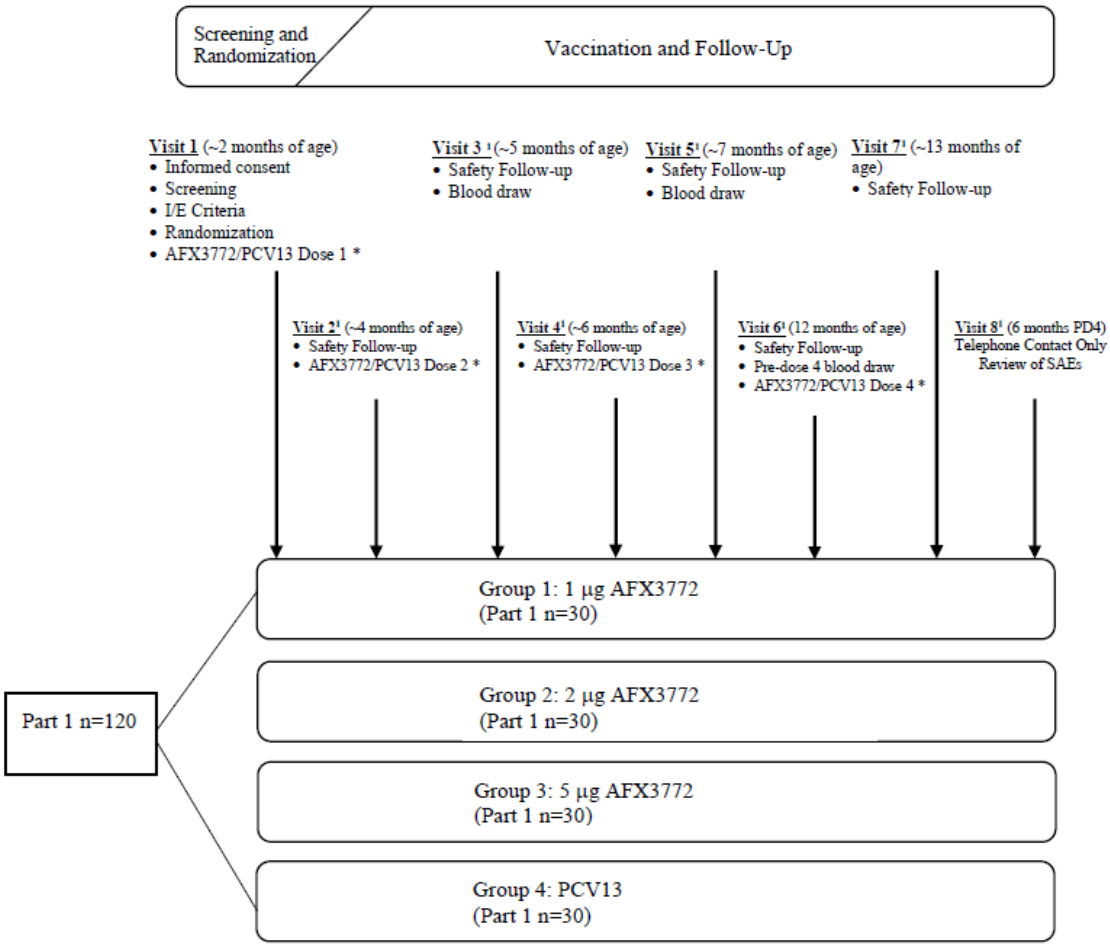
DMC: Data Monitoring Committee; PCV13: Prevnar 13

†DMC reviews safety data through Day 7 for each cohort (approximately 40 participants) after all participants have completed eDiary safety assessments through Day 7 postvaccination. For Cohorts 2 and 3, DMC will also review all safety data available for prior cohorts.

Subsequent cohorts start after DMC recommendation to dose escalate.

Infants enrolled in Part 1 follow the same visit schedule as depicted in Figure 2.

Figure 2 Study Schema Part 1



PCV13: Prevnar 13

*Telephone contact occurs on Day 8 postvaccination for safety follow-up and eDiary review

Part 2 starts after DMC review of Cohort 3 safety data through Day 7, plus cumulative safety data from prior cohorts of Part 1 and agreement to proceed to full enrolment.

Concomitant vaccines are permitted as per local requirements.

[1] Applicable only for Part 1 participants affected by Urgent Safety Measure i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines following the decision to halt study vaccinations and the Dear-Investigator-Letter (DIL) sent on 24 February 2023:

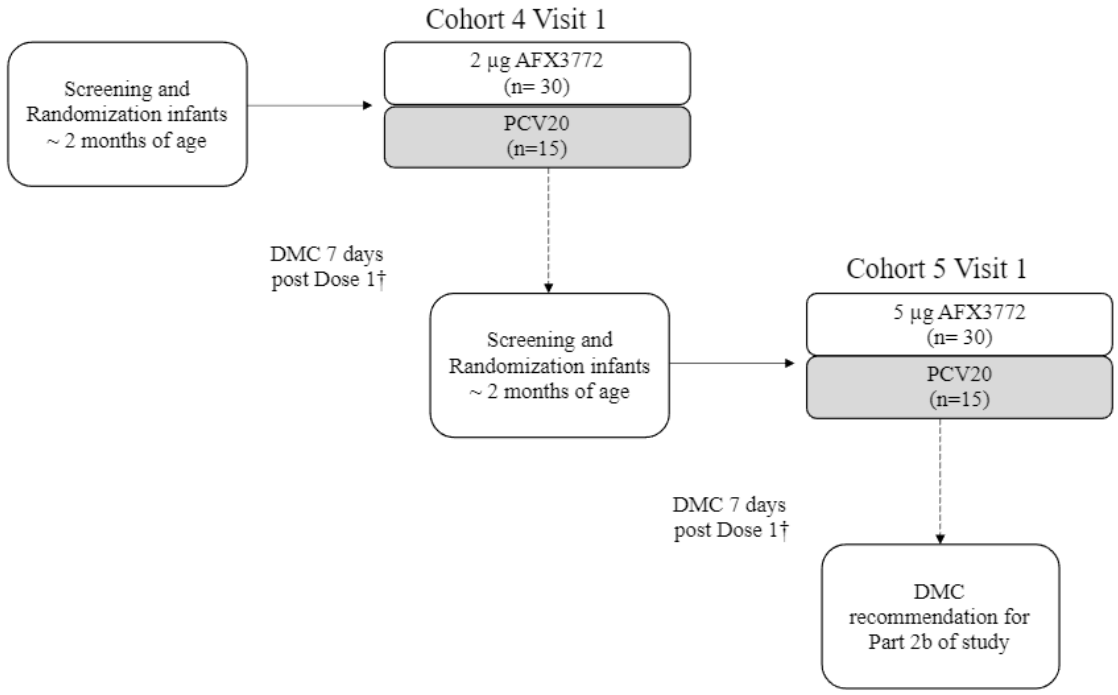
Timepoints impacted by protocol amendment 2:

- Visit 2 – SoC pneumococcal vaccine to be administered, if not received prior 24 February 2023
- Visit 3 – not applicable for participants who received SoC pneumococcal vaccine as Dose 2.
- Visit 4 – SoC pneumococcal vaccine to be administered, if not received prior 24 February 2023
- Visit 6 –is 6-months post Dose 3 safety follow-up and timepoint for administration of SoC pneumococcal vaccine booster dose for all Part 1 participants (first subject's booster dose expected in April 2023). Visit 6 is to be performed at study site if this option is exercised by participants' LARs or via TC if participants' LARs opt to receive the booster dose at their primary care center.

- Visit 7 and 8 are not applicable for participants who receive SoC pneumococcal vaccine as booster dose, no specific safety and immunogenicity assessment will be performed following the booster dose with SoC

Note: Visit 5 (1-month post Dose 3) includes blood sampling and is maintained for all Part 1 participants (including those who received SoC pneumococcal vaccine) to assess a need for rescue vaccination.

Figure 3 Study Schema Part 2a – Dose escalation



DMC: Data Monitoring Committee; PCV20: Prevnar 20

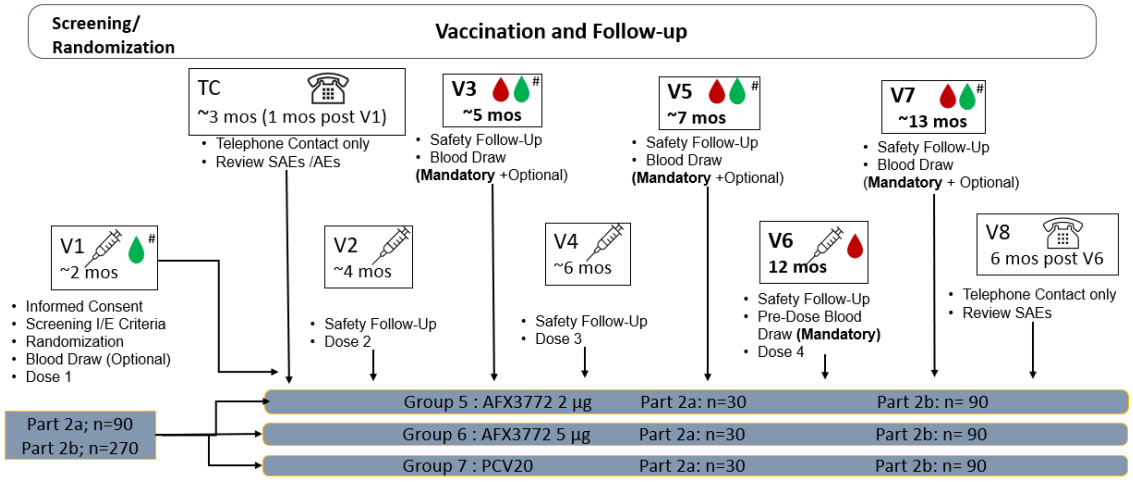
†DMC reviews safety data through Day 7 post-dose 1 for each cohort (approximately 45 participants), after all participants have completed eDiary safety assessments through Day 7 postvaccination.

Part 2b starts after DMC review of Part 2a Cohort 5 safety data through Day 7 plus cumulative safety data of Part 2a and agreement to proceed to full enrollment.

Subsequent cohort starts after DMC recommendation to dose escalate.

Infants enrolled in Part 2 will follow the same visit schedule as depicted in Figure 4.

Figure 4 Study Schema Part 2



n: number of participants; mos: months; PCV20: Prevnar 20

TC = Safety follow-up telephone contact at 1-month post-Dose 1,

Part 2b start after DMC review of Part 2a Cohort 2 safety data through Day 7 post-dose 1 and agreement to proceed to full enrollment.

Applicable only for Part 2 participants affected by FDA clinical hold on 31 October 2024, i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines following the decision to halt study vaccinations and the Dear-Investigator-Letter (DIL) sent on 01 and 04 November 2024:

Timepoints impacted by protocol amendment 5:

- ***Visit 2 – SoC pneumococcal vaccine to be administered, if not received prior 31 October 2024***
- ***Visit 4 – SoC pneumococcal vaccine to be administered, if not received prior 31 October 2024***
- ***Visit 6 –is 6-months post Dose 3 safety follow-up and timepoint for administration of SoC pneumococcal vaccine booster dose for all Part 2 participants. Visit 6 is to be performed at study site if this option is exercised by participants' LARs or via TC if participants' LARs opt to receive the booster dose at their primary care center as per local practice; this will be outside the scope of the study.***
- ***No specific safety and immunogenicity assessment will be performed before or following the booster dose with SoC.***
- ***No collection on solicited AEs on Day 1 to Day 7 following vaccination with SoC vaccine.***
- ***Collection of unsolicited AEs from Day 1 through Day 30 following vaccination with each SoC vaccine.***
- ***Immunogenicity to be done only at Visit 5 to assess need for rescue vaccination.***
- ***SAEs will be collected for 6 months post Dose 3 (primary series).***
- ***Collection of eDiary (solicited) safety data will be halted.***
- ***Collection of optional blood draws will be discontinued.***

1.3. Schedule of Assessments

Table 1 Schedule of Assessments (SoA) for Part 1

Assessments	Intervention										Follow-up		Comments
Visit Number	**1**	**TC1**	**2[4]**	**TC2[4]**	**3[1,4]**	**4[4]**	**TC3[4]**	**5[1,4]**	**6[4]**	**TC4[4]**	**7[1,4]**	**8[4]**	
Scheduled Time	**Dose 1 (~2 months of age)**	**1-week post Dose 1**	**Dose 2 (~4 months of age)**	**1 week post Dose 2**	**1 month post Dose 2 (~5 months of age)**	**Dose 3 (~6 months of age)**	**1 week post Dose 3)**	**1 month post Dose 3 (~7 months of age)**	**Dose 4 (~12 months of age)**	**1 week post Dose 4**	**1 month post Dose 4**	**6-months post Dose 4**	
Visit Window[2]	**≥42 to ≤90 Days of age**	**Day 8 to Day 10 after Dose 1**	**49 to 70 days after Dose 1**	**Day 8 to Day 10 after Dose 2**	**Day 28 to Day 42 after Dose 2**	**49 to 70 days after Dose 2**	**Day 8 to Day 10 after Dose 3**	**Day 28 to Day 42 after Dose 3**	**365 to 392 days of age**	**Day 8 to Day 10 after Dose 4**	**Day 28 to Day 42 after Dose 4**	**Day 166 to Day 194 after Dose 4**	
Informed Consent	X												Informed consent must be obtained prior to the performance of any study-related procedure and before randomization.
Inclusion/ Exclusion Criteria	X†												
Demographics	X†												
Medical History	X†												Clinically significant medical history and vaccination history after birth will be collected at screening prior to vaccination in order to assess inclusion/ exclusion criteria.

Assessments	Intervention										Follow-up		Comments
Visit Number	**1**	**TC1**	**2[4]**	**TC2[4]**	**3[1, 4]**	**4[4]**	**TC3[4]**	**5[1, 4]**	**6[4]**	**TC4[4]**	**7[1, 4]**	**8[4]**	
Scheduled Time	**Dose 1 (~2 months of age)**	**1-week post Dose 1**	**Dose 2 (~4 months of age)**	**1 week post Dose 2**	**1 month post Dose 2 (~5 months of age)**	**Dose 3 (~6 months of age)**	**1 week post Dose 3)**	**1 month post Dose 3 (~7 months of age)**	**Dose 4 (~12 months of age)**	**1 week post Dose 4**	**1 month post Dose 4**	**6-months post Dose 4**	
Visit Window[2]	**≥42 to ≤90 Days of age**	**Day 8 to Day 10 after Dose 1**	**49 to 70 days after Dose 1**	**Day 8 to Day 10 after Dose 2**	**Day 28 to Day 42 after Dose 2**	**49 to 70 days after Dose 2**	**Day 8 to Day 10 after Dose 3**	**Day 28 to Day 42 after Dose 3**	**365 to 392 days of age**	**Day 8 to Day 10 after Dose 4**	**Day 28 to Day 42 after Dose 4**	**Day 166 to Day 194 after Dose 4**	
Prior/Concomitant Medication	X†	X	X†	X	X	X†	X	X	X†	X	X	X	All concomitant medications or therapies during the eDiary reporting period or associated with SAEs through Visit 8 will be collected. This also includes concomitant vaccinations. Refer to Section 6.8.1.
Complete Physical Examination	X†												
Targeted Physical Examination			X†			X†			X†				
Body Temperature	X†		X†		X	X†			X†				Rectal or axillary temperature will be taken at the site prior to study vaccination according to the site's standard of care. Participants who have febrile illness (rectal

Assessments	Intervention										Follow-up		Comments
Visit Number	1	TC1	2[4]	TC2[4]	3[1, 4]	4[4]	TC3[4]	5[1, 4]	6[4]	TC4[4]	7[1, 4]	8[4]	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
													temperature ≥38.1°C [≥100.5°F] or axillary temperature ≥37.8°C [≥100.0°F]) occurring at or within 72 hours of Visits 1, 2, 4 and 6 must be rescheduled.
Blood Sample (***4 mL***)					X			X[3]	X†		X		
Randomization	X†												

Assessments	Intervention										Follow-up		Comments
Visit Number	1	TC1	2[4]	TC2[4]	3[1,4]	4[4]	TC3[4]	5[1,4]	6[4]	TC4[4]	7[1,4]	8[4]	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
AFX3772 or PCV13 Administration (Blinded)	X		X			X			X				Before vaccine administration, the investigator (or designee) must review medical history to ensure that the participant has no new contraindication to the vaccine. This information must be documented in the participant's chart. Any concomitant vaccinations must not be administered in the same limb as the study vaccine.
Provide eDiary	X												The participant's LAR will be instructed to measure reactogenicity/tolerability and temperature daily from Day 1 through Day 7 following each vaccination, and to record observed events

Assessments	Intervention										Follow-up		Comments
Visit Number	1	TC1	2[4]	TC2[4]	3[1,4]	4[4]	TC3[4]	5[1,4]	6[4]	TC4[4]	7[1,4]	8[4]	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
													in the eDiary. LAR will also be instructed to contact the study site in case of any hospitalization or emergency room visit or if the participant experiences a severe intensity solicited event, as prompted by the eDiary.
30-minute[5] Postvaccination Observation Period	X		X			X			X				To be performed by blinded study site personnel only.
Review eDiary with participant's LAR		X		X			X			X			On Day 8 postvaccination, the site will contact the participant's LAR via telephone for safety follow-up, concomitant medications and eDiary review.

Assessments	Intervention										Follow-up		Comments
Visit Number	1	TC1	2[4]	TC2[4]	3[1,4]	4[4]	TC3[4]	5[1,4]	6[4]	TC4[4]	7[1,4]	8[4]	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
Collect eDiary from participant											X		
AE monitoring	⟷												AEs (non-serious) are to be reported Day 1 through Day 30 following each vaccination. SAEs and deaths are to be reported within 24 hours of awareness throughout the duration of an individual's study participation.

AE: adverse event; CP1: fusion protein 1; IgG: immunoglobulin G; IRT: interactive response technology; LAR: legally acceptable representative; PCV13: Prevnar 13; PD: post-dose: SAE: serious adverse event; TC: telephone contact

†To be performed prior to administration of study vaccine by the investigator or a medically qualified designee.

1. Refer to Section 10.6 Appendix 6: Clinical Study Continuity for alternative visit procedures allowed at the noted visits in the case of a crisis.
2. For calculating the visit windows, the day of vaccination is considered Day 1. To calculate visit windows for subsequent vaccinations, confirm participant date of birth and ensure the age of the participant will fall within the appropriate age range for each study visit.
3. Following analysis of PD3 immune responses, investigators will be informed by the Sponsor if participants met rescue criteria (defined as the pneumococcal serotype-specific IgG concentration below 0.35 µg/mL for 3 or more of the 13 serotypes contained in PCV13).
4. Applicable only for Part 1 participants affected by Urgent Safety Measure i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines following the decision to halt study vaccinations and the Dear-Investigator-Letter (DIL) sent on 24 February 2023:

Study timepoints impacted by amendment 2:

- Visit 2 – SoC pneumococcal vaccine is administered, if not received prior 24 February 2023
- Visit 3 –not applicable for participants who received SoC pneumococcal vaccine as Dose 2
- Visit 4 – SoC pneumococcal vaccine is administered, if not received prior 24 February 2023
- TC2, TC3 and TC4 are not applicable for participants who receive the SoC pneumococcal vaccine.
- Visit 6 – is 6-months post Dose 3 safety follow-up and timepoint for administration of SoC pneumococcal vaccine booster dose for all Part 1 participants (first subject's booster dose expected in April 2023). Visit 6 is to be performed at study site if this option is exercised by participants' LARs or via TC if participants' LARs opt to receive the booster dose at their primary care center.
- Visit 7 and Visit 8 – not applicable for participants who receive SoC pneumococcal vaccine as booster dose. no specific safety and immunogenicity assessment will be performed following the booster dose with SoC.

Note: Visit 5 (1-month post Dose 3) includes blood sampling and is maintained for all Part 1 participants (including those who received SoC pneumococcal vaccine) to assess a need for rescue vaccination.

5. Applicable following administration of SoC pneumococcal conjugate vaccines: Duration of observation period as required by standard medical practice [CDC., 2023].
6. All unscheduled visits and telephone contacts must be reported in the eCRF.

Table 2 Schedule of Assessments (SoA) for Part 2

Assessments	Intervention											Follow-up		Comments
Visit Number	1	TC1	TC2	2	TC3	3[1]	4	TC4	5[1]	6	TC5	7[1]	TC6	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	1 month post Dose 1 (~3 months of age)	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	Day 28 to Day 42 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
Informed Consent	X													Informed consent must be obtained prior to the performance of any study-related procedure and before randomization.
Inclusion/ Exclusion Criteria	X†													
Demographics	X†													
Medical History	X†													Clinically significant medical history and vaccination history after birth will be collected at screening prior to vaccination in order to assess inclusion/ exclusion criteria.

Assessments	Intervention											Follow-up		Comments
Visit Number	1	TC1	TC2	2	TC3	3[1]	4	TC4	5[1]	6	TC5	7[1]	TC6	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	1 month post Dose 1 (~3 months of age)	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	Day 28 to Day 42 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
Prior/ Concomitant Medication	X†	X	X	X†	X	X	X†	X	X	X†	X	X	X	All concomitant medications or therapies during the eDiary reporting period or associated with SAEs through Visit 8 will be collected. This also includes concomitant vaccinations. Refer to Section 6.8.1.
Complete Physical Examination	X†													Body weight will be collected at Visit 1.
Targeted Physical Examination				X†			X†			X†				
Body Temperature	X†			X†		X	X†			X†				Rectal or axillary temperature will be taken at the site prior to study vaccination according to the site's standard of care.

Assessments	Intervention											Follow-up		Comments
Visit Number	1	TC1	TC2	2	TC3	3[1]	4	TC4	5[1]	6	TC5	7[1]	TC6	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	1 month post Dose 1 (~3 months of age)	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	Day 28 to Day 42 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
														Participants who have febrile illness (rectal temperature ≥38.0°C [≥100.4°F] or axillary temperature ≥37.5°C [≥99.1°F]) occurring at or within 72 hours of Visits 1, 2, 4 and 6 must be rescheduled.
Blood Sample (For Part 2) (Mandatory)						X			X[3]	X†		X		For details of amount of blood draw, see Table 8.
Blood Sample (For Part 2b) (Optional)	X					X			X			X		For details of amount of blood draw, see Table 8.
Randomization	X†													

Assessments	Intervention											Follow-up		Comments
Visit Number	1	TC1	TC2	2	TC3	3[1]	4	TC4	5[1]	6	TC5	7[1]	TC6	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	1 month post Dose 1 (~3 months of age)	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	Day 28 to Day 42 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
AFX3772 or PCV20 Administration (Blinded)	X			X			X			X				Before vaccine administration, the investigator (or designee) must review medical history to ensure that the participant has no new contraindication to the vaccine. This information must be documented in the participant's chart. Any concomitant vaccinations must not be administered in the same limb as the study vaccine.
Provide eDiary	X													The participant's LAR will be instructed to measure reactogenicity/tolerability and temperature daily

Assessments	Intervention											Follow-up		Comments
Visit Number	1	TC1	TC2	2	TC3	3[1]	4	TC4	5[1]	6	TC5	7[1]	TC6	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	1 month post Dose 1 (~3 months of age)	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	Day 28 to Day 42 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
														from Day 1 through Day 7 following each vaccination, and to record observed events in the eDiary. LAR will also be instructed to contact the study site in case of any hospitalization or emergency room visit or if the participant experiences a severe intensity solicited event, as prompted by the eDiary.
30-minute[5] Postvaccination Observation Period	X			X			X			X				To be performed by blinded study site personnel only.
Review eDiary with		X	X		X			X			X			On Day 8 postvaccination, the site will contact the

Assessments	Intervention											Follow-up		Comments
Visit Number	1	TC1	TC2	2	TC3	3[1]	4	TC4	5[1]	6	TC5	7[1]	TC6	
Scheduled Time	Dose 1 (~2 months of age)	1-week post Dose 1	1 month post Dose 1 (~3 months of age)	Dose 2 (~4 months of age)	1 week post Dose 2	1 month post Dose 2 (~5 months of age)	Dose 3 (~6 months of age)	1 week post Dose 3)	1 month post Dose 3 (~7 months of age)	Dose 4 (~12 months of age)	1 week post Dose 4	1 month post Dose 4	6-months post Dose 4	
Visit Window[2]	≥42 to ≤90 Days of age	Day 8 to Day 10 after Dose 1	Day 28 to Day 42 after Dose 1	49 to 70 days after Dose 1	Day 8 to Day 10 after Dose 2	Day 28 to Day 42 after Dose 2	49 to 70 days after Dose 2	Day 8 to Day 10 after Dose 3	Day 28 to Day 42 after Dose 3	365 to 392 days of age	Day 8 to Day 10 after Dose 4	Day 28 to Day 42 after Dose 4	Day 166 to Day 194 after Dose 4	
participant's LAR[6]														participant's LAR via telephone for safety follow-up, concomitant medications and eDiary review.
Collect eDiary from participant												X		
AE monitoring	←												→	AEs (non-serious) are to be reported Day 1 through Day 30 following each vaccination. SAEs and deaths are to be reported within 24 hours of awareness throughout the duration of an individual's study participation.

AE: adverse event; CP1: fusion protein 1; IgG: immunoglobulin G; IRT: interactive response technology; LAR: legally acceptable representative; PCV20: Prevnar 20; PD: post-dose: SAE: serious adverse event; TC: telephone contact

†To be performed prior to administration of study vaccine by the investigator or a medically qualified designee.

1. Refer to Section 10.6 Appendix 6: Clinical Study Continuity for alternative visit procedures allowed at the noted visits in the case of a crisis.
2. For calculating the visit windows, the day of vaccination is considered Day 1. To calculate visit windows for subsequent vaccinations, confirm participant date of birth and ensure the age of the participant will fall within the appropriate age range for each study visit.
3. Following analysis of PD3 immune responses, investigators will be informed by the Sponsor if participants met rescue criteria (defined as the pneumococcal serotype-specific IgG concentration below 0.35 µg/mL for 5 or more of the 20 serotypes contained in PCV20).
4. All unscheduled visits and telephone contacts must be reported in the eCRF. See Section 8.1.1 for unscheduled visit assessments.
5. Applicable following administration of SoC pneumococcal conjugate vaccines: Duration of observation period as required by standard medical practice [CDC., 2023].
6. In-App reminders in the eDiary. There will be a call from the site to the LAR in case of missing data for the day. Also, there will be training for the LAR and sites for eDiary completion.

Note: The optional blood draws will be collected in Part 2b only. The mandatory blood samples are collected in Part 2a and Part 2b throughout the study.

Note: Applicable only for Part 2 participants affected by FDA clinical hold on 31 October 2024, i.e., the replacement of study vaccines with local SoC pneumococcal conjugate vaccines following the decision to halt study vaccinations and the Dear-Investigator-Letter (DIL) sent on 01 and 04 November 2024:

Study timepoints impacted by amendment 5:

- ***Visit 2 – SoC pneumococcal vaccine is administered, if not received prior 31 October 2024***
- ***Visit 4 – SoC pneumococcal vaccine is administered, if not received prior 31 October 2024***
- ***Visit 6 – is 6-months post Dose 3 safety follow-up and timepoint for administration of SoC pneumococcal vaccine booster dose for all Part 2 participants. Visit 6 is to be performed at study site if this option is exercised by participants' LARs or via TC if participants' LARs opt to receive the booster dose at their primary care center as per local practice; this will be outside the scope of the study.***
- ***No specific safety and immunogenicity assessment will be performed before or following the booster dose with SoC.***
- ***No collection on solicited AEs on Day 1 to Day 7 following vaccination with SoC vaccine.***
- ***Collection of unsolicited AEs from Days 1 through 30 following vaccination with each SoC vaccine.***
- ***Immunogenicity to be done only at Visit 5 to assess need for rescue vaccination.***
- ***SAEs will be collected for 6 months post Dose 3.***
- ***Collection of eDiary (solicited) safety data will be halted.***
- ***Collection of optional blood draws will be discontinued.***

2. INTRODUCTION

Affinivax has developed an investigational 24-valent pneumococcal vaccine (referred to as AFX3772) for the prevention of pneumococcal disease caused by the serotypes contained in the vaccine. AFX3772 contains 24 serotypes of *Streptococcus pneumoniae* and a unique fusion protein (CP1) created by the fusion of 2 pneumococcal proteins (sp1500 and sp0785), which has the potential to induce T- and B-cell responses and provide extended protection. This novel investigational pneumococcal vaccine uses the MAPS ***technology*** [Zhang, 2013] containing pneumococcal capsular polysaccharides (PS) individually biotinylated and complexed with CP1. It contains 11 additional PS (serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F) compared to the pneumococcal conjugate vaccine Prevnar13 (PCV13) (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) or 4 additional PS (serotypes 2, 9N, 17F and 20B) compared the Prev***(e)***nar 20 (PCV20) (serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F).

2.1. Study Rationale

This clinical trial, to be conducted in healthy infants approximately 2 months of age (42 to 90 days), is a Phase 2, proof-of-concept study to support advancement of AFX3772 into Phase 3 pediatric clinical development. AFX3772-003 will evaluate the safety, tolerability, and immunogenicity of different AFX3772 dose levels (1, 2 and 5 µg, with 1 µg used only in Part 1) administered as a 3+1 regimen (at 2, 4 and 6 months of age for the primary series and 12 months of age for the booster dose). Data in adults and toddlers demonstrated that AFX3772 has an acceptable safety and tolerability profile and generates antibody responses to all 24 serotypes contained in the vaccine [***Borys, 2024; Borys, 2025; Chichili, 2024***].

2.2. Background

S. pneumoniae is a gram-positive encapsulated bacterium that is a major cause of human disease, ranging from acute otitis media to meningitis and bacteremia. The capsular PS on the cell surface is a major virulence factor, and antibodies to the capsule are protective. Currently, over 90 different pneumococcal serotypes have been identified.

S. pneumoniae can cause both invasive and noninvasive disease. Invasive pneumococcal disease (IPD) is defined as *S. pneumoniae* isolated from a normally sterile anatomic site (e.g., cerebrospinal fluid, blood, joint fluid, pleural fluid or peritoneal fluid). The highest incidence of IPD occurs in elderly adults and in young children < 2 years of age. Noninvasive or mucosal disease is more common than IPD and includes acute otitis media, sinusitis and nonbacteremic pneumonia.

Pneumococcal conjugate vaccines use a chemical process to link pneumococcal PS to a carrier protein that creates a PS-protein complex capable of inducing T-cell-dependent antibody responses. Prevnar 13 has been approved for children 6 weeks to 17 years of age

for the prevention of IPD caused by the 13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) contained in the vaccine and for the prevention of acute otitis media in children up to 6 years of age for the 7 serotypes in Prevnar (4, 6B, 9V, 14, 18C, 19F, 23F).

PCV20 has been approved for children 6 weeks of age and older for the prevention of IPD caused by the 20 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F) contained in the vaccine and for the prevention of otitis media in children 6 weeks to 5 years of age for the 7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) in Prevnar.

Widespread use of PCVs has reduced the burden of pneumococcal disease due to the serotypes contained in the vaccines in vaccinated children, as well as unvaccinated individuals through herd protection [Pilishvili, 2010; Feikin, 2013; CDC, 2008; Moore, 2015]. However, an increase in the burden of IPD caused by serotypes not contained in currently available vaccines has been observed in settings where PCV vaccines were routinely implemented.

AFX3772 is a 24-valent pneumococcal vaccine (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F) being developed for the prevention of pneumococcal disease in children 6 weeks to 17 years of age and adults 18 years of age and older. AFX3772 uses a novel MAPS ***technology*** in which high-affinity, non-covalent binding between biotin and rhizavidin links pneumococcal PS to CP-1 made up of the fusion of 2 conserved pneumococcal proteins (SP1500 and SP0785) [AFX3772 Investigator's Brochure].

AFX3772 aims to provide broad and durable protection by enabling both B- and T-cell responses to pneumococcal PS and proteins [Lu, 2018].

Refer to the AFX3772 Investigator's Brochure (IB) for detailed background, including pneumococcal epidemiology and completed preclinical and clinical trials conducted with AFX3772.

2.2.1. Information on Other Study-related Therapy

Refer to the approved labelling for detailed background information on Prevnar 13 ***and Prevnar 20***.

Prevnar 13 (PCV13) contains 13 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) and is approved in the United States (US) and elsewhere for use in infants 6 weeks of age and older [Prevnar 13 Product information, 2019]. The US Advisory Committee on Immunization Practices (ACIP) recommends PCV13 be given to infants as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a booster dose given at 12 months of age [Nuorti, 2010]. Prevnar 13 can be given concomitantly with other recommended childhood vaccines.

PCV20 contains 20 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F) and is approved in the United States (US) for the prevention of pneumococcal disease in individuals 6 weeks of age and older [Prevnar 20 Product information, 2022].

2.3. Benefit/Risk Assessment

It is unknown if AFX3772, as an investigational vaccine, will have the same benefit/risk profile as PCV13 or PCV20. Because clinical trials are designed to provide information about the safety and efficacy/immunogenicity of an investigational medicine or vaccine, participants in a clinical trial may not directly benefit from study participation.

Approximately 25% (Part 1) and 33% (Part 2) of participants will receive either PCV13 in Part 1 or PCV20 in Part 2, as the comparator in this trial and 75% will receive 1 of 3 different dose levels of AFX3772 (1, 2 or 5 µg) in Part 1 and 67% 2 different dose levels of AFX3772 (2 or 5 µg) in Part 2. Safety data from previous studies in adults and toddlers demonstrate to be evaluated in this study (1, 2 and 5 µg), and no clinically significant findings following administration of AFX3772 have been observed. Based on data in adults and toddlers, AFX3772 is predicted to have a safety and immunogenicity profile comparable to PCV for the shared serotypes while providing additional coverage for the unique serotypes in AFX3772.

At the time this study was being started, there was a COVID-19 global pandemic. The sites were expected to implement risk mitigation strategies for the participant and his or her legally acceptable representative (LAR). Some data can be collected remotely via telephone. A summary of clinically significant risks and proposed mitigations strategies is presented in Table 3.

Table 3 Summary of Clinically Significant Risks and Mitigation Strategies

Clinically Significant Risks	Mitigation Strategies
Study Intervention	
• No clinically significant findings following receipt of AFX3772 (1, 2, or 5 µg) have been observed in previous studies in adults and toddlers. • Based on data in adults and toddlers, AFX3772 is predicted to have a safety and immunogenicity profile comparable to PCV13 for the 13 shared serotypes while providing additional coverage for the 11 unique serotypes in AFX3772	• An independent DMC will review all available data at least every 6 months throughout the study to ensure the benefit/risk profile continues to support the study of AFX3772 in infants. • 30-minute observation period following each dose, plus daily safety/tolerability monitoring for 7 days and collection of adverse events for 30 days following each vaccination. • To mitigate the impact of potential lack of immunogenicity in AFX3772 recipients, immunogenicity data will be evaluated for all participants following completion of the primary series. Rescue vaccination with PCV* will be offered within 3 weeks of site notification. Summary of participants of Part 1 ***and Part 2*** meeting criteria for rescue vaccination will be evaluated by DMC. • Potential for broader serotype coverage from pneumococcal disease.
Study Procedures	

Clinically Significant Risks	Mitigation Strategies
• Discomfort and local reactions associated with blood draws • Burden to families associated with study-specific procedures	• Use of pediatric phlebotomists. • Additional procedures minimized to include only those required to ensure safety and to accomplish objectives of the study. • Potential for more attentive clinical care and safety follow-up. • Make sure the baby is well-hydrated. This helps with finding a vein AND with increasing the serum content in the sample. Qualified staff should be drawing blood (e.g., pediatric phlebotomist). • EMLA cream is permitted per protocol for blood draws and vaccinations and should be used as per site standard of care. • Vein finders can be used as per site standard of care. • Warm compresses can be used as per site standard of care to aid in successful blood draw. • Breastfeeding during the procedure can be a comfort to infants during blood draws and vaccinations. • Vibrating icepack provided to all sites as distraction and pain management tool.

PCV13: Prevnar 13; PCV20: Prevnar 20; DMC: Data Monitoring Committee
*PCV13 (Part 1) and PCV20 (Part 2)

Additional information regarding assessments that are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study required assessments and procedures if study activities are unable to be performed due to a crisis is available in Section 10.6.

3. OBJECTIVES, ENDPOINTS, AND ESTIMANDS

Objectives	Endpoints
Primary	
• To describe the safety and tolerability profiles of different dose levels (1**, 2 and 5 µg) of AFX3772 compared with PCV* with respect to the proportion of participants with AEs	Following any vaccination with AFX3772 or PCV* • Solicited injection site AEs (tenderness, redness/erythema and swelling) from Day 1 through Day 7 post-vaccination • Solicited systemic AEs (irritability, fever, decrease of appetite and increased sleep, and decrease in sleep) from Day 1 through Day 7 • AEs Day 1 through Day 30 • SAEs through completion of study participation (6 months post last study vaccination)
Secondary	
• To evaluate the immunogenicity of different dose levels of AFX3772 (1**, 2 and 5 µg) compared with PCV*	• The proportion of participants with a pneumococcal serotype-specific IgG concentration ≥ 0.35 µg/mL or corresponding threshold at 30 days PD2, 30 days PD3, pre- Dose 4 and 30 days PD4 • Serotype-specific IgG GMC at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4

Objectives	Endpoints
Exploratory	
CCI [redacted]	
AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; OPA: opsonophagocytic activity; PCV13: Prevnar 13; PCV20: Prevnar 20; PD: post-dose; SAE: serious adverse event *PCV13 [Part 1] and PCV20 [Part 2] ** in Part 1 only	
Estimands	
Primary Estimand Description: Percentage of pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age who receive at least 1 vaccination with either AFX3772, or PCV*(PCV13 will be used in Part 1 and PCV20 will be used in Part 2) and develop a solicited local or systemic AE within 7 days of each vaccination, AEs through Day 30 after each vaccination, and SAEs through completion of study participation. • A treatment policy strategy is used for assessing safety irrespective of missed vaccination or taking of prohibited medication/vaccination.	

Primary Estimand

The primary safety estimand is defined by the following 5 attributes:

Treatment: A total of 4 vaccinations 0.5 mL each of either AFX3772 (1, 2 or 5 µg) or PCV administered as 4 separate vaccinations with a 3-dose primary series at approximately 2 (no earlier than 6 weeks of age), 4 and 6 months of age + 1 booster vaccination at 12 months of age.

Population: Pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age at enrollment defined by the inclusion/exclusion criteria for the study who will receive at least 1 vaccination with either AFX3772 or PCV during this study.

Endpoints: See above.

Intercurrent events and strategies: A treatment policy strategy will be used to address intercurrent events of missed vaccination and taking prohibited medication/vaccine.

Population level summary: The percentage of participants who develop each type of AE.

Secondary Estimand(s)

The secondary estimand is defined by the following 5 attributes:

Treatment:

A total of 4 vaccinations 0.5 mL each of either AFX3772 (1, 2 or 5 μg) or PCV administered as 4 separate vaccinations with a 3-dose primary series at approximately 2 (no earlier than 6 weeks of age), 4 and 6 months of age + 1 booster vaccination at 12 months of age.

Population: Pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age at enrollment defined by the inclusion/exclusion criteria for the study.

Endpoints: See above.

Intercurrent events (ICE) and their corresponding strategies: The immunogenicity data after the following ICEs are considered not relevant for assessing the vaccine effect and data post ICE will not be considered for the analysis of the outcome measure.

The following intercurrent events will lead to data exclusion from assessments of immunogenicity:

- Immunogenicity data collected after a participant misses a scheduled vaccination will be excluded.
- If the blood draw occurs outside the visit window, immunogenicity data from that blood draw will be excluded.
- Immunogenicity data collected after a participant receives prohibited systemic corticosteroids at specified doses and duration outlined in the exclusion criteria will be excluded for that assessment only.
- Immunogenicity data collected after a participant has newly diagnosed immunologic abnormality occurring within the first 2 weeks after receiving the study vaccine will be excluded.

Population level summary: Summaries will be provided after the second vaccination, after the third vaccination and pre- and post-booster vaccination. By-treatment summaries for each of the endpoints listed above are:

- The percentage of participants with an IgG concentration ≥0.35 μg/mL for each serotype, the 95% confidence interval (CI) and the difference (95% CI) between each AFX3772 dose level and PCV, the geometric mean concentration (GMC) (95% CI) for each serotype-specific IgG concentration and the ratio of the GMCs (95% CI) (each AFX3772 dose level/PCV). The antibody GMCs and the corresponding 2-sided 95% CIs will be calculated using logarithmically transformed values and the Student t distribution for the CIs.

4. STUDY DESIGN

4.1. Overall Design

This is a Phase 2, randomized, double-blind, multi-dose, dose finding study in infants approximately 2 months of age at the time of first vaccination who are naïve to licensed or investigational pneumococcal vaccines. The safety, tolerability and immunogenicity of different dose levels of AFX3772 will be studied and compared to PCV (active control).

This study will be conducted at approximately 65-75 study sites in the US including Puerto Rico. Up to 480 healthy infants are planned to be enrolled in this study with an initial enrollment of 120 infants in Part 1, followed by up to 360 infants in Part 2.

Vaccinations with either AFX3772 (1, 2 or 5 µg, with 1 µg in Part 1 only) or PCV are scheduled at approximately 2, 4, and 6 months of age for the primary series and 12 months for the booster dose. Subsequent immunogenicity assessments are scheduled at 30 days PD2, 30 days PD3, pre-dose 4 and 30 days PD4. In rare cases, if the participant is unable to return to the site, study data may be collected by telephone and blood collected at a remote location, following study-specific procedures that have been approved by the Sponsor (refer to Section 10.6 Appendix 6 Clinical Study Continuity for alternative visit procedures in the case of a crisis). The follow-up visit will occur 6 months following the booster dose.

On Day 1 to Day 7 following each vaccination, the participant's LAR will record body temperature and solicited local and systemic AEs in an eDiary. Unsolicited AEs will be collected for 30 days following each vaccination. Serious adverse events (SAEs) and deaths, regardless of whether the events are considered to be vaccine-related by the investigator, will be collected from the time consent is signed through an individual's completion of participation in the study.

Immunogenicity data from the primary series (after the third dose of study vaccine) will be used to determine if any participant meets criteria for rescue vaccination, defined as a serotype-specific IgG concentration below 0.35 µg/mL for ≥ 3 of the 13 serotypes contained in PCV13 [Part 1] and 5 out of 20 serotypes contained in PCV20 [Part 2]. These assessments will be performed by the Sponsor in a timely manner, and the investigator or sub-investigator(s) will be informed of any participants meeting rescue criteria. Participants will be offered a rescue vaccination with PCV13 (Part 1) or PCV20 (Part 2) within 3 weeks of site notification.

Additional blood volumes will be collected from study participants for the development, validation, and maintenance of assays measuring responses to the pneumococcal vaccine needed for the vaccine clinical development program.

Part 1 Dose Escalation

Cohorts 1, 2 and 3 consist of 40 participants per cohort to be randomized 3:1 to receive either AFX3772 (1, 2 or 5 µg) or PCV13 as noted in Table 4 and Figure 1. Following a review of the most recent cohort safety data through Day 7 postvaccination and any additional safety data from earlier cohorts, the DMC will determine whether to initiate enrollment in the next cohort. The DMC will review all available safety data from Cohorts 1, 2 and 3 to determine if the study will proceed into Part 2 as planned. Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 1, approximately 120 participants per dose level (approximately 360 infants if all dose levels are evaluated in Part 2) will be enrolled and randomized equally to receive either PCV20 or AFX3772 at dose levels (2 or 5 µg) approved for Part 2. If the DMC does not recommend evaluating all AFX3772 dose levels in Part 2, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to Part 2.

A description of the structure, function, and guidelines for decision-making by the DMC, along with the timing and content of the safety data collection is provided in Section 10.1.5.

Table 4 Number of Participants by Vaccination Group for Part 1

Cohort	AFX3772	Number of Participants	Comparator	Number of Participants
1	1 µg	30	PCV13	10
2	2 µg	30		10
3	5 µg	30		10

PCV13: Prevnar 13

Prior start of enrolment to Part 2, DMC has reviewed the data from Part 1 of the study, and GSK has decided to drop the 1 µg dose level of AFX3772 from Part 2. In addition, the ACIP has recommended the use of PCV20 for routine infant immunization. Part 2 will start with target recruitment of a total of 360 healthy infants, i.e., 120 participants in each of treatment arm (AFX3772 2 µg or AFX3772 5 µg or PCV20).

Part 2a – Dose escalation safety lead-in

Part 2a Cohorts 4 and 5 consist of 45 participants per cohort to be randomized 2:1 to receive either AFX3772 (2 or 5 µg) or PCV20 as noted in Table 5 and Figure 3.

Following a review of the tolerability data through Day 7 post-vaccination of 1st dose and cumulative safety data from Cohort 4, the DMC will determine whether to initiate enrollment in the Cohort 5. The DMC will review all available safety data from Cohorts 4 and 5 to determine if the study will proceed into Part 2b as planned.

If the DMC does not recommend evaluating 2 AFX3772 dose levels in Part 2b, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to Part 2b.

A description of the structure, function, and guidelines for decision-making by the DMC, along with the timing and content of the safety data collection is provided in Section 10.1.5.

Part 2b Full Enrollment

Following DMC review and agreement to proceed to Part 2b, up to 270 participants (~90 per dose level) will be randomized equally to receive approved AFX3772 dose levels (2 µg or 5 µg) or PCV20 (Figure 4). The DMC will continue to review safety data as outlined in the DMC charter throughout the study. If the number of dose groups is reduced, the number of participants enrolled in the study overall will be reduced, with the number of participants in each dose group remaining at approximately 90.

Table 5 Number of Participants by Vaccination Group for Part 2a

Cohort	AFX3772	Number of Participants	Comparator	Number of Participants
4	2 µg	30	PCV20	15
5	5 µg	30		15

PCV20: Prevnar 20

Table 6 Number of Participants by Vaccination Group for Part 2b

AFX3772 2 µg	AFX3772 5 µg	Comparator PCV20
90	90	90

PCV20: Prevnar 20

4.1.1. Dose Escalation and Stopping Criteria

After all vaccinated participants in a dose escalation cohort have completed study safety procedures through Day 7 postvaccination, the DMC will recommend whether to initiate enrollment in the next cohort based on the review of all available safety data from the current and preceding cohorts. Once the Day 7 safety assessments in the final cohort (Cohort 3) of Part 1 are complete, the DMC will review all available safety data from the Cohorts 1, 2 and 3 to recommend if the study should proceed into Part 2 and, if so, which AFX3772 dose level(s) will be recommended for continued evaluation in Part 2.

Once the Day 7 safety assessments in the final cohort (Cohort 5) of Part 2a are complete, the DMC will review all available safety data from the Cohorts 4 and 5 to recommend if the study should proceed into Part 2b and, if so, which AFX3772 dose level(s) will be recommended for continued evaluation in Part 2b (Refer to Figure 3 and Figure 4).

Depending on the safety data observed in the study, the DMC will recommend whether to proceed with or stop dose escalation. The Sponsor will make the final decision based on all the data available and the DMC's recommendation(s). A stopping rule is met if any of the following situations occur after administration of AFX3772. Reactogenicity eDiary data confirmed by the investigator as being entered by the LAR in error will not contribute to the stopping rule. Dose escalation may proceed unless the following applies to participant(s) receiving AFX3772:

- Two or more participants vaccinated with AFX3772 at any dose level report the same or similar severe (Grade 3) local or systemic AE (see Section 10.2 Appendix 2 Assessment of Intensity) that is possibly related to AFX3772.
- Two or more participants vaccinated with AFX3772 develop fever >104°F (>40°C) for at least 1 daily measurement after vaccination that is assessed as possibly related to AFX3772, or for which there is no alternative, plausible, attributable cause.
- If any participant vaccinated with AFX3772 at any dose level develops a Grade 4 local or systemic AE that is assessed as possibly related to AFX3772 or for which there is no alternative, plausible, attributable cause.
- ≥1 participant(s) experience an SAE, including death, possibly related to AFX3772, or for which there is no alternative, plausible, attributable cause.

  Note: Any events that have been previously reported and deemed related to licensed PCVs or other vaccines administered concomitantly to participants will not be considered to meet the halting criteria.

Please note: Reporting of AEs and SAEs occurring in the trial are to follow guidelines detailed in Sections 10.3.3 and 10.3.4.

The DMC will review the event(s) and may decide to unblind the participant(s) treatment assignment. After review of all information, the DMC will recommend whether to resume the study and/or dose escalation. If the DMC does not recommend evaluating all AFX3772 dose levels, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to the next step.

4.2. Scientific Rationale for Study Design

This study will be conducted in healthy infants approximately 2 months of age (42 to 90 days of age). Infants are at increased risk for pneumococcal disease and its associated morbidity and mortality [Drijkoningen, 2014]. In addition, the enrollment of infants in this study is intended to assess safety, tolerability and immunogenicity in a population that is representative of children receiving licensed PCVs in the US and elsewhere.

This study is designed to evaluate the safety, tolerability, and immunogenicity of AFX3772 in healthy infants who are naïve to licensed or investigational pneumococcal vaccines. The study design is consistent with the clinical development for PCV and addresses the guidelines set for the evaluation of next generation pneumococcal vaccines by the World Health Organization (World Health Organization [WHO, 2013]) and adopted by global regulatory agencies. The proposed safety, tolerability (including reactogenicity) and immunological assessments (i.e., immunological endpoints [0.35 µg/mL or corresponding threshold value, IgG GMCs and opsonophagocytic activity (OPA) geometric mean titers (GMTs)]) are also consistent with standard PCV pediatric clinical development endpoints.

The study will implement a 3+1 pneumococcal vaccination schedule for infants as recommended by the US ACIP. In order to evaluate other vaccination schedules (i.e., 2 + 1 schedule used in Europe and elsewhere), immunogenicity will also be assessed after the second vaccination. A dose escalation approach is being implemented in this trial to ensure adequate safety review following the first administration of each dose level prior to initiating enrollment into the next dose level. Once dose escalation has been completed and DMC recommendation has been given and followed by the Sponsor's decision to continue the study, enrollment in Part 2 will be initiated.

The objective of collecting additional blood volumes from study participants at different timepoints is to obtain a panel of serum samples with an intent to cover adequately the analytical range of the respective assays during further assay development and validation. ***For participants affected by clinical hold on 31 October 2024, optional blood samples will no longer be collected.***

4.2.1. Rationale for Endpoints

4.2.1.1. Rationale for Immunogenicity Endpoints

Sera collected from participants will be used to measure vaccine-induced serotype-specific IgG concentrations for the 24 serotypes i.e., 13 serotypes shared with PCV13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) as well as the 11 additional serotypes in AFX3772 (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F, and 33F) in Part 1, and the 20 serotypes shared with PCV20 (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F) and the 4 unique serotypes (2, 9N, 17F and 20B) in AFX3772 in Part 2, at post Dose 2 (PD2), PD3, pre-dose 4 and PD4. Blood

sampling at pre-dose 4 and PD4 will be used to evaluate the persistence of protective immunity and the anamnestic antibody responses.

Details on the immunogenicity endpoints evaluated in this study can be found in Section 3.

4.2.1.2. Rationale for Safety Endpoints

The safety endpoints evaluated in this study were selected based on the product's safety profile demonstrated in previous studies, published data from marketed PCVs, and feedback received from regulatory agencies during product development. The eDiary used to record AEs during the postvaccination periods, as defined in Section 10.3.3, was structured as recommended in the final Food and Drug Administration Patient Reported Outcome Guidance (Food and Drug Administration [FDA, 2009]).

Details on the safety endpoints evaluated in this study can be found in Section 9.3.5.

Details on AEs, including definitions and reporting requirements, can be found in Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

4.2.2. Rationale for Use of a Comparator

Placebo-controlled clinical studies for new PCVs are no longer ethical given the proven clinical efficacy, public health impact and widespread use of licensed PCVs worldwide.

Prevnar 13 (Part 1) and Prevnar 20 (Part 2) will be the active comparators for use in this trial and are the most widely recommended vaccines for the prevention of pneumococcal disease in infants in many countries worldwide, including the US.

Refer to approved labeling for more details on Prevnar 13 and Prevnar 20.

4.3. Justification for Dose

The proposed initial dose levels of 1, 2 and 5 µg for clinical development of AFX3772 were chosen based on the PS levels contained in marketed PCV, which range from 1.1 to 4.4 µg for each of the serotypes, and the AFX3772 nonclinical pharmacology and toxicology studies. Clinical studies using all 3 dose levels have been conducted in adults 18 to 64 years of age, 65 to 85 years of age, and toddlers 12 to 15 months of age.

AFX3772 has been evaluated in adults in a dose escalation, dose finding study (3772-CL-1001). AFX3772 was immunogenic and induced both pneumococcal serotype-specific IgG responses and antibody-mediated serotype-specific opsonophagocytic activity (OPA) titers to all 24 serotypes included in the vaccine, and at all 3 dose levels (1, 2 and 5 µg) tested in healthy adults aged 18 to 64 years and 65 to 85 years (3772-CL-1001).

The same doses (1, 2 and 5 µg) were evaluated in toddlers aged 12 to 15 months of age (study 3772-CL- 2001) and were demonstrated to have an acceptable safety/tolerability profile and were immunogenic for all 24 serotypes contained in the vaccine (3772-CL-2001).

In Part 1 of this study, 3 dose levels (1, 2 and 5 µg) were assessed and preliminary safety and immunogenicity data became available prior start of Part 2. AFX3772 vaccine formulations at 1 µg, 2 µg, and 5 µg dose levels elicited immune responses in terms of serotype-specific IgG and OPA titers for both the 13 common and 11 unique serotypes in infants for whom a 3-dose primary series was initiated at the age of approximately 2 months. No safety signals were identified for any of the 3 dose levels of AFX3772 administered in infants aged approximately 2 months at the time the 3-dose primary vaccination series was initiated. Following DMC review of data from Part 1, GSK decided to discontinue further investigation of the 1 µg dose level due to observation of a trend for lower immunogenicity following vaccination with 1 µg dose level versus other dose levels.

Refer to the IB for details about completed clinical trials.

4.4. End of Study Definition

The overall study begins when the written informed consent is provided for the first participant. The overall study ends when the last participant completes the last study-related telephone call or visit, withdraws from the study or is lost to follow-up (i.e., the investigator is unable to contact the participant's LAR).

4.4.1. Clinical Criteria for Early Trial Termination

The clinical trial may be terminated early if the risk/benefit profile to the study population as a whole becomes unacceptable. In addition, further recruitment in the study or at (a) particular study site(s) may be stopped due to insufficient compliance with the protocol, Good Clinical Practice (GCP), and/or other applicable regulatory requirements, procedure-related problems or an unacceptably high number of discontinuations or withdrawals due to administrative reasons.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

Healthy male and female infants approximately 2 months of age (42 to 90 days inclusive) will be enrolled in this study.

All screening assessments must be completed and reviewed to confirm the potential participant meets all eligibility criteria.

5.1. Inclusion Criteria

Participant is eligible for participation in the study only if all of the following apply:

1. Participant has an LAR who understands study procedures, alternative treatments and risks involved in the study and who voluntarily agrees to participate by giving informed consent.
2. Participant is a full-term (≥37 weeks gestation at birth) infant approximately 2 months of age (42 to 90 days, inclusive) at time of obtaining the informed consent.
3. Participant is healthy based on the clinical judgment of the investigator following review of medical history and physical examination.

5.2. Exclusion Criteria

Participant will be excluded from participation in the study if any of the following apply:

1. Participant had prior administration of any pneumococcal vaccine.
2. Participant has a known or suspected hypersensitivity to AFX3772, PCV13, PCV20 or any components of the formulations used.
3. Participant has a known or suspected immunodeficiency (including autoimmune disease) or other conditions associated with immunosuppression that may require immunosuppressive drugs including but not limited to HIV infection, malignancy or organ or bone marrow transplant.
    a. In addition, the participant's biological mother has known HIV infection or known to be hepatitis B surface antigen positive.
4. Participant has any clinically significant allergic condition or history (including anaphylactic reactions, but excluding untreated, asymptomatic, seasonal allergies) prior to the first vaccination for primary immunization series.
5. Participant has functional asplenia.
6. Participant has medical history of epilepsy or febrile convulsion.
7. Participant weighed <2000 g at birth.

8. Participant has known neurological or cognitive behavioral disorders including clinically significant developmental disorder and related disorders.
9. Participant has any active malignancy or history of malignancy.
10. Participant has a history of microbiologically proven invasive disease caused by *S. pneumoniae*.
11. Participant has cochlear implant or cerebrospinal fluid leakage.
12. Has received other licensed non-live vaccines within the 14 days or a licensed live virus vaccine within the 30 days prior to receipt of study vaccine.
13. Participant has received immunoglobulins. **Note:** This does not include monoclonal antibodies, such as palivizumab or nirsevimab.
14. Participant has a bleeding diathesis or condition associated with prolonged bleeding that would contraindicate intramuscular injection.
15. Participant has received systemic corticosteroids (equivalent of >2 mg/kg total daily dose of prednisone or >20 mg/day for persons weighing >10 kg) for a period of more than 14 days and has not completed the treatment for at least 30 days before study vaccine. Topical, ophthalmic, and inhaled steroids are allowed.
16. Participant has febrile illness (rectal temperature ≥38.1°C [100.5°F] or axillary temperature ≥37.8°C [100.0°F]) in Part 1 and Part 2 prior to the effective date of protocol amendment 4. After protocol amendment 4 becomes effective, febrile illness is defined as rectal temperature ≥38.0°C [100.4°F] or axillary temperature ≥37.5°C (99.1°F) in Part 2. Upon resolution of symptoms, participant can be rescreened.
17. Participant has received any investigational therapy within 28 days or 5 half-lives, whichever is longer, prior to screening.
18. Participant has any condition which, in the investigator's opinion, makes the participant unsuitable for study participation.
19. LARs that are study personnel or their immediate dependents, family, or household members.

5.3. Screen Failures

Screen failures are defined as participants whose LAR provides consent to participate in the clinical study but who are not subsequently randomized and vaccinated. A minimal set of screen failure information is required to ensure transparent reporting to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria and any SAEs meeting reporting requirements as outlined in the data entry guidelines.

For each study participant, a unique identifier (Subject ID) is assigned at Screening when the site completes the Enrollment eCRF page. The eCRF Completion Guidelines will describe what data points need to be entered on the Enrollment eCRF page. If screening criteria are not met, the participant will be considered a screen failure.

Rescreens

The participant can return for a rescreen visit once within 28 days of the initial screening visit, upon which a new informed consent will be obtained and a new Subject ID assigned. If the participant does not meet all inclusion criteria at the rescreening visit, the participant will be documented as a screen failure.

5.4. Criteria for Temporarily Delaying Enrollment and Administration of Study Intervention

Enrollment and study intervention administration may be postponed within the permitted time interval until transient conditions cited below are resolved:

The following condition(s) are temporary or self-limiting and participant may be vaccinated and/or blood draw at the visit can be performed, once the condition(s) has/have resolved and no other exclusion criteria are met and should other requirements such as appropriate window for study intervention Administration allows.

1. Acute illness or fever at the time of enrollment and/or study intervention administration. Refer to the SoA for definition of fever and preferred location for measuring temperature in this study.
2. Participant with a minor illness (such as mild diarrhea, mild respiratory infection) without fever may be enrolled and/or vaccinated at the discretion of the investigator.
3. Delay is warranted in order to meet the following conditions: Influenza vaccine, SARS-CoV-2 (COVID-19) vaccine should be administered either 7 days prior to or 15 days after the administration of the study vaccine. Non-live vaccines not given concomitantly (i.e., on the same day as study vaccine) should be given at least 7 days prior or at least 15 days after administration of study vaccine. Live vaccines not given concomitantly should be given at least 28 days prior or at least 28 days after study vaccine. Refer also to the Sections 6.8.1 Permitted Concomitant Therapy and Section 6.8.2 Prohibited Concomitant Therapy, and Section 10.4: Appendix 10.4 List of Excluded Concomitant Medications.
4. There is a clinical circumstance that warrants delay of blood collection for immunogenicity assessment. In the event that a participant meets this criterion for delay of blood collection, blood collection can proceed once the window for delay has passed.
5. Participants has been found to have clinically significant hematologic/biochemical values out of normal range (evident in tests performed for any reason outside of study procedures) which are expected to be temporary and warrant re-screening per PI's judgement and dosing within the allowed time interval per protocol.

6. STUDY INTERVENTION(S) AND CONCOMITANT THERAPY

6.1. Study Intervention(s) Administered

The vaccines to be used in this trial are outlined below in Table 7.

Table 7 Study Intervention(s) Administered

Intervention Label	AFX3772	Prevnar 13*	Prevnar 20*
Intervention Name	AFX3772	PCV13	PCV20
Intervention Description	AFX3772 is administered as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a booster dose given at 12 months of age.	PCV13 is administered as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a booster dose given at 12 months of age.	PCV20 is administered as a 4-dose regimen, with 3 doses given at 2, 4, 6 months of age as part of a primary series and a booster dose given at 12 months of age.
Type	Combination product	Combination product	Combination product
Dose Formulation	Vial (Part 1)# Prefilled syringe (Part 2)#	Prefilled syringe (Part 1)	Prefilled syringe (Part 2)
Unit Dose Strength(s)	0.5 mL each administered as 4 separate vaccinations	0.5 mL each administered as 4 separate vaccinations	0.5 mL each administered as 4 separate vaccinations
Dosage Level(s)	1 µg (Part 1 only) 2 µg 5 µg	See PCV13 label for details	See PCV20 label for details
Route of Administration	Intramuscular injection	Intramuscular injection	Intramuscular injection
Use	Investigational vaccine	Comparator vaccine	Comparator vaccine
IMP and *Authorized* AxMP	IMP	Authorized AxMP	Authorized AxMP
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor	Provided centrally by the Sponsor
Packaging and Labeling	AFX3772 provided as a sterile suspension in single-dose vials (Part 1) / Pre-filled syringe (Part 2)# and appears as a grey/white suspension. Each vial (Part 1)/ Pre-filled syringe (Part 2) is labeled as required per country requirement.	PCV13 provided in a single-dose 0.5 mL prefilled syringe along with instructions for storage, handling and use. Each syringe is labeled as required per country requirement.	PCV20 provided in a single-dose 0.5 mL prefilled syringe along with instructions for storage, handling and use. Each syringe is labeled as required per country requirement.

AxMP: auxiliary medicinal product; IMP: investigational medicinal product; PCV13: Prevnar 13; PCV20: Prevnar 20.
#Vials of AFX3772 used in Part 1 and Pre-filled syringes containing AFX3772 used in Part 2.
*Refer to Figure 2 for Part 1, Figure 3 and Figure 4 for Part 2.

6.1.1. Medical Devices

- The Sponsor-manufactured medical devices provided for use in this study is AFX3772.
- Other medical devices (not manufactured by or for the Sponsor) provided for use in this study are PCV13 and PCV20.
- Instructions for medical device use are provided in Pharmacy Manual.

All device deficiencies (including malfunction, use error and inadequate labeling) shall be documented and reported by the investigator throughout the clinical investigation (see Section 8.4.8 and Section 10.7) and appropriately managed by GSK.

6.2. Preparation, Handling, Storage, and Accountability

6.2.1. Dose Preparation

There are no specific calculations or evaluations required to be performed in order to administer the proper dose to each participant. The rationale for selection of doses to be used in this study is provided in Section 4.3.

6.2.2. Handling, Storage, and Accountability

The investigator or designee must confirm that study vaccine deliveries from the Sponsor have been maintained during transit for all study interventions received and any discrepancies are reported and resolved before use of study vaccine. The investigator or designee will also ensure that:

- Deliveries of study vaccines are recorded.
- Study vaccine is handled and stored according to labeled storage instructions.
- Study vaccine has appropriate expiry/retest and is only dispensed to study participants in accordance with the protocol.

Study vaccine inventory and accountability records will be kept by the pharmacist/study site personnel who are responsible for inventory and preparation of study vaccine. In addition, the following apply:

- The investigator or delegates agree not to supply study vaccines to any persons except participants in this study in accordance with the protocol.
- The investigator or delegates will keep the study vaccines in a locked and secure storage facility under controlled storage conditions, accessible only to those authorized by the investigator to dispense study vaccines.

- At the conclusion or termination of this study, investigator or delegate agrees to conduct a final vaccine supply inventory and to record the results of this inventory in the interactive response technology (IRT) system.
- Remaining study vaccine must be returned to the Sponsor at the end of the site's participation in the study or upon expiration, unless otherwise approved by the Sponsor.

6.3. Measures to Minimize Bias: Randomization and Blinding

6.3.1. Intervention Assignment

Treatment allocation/randomization will occur centrally using an IRT system.

In Part 1, 40 participants per cohort were enrolled and randomized 3:1 to receive AFX3772 (1, 2 or 5 µg) or PCV13. In Part 2a, 45 participants per cohort will be enrolled and randomized 2:1 to receive AFX3772 (2 or 5 µg) or PCV20. If continuation into Part 2b is recommended, approximately 270 additional participants will be randomized 1:1:1 (90 per group) to receive either 1 of up to 2 AFX3772 dose levels (2 or 5 µg), or PCV20 as agreed to for evaluation in Part 2b. At Visit 1, after the ICF signature, study site personnel will access the electronic data collection (EDC) system and enter the participant's required information as described in the eCRF Completion Guidelines. Upon completion of the Randomization eCRF page, the EDC system will assign a unique Subject Randomization Number to each randomized participant. Upon completion of the Vaccine Administration eCRF page, the IRT system will assign a unique kit to each randomized participant. An unblinded pharmacist will access the IRT to find out which investigational product kit was assigned to a randomized participant.

If a participant is randomized but does not receive study vaccine, the kit number will not be used again. In this instance, the study vaccine assignment should only be known by the unblinded pharmacist/study site personnel who are responsible for inventory and preparation of study vaccine, or designee.

Specific procedures for randomization through the IRT are contained in the IRT and pharmacy manual.

6.3.2. Stratification

No stratification based on age, sex or other characteristics will be used in this trial.

6.3.3. Blinding

A double-blinded technique will be used. The participant's LAR, the investigator, and all site personnel responsible for the clinical evaluation of participants will remain blinded until study completion.

The laboratory in charge of the sample testing will be blinded to the intervention assignment. Codes will be used to link the participant and study (without any link to the intervention attributed to the participant) to each sample.

Because AFX3772 and Prevnar 13/Prevnar 20 have different appearances, a member of the study staff (and back-up) will be unblinded for purposes of receiving, maintaining, preparing, and administering study vaccines. The syringes will be masked prior to dosing to maintain the blind for the participant's LAR and all other personnel. In order to avoid bias, the unblinded study personnel will not perform any safety assessments after administration of study vaccines. Additionally, blinded site personnel will not be present in the examination room when study vaccines are administered.

The safety and tolerability data for dose escalation by the DMC will be reviewed under blinded conditions. If a participant(s) experiences an AE(s) that meets stopping criteria or that could prohibit dose escalation (Section 7.1), the study vaccine assignment for those participants may be unblinded, if requested by the DMC to determine if these events are possibly related to AFX3772 and of clinical concern. An independent statistician will provide the treatment assignment, and the unblinding will be documented.

Details regarding study blinding will be described in the IRT/pharmacy manual.

Breaking the Treatment Code for Emergency

The study vaccine assignment code for each randomized participant will be provided by the IRT in the event of a medical emergency requiring knowledge of the study vaccine assigned to the participant. The IRT will be programmed with blind-breaking instructions that may only be requested by the investigator or sub-investigator(s) designated to have access to perform blind break. In case of a medical emergency, the investigator has the sole responsibility for determining if unblinding of participant's vaccine assignment is warranted. Participant safety must always be the first consideration in making such determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the Sponsor prior to unblinding a participant's vaccine assignment unless this could delay emergency treatment for the participant.

The investigator must have confirmed functionality to access code-break through the IRT system and must have a designated back-up (e.g., redundant processes) to support emergency unblinding requirements.

Prior to randomization, the participant's LAR should be provided with information that includes the study site emergency contact number and back-up contact number in case of a medical emergency. Any unblinding by the study site personnel must be reported immediately to the Sponsor and include an explanation of why the study vaccine was unblinded. If unblinding is associated with an SAE the investigator is to follow the instructions in Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

Care should be taken to limit knowledge of the treatment assignment; in case this could affect the blinding of other participants or future study assessment for the participant.

Breaking the Treatment Code by the Sponsor

The Sponsor and DMC may break the study vaccine assignment code for participants who experience a suspected unexpected serious adverse reaction (SUSAR), in order to determine if the individual case or a group of cases requires expedited regulatory reporting. In addition, the Sponsor's safety staff may unblind the intervention assignment for any participant with an SAE that is fatal or life-threatening. Individual emergency codes will be provided to the limited personnel who are responsible to break the codes for all SUSAR cases for reporting purposes.

6.4. Study Intervention Compliance

Immunization compliance will be recorded at the time of each study immunization. Study immunization will take place at the study site. The exact day and time of study vaccine administration will be documented.

Interruptions from the protocol-specified vaccine schedule outlined in the schedule of assessments (Table 1 and Table 2) requires consultation between the investigator and Sponsor.

6.5. Dose Modification

Dose modification for individual participants is not permitted.

6.6. Continued Access to Study Intervention after the End of the Study

After the conclusion of the study, participants will not have further access to AFX3772.

6.7. Treatment of Overdose

In this study, an overdose is defined as administration of more than 1 dose of any individual study vaccine in a 24-hour period. There is no specific information available on the treatment of overdose.

In the event of an overdose, the investigator/treating physician should:

- Evaluate the participant to determine, in consultation with the medical monitor, if possible, whether study intervention should be interrupted or whether the next dose should be reduced.
- Closely monitor the participant for any AE/SAE.
- Document the quantity of the excess dose.

Refer to Section 10.3.5.1 for reporting requirements for suspected overdose or other medication error.

6.8. Concomitant Medications/Vaccines

6.8.1. Permitted Concomitant Therapy

Routine infant vaccinations licensed for use in the US are permitted in this study with the exception of PCVs. The schedule for routine childhood vaccines given outside the protocol is at the investigator's discretion. During influenza season, it is anticipated that participants 6 months of age and older may be given an influenza vaccine. Influenza vaccine should be administered either 7 days prior to or 15 days after the administration of the study vaccine. SARS-CoV-2 (COVID-19) vaccine is permitted if administered either 7 days prior to or 15 days after administration of the study vaccine. Non-live vaccines not given concomitantly (i.e., on the same day as study vaccine) should be given at least 7 days prior or at least 15 days after administration of study vaccine. Live vaccines not given concomitantly should be given at least 28 days prior or at least 28 days after study vaccine.

Concomitant vaccines (oral or injectable) should be administered on the same day as trial vaccine. Concomitant oral vaccines must be administered prior to other injectable concomitant vaccines followed by trial vaccine to prevent choking. To avoid any confounding local reactogenicity results, non-trial injectable vaccines must not be administered in the same limb as trial vaccine.

Documentation of limb used for the administration of trial vaccine must be recorded in the participant's medical record, and the LAR should be instructed on the appropriate limb to monitor for AEs. Injection site AEs for concomitantly administered non-study vaccines will not be collected.

Acetaminophen and nonsteroidal anti-inflammatory drugs are permitted during the study. The LAR will be instructed to record antipyretic medications and the reason for use in the provided eDiary on Day 1 to Day 7 following each vaccination. ***The LAR will be instructed to record the exact time of use of antipyretic medications on Day 1***.

6.8.2. Prohibited Concomitant Therapy

Medications and therapies at doses, timing and duration specifically prohibited in the exclusion criteria are prohibited during study participation. If there is a clinical indication for any prohibited medication or vaccination, discontinuation from study intervention may be required. The investigator should discuss any questions regarding this with the Sponsor. The participant's medical treatment is the responsibility of the investigator and/or the participant's primary physician. However, the decision for the participant to continue in the trial requires the mutual agreement of the investigator, the Sponsor and the participant's LAR.

Administration of the excluded concomitant medication will be recorded as a protocol deviation.

All concomitant medication including prescription and nonprescription, vitamins and natural and herbal remedies associated with AEs or SAEs during the respective reporting periods will be collected.

No other investigational compounds or devices may be administered at any time during this trial without prior approval by the Sponsor.

A list of excluded concomitant medications is provided in Section 10.4 Appendix 4: List of Excluded Concomitant Medications.

6.8.3. Rescue Vaccination

Investigators will be informed if a participant met criteria for rescue vaccination (defined as the pneumococcal serotype-specific IgG concentration below 0.35 µg/mL for 3 or more of the PCV13 [Part 1] and 5 or more of the PCV20 [Part 2] serotypes after completion of the primary series), so that a rescue dose of PCV can be administered within 3 weeks following site notification. The serologic threshold value of 0.35 µg/mL was recommended by a WHO expert panel as an acceptable threshold value for evaluating the clinical performance of next generation PCVs in infants [Siber, 2007]; World Health Organization [WHO, 2013]).

All participants who meet rescue criteria will be discontinued from further study intervention prior to administering the rescue dose of a licensed PCV as outlined in Section 7.1 The discontinuation of study intervention date should be the date the LAR was notified of the participant meeting rescue criteria. Adverse events or changes to medical history occurring during the AE and SAE reporting period following Dose 3 will be collected for up to 6 months for all infants meeting rescue criteria. No additional immunogenicity blood samples will be drawn from these participants following discontinuation from study intervention.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

Refer to Section 10.1.8 Study and Site Start and Closure regarding discontinuation of study sites or of the study as a whole.

7.1. Discontinuation of Individual Participant(s) From Study Intervention

Discontinuation of study intervention does not represent withdrawal from the study. A participant may discontinue from study intervention but continue to participate in

protocol-specified AE-monitoring activities (see Section 8.4 for details). A participant may discontinue study intervention for any of the following reasons:

- The participant's LAR requests to stop vaccination.
- Any clinical AE, laboratory abnormality, medical condition, or intercurrent illness, which, in the opinion of the investigator, indicates continued vaccination is not in the best interest of the participant.
- If rescue vaccination is needed, the participants will be discontinued from study intervention but will be asked to continue to participate in safety follow-up assessments for up to 6 months PD3.

The primary reason for premature discontinuation of the study intervention will be documented in the eCRF based on the list below:

- Adverse Event
- Rescue Medication
- Protocol Deviation Affecting Efficacy and/or Safety
- Investigator Decision
- Lost to Follow-up
- Withdrawal of Consent by Parent/Guardian
- Death
- Other (specify)

7.2. Discontinuation of Individual Participant(s) from Study

Individual participant(s) will be withdrawn from the study if the principal investigator deems it clinically appropriate for any of the following reasons:

- Participant is lost to follow-up despite reasonable efforts by the investigator to locate the participant.
- LAR voluntarily withdraws the participant from the study at any time for any reason.

The reason for discontinuation from study must be documented in the participant's medical records and eCRF.

Any participant who has received study vaccine and discontinues early for any reason, including withdrawal of consent, will be asked to participate in the remaining safety assessments.

Investigators will attempt to contact participants who do not return for scheduled visits or follow-up.

The participant will be permanently discontinued from the study intervention and the study at that time.

All data and samples collected up to and including the date of withdrawal of/last contact with the participant will be included in the study analyses. If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, the participant may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

The primary reason for participant discontinuation/ withdrawal from the study will be documented in the eCRF based on the list below:

- Adverse Event
- Rescue Medication
- Protocol Deviation Affecting Efficacy and/or Safety
- Investigator Decision
- Lost to Follow-up
- Withdrawal of Consent by Parent/Guardian
- Death
- Other (specify)

Participants who are withdrawn from the study because of AEs/SAEs must be clearly distinguished from participants who are withdrawn for other reasons. Investigator will follow participants who are withdrawn from the study due to an AE/SAE until the event is resolved (see Section 8.4.3).

7.3. Lost to Follow-up

Every reasonable effort should be made to contact any participant lost to follow-up during the course of the study to complete study-related assessments and record outstanding data (e.g., telephone calls and/or a certified letter to the LAR's last known mailing address). These contact attempts should be documented in the participant's medical record.

Note: A participant is not considered lost to follow-up until after the last scheduled visit for the individual participant. The missing data for the participant will be managed via the prespecified statistical data handling and analysis guidelines.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant. These contact attempts should be documented in the participant's medical record.

8. STUDY ASSESSMENTS AND PROCEDURES

- Participants who have signed informed consent but are not eligible to proceed should be recorded in the eCRF with a status of 'screen failure'.
- Procedures conducted as part of the participant's routine clinical management and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.
- In the event of a significant study-continuity issue (e.g., caused by a pandemic), alternate strategies for participant visits, assessments, study intervention distribution and monitoring may be implemented by the Sponsor or the investigator, as per local health authority/ethics requirements.

8.1. Administrative and General Procedures

Immunogenicity results that could unblind the study will not be reported to study sites or other blinded personnel until the study has been unblinded.

Study procedures and their timing are summarized in the schedule of assessments (Table 1 and Table 2). Adherence to the study design requirements, including those specified in the schedule of assessments, is essential and required for study conduct. Prospective protocol waivers or exemptions are not allowed.

The investigator is responsible for ensuring that procedures are conducted by appropriately qualified or trained staff. Delegation of study site personnel responsibilities will be documented by the site.

Any change, divergence or departure from the study design or procedures identified in the protocol is considered a protocol deviation. All deviations from the protocol will be recorded and reported to the IRB of record in accordance with the IRB-specific protocol deviation reporting guidelines.

Immediate safety concerns should be discussed with the Sponsor upon occurrence or awareness to determine if the participant should continue or discontinue the study.

All screening evaluations must be completed and reviewed by the investigator or qualified delegate to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable. Participants who have signed informed consent but are not eligible to proceed should be recorded in the eCRF with a status of 'screen failure'.

A participant also can be seen for an unscheduled visit at any time during the study. An unscheduled visit may be prompted by reactogenicity issues, new or ongoing AEs, or other reasons per investigator discretion. The site also has the discretion to make reminder telephone calls or send text messages to inform the participant about visits, review eDiary requirements, or follow-up on ongoing or outstanding issues. All unscheduled visits and telephone contacts must be reported in the eCRF.

Procedures conducted as part of the participant's routine clinical management and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.

In the event of a significant study-continuity issue (e.g., caused by a pandemic), alternate strategies for participant visits, assessments, study intervention distribution and monitoring may be implemented by the Sponsor or the investigator, as per local health authority/ethics requirements.

8.1.1. All Unscheduled Visits

During unscheduled visits, investigator should record the date of visit, reason for the visit, any investigator assessment(s), the basis for assessment i.e., real time or near real time (within 24-36h) symptomatology or participant recall, timing of participant recall in relation to the visit, if applicable, and the intensity grading scale used by investigator for AEs occurring within 7 days post-vaccination and recorded in the eDiary. ***For participants in Part 2 affected by clinical hold on 31 October 2024, eDiary safety data collection will no longer be collected.*** The severity of the local reactions and systemic events should ideally be graded using the AE severity scale in Section 10.3.3.

8.2. Immunogenicity Assessments

Planned timepoints for all immunogenicity assessments are provided in the SoA.

The following measures will be used to characterize the immunological response in infants following administration of either AFX3772 or PCV.

Considering the mechanism of action, AFX3772 is expected to induce an immune response to serotype-specific pneumococcal polysaccharides and to the fusion protein, CP1. Blood samples to assess immunogenicity will be collected as indicated in the schedule of assessments (Table 1 and Table 2). Priority ranking of the use of blood

samples, as well as optional additional blood volume collected are described in Section 8.6.

Immunogenicity of AFX3772 and PCV will be evaluated by measuring pneumococcal serotype-specific anti-capsular polysaccharide IgG antibody concentrations by 24-valent multiplex direct Luminex immunoassays (dLIAs) or alternative assay format (e.g., 24-valent electrochemiluminescent [ECL] multiplexed assay).

Immunogenicity of AFX3772 or PCV will be also evaluated by measuring the antibody-mediated pneumococcal serotype-specific opsonophagocytic activity (OPA) titers by multiplexed opsonophagocytic assay (MOPA) targeting the 24 AFX3772 vaccine serotypes. The MOPA measures the functional opsonic activity of antibodies against the pneumococcal serotypes that results in bacteria killing by phagocytic cells in vitro.

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Additional optional blood volumes will be collected from study participants for the development, validation, and maintenance of assays measuring responses to the pneumococcal vaccine needed for the vaccine clinical development program.

8.3. Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA (Table 1 and Table 2). Details of definitions and reporting of AEs and SAEs are provided in Section 8.4.

8.3.1. Body Temperature

At the dosing visit (Day 1), a rectal or axillary temperature will be taken prior to administration of study vaccine. The method used by the site will be based on their standard of care for temperature measurement. Participants who have febrile illness (rectal temperature ≥38.0°C [≥100.4°F] or axillary temperature ≥37.5°C [≥99.1°F]) should have the visit rescheduled.

The participant's LAR will be given a thermometer and instructed by study site personnel on how to measure body temperature and record it to 1 decimal place in the eDiary. After each vaccination, the participant's LAR will measure body temperature from Day 1 through Day 7 and will record it daily in the eDiary. Temperature will be collected in the eDiary in the evening as well as at any time when an elevated temperature is suspected during the 7-day collection period.

Axillary (underarm) is the preferred method for the LAR to obtain the participant's temperature. Details for collection and recording of body temperature are in Section 10.2.2.

8.3.2. Physical Examinations

A complete physical examination will be performed at Visit 1. A targeted physical examination will be performed at subsequent vaccination visits (Visits 2, 4, and 6). Any clinically significant abnormality will be recorded on the appropriate eCRF.

The full and targeted physical examination procedures both include obtaining vital signs (heart rate, respiratory rate, and axillary or rectal temperature), auscultation of the heart and lung, and examination of the abdomen. In addition, a full physical examination will include an assessment of the head, eyes, ears, nose and throat (HEENT), skin, lymph nodes, neurological system, and musculoskeletal system.

Findings related to the physical examinations should be documented in the participant's chart/source documentation.

8.3.3. Reactogenicity

Participants will remain at the study site for at least 30 minutes following vaccination to assess for immediate hypersensitivity reactions; participants do not need direct supervision. If any immediate AEs are observed during this period, the time as well as the event itself, any concomitant medications that were administered, and resolution of the event must be recorded on the appropriate eCRF.

The study site personnel will instruct the participant's LAR on how to measure body temperature and observe reactogenicity and tolerability and how to record observed events in the eDiary. The participant's LAR will be instructed to contact the study site in case of any hospitalization or emergency room visit or if the participant experiences an AE with severe intensity, as prompted by the eDiary. All local and systemic AEs with a severe intensity require evaluation by a health care professional.

The participant's LAR will use the eDiary to document the following information after each vaccination:

- Axillary temperatures Day 1 to Day 7
- Solicited injection site AEs (tenderness, redness/erythema and swelling) Day 1 to Day 7 Note: Only injection-site AEs from AFX3772 or PCV and not from concomitant injectable vaccines will be collected. The location of AFX3772 or PCV administration can be used by the participant's LAR to monitor the appropriate limb for injection-site AEs related to AFX3772 or PCV
- Solicited systemic AEs (irritability, fever (body temperature), decrease of appetite, increased sleep and decrease in sleep) Day 1 to Day 7

If any reactogenicity extends beyond the 7-day period, it will be recorded as a solicited AE in the e-Diary by the LAR on a daily basis until resolution.

Local and systemic solicited AEs will be graded per Section 10.2 Appendix 2: Assessment of Intensity.

Note that as part of protocol amendment 2, following the administration of SoC, participants should remain at the study site for a period of time in line with the standard medical practice for vaccination with SoC pneumococcal vaccines. In addition, LARs are no longer asked to record reactogenicity events and body temperature in the eDiary following the vaccination with SoC pneumococcal vaccines.

Note that as part of protocol amendment 5, following the administration of SoC, participants should remain at the study site for a period of time in line with the standard medical practice for vaccination with SoC pneumococcal vaccines. In addition, eDiary devices will be inactivated and returned to site, and LARs will no longer be expected to record reactogenicity events and body temperature in the eDiary following the vaccination with SoC pneumococcal vaccines.

8.3.4. Order of Assessments

The following order is preferred when more than one assessment is required:

- Body temperature
- Blood collection
- Study vaccination

8.4. Adverse Events, Serious Adverse Events and Other Safety Reporting

The definitions of an AE or SAE can be found in Sections 10.3.1 and 10.3.2, respectively.

The investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study vaccine and comparator, or that caused the participant to discontinue the study vaccine or comparator and/or study (see Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting). This includes events reported by the participant's legally acceptable representative.

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

8.4.1. Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected at the time points specified in the SoA.

SAEs and deaths are to be reported within 24 hours of awareness throughout the duration of an individual's study participation.

AEs (non-serious) are to be reported Day 1 through Day 30 following each vaccination. Immediate AEs are to be reported within 30 minutes of vaccination and recorded on AE page in the eCRF.

All SAEs will be recorded and reported to the Sponsor within 24 hours of learning of the event, as indicated in Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting. The investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

If the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study vaccine or comparator or study participation, the investigator must promptly notify the Sponsor.

The Reference Safety Information that will be used to determine expectedness of an SAE for this study is the IB.

For all Part 1 participants, SAEs will be collected for 6 months after completion of the primary vaccination series, i.e., 6 months post Dose 3. No specific safety follow-up will be performed following administration of the booster dose with SoC pneumococcal vaccines. Instead, routine PV reporting should be performed in line with local medical practice.

For all Part 2 participants, SAEs will be collected for 6 months after completion of the primary vaccination series, i.e., 6 months post Dose 3 including SoC. No specific safety follow-up will be performed following administration of the booster dose with SoC pneumococcal vaccines as booster is not in scope of the study for the participants affected by clinical hold on 31 October 2024. Instead, routine pharmacovigilance (PV) reporting should be performed in line with local medical practice.

8.4.2. Method of Detecting AEs and SAEs

The methods of recording, evaluating and assessing seriousness, causality and intensity of AEs and SAEs are described in Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.4.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and AEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to

follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

If after the protocol-defined AE collection period (see Section 8.4.1), an AE progresses to an SAE, or the investigator learns of any (S)AE including death, where he/she considers there is reasonable possibility it is related to the study vaccine or comparator or study participation, the investigator must promptly notify the Sponsor.

8.4.4. Regulatory Reporting Requirements for SAEs

1. Prompt notification by the investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study vaccine under clinical investigation are met.
2. The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study vaccine under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB and investigators.
3. For SAEs, the investigator must always provide an assessment of causality at the time of the initial report, as defined in the Section 10.3.4.
4. An investigator who receives an investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.
5. Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and Sponsor policy and forwarded to investigators as necessary.
6. An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and file it along with the IB and will notify the IRB, if appropriate according to local requirements.

8.4.5. Special Situations

Certain special situations observed in association with the study vaccines, such as incorrect administration (e.g., wrong dose of study vaccine or comparator) are reported as protocol deviations and/or may require special reporting, as described below. These special situations are not considered AEs, but do require communication to the Sponsor as per the timelines defined below.

If a special situation is associated with, or results in, an AE, the AE is to be assessed separately from the special situation and captured as an AE in the eCRF. If the AE meets the definition of an SAE, the SAE is to be reported as described in Section 10.3.4. New

or updated information will be recorded in the originally completed eCRF and the details of the associated special situation are to be included in the clinical description on the SAE worksheet.

The special situations are:

- Medication error, overdose and use outside protocol
- Occupational exposure

Instructions and procedures for reporting special situations are provided in Section 10.3.5 Reporting Procedures for Special Situations.

8.4.6. Supply of New Information Affecting the Conduct of the Study

When new information becomes available that is necessary for conducting the study properly, the Sponsor will inform all investigators involved in the study as well as the appropriate regulatory authorities. Investigators should inform the IRB of such information when needed.

The investigator will also inform the participant's LAR, who may be required to sign an updated IRB- approved ICF in order for the participant to continue in the study.

8.4.7. Adverse Events of Special Interest

There are no AEs of special interest in this study.

8.4.8. Medical Device Deficiency

Medical devices are being provided for use in this study as the study intervention. To fulfill regulatory reporting obligations worldwide, the investigator is responsible for the detection and documentation of events meeting the definitions of device deficiency that occur during the study with such devices.

If the site(s) uses non-sponsor medical devices, i.e., medical devices not provided by GSK, then the investigators are obligated to report any device deficiencies to the legal manufacturer of the devices directly.

The definition of a medical device deficiency can be found in Section 10.7.

Note: Deficiencies fulfilling the definition of an AE/SAE will follow the processes outlined in Section 10.7 of the protocol.

8.4.8.1. Time period for detecting medical device deficiencies

- Medical device deficiencies that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

- If the investigator learns of any device deficiency at any time after a participant has been discharged from the study, and such a deficiency is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.

The method of documenting medical device deficiencies is provided in Section 10.7.

8.4.8.2. Follow-up of medical device deficiencies

- Follow-up applies to all participants, including those who discontinue study intervention.
- The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.
- New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.4.8.3. Prompt reporting of device deficiencies to the Sponsor

- Device deficiencies will be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device deficiency.
- The medical device deficiency report form will be sent to the sponsor by via email (please refer to Pharmacy Manual). If email is unavailable, then fax should be utilized. This information will also be recorded in EDC and alternative method is a paper form faxed to sponsor.
- The sponsor will be the contact for the receipt of device deficiency reports.

8.4.8.4. Regulatory reporting requirements for device deficiencies

- The investigator will promptly report all device deficiencies occurring with any medical device provided for use in the study in order for the sponsor to fulfill the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.
- The investigator, or responsible person according to local requirements (e.g., the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.

8.5. Pharmacokinetics

Not applicable.

8.6. Total Amount of Blood

Blood should be drawn at visits mentioned in Table 1 and Table 2.

The approximate total amount of blood to be collected per participant for laboratory assessment and/or for assay development, validation, and maintenance is presented in Table 8.

Table 8 Blood Sampling

Study Part	*Component or additional intend to use blood samples, per priority ranking*[∞]	*Maximum* Number of Samples *[including optional]*	*Approx.* Sample Volume (mL) *[including optional in Part 2b only]*	*Approx.* Total Volume (mL) *[including optional]*
Part 1*	1. Pneumococcal serotype-specific anti-capsular PS IgG 2. Pneumococcal serotype-specific OPA titers	4	4.0	16.0*
Part 2	1. Pneumococcal serotype-specific anti-capsular PS IgG 2. Assay validation and/or maintenance 3. Pneumococcal serotype-specific OPA titers 4. Anti-CP1 IgG	4 [or 5]	Visit 1: [Up to 2.5] Visit 3: 5.0 [up to 7.5] Visit 5: 5.0 [up to 7.5] Visit 6: 5.0 Visit 7: 5.0 [up to 10.0]	20.0 [up to 32.5]

CP1: fusion protein; IgG: immunoglobulin G; OPA: opsonophagocytic activity

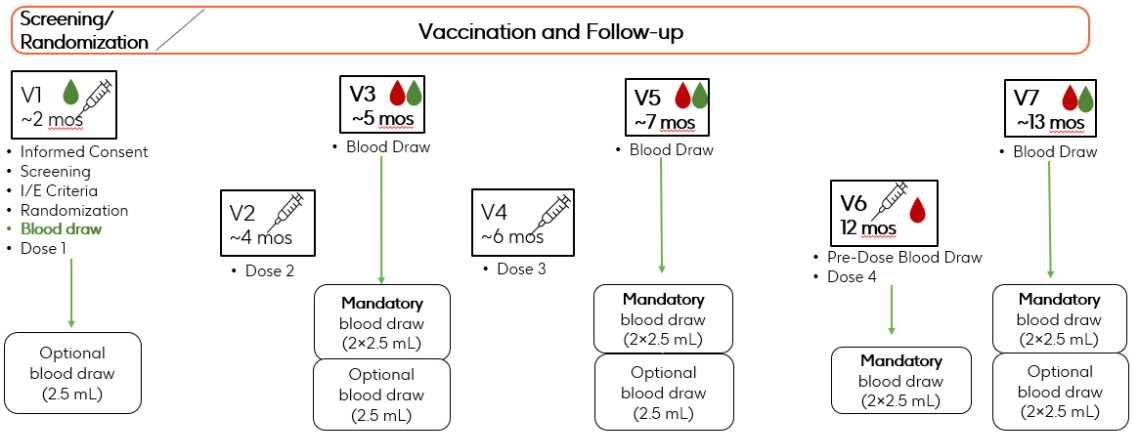
Note: the maximum blood volume drawn from the participant will not exceed 9.5 mL per kg in an 8-week period for the first year of life.

[∞] In case of insufficient blood sample volume to perform assays for all endpoints or for assay development/validation, the samples will be used according to the described priority ranking, 1 being the highest priority

* The total volume of blood collected from Part 1 participants who complete the primary series including post Dose 2 and post Dose 3 sampling, will be 8.0 mL, while for those who receive SoC pneumococcal vaccines as Dose 2 will be 4.0 mL. Note that for all Part 1 participants, pre and post booster blood sampling has been cancelled.

Note: Post protocol amendment 5 implementation, optional blood samples will not be taken from the participants. Note that for all Part 2 participants affected by clinical hold on 31 October 2024, pre and post booster blood sampling has been cancelled.

Figure 5 Blood Sampling Schedule for Part 2b



Post protocol amendment 5 implementation, optional blood samples will not be taken from the participants. Blood samples for immunogenicity to be collected only at Visit 5 to assess need for rescue vaccination.

Repeat and additional blood samples may be taken if required. Maximum blood drawing is calculated based on the 5th percentile of infant girl body weight in the US population, such as that no more than 5 mL/kg body weight could be collected from children for research purposes in a single day and no more than 9.5 mL/kg over any 8-week period.

Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.

CCI [redacted]

8.7. Genetics

Not applicable.

8.8. Biomarkers

Not applicable.

9. STATISTICAL CONSIDERATIONS

In general, continuous data will be summarized with descriptive statistics (number of participants [n], mean, standard deviation, geometric mean and geometric standard deviation where applicable, minimum, median and maximum), and categorical data will be summarized by frequency and percentage. Percentages by categories will be based on the number of participants with no missing data, i.e., the percentages will sum to 100%.

Estimates of a treatment difference between AFX3772 treatment groups and PCV will be conducted for some immunogenicity measures.

All analyses will be performed and summarized separately for Part 1 and Part 2.

Baseline will be defined as the last observation prior to first dose, unless otherwise specified.

9.1. Statistical Hypotheses

There is no formal hypothesis testing planned in this Phase 2 study for the primary endpoints. For the secondary endpoints, a 1-way analysis of variance (ANOVA) will be used to test for overall treatment differences in the geometric means. A t-test will be used for paired comparisons between each AFX3772 dose and PCV. There will be no multiplicity adjustment. These comparisons will be used to aid in dose selection.

The study will provide estimates of safety and immunogenicity for 3 dose levels of AFX3772 and PCV13 in Part 1, 2 dose levels of AFX3772 and PCV20 in Part 2.

Estimates of differences in response following AFX3772 relative to response following PCV administration will be used to determine sample size for hypothesis testing for future studies.

9.2. Analysis Sets

For each AFX3772 or PCV immunization group, the number and percentage of participants will be summarized for all randomized participants (AFX3772 or PCV), the SAF and the PPS.

The following populations are defined:

Population	Description
Randomized	All participants who are randomized to receive either AFX3772 or PCV*.
PPS	Pneumococcal vaccine-naïve healthy infants 42 to 90 days of age defined by the inclusion/exclusion criteria for the study who receive at least 2 vaccinations with either AFX3772 or PCV* during this study and do not have exclusionary intercurrent events defined in Section 3

Population	Description
	The following participants will have their data excluded from assessments of immunogenicity for the PPS, the primary analysis set for immunogenicity. 1. Participants who have their 30-day blood draw not earlier than 28 days and no more than 50 days postimmunization scheduled blood collection for that immunogenicity assessment only 2. Participants who receive systemic corticosteroids at the doses, duration and timing noted in Section 10.4 Appendix 4: List of Excluded Concomitant Medications and for that immunogenicity assessment only. 3. Participants who have newly diagnosed immunologic abnormalities within the first 2 weeks after receiving the study vaccine will be excluded from all immunogenicity assessments. Participants who receive rescue vaccination (an intercurrent event) will be discontinued from study intervention and not included in subsequent immunogenicity assessments.
SAF	All participants who are randomized and receive at least 1 vaccination with either AFX3772 or PCV*. Participants will be analyzed according to the vaccine received.

PPS: Per Protocol Set; PCV13: Prevnar 13; PCV20: Prevnar 20; SAF: Safety Analysis Set
*PCV13 [Part 1] and PCV20 [Part 2]

9.3. Statistical Analyses

A statistical analysis plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before the database lock. Any changes from the analyses planned in the SAP will be justified in the clinical study report.

9.3.1. General Considerations

In general, data will be summarized with descriptive statistics for continuous endpoints, and frequency and percentage for categorical endpoints, unless otherwise specified. Percentages by categories will be based on the number of participants with no censored data (i.e., will add up to 100%).

Demographics and baseline characteristics (age, sex, race, ethnicity, and weight) will be summarized by treatment group for AFX3772 (1 µg, 2 µg, or 5 µg) and immunization group (AFX3772, or PCV).

The number and percentage of participants who completed the treatment, and for those who did not, the reasons for treatment discontinuation (AE, death, lost-to-follow-up, protocol deviation, rescue vaccination, withdrawal by participant, non-compliance with study, other) will be provided for the SAF.

The number and percentage of participants who completed the study, those who discontinued, and the reasons for study discontinuation (AE, death, lost to follow-up, protocol deviation, rescue vaccination, withdrawal by the LAR, non-compliance with study, other) will be presented for the SAF. All disposition details and dates of first and last evaluations for each participant will be listed. The number and percentage of

participants who receive rescue vaccination will be provided by immunization group (AFX3772 [1 µg, 2 µg and 5 µg] or PCV). Participants who are administered rescue vaccination will be encouraged to remain in the study for safety follow-up.

For antibody titer and concentration, values above the LLOQ are considered accurate and their quantitated values will be reported. Values below the LLOQ will be set to ½ LLOQ for calculation of geometric means and set to LLOQ for calculation of fold changes.

9.3.2. Primary Endpoint(s)/Estimand(s) Analysis

The primary analysis for safety, the primary endpoint, will be conducted on the SAF.

9.3.2.1. Primary Vaccination Series Assessments

In the event there is an AE that is of concern to the DMC, the treatment assignment may be requested for that participant. The independent statistician will provide the treatment assignment and the unblinding will be documented. All other personnel involved with the study will remain blinded to vaccination assignment until the study has completed.

The primary data analysis for safety (through 30 days after the primary series) and immunogenicity will occur after the last participant completes the primary series. See Section 9.4.1 for full details of analyses. The purpose is to allow proceeding with dose selection for Phase 3 clinical trial material planning.

Reassessment of the dose will be confirmed once the booster vaccination assessment is available.

In Part 1 participants, an interim analysis of safety and immunogenicity following primary vaccination series will be performed. This will include the analysis of all available safety and immunogenicity data for all three cohorts post study intervention. Section 9.4.1 for full details of analyses.

In Part 1 participants, the final assessment of safety and immunogenicity will be performed after the last participant completes the study i.e., approximately 6 months after the last participant has completed the primary series and the results will be unblinded at individual-level.

9.3.2.2. Booster Vaccination Assessments

Since Part 1 ***and Part 2*** participants should receive SoC for the booster vaccination and no safety and immunogenicity assessment is planned for SoC pneumococcal vaccines, no analysis will be performed for the Part 1 ***and Part 2*** participants for booster series.

9.3.2.3. Final Safety Assessment

The final safety assessment (SAEs) will be after the last participant completes the study, approximately 6 months after the last participant has received the ***third dose of the primary series standard of care vaccine (PCV20)***.

See Section 9.4.3 for full details of analysis.

The primary endpoints for this Phase 2 study address safety and will be assessed in participants who have received at least 1 vaccination. Summaries by treatment for each vaccination and overall will be summarized by n and percent.

The percentage of participants with any local AE, any systemic AE, and each local or systemic AE will be summarized with n, percent, and the 95% CI. Summaries will be provided for each vaccination in the primary series, for the booster vaccination, for the primary series, and for the 4-dose series. The solicited reactions will be summarized by intensity, onset day and duration for each treatment group.

AEs and SAEs will be summarized by treatment group with n and percent. All AEs reported from the time of consent will be listed. Participants who do not have the full 4-dose regimen will have all AEs through 30 days after the last vaccination included in AEs. All data through 6 months after their last vaccination will be included in the summaries of SAEs.

Safety data used for dose escalation decisions will be provided for each dose cohort.

9.3.3. Secondary Endpoint(s)/Estimand(s) Analysis

Key secondary endpoints for this Phase 2 study are assessments of immunogenicity 30 days after the second and third dose of the primary infant 3-dose series with either AFX3772 or PCV13 for Part 1 and 30 days after the second and third dose of the primary infant 3-dose series and before and 30 days after the booster vaccination with either AFX3772 or PCV20 for Part 2.

The proportion of participants with pneumococcal serotype-specific IgG concentration ≥0.35 µg/mL or corresponding threshold, the 95% CI (Clopper-Pearson) for each treatment, the difference between each AFX3772 dose level and PCV (AFX3772-PCV) at the defined timepoints and associated 95% CI [Miettinen, 1985] will be calculated for each serotype. These exploratory comparisons in this Phase 2 study are for estimating the difference between AFX3772 and PCV and for sample size estimation for future studies.

9.3.3.1. IgG concentration

Geometric means for the IgG concentration will be provided for each dose level of AFX3772 (1, 2 and 5 µg) and the combined PCV group after each concentration has been logarithmically transformed. Two-sided 95% CIs for the GMC will be constructed after back transforming the CIs for the mean of the logarithmically transformed assays computed using Student t distribution. The ratio of the IgG GMC (each AFX3772 dose level/PCV) and the 95% CI will be provided.

9.3.4. Exploratory Endpoint(s)/Estimand(s) Analysis

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[redacted]

9.3.5. Safety Analyses

An AE is defined as an AE observed after the first study immunization and up to 30 days following each dose. See also Section 9.3.2.

9.3.5.1. Adverse Events

AEs will be collected from Day 1 to Day 30 following each vaccination. A vaccine-related AE is defined as any AE with a causal relationship assessed as "yes" by the investigator. AEs will be coded using the latest version of MedDRA. SAEs, including deaths, will be collected throughout the duration of an individual's study participation.

Summary tables by treatment group will be provided for:

Number and percentage of participants with AEs

Number and percentage of participants with serious AEs (SAEs)

Number and percentage of participants with vaccine-related AEs

Number and percentage of participants with vaccine-related SAEs

Number of deaths.

All AEs and SAEs will be listed.

9.3.5.2. Body Temperature

Descriptive statistics for temperature will be tabulated by treatment group and scheduled timepoint. Summaries will include n, mean, median, standard deviation, minimum and maximum for the temperature collected at each scheduled time point and the change from baseline.

Proportion of participants with maximum temperature measurements meeting the Brighton Collaboration cut points Day 1 through Day 7 following any vaccination with AFX3772 or PCV will be listed.

9.3.5.3. Reactogenicity

See Section 9.3.2 Primary Analysis. Immediate reactions will be collected by the site staff for 30 minutes after each vaccination and recorded on the appropriate eCRF. The LARs will complete the eDiary for 7 days following each vaccine administration. After the 7-day eDiary has been completed, the site personnel will review the data with the participant's LAR. ***Reactogenicity ongoing beyond 7 days will be recorded as a solicited AE in the e-Dairy by the LAR on a daily basis until resolution.*** Reactogenicity data will be summarized.

9.3.5.4. Safety Consideration for Dose Selection

Solicited AEs (i.e., reactogenicity), AEs and SAEs will be considered when selecting a dose.

9.4. Interim Analyses

A total of ***4*** analyses will be performed in the study:

In Part 1 participants, 1 primary vaccination series assessment and a final assessment.

In Part 2 participants ***affected by the clinical hold on 31 October 2024, an interim analysis of safety, reactogenicity, and immunogenicity may be performed for the purpose of internal decision making primary vaccination series assessments, and a final analyses of all the study objectives will be performed when all the participants have completed the last study visit***.

9.4.1. Primary Vaccination Series Assessments

In Part 1 participants, the first analysis, an interim analysis of the primary vaccination series will be performed. This will include the analysis of all available safety data for all three cohorts post study intervention and available immunogenicity data for the 1 µg, 2 µg and 5 µg cohorts. A second analysis, i.e., the final assessment will be performed after the last participant in Part 1 completes the study, approximately 6 months after the last participant has received the primary series. ***The results of this analysis will be unblinded at individual-level.***

An interim analysis of the primary vaccination series of Part 2 ***for the purpose of internal decision making may*** be performed when ***the*** participants, have completed 30 days after the primary series and the immunogenicity results become available.

9.4.2. Booster Vaccination Assessments

Since Part 1 ***and Part 2*** participants should receive SoC for the booster vaccination and no safety and immunogenicity assessment is planned for SoC pneumococcal vaccines, no analysis will be performed for booster series.

9.4.3. Final Safety Assessment

The ***final*** analysis will be performed to report ***all*** the final safety assessment (SAEs) of Part 2***,*** after the last participant completes the study, approximately 6 months after the vaccination in the last participant.

This analysis will include all the data regarding immunogenicity, reactogenicity and safety objectives of the study.

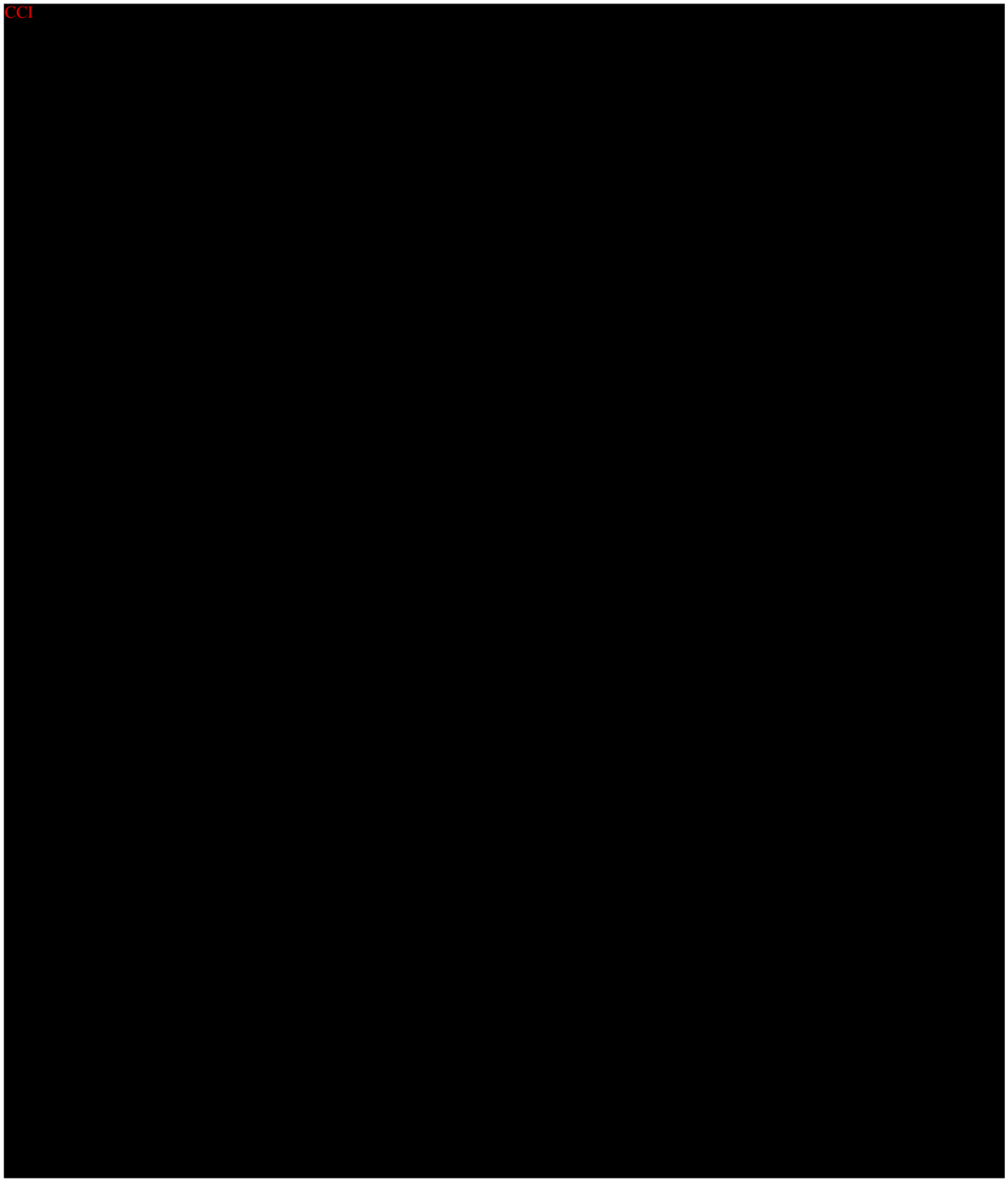
9.5. Sample Size Determination

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[redacted]

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9.6. Additional Conventions

As a general principle, no imputation of missing data will be done. Exceptions will be detailed in the SAP.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

1. This study will be conducted in accordance with the protocol and with the following:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences international ethical guidelines
    - Applicable ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) GCP guidelines
    - Applicable laws and regulations
2. The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB by the investigator and reviewed and approved by the IRB before the study is initiated.
3. Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
4. The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB
    - Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB and all other applicable local regulations

10.1.2. Financial Disclosure

Investigators and sub-investigators listed on Form FDA 1572-Statement of Investigator will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for

providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the participant's LAR and answer all questions regarding the study.

The information provided shall be provided in writing and shall:

Enable the participant's LAR to understand:

- The nature, objectives, benefits, implications, risks and inconveniences of the clinical study;
- The conditions under which the clinical study is to be conducted, including the expected duration of the participant's participation in the clinical study; and
- The possible treatment alternatives, including the follow-up measures if the participation of the participant in the clinical study is discontinued;

Be kept comprehensive, concise, clear, relevant, and understandable to a layperson;

Be provided in a prior interview with an appropriately qualified member of the study team. Special attention shall be paid to the information needs of specific patient populations and of individual participants, as well as to the methods used to give the information. Care should be taken to verify that the participant has understood the information;

Include information about the applicable damage compensation system; and

Include the international study number (ISN) and information about the future availability of the clinical study results in terms understandable to a layperson.

Participant LARs must be informed that their participation is voluntary and shall have their protective rights and guarantees explained. In particular their right to refuse to participate and the right to withdraw from the clinical study at any time without any resulting detriment and without having to provide any justification shall be explained.

Participant LARs will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB or study center.

Sample testing will be done in accordance with the recorded consent of the individual participant's parent(s)/LAR(s).

By default, collected samples for the study will be stored for a maximum of 20 years. This storage period begins when the last participant completes the last study visit. This

timeline can be adapted based on local laws, regulations or guidelines requiring different timeframes or procedures. In all cases, the storage period should be aligned with participant's parent(s)/LAR(s) consent. These additional requirements must be formally communicated to, discussed and agreed with GSK.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participant LARs must be reconsented with the most current IRB-approved version of the ICF(s) during their participation in the study, if updates are made.

A signed copy of the ICF(s) must be provided to the participant's LAR**.**

10.1.4. Data Protection

The Sponsor will use the personal data collected from participants in order to run the study and to use and publish the results of the study.

The Sponsor relies on the permission (or "consent") in order to use the data of the participant's LAR and their permission is obtained by signing the ICF.

The investigator and/or the site personnel will record information from the medical file of study participants in the eCRF and in an external (electronic) data file (e.g., central laboratory data). These records will identify the participants with a code instead of their name or other personal data. Only the investigator and the site personnel can match the code with the name of the study participant.

The Sponsor collects information from the participants during the study that may be used for:

Submission to government regulatory authorities and IRB

Use in reports or public scientific presentations

Use in research, now or in the future.

However, the identity of participants will not be revealed if study participants' personal data are shared for these purposes.

The personal data of participants may be shared with:

The Sponsor and its present or future affiliates,

Research, collaboration and licensing partners and/or service providers (such as laboratories conducting tests on behalf of the Sponsor) and authorized representatives of the Sponsor,

Study monitors appointed by the Sponsor or the Sponsor's service providers, and

Auditors/inspectors appointed by the Sponsor or the Sponsor's service providers or by health and regulatory authorities for GCP inspections.

The Sponsor will inform the participant's LAR about their privacy rights and how to exercise them under the ICF which participant's LAR will sign in order to participate in the study. The Sponsor will provide the investigator and study staff with a privacy notice explaining how their personal data will be used and how to exercise their privacy rights.

The Sponsor will comply and process personal data in accordance with all applicable privacy laws and regulations.

The Sponsor has performed a privacy impact assessment to describe the data processing activities, assess their necessity and proportionality and manage the risks to the rights and freedoms of data participants resulting from the processing of personal information by assessing them and determining the measures or mitigation actions to address them. The Sponsor has assessed, among others, how the data are collected and the purposes they are used, the impact on data participant rights, the security mechanisms including the safeguards of potential transfer of data outside the US and the access rights to data. The Sponsor has taken actions to mitigate the risks to the rights and freedoms of data participants resulting from the processing of their data as part of their participation in the study.

The participant' LAR(s) must be informed that their child's medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

The contract between sponsor and study sites specifies responsibilities of the parties related data protection, including handling of data security breaches and respective communication and cooperation of the parties.

Information technology systems used to collect, process, and store study-related data are secured by technical and organizational security measures designed to protect such data against accidental or unlawful loss, alteration, or unauthorized disclosure or access. GSK, third parties working on behalf of GSK, and/or institutions working with GSK for the purposes of this study are contractually bound to protect participant coded data. GSK will protect participant coded data and will only share it as described in the ICF.

GSK has a global, internal policy that requires all GSK staff and complementary workers to report data incidents or breaches immediately, using dedicated tools. Clear procedures are defined for assessing and investigating data breaches to identify and to take appropriate remediation steps, to contain and to mitigate any risks for individuals resulting from a breach, in compliance with applicable laws.

10.1.5. Committees Structure

10.1.5.1. Data Monitoring Committee

The study design includes close monitoring of participant safety by study investigators and the Sponsor. A DMC, which includes external safety and statistical experts, will review all available data at regular intervals to ensure the benefit/risk profile continues to support the study of AFX3772 in infants. The DMC members will be selected based on expertise in vaccine clinical studies and other considerations such as the disease area and age of the participants (e.g., infants). A DMC charter will detail the roles, responsibilities, and procedures for safety reviews.

Responsibilities of the DMC will include:

Making recommendations on all cohort dose escalation decisions during Part 1 and Part 2a. After the last participant in a dose cohort completes Dose 1 and the 7-day eDiary reporting period and prior to initiating the next dose level, the DMC will review safety data from the current cohort and the overall safety of all participants of the study.

Recommending dose escalation as appropriate

Recommending doses to be evaluated in Part 2

Periodic monitoring of all relevant safety findings throughout the study, and

Recommending an alternative design of Part 2 of the study if it recommends not proceeding as originally planned

10.1.5.2. Internal Data Review Committee

The internal data review committee (iDRC) is a multidisciplinary committee including immunogenicity, safety, clinical and statistics experts from GSK. The role of iDRC is to oversee interim analyses and to determine the composition of the investigational study intervention formulation (selection of dose levels 2 µg or 5 µg and aluminum content or any combination of those) to be assessed in Phase 3. Internal DRC will review data for making a dose escalation and dose selection recommendation. The iDRC charter will describe the required data review and documentation for making a dose escalation and dose selection recommendation.

10.1.5.3. Dissemination of Clinical Study Data

ICH E3 guidelines recommend, and European Union Directive 2001/83/EC requires that a final clinical study report that forms part of a marketing authorization application, be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator(s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study.

The representative for the coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the Sponsor prior to database lock.

10.1.6. Data Quality Assurance

All participant data relating to the study will be recorded on the eCRF unless transmitted to the Sponsor or designee electronically in an external data file (e.g., central laboratory data). The investigator is responsible for verifying that data entries in the eCRF are accurate and correct by physically or electronically signing the eCRF.

Guidance on completion of eCRFs will be provided in a separate document.

The investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy, including definition of study critical data items and processes (e.g., risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities, and requirements, including handling of non-compliance issues and monitoring techniques (central, remote or on-site monitoring) are provided in the Monitoring Plan.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data.

The Sponsor assumes accountability for actions delegated to other individuals (e.g., CROs).

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator according to ICH or applicable local regulatory requirements, whichever is longer, after study completion. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

10.1.7. Source Documents

Source data must be available at the study site to document the existence of the participants and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are handwritten on

paper or entered electronically. If source data are created (first entered), modified, maintained, archived, retrieved, or transmitted electronically via computerized systems (and/or other kind of electronic devices) as part of regulated study activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol-related assessments, AE tracking, electronic clinical outcome assessment and/or drug accountability.

Paper records from electronic systems used in place of electronic format must be certified copies. A certified copy must be an exact copy and must have all the same attributes and information as the original. Certified copies must include signature and date of the individual completing the certification. Certified copies must be a complete and chronological set of study records (including notes, attachments, and audit trail information, if applicable). All printed records must be kept in the participant file and be available for archiving.

Study monitors will perform ongoing source data review to confirm that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

The documents of the efficacy and safety evaluation committee (minutes and standard operating procedures [SOPs] and others) and the judgment committee outside the study sites (minutes and SOPs and others) shall be retained by the Sponsor.

10.1.8. Study and Site Start and Closure

The study start date is the date the first participant's LAR signs the ICF for the study (excluding screen failures).

The Sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or investigator may include but are not limited to:

For study termination:

Discontinuation of further study test product development.

For site termination:

Failure of the investigator to comply with the protocol, the requirements of the IRB or local health authorities, the Sponsor's procedures or GCP guidelines

Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator

If the study is prematurely terminated or halted, the Sponsor or designee shall promptly inform the investigators, the IRBs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or halting, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant's LAR and should ensure appropriate participant therapy and/or follow- up.

10.1.8.1. Arrangement for Use of Information and Publication of the Study

Information concerning the test product, patent applications, processes, unpublished scientific data, the Investigator's Brochure and other pertinent information is confidential and remains the property of the Sponsor. Details may only be disclosed to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the Sponsor will use the information obtained during the study in connection with the development of the product and therefore may disclose it as required to other clinical investigators or to regulatory agencies.

In order to allow for the use of the information derived from this study, the investigator understands that he/she has an obligation to provide the Sponsor with all data obtained during the study.

Publication of the study results is discussed in the study agreement.

10.1.9. Quality Assurance

The Sponsor is implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP and applicable regulatory requirement(s). Where applicable, the QA and QC systems and written SOPs of the CRO will be applied.

The Sponsor or Sponsor's designee may arrange to audit the study at any or all study sites and facilities. The audit may include on-site review of regulatory documents, eCRFs and source documents. Direct access to these documents will be required by the auditors.

To support quality around participant safety and reliability of study results, quality tolerance levels (QTLs) are defined and monitored. QTLs represent the acceptable variation of study data, taking into consideration the current state of medical and statistical knowledge about the variables to be analyzed, as well as the statistical design of the study. It is a level, point, or value associated with a parameter that should trigger an

evaluation if a deviation is detected to determine if there is a possible systematic issue (i.e., a trend has occurred).

QTL Management:

For detailed information on QTLs and control of risks associated with QTLs, refer to the Study Monitoring Plan.

10.2. Appendix 2: Assessment of Intensity

10.2.1. Assessment of Intensity for Local AEs

The participant's LAR will observe reactogenicity and tolerability from Day 1 through Day 7 and record observed events daily in the eDiary. Study site personnel will instruct the participant's LAR on how to observe reactogenicity and tolerability and how to record observed events daily in the eDiary. A measurement tool will be provided to the participants to measure the diameter of erythema/redness and swelling if it occurs. Any treatment of reactogenicity will be recorded in the e-Diary. Injection site redness and swelling will be evaluated by maximum size and then categorized during analysis as mild, moderate, or severe based on the grading scale in Table 11 below.

Note: As described in Section 8.3.3, reactogenicity will not be applicable for participants in Part 2 receiving SoC. Grade 3 and Grade 4 events after SoC will follow spontaneous reporting on AE page.

Table 11 *Local* Adverse Event Grading Scale

Local Reaction to Injectable Product	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Grade 4[b]
Pain/Tenderness	Hurts if gently touched (e.g., whimpers, winces, protests, or withdraws)	Hurts if gently touched, with crying	Interferes with limb movement	Emergency room visit or hospitalization for severe pain (tenderness) at injection site
Induration/Swelling	0.5 to 2.0 cm	>2.0 to 7.0 cm	>7 cm[a]	Necrosis
Erythema/redness	0.5 to 2.0 cm	>2.0 to 7.0 cm	>7 cm[a]	Necrosis

Note: If the size of the redness and/or swelling falls between 2 measuring device units, the higher measuring device unit number will be recorded in the eDiary.

a. Legally acceptable representatives of the participants experiencing local reactions >7.0 cm are to be contacted by the study site. An unscheduled visit may be required for targeted physical examination and symptom driven assessment based on PI's judgement.

b. Grade 4 assessment should be made by the investigator. Grade 4 will not be collected in the eDiary but as an AE on the eCRF. The severity of the local reaction should be graded using the AE severity scale in Section 10.3.3.

10.2.2. Grading of Signs of Systemic Reactogenicity

For the first 7 days following each vaccination, the participant's LAR will be asked to assess decreased appetite, increased sleep, irritability and decrease in sleep and to record the symptoms in the eDiary.

The symptoms will be assessed by the participant's LAR as mild, moderate, or severe according to the grading scale in Table 12 below. The participant's LAR will also be instructed to contact site staff if the participant experiences any possible Grade 4 prompted systemic event (i.e., emergency room visit or hospitalization for severe decreased appetite, severe drowsiness/increased sleep, or severe irritability) within 7 days after vaccination. Study staff may also contact the participant's LAR to obtain additional information on Grade 3 events entered into the eDiary.

Only an investigator is able to classify a participant's systemic event as Grade 4, after physical examination of the participant or documentation from another medically qualified source (e.g., emergency room or hospital record) or telephone contact with the participant's LAR. If a participant experiences a Grade 4 systemic event, the investigator must immediately notify the Sponsor.

Table 12 System Reactogenicity Grading Scale

	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Grade 4[a]
Decreased appetite	Decreased interest in eating	Decreased oral intake	Refusal to feed	Emergency room visit or hospitalization for severe decreased Appetite
Increased sleep	Increased or prolonged sleeping Bouts	Slightly subdued, interfering with daily activity	Disabling, not interested in usual daily activity	Emergency room visit or hospitalization for severe increased sleep
Irritability	Easily consolable	Requiring increased attention	Inconsolable; crying cannot be comforted	Emergency room visit or hospitalization for severe irritability
Decrease in sleep	Needing more time to fall asleep	Interfering with daily activity	Disabling, not interested in daily activity	Emergency room visit or hospitalization for severe decreased sleep

a. Grade 4 assessment should be made by the investigator; Grade 4 will not be collected in the eDiary but will be collected as an AE on the eCRF. The severity of the systemic event should be graded using the AE severity grading scale in Section 10.3.3.

Body temperature will be measured prior to administration of study vaccine. The participant's LAR will be given a thermometer and instructed by study site personnel on how to measure body temperature and record it to 1 decimal place in the eDiary device. Temperature will be collected in the eDiary in the evening as well as at any time when an elevated temperature is suspected during the 7-day collection period. Fever is defined as a temperature of ≥100.4°F (≥38.0°C). The highest temperature for each day will be recorded in the eDiary. In the event of a fever on Day 7, temperature will be collected daily until fever has resolved (1 day of temperature less than 100.4°F [38.0°C]) in order to collect a stop date in the eCRF. The LAR will be prompted to contact the investigator if the participant experiences a fever ≥104.0°F (≥40.0°C) to perform an unscheduled assessment. If a fever ≥102.1°F (39.0°C) is reported in the eDiary, a TC should occur to ascertain further details and determine if a site visit is clinically indicated. Only an investigator or medically qualified designee can confirm a participant's fever as ≥104.0°F (≥40.0°C). If a participant experiences a confirmed fever ≥104.0°F (≥40.0°C), the investigator must immediately notify the Sponsor.

Body temperature will be grouped into ranges for the analysis based on Table 13.

Table 13 Fever Ranges for Grouped Analysis

Body Temperature Range
100.4°F to 101.1°F (38.0°C to 38.4°C)
101.2°F to 102.0°F (38.5°C to 38.9°C)
102.1°F to 104.0°F (39.0°C to 40.0°C)
>104.0°F (>40.0°C)

Grading for intensity will be performed within 7 days for solicited events and within 30 days for unsolicited events post vaccination by PI in addition to LAR in appendix 2, for any intensity including Grade 4.

10.3. Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1. Definition of AE

AE Definition
1. An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. 2. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.
Definition of Unsolicited and Solicited AE
1. An unsolicited adverse event is an adverse event that was not solicited using a participant eDiary and that is communicated by a participant's LAR(s) who has signed the informed consent. Unsolicited AEs include serious and non-serious AEs. 2. Potential unsolicited AEs may be medically attended (i.e., symptoms or illnesses requiring a hospitalization, emergency room visit, or visit to/by a healthcare provider). The participant's LAR(s) will be instructed to contact the site as soon as possible to report medically attended event(s), as well as any events that, though not medically attended, are of participant's LAR(s) concern. Detailed information about reported unsolicited AEs will be collected by qualified site personnel and documented in the participant's records. 3. Unsolicited AEs that are not medically attended nor perceived as a concern by the participant's LAR(s) will be collected during an interview with the participant's LAR(s) and by review of available medical records at the next visit. 4. Solicited AEs are predefined local at the injection site and systemic events for which the participant is specifically questioned, and which are noted by the participant in their eDiary.
Events Meeting the AE Definition
1. Any abnormal safety assessments (e.g., ECG, radiological scans, body temperature measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). 2. Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. 3. Reactogenicity events that extend beyond the 7-day period will be recorded in the eDiary. 4. New conditions detected or diagnosed after study vaccine or comparator administration even though it may have been present before the start of the study.

Events NOT Meeting the AE Definition
1. Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
2. Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
3. Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of SAE

An SAE is defined as any untoward medical occurrence that, at any dose, meets one or more of the criteria listed:
a. Results in death
b. Is life threatening The term life threatening in the definition of serious refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization 1. In general, hospitalization signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious. 2. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in persistent or significant disability/incapacity 3. The term disability means a substantial disruption of a person's ability to conduct normal life functions. 4. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza-like illness, and accidental trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Other situations: 5. Medical or scientific judgment should be exercised by the investigator in deciding whether SAE reporting is appropriate in other situations such as significant medical events that may jeopardize the participant or may require

medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

- Examples of such events include invasive or malignant cancers, intensive treatment for allergic bronchospasm, blood dyscrasias, convulsions or development of intervention dependency or intervention abuse.

6. If an event is not an AE per definition in Section 10.3.1, then it cannot be an SAE even if serious conditions are met.

10.3.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.

The investigator will then record all relevant AE/SAE information in the eCRF.

It is not acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the eCRF.

There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the Sponsor.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

An eDiary will be used in this study to capture solicited administration site or systemic events. The participant’s LAR(s) should be trained on how and when to complete the eDiary.

Anyone who measures administration site or systemic events and who will record the event in the eDiary should be trained on using the eDiary. This training must be documented in the participant’s source record.

For each solicited and unsolicited AE the participant experiences, the participant’s LAR(s) will be asked if their child received medical attention (defined as unscheduled visit to or from medical personnel for any reason, including emergency room visits). This information will be recorded in the participant’s eDiary and in the participant’s eCRF as part of normal AE reporting (for solicited and unsolicited AEs). If the collection of solicited AEs was not possible for any reasons via the eDiary and solicited AEs were reported to the investigator by the participant’s LAR(s), then it would be possible for it to be reported directly to the site staff and submitted (e.g., via the eCRF). Medical attention

received for SAEs/AESIs will have to be reported using the normal AE reporting process in the eCRF. If any individual other than the participant's LAR(s) is making entries in the eDiary, their identity must be documented in the participant's source record. All solicited events that occur during the 7 days following administration of each dose of study intervention (Day 1 to Day 7 post-Dose) must be recorded into the eDiary, irrespective of intensity. An automatic reminder to complete the eDiary will be sent to the participants during this time frame. Daily eDiary compliance will be checked by the investigator or delegate on the eDiary portal. In case of non-compliance, the investigator should contact the participant to remind the importance of daily entries. All other AEs occurring within this time frame should be recorded into the appropriate section of the eCRF, irrespective of their intensity or whether or not they are considered related to the study intervention. After review and verbal discussion of the e-Diary entries with the participant's LAR(s), if there are eDiary errors, the investigator will complete his/her own assessment in the relevant sections of the eCRF.
Assessment of Intensity
The investigator will use the following definitions to rate the severity of each AE: Mild: No disruption of normal daily activities Moderate: Affect normal daily activities Severe: Inability to perform daily activities Reactogenicity grading scale will assess local and systemic injection reactions
Assessment of Causality
The investigator is obligated to assess the relationship between study vaccine or comparator, study procedures and each occurrence of each AE/SAE. A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study vaccine or comparator administration will be considered and investigated.

The investigator will also consult the Investigator's Brochure and/or product information, for marketed products, in his/her assessment.

For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always ***assesses*** causality for every event before the initial transmission of the SAE data to the Sponsor.

The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

The causality assessment between study vaccine or comparator is one of the criteria used when determining regulatory reporting requirements.

Following a review of the relevant data, the causal relationship between the study vaccine or comparator and each (S)AE will be assessed by answering “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the study vaccine or comparator?”

When assessing causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a “reasonable possibility” that an (S)AE may have been caused by the study vaccine or comparator (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

Has the participant been administered the study vaccine or comparator?

Plausibility (i.e., Could the event have been caused by the suspect study vaccine or comparator? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and study data, etc.)

Available alternative explanations independent of the study vaccine or comparator exposure; such as other concomitant drugs, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc., and strength of the alternative explanation.

Temporal relationship between exposure to the study vaccine or comparator and (S)AE onset and/or resolution. Did the (S)AE occur in a reasonable temporal relationship to the administration of the study vaccine or comparator?

Finally, judging which are more likely based on all the above contents, factors of reasonable possibility or confounding factors, comprehensive judgment of plausible will be provided.

There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to the Sponsor. While it is very important that the investigator always assesses causality for every event before the initial transmission of the SAE data to the Sponsor, the initial report should be submitted without delay (i.e., within 24 hours of awareness). With limited or insufficient information about the event to make an informed judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of "no" is to be considered. In such instance, the investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified investigator may revise his/her assessment of causality in light of new information regarding the SAE and shall send an SAE follow-up report and update the eCRF with the new information and updated causality assessment.
Follow-up of AEs and SAEs
The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the Sponsor with a copy of any postmortem findings including histopathology. New or updated information will be recorded in the originally completed eCRF. The investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.3.4. Reporting of SAEs

SAE Reporting via Paper Data Collection Tool
Investigator/sites are to enter all initial SAE information and follow-up information into the Electronic Data Collection (EDC) within 24 hours of awareness. The paper-based method is a secondary method of reporting that is to be used only when the EDC is unavailable. The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the Sponsor (or designee) by email immediately (within 24 hours of awareness) if the EDC is not accessible. The SAE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE data, as well as the investigator causality assessment including the explanation for the causality assessment. Syneos Health Inc. Email: safetyreporting@syneoshealth.com Fax: +1-877-464-7787 Follow-up information for the event should be sent promptly (as soon as available, within 7 days of the initial notification) Full details of the SAE should be recorded in the medical records, SAE/special situation worksheet and on the eCRF The following minimum information is required: ISN/study number • Participant number, sex and age • Date of report • Description of the SAE (event and seriousness criteria) • Causal relationship to the study vaccine (including rationale) • Drug provided (if any) The Sponsor or Sponsor's designee will medically evaluate the SAE and determine if the report meets the requirements for expedited reporting based on seriousness, causality, and expectedness of the events (e.g., SUSAR reporting) according to current local/regional regulatory requirements. The Sponsor or Sponsor's designee will submit expedited safety reports to competent authorities and concerned ethics committee per

SAE Reporting via Paper Data Collection Tool
current local regulations and will inform the investigators of such regulatory reports as required. Investigators must submit safety reports as required by their IRB within timelines set by regional regulations (e.g., FDA) where required. Documentation of the submission to and receipt by the IRB of expedited safety reports should be retained by the study site. In the US, FDA expedited IND reporting guidelines will be followed. The Sponsor will notify all investigators responsible for ongoing clinical studies with the test product of all SUSARs, which require submission per local requirements IRB. The investigators or designee should provide written documentation of IRB notification for each report to the Sponsor.

10.3.5. Reporting Procedures for Special Situations

10.3.5.1. Medication Error, Overdose and "Off-label Use"

Medication Error, Overdose and "Off-label Use"
If a medication error (defined as an unintended failure in the treatment process that leads to, or has the potential to lead to, harm to the participant), overdose or "off-label use" (i.e., use outside of the target disease defined in the protocol) is suspected, refer to Section 6.7 Treatment of Overdose. Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in Section 10.3.4 Reporting of SAEs together with the details of the medication error, overdose and/or "off-label use."

10.3.5.2. Misuse/Abuse

Misuse/Abuse
Definition of misuse: Situations where the study vaccine or comparator are intentionally and inappropriately used not in accordance with the intended use as defined in the protocol. Definition of abuse: Persistent or sporadic, intentional excessive use of medicinal products which is accompanied by harmful physical or psychological effects. If misuse or abuse of the study vaccine or comparator is suspected, the investigator must forward the special situation worksheet to the Sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in Section 10.3.4 Reporting of SAEs with details of the misuse or abuse of the study vaccine or comparator.

10.3.5.3. Occupational Exposure

Occupational Exposure
If occupational exposure (e.g., inadvertent exposure to the study vaccine of study site personnel while preparing it for administration to the participant) to the study vaccine occurs, the investigator must forward the special situation worksheet to the Sponsor by fax or email immediately (within 24 hours of awareness [Section 10.3.4]). Any associated (S)AEs occurring to the individual associated with or resulting from the special situation are to be reported on the special situations worksheet.

10.3.5.4. (Suspicion of) Transmission of Infectious Agent

(Suspicion of) Transmission of Infectious Agent
If transmission of an infectious agent associated with the study vaccine is suspected, the investigator must forward the special situation worksheet to the Sponsor by fax or email immediately (within 24 hours of awareness) and any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in Section 10.3.4 Reporting of SAEs together with the details of the suspected transmission of infectious agent.

10.3.5.5. Suspected Drug-drug Interaction

Suspected Drug-drug Interaction
If a drug-drug interaction associated with the study vaccine is suspected, the investigator must forward the special situation worksheet to the Sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in Section 10.3.4 Reporting Procedures for Serious Adverse Events together with details of the suspected drug-drug interaction.

10.3.6. Supply of New Information Affecting the Conduct of the Study

Supply of New Information Affecting the Conduct of the Study
When new information becomes available that is necessary for conducting the study properly, the Sponsor will inform all investigators involved in the study, as well as the appropriate regulatory authorities. Investigators should inform the IRB of such information when needed. The investigator will also inform the participants, who will be required to sign an updated ICF in order to continue in the study.

10.3.7. Urgent Safety Measures

Urgent Safety Measures
An urgent safety measure (USM) is an intervention that is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the Sponsor, relevant competent authorities (CA), IRB, where applicable, in order to protect participants from any immediate hazard to their health and/or safety. Either the investigator or the Sponsor can initiate a USM. The cause of a USM can be safety-, product- or procedure-related.

10.3.8. Reporting Urgent Safety Measures

Reporting Urgent Safety Measures
In the event of a potential USM, the investigator must contact the Sponsor at safetyreporting@syneoshealth.com (within 24 hours of awareness). Full details of the potential USM are to be recorded in the participant's medical records. The Sponsor may request additional information related to the event to support their evaluation. If the event is confirmed to be a USM, the Sponsor will take appropriate action to ensure the safety and welfare of the participants. These actions may include but are not limited to a change in study procedures or study treatment, halting further enrollment in the study, or stopping the study in its entirety. The Sponsor or Sponsor's designee will notify the relevant competent authorities and concerned ethics committee within the timelines required per current local regulations, and will inform the investigators, as required. When required, investigators must notify their IRB within timelines set by regional regulations

10.4. Appendix 4: List of Excluded Concomitant Medications

Participants must not use any of the following during the study:

Blood products, including immunoglobulins. **Note:** This does not include monoclonal antibodies, such as palivizumab and nirsevimab.

Systemic corticosteroids equivalent to > 2 mg/kg total daily dose of prednisone (or > 20 mg/day for persons weighing > 10 kg) for a period of more than 14 days. Topical, ophthalmic and inhaled steroids are allowed.

Immunosuppressive drugs required for conditions including but not limited to HIV infection, malignancy or organ or bone marrow transplant.

Influenza vaccine is permitted if administered either 7 days prior or 15 days after administration of the study vaccine.

SARS-CoV-2 (COVID-19) vaccine is permitted if administered either 7 days prior or 15 days after administration of the study vaccine.

10.5. Appendix 5: Clinical Laboratory Assessments

Laboratory tests will be performed according to the schedule of assessments (Table 1 and Table 2) and sent for analysis to a laboratory designated by the Sponsor.

Immunogenicity of AFX3772 will be evaluated by the different assays listed in Table 14.

Table 14 Clinical Laboratory Tests

System	Component	Method	Testing Lab	Unit
Serum	Pneumococcal serotype-specific anti-capsular polysaccharide IgG*	dLIA or ECL	GSK or GSK designated laboratory	ng/mL µg/mL
Serum	Anti-pneumococcal serotype-specific opsonophagocytic activity*	MOPA		dil^{-1}
Serum	Anti-CP1 IgG	ECL		AU/mL

Ab: antibody; CP1: fusion protein; dLIA: direct Luminex immunoassay; IgG: immunoglobulin G; MOPA: multiplexed opsonophagocytic assay; dil^{-1}: reciprocal of the dilution; µg = microgram; mL = milliliter; ng: nanogram; ECL: electrochemiluminescent assay.

* Testing for each of the 24 pneumococcal AFX3772 vaccine serotypes (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F).

10.6. Appendix 6: Clinical Study Continuity

10.6.1. INTRODUCTION

The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during any crisis (e.g., natural disaster, pandemic).

10.6.2. BENEFIT-RISK RATIONALE

Maintaining the safety of clinical study participants and delivering continuity of care in the clinical study setting is paramount during any crisis. The site is expected to follow the protocol and associated schedule of assessments (Table 1 and Table 2) unless the site principal investigator discusses the need with the Sponsor's medical monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study required assessments and procedures if study activities are unable to be performed as described in Section 8 due to a crisis.

10.6.3. INFORMED CONSENT

The LAR of participants who need to follow any or all of the alternate measures outlined in this appendix will be required to provide informed consent, which explicitly informs them of the nature of and rationale for these changes and gains their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the principal investigator or designee will obtain oral agreement from the participant followed by written documentation as soon as is feasible. A separate addendum to the study informed consent will be provided to document the participant's consent of the changes.

10.6.4. PARTICIPANT PROCEDURES ASSESSMENT

Sites with participants who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if one or more of the following conditions are met due to the crisis:

Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible.

Site facilities have been closed for clinical study conduct.

Site has been restricted to treating participants with conditions outside of the scope of the study.

Site personnel have temporarily relocated the conduct of the study to a location that places a burden on the participant with respect to time and travel.

Participant(s) have temporarily relocated from the current study site to an alternate study site to avoid placing a burden on the participant with respect to travel.

Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel.

Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety.

10.6.5. ALTERNATIVES TO THE SCHEDULE OF ASSESSMENTS

Adherence to the original protocol as reflected in the schedule of assessments (Table 1 and Table 2) is expected, where plausible, in the case of a crisis. If one or more of the alternate measures noted below is implemented for a participant, the site should document in the participant's source document the justification for implementing the alternate measure and the actual alternate measures that were implemented, along with the corresponding time point(s).

In a crisis situation, all vaccinations need to be administered by the trained site personnel as previously described in the schedule of assessments (Table 1 and Table 2). Blood collections at Visits 3, 5 and 7 may be completed at a local laboratory. This is to allow for continuity of receiving IMP and maintaining critical safety and efficacy assessments for participants in the study at a time of crisis.

10.6.6. INVESTIGATIONAL PRODUCT SUPPLY

If any of the conditions outlined above in the Participants Procedures Assessment are met, the following mitigating strategy will be employed, as needed, to ensure continuity of study vaccine supply to the participants:

Increase stock of study vaccine on site to reduce number of shipments required, if site space will allow.

10.6.7. DATA COLLECTION REQUIREMENTS

Additional data may be collected in order to indicate how participation in the study may have been affected by a crisis and to accommodate data collection resulting from alternate measures implemented to manage the conduct of the study and participant safety.

Critical assessments for safety and efficacy based on study endpoints to be identified as missing or altered (performed virtually, at alternative locations, out of window or other modifications) due to the crisis.

10.7. Appendix 7: Medical Device AEs, ADEs, SAEs, SADEs, USADEs and Device Deficiencies: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting in Medical Device Studies

- Both the investigator and the sponsor will comply with all local reporting requirements for medical devices.
- The detection and documentation procedures described in this protocol apply to all sponsor medical devices provided for use in the study. See Section 6.1.1 for the list of sponsor medical devices.

10.7.1. Definition of medical device AE and ADE

Medical device AE and ADE definition
• A medical device AE is any untoward medical occurrence, in a clinical study participant, users, or other persons, temporally associated with the use of study intervention whether or not considered related to the investigational medical device. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated)

temporally associated with the use of an investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices. • An ADE is defined as an AE related to the use of an investigational medical device. This definition includes any AE resulting from insufficient or inadequate instructions for use or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.

10.7.2. Definition of medical device SAE, SADE, and USADE

A medical device SAE is any serious AEs that:
a. Led to death.
b. Led to serious deterioration in the health of the participant, that either resulted in: • **A life-threatening illness or injury. The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.** • **A permanent impairment of a body structure or a body function.** • **Inpatient or prolonged hospitalization. Planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE.** • **Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.** • **Chronic disease (MDR 2017/745).**
c. Led to fetal distress, fetal death or a congenital abnormality or birth defect.
d. Is a suspected transmission of any infectious agent via a medicinal product.
SADE definition
• A SADE is defined as an ADE that has resulted in any of the consequences characteristic of an SAE. • Any device deficiency that might have led to an SAE if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate.
Unanticipated SADE (USADE) definition

- An USADE (also identified as UADE in US Regulations 21 CFR 813.3), is defined as a serious ADE that by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report/IB (see Section 2.3).

10.7.3. Definition of device deficiency

Device deficiency definition
• A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequacy of the information supplied by the manufacturer.

10.7.4. Recording and follow-up of medical device AEs and/or SAEs and device deficiencies

10.7.4.1. Medical device AE, SAE, and device deficiency recording

- When an AE/SAE/device deficiency occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE/device deficiency information in the participant's medical records, in accordance with the investigator's normal clinical practice and on the appropriate form.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the Sponsor AE/SAE/device deficiency form.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the Sponsor.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- For device deficiencies, it is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the deficiency.

A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of a device deficiency. This includes any amendment to the device design to prevent recurrence.

- If the site during the course of the study becomes aware of any serious, nonserious incident (including device deficiencies and malfunctions) related to any GSK

product that is not part of the study design, they will report these events to GSK or to the concerned CA via the national spontaneous reporting system. These will be classified as spontaneous ICSRs.

10.7.4.2. Assessment of intensity

See Section 10.3.3.

10.7.4.3. Assessment of causality

See Section 10.3.3.

10.7.4.4. Follow-up of medical device AE/SAE and device deficiency

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE/SAE/device deficiency as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the Sponsor with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed form.
- The investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.7.5. Reporting of medical device SAEs

Medical Device SAE Reporting to the Sponsor via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to the Sponsor will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next table) to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken offline to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken offline, then the site can report this information on a paper SAE form (see next table) or to the medical monitor by telephone.

- If the site during the course of the study or poststudy becomes aware of any serious, nonserious AEs, pregnancy exposure, related to any GSK device they will report these events to GSK or to the concerned CA via the national spontaneous reporting system. These will be classified as spontaneous ICSRs.

Medical device SAE reporting to the Sponsor via paper data collection tool

- Investigator/sites are to enter all initial medical device SAE information and follow-up information into the Electronic Data Collection (EDC) within 24 hours of awareness. The paper-based method is a secondary method of reporting that is to be used only when the EDC is unavailable.
- The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the Sponsor (or designee) by email immediately (within 24 hours of awareness) if the EDC is not accessible.
- The SAE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE data, as well as the investigator causality assessment including the explanation for the causality assessment.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE paper data collection tool within the designated reporting time frames.
- Please see Section 10.3.4 for contact details.

10.7.6. Reporting of SADEs

SADE Reporting to the Sponsor

Note: There are additional reporting obligations for medical device deficiencies that are potentially related to SAEs that must fulfill the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

- Any device deficiency that is associated with an SAE must be reported to the sponsor within 24 hours after the investigator determines that the event meets the definition of a device deficiency.
- The sponsor will review all device deficiencies and determine and document in writing whether they could have led to an SAE. These device deficiencies will be reported to the regulatory authorities and IRBs/IECs as required by national regulations.

10.7.7. Reporting of medical device deficiencies for associated person

- **Reporting to GSK**

If an Associated Person (e.g., spouse, caregiver, site staff) experiences a device deficiency, the medical device deficiency information, and any associated AE/SAE information will be reported to GSK. The associated person will be provided with the authorization to contact physician letter.

If follow-up information is required, authorization to contact physician (or other licensed medical practitioner) must be signed to obtain consent.

- Medical device deficiencies that are not related to an AE or SAE should be reported via email to gsk-rd.complaints@gsk.com, using the medical device deficiency report form.
- If the medical device deficiency is related to a nonserious AE and not linked to an SAE, please send the medical device deficiency report form with details of the associated AE via email to gsk-rd.complaints@gsk.com only.
- If the device incident is linked to an SAE, please email the medical device deficiency report form, within 24 hours.
- GSK will review all device deficiencies and determine and document in writing whether they could have led to an SAE. These device deficiencies will be reported to the regulatory authorities and IRBs/IECs as required by national regulations.

10.8. Appendix 8: Protocol Amendment History



CCI

[redacted]

CCI

[redacted]

CCI

[redacted]

CCI

[redacted]

CCI

[redacted]

CCI [redacted]

1. Protocol summary

1.1. Synopsis

Objectives, Endpoints, and Estimands

Objectives	Endpoints
Primary	
• To describe the safety and tolerability profiles of different dose levels (1**, 2 and 5 µg) of AFX3772 compared with PCV* with respect to the proportion of participants with AEs	Following any vaccination with AFX3772 or PCV*: • Solicited injection site AEs (tenderness, redness/erythema and swelling) from Day 1 through Day 7 post-vaccination • Solicited systemic AEs (irritability, fever, decrease of appetite, increased sleep, and decrease in sleep) from Day 1 through Day 7 • AEs Day 1 through Day 30 • SAEs through completion of study participation (6 months post last study vaccination)
Secondary	
• To evaluate the immunogenicity of different dose levels of AFX3772 (1**, 2 and 5 µg) compared with PCV*	• The proportion of participants with a pneumococcal serotype-specific IgG concentration ≥ 0.35 µg/mL ***or corresponding threshold*** at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4 • Serotype-specific IgG GMC at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4
Exploratory	
CCI [redacted]	

AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; ***OPA: opsonophagocytic activity;*** PCV13: Prevnar 13; PCV20: Prevnar 20; PD: post-dose; SAE: serious adverse event

*:PCV13 [Part 1] and PCV20 [Part 2]. ** in Part 1 only
Estimands
Primary Estimand description: Percentage of pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age who receive at least 1 vaccination with either AFX3772, or PCV*(*PCV13 [Part 1] and PCV20 [Part 2]) and develop a solicited local or systemic AE within 7 days of each vaccination, AEs through Day 30 after each vaccination, and SAEs through completion of study participation. • A treatment policy strategy is used for assessing safety irrespective of missed vaccination or taking of prohibited medication/vaccination.

Brief Summary

~~Approximately~~ ***Up to*** 480 healthy infants approximately 2 months of age are planned to be enrolled and receive 4 doses of study vaccine over 8 protocol-defined visits spanning a duration of approximately 18 to 21 months. Part 1 is the dose escalation, lead-in portion of the study in which 40 infants at each dose level were randomized 3:1 in sequential cohorts of increasing doses of AFX3772 (Cohort 1: 1 µg; Cohort 2: 2 µg and Cohort 3: 5 µg) or PCV13. Enrollment in Cohorts 2 and 3 proceeded following Data Monitoring Committee (DMC) review of cumulative safety and tolerability data through Day 7 from preceding cohorts. Prior to the completion of vaccination series in Part 1, the study vaccination was halted and urgent safety measures, i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines, were implemented for participants of Part 1 and the start of recruitment in Part 2 was paused at the same time.

Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 1, Part 2 will start with target recruitment of a total ***of*** 360 healthy infants. In the dose escalation lead-in ~~***the***~~ step of Part 2 (***i.e.,*** Part 2a**)** ~~i.e., 45 infants)~~, ***at each dose level, 45 infants*** will be randomized 2:1 into 2 cohorts of increasing dose levels of AFX3772 (Cohort 4: 2 µg and Cohort 5: 5 µg) or PCV20.

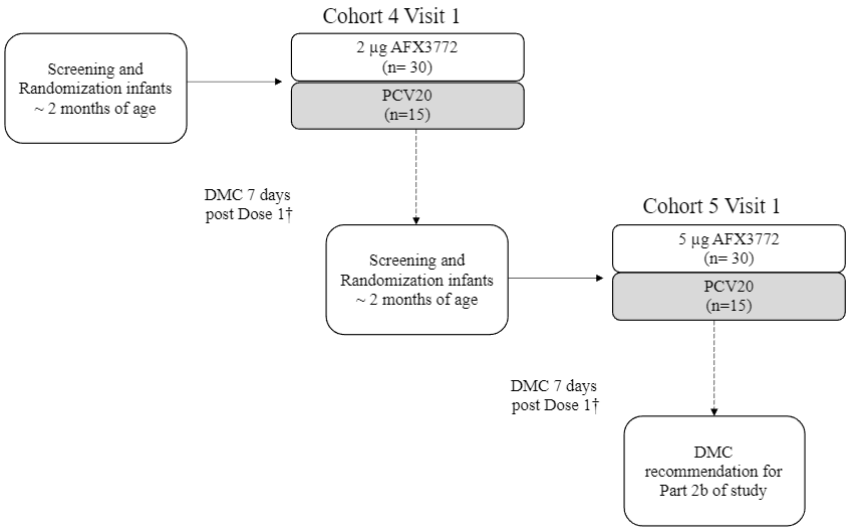
Number of Participants: ~~Approximately~~ ***Up to*** 480 healthy infants are planned to be enrolled in this study with a subset of 120 initially enrolled (Part 1) followed by ~~the remaining~~ 360 participants (Part 2). Accounting for an 85% evaluable rate, such a sample size predicts approximately 102 evaluable participants will be available for analysis in each treatment arm in Part 2.

CONFIDENTIAL

Internal Data Review Committee: Yes

1.2. Schema

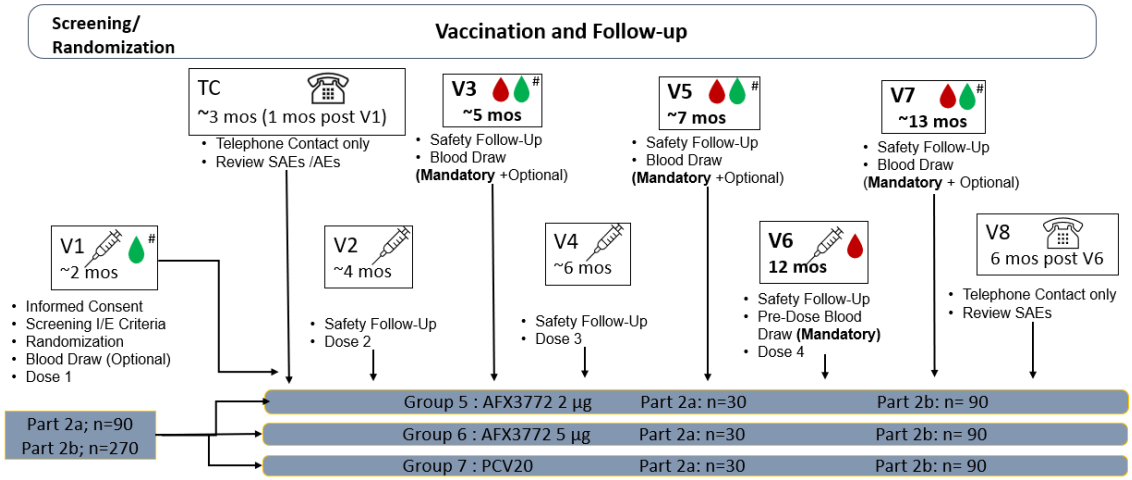
Figure 3 Study Schema Part 2a – Dose escalation



DMC: Data Monitoring Committee; PCV20: Prevnar 20
†DMC reviews safety data through Day 7 ***post-dose 1*** for each cohort (approximately 45 participants), after all participants have completed eDiary safety assessments through Day 7 postvaccination.
Part 2b starts after DMC review of Part 2a Cohort 5 safety data through Day 7 plus cumulative safety data of Part 2a and agreement to proceed to full enrollment.
Subsequent cohort starts after DMC recommendation to dose escalate.

Infants enrolled in Part 2 will follow the same visit schedule as depicted in Figure 4.

Figure 4 Study Schema Part 2



n: number of participants; mos: months; PCV20: Prevnar 20
Telephone contact occurs ***on*** Day 8 post-vaccination for safety follow-up and eDiary review,
TC = Safety follow-up telephone contact at 1-month post-Dose 1,

#In addition to the mandatory blood draws at Visits 3, 5, 6, and 7, optional blood sampling will be proposed in Part 2b at Visits 1, 3, 5, and 7.
Approximately 2.5 mL will be proposed as optional blood sampling at Visits 1, 3, and 5, and approximately 5 mL will be proposed as optional blood sampling at Visit 7.
Part 2b start after DMC review of Part 2a Cohort 2 safety data through Day 7 ***post-dose 1*** and agreement to proceed to full enrollment.
Concomitant vaccines are permitted as per local requirements.

1.3. Schedule of Assessments

Table 2 Schedule of Assessments (SoA) for Part 2

Body Temperature	X†			X†		X	X†			X†				Rectal or axillary temperature will be taken at the site prior to study vaccination according to the site's standard of care. Participants who have febrile illness (rectal temperature ≥38.***0***°C [≥100.***4***°F] or axillary temperature ≥37.***5***°C [≥***99.1***°F]) occurring at or within 72 hours of Visits 1, 2, 4 and 6 must be rescheduled.
Blood Sample ***(For Part 2) (Mandatory)***						X			X[3]	X†		X		***For details of amount of blood draw, see Table 8.***
Blood Sample (For Part 2b) (Optional)	***X***					***X***			***X***			***X***		***For details of amount of blood draw, see Table 8.***
Review eDiary with participant's LAR[6]		X	X		X			X			X			On Day 8 postvaccination, the site will contact the participant's LAR via telephone for safety follow-up, concomitant medications and eDiary review.

4. All unscheduled visits and telephone contacts must be reported in the eCRF. ***See Section 8.1.1 for unscheduled visit assessments.***

6. In-App reminders in the eDiary. There will be a call from the site to the LAR in case of missing data for the day. Also, there will be training for the LAR and sites for eDiary completion.

Note: The optional blood draws will be collected in Part 2b . The mandatory blood samples are collected in Part 2a and Part 2b throughout the study.

2. Introduction

Affinivax has developed an investigational 24-valent pneumococcal vaccine (referred to as AFX3772) for the prevention of pneumococcal disease caused by the serotypes contained in the vaccine. AFX3772 contains 24 serotypes of *Streptococcus pneumoniae* and a unique fusion protein (CP1) created by the fusion of 2 pneumococcal proteins (sp1500 and sp0785), which has the potential to induce T- and B-cell responses and provide extended protection. This novel investigational pneumococcal vaccine uses the CCI [redacted] (MAPS) platform [Zhang, 2013] containing pneumococcal capsular polysaccharides (PS) individually biotinylated and complexed with CP1. It

contains 11 additional PS (serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F) compared to the pneumococcal conjugate vaccine Prevnar13 (PCV13) (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) or 4 additional PS (serotypes 2, 9N, 17F and 20B) compared the ~~***Prevnar 20 (PCV20)/Apexxnar***~~ Prev***(e)***nar 20 (PCV20) (serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F).

2.3. Benefit/Risk Assessment

At the time this study ~~is~~***was*** being ~~conducted~~***started***, there ~~is~~***was*** a COVID-19 global pandemic. The sites ~~are~~***were*** expected to implement risk mitigation strategies for the participant and his or her legally acceptable representative (LAR). Some data can be collected remotely via telephone. A summary of clinically significant risks and proposed mitigations strategies is presented in Table 3.

Table 3 Summary of Clinically Significant Risks and Mitigation Strategies

Clinically Significant Risks	Mitigation Strategies
Study Procedures	
• Discomfort and local reactions associated with blood draws • Burden to families associated with study-specific procedures	• Use of pediatric phlebotomists. • Additional procedures minimized to include only those required to ensure safety and to accomplish objectives of the study. • Potential for more attentive clinical care and safety follow-up. • ***Make sure the baby is well-hydrated. This helps with finding a vein AND with increasing the serum content in the sample. Qualified staff should be drawing blood (e.g., pediatric phlebotomist).*** • ***EMLA cream is permitted per protocol for blood draws and vaccinations and should be used as per site standard of care.*** • ***Vein finders can be used as per site standard of care.*** • ***Warm compresses can be used as per site standard of care to aid in successful blood draw.*** • ***Breastfeeding during the procedure can be a comfort to infants during blood draws and vaccinations.*** • ***Vibrating icepack provided to all sites as distraction and pain management tool.***

PCV13: Prevnar 13; PCV20: Prevnar 20; DMC: Data Monitoring Committee
*PCV13 (Part 1) and PCV20 (Part 2)

3. Objectives, endpoints, and Estimands

Objectives	Endpoints
Secondary	
• To evaluate the immunogenicity of different dose levels of AFX3772 (1**, 2 and 5 µg) compared with PCV*	• The proportion of participants with a pneumococcal serotype-specific IgG concentration ≥ 0.35 µg/mL ***or corresponding threshold*** at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4 • Serotype-specific IgG GMC at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4
Exploratory	

CCI

AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; ***OPA: opsonophagocytic activity;*** PCV13: Prevnar 13; PCV20: Prevnar 20; PD: post-dose; SAE: serious adverse event
*:PCV13 [Part 1] and PCV20 [Part 2].
** in Part 1 only

Estimands

Primary Estimand description: Percentage of pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age who receive at least 1 vaccination with either AFX3772, or PCV*(*PCV13 [Part 1] and PCV20 [Part 2]) and develop a solicited local or systemic AE within 7 days of each vaccination, AEs through Day 30 after each vaccination, and SAEs through completion of study participation.

- A treatment policy strategy is used for assessing safety irrespective of missed vaccination or taking of prohibited medication/vaccination.

4. Study Design

4.1. Overall Design

This study will be conducted at approximately 65-75 study sites in the US including Puerto Rico. ~~Approximately~~ ***~~480~~Up to*** 480 healthy infants are planned to be enrolled in this study with an initial enrollment of 120 infants in Part 1, followed by up to 360 infants in Part 2.

Additional blood volumes will be collected from study participants for the development ***~~and~~***, validation, ***and maintenance*** of assays measuring responses to the pneumococcal vaccine needed for the vaccine clinical development program.

4.1.1. Dose Escalation and Stopping Criteria

Please note: Reporting of AEs and SAEs occurring in the trial are to follow guidelines detailed in Sections 10.3.3 and 10.3.4.

4.2. Scientific Rationale for Study Design

This study is designed to evaluate the safety, tolerability, and immunogenicity of AFX3772 in healthy infants who are naïve to licensed or investigational pneumococcal vaccines. The study design is consistent with the clinical development for PCV and addresses the guidelines set for the evaluation of next generation pneumococcal vaccines by the World Health Organization (World Health Organization [WHO, 2013]) and

adopted by global regulatory agencies. The proposed safety, tolerability (including reactogenicity) and immunological assessments (i.e., immunological endpoints [0.35 µg/mL ***or corresponding*** threshold value, IgG GMCs and opsonophagocytic activity (OPA) geometric mean titers (GMTs)]) are also consistent with standard PCV pediatric clinical development endpoints.

The study will implement a 3+1 pneumococcal vaccination schedule for infants as recommended by the US ACIP. In order to evaluate other vaccination schedules (i.e., 2 + 1 schedule used in Europe and elsewhere), immunogenicity will also be assessed after the second vaccination. A dose escalation approach is being implemented in this trial to ensure adequate safety review following the first administration of each dose level prior to initiating enrollment into the next dose level. Once dose escalation has been completed and DMC recommendation has been given and followed by the Sponsor's decision to continue the study, enrollment in Part 2 ~~will be initiated.~~

The objective of collecting additional blood volumes from study participants at different timepoints is to obtain a panel of serum samples with an intent to cover adequately the analytical range of the respective assays during further assay development and validation.

4.4. End of Study Definition

~~A participant is considered to have completed the study if the participant has completed all periods of the study including the last study-related telephone call or visit or the Sponsor receives the last laboratory result.~~

~~For purposes of analysis and reporting, the end of study occurs when the Sponsor receives the last laboratory result.~~

5.2. Exclusion Criteria

13. Participant has received immunoglobulins. **Note:** This does not include monoclonal antibodies, such as palivizumab ***or nirsevimab***.

16. Participant has febrile illness (rectal temperature ≥38.1°C [100.5°F] or axillary temperature ≥37.8°C [100.0°F]) ~~at Visit~~ ***in Part 1 and Part 2 prior to the effective date of protocol amendment 4. After protocol amendment 4 becomes effective, febrile illness is defined as rectal temperature ≥38.0°C [100.4°F] or axillary temperature ≥37.5°C (99.1°F) in Part 2.*** Upon resolution of symptoms, participant can be rescreened.

5.4. Criteria for Temporarily Delaying Enrollment *and Administration of Study Intervention*

~~Not applicable.~~

Enrollment and study intervention administration may be postponed within the permitted time interval until transient conditions cited below are resolved:

The following condition(s) are temporary or self-limiting and participant may be vaccinated and/or blood draw at the visit can be performed, once the condition(s) has/have resolved and no other exclusion criteria are met and should other requirements such as appropriate window for study intervention Administration allows.

1. Acute illness or fever at the time of enrollment and/or study intervention administration. Refer to the SoA for definition of fever and preferred location for measuring temperature in this study.

2. Participant with a minor illness (such as mild diarrhea, mild respiratory infection) without fever may be enrolled and/or vaccinated at the discretion of the investigator.

3. Delay is warranted in order to meet the following conditions: Influenza vaccine, SARS-CoV-2 (COVID-19) vaccine should be administered either 7 days prior to or 15 days after the administration of the study vaccine. Non-live vaccines not given concomitantly (i.e., on the same day as study vaccine) should be given at least 7 days prior or at least 15 days after administration of study vaccine. Live vaccines not given concomitantly should be given at least 28 days prior or at least 28 days after study vaccine. Refer also to the Sections 6.8.1 Permitted Concomitant Therapy and Section 6.8.2 Prohibited Concomitant Therapy, and Section 10.4: Appendix 10.4 List of Excluded Concomitant Medications.

4. There is a clinical circumstance that warrants delay of blood collection for immunogenicity assessment. In the event that a participant meets this criterion for delay of blood collection, blood collection can proceed once the window for delay has passed.

5. Participants has been found to have clinically significant hematologic/biochemical values out of normal range (evident in tests performed for any reason outside of study procedures) which are expected to be temporary and warrant re-screening per PI's judgement and dosing within the allowed time interval per protocol.

Section 6.1. Study Intervention(s) Administered

Table 7 Study Intervention(s) Administered

Type	*Combination product*	*Combination product*	*Combination product*
IMP and *Authorized* ~~**NIMP**~~**/AxMP**	IMP	***Authorized AxMP*** ~~**IMP**~~	***Authorized AxMP*** ~~**IMP**~~

AxMP: auxiliary medicinal product; IMP: investigational medicinal product; ~~NIMP: non-investigational medicinal product;~~ PCV13: Prevnar 13; PCV20: Prevnar 20.

6.1.1. Medical Devices

- ***The Sponsor-manufactured medical devices provided for use in this study is AFX3772.***

- ***Other medical devices (not manufactured by or for the Sponsor) provided for use in this study are PCV13 and PCV20.***

- ***Instructions for medical device use are provided in Pharmacy Manual.***

All device deficiencies (including malfunction, use error and inadequate labeling) shall be documented and reported by the investigator throughout the clinical investigation (see Section 8.4.8 and Section 10.7) and appropriately managed by GSK.

6.3.1. Intervention Assignment

In Part 1, 40 participants per cohort were enrolled and randomized 3:1 to receive AFX3772 (1, 2 or 5 µg) or PCV13. In part 2a, 45 participants per cohort will be enrolled and randomized 2:1 to receive AFX3772 (2 or 5 µg) or PCV20. If continuation into Part 2b is recommended, approximately 270 additional participants will be randomized 1:1:1 (90 per group) ~~are~~ to receive either 1 of up to 2 AFX3772 dose levels (2 or 5 µg), or PCV20 as agreed to for evaluation in Part 2b. At Visit 1, after the ICF signature, study site personnel will access the electronic data collection (EDC) system and enter the participant's required information as described in the eCRF Completion Guidelines. Upon completion of the Randomization ***e***CRF page, the EDC system will assign a unique Subject Randomization Number to each randomized participant. Upon completion of the Vaccine Administration ***e***CRF page, the IRT system will assign a unique kit to each randomized participant. An unblinded pharmacist will access the IRT to find out which investigational product kit was assigned to a randomized participant.

6.8.1. Permitted Concomitant Therapy

Acetaminophen and nonsteroidal anti-inflammatory drugs are permitted during the study. The LAR will be instructed to record antipyretic medications and the reason for use in the provided eDiary on Day 1 to Day 7 following each vaccination. ***The LAR will be instructed to record the exact time of use of antipyretic medications on Day 1***.

8.1.1. All Unscheduled Visits

During unscheduled visits, investigator should record the date of visit, reason for the visit, any investigator assessment(s), the basis for assessment i.e., real time or near real time (within 24-36h) symptomatology or participant recall, timing of participant recall in relation to the visit, if applicable, and the intensity grading scale used by investigator for AEs occurring within 7 days post-vaccination and recorded in the eDiary. The severity of the local reactions and systemic events should ideally be graded using the AE severity scale in Section 10.3.3.

8.2. Immunogenicity Assessments

Considering the mechanism of action, AFX3772 is expected to induce an immune response to serotype-specific pneumococcal polysaccharides and to the fusion protein, CP1. Blood samples to assess immunogenicity will be collected as indicated in the schedule of assessments (Table 1). ***Priority ranking of the use of blood samples, as well as optional additional blood volume collected are described in Section 8.6.***

Immunogenicity of AFX3772 and PCV will be evaluated by measuring pneumococcal serotype-specific anti-capsular polysaccharide IgG antibody concentrations by ***24-valent***

multiplex direct Luminex immunoassays (dLIAs) or alternative assay format (e.g., ***24-valent*** Electrochemiluminescent (ECL) multiplexed assay).

Immunogenicity of AFX3772 or PCV ~~***vaccines***~~ will be also evaluated by measuring the antibody-mediated pneumococcal serotype-specific opsonophagocytic activity (OPA) titers by multiplexed opsonophagocytic assay (MOPA) targeting the 24 AFX3772 vaccine serotypes. The MOPA measures the functional opsonic activity of antibodies against the pneumococcal serotypes that results in bacteria killing by phagocytic cells in vitro.

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Additional ***optional*** blood volumes will be collected from study participants for the development ~~***and***~~ validation, ***and maintenance*** of assays measuring responses to the pneumococcal vaccine needed for the vaccine clinical development program.

8.3.1. Body Temperature

At the dosing visit (Day 1), a rectal or axillary temperature will be taken prior to administration of study vaccine. The method used by the site will be based on their standard of care for temperature measurement. Participants who have febrile illness (rectal temperature ≥38.~~1~~***0***°C [≥100.~~5~~***4***°F] or axillary temperature ≥37.~~8~~***5***°C [≥~~100.0~~***99.1***°F]) should have the visit rescheduled.

8.3.3. Reactogenicity

If any reactogenicity ~~reaction~~ extends beyond the 7-day period, it will be recorded ***as*** ~~***an***~~***a solicited AE*** ~~(on~~ ***in the*** ~~AE eCRF page) and followed~~ ***e-Diary by the LAR on a daily basis until resolution*** ~~or deemed medically stable~~.

8.4.1. Time Period and Frequency for Collecting AE and SAE Information

AEs (non-serious) are to be reported Day 1 through Day 30 following each vaccination. ***Immediate AEs are to be reported within 30 minutes of vaccination and recorded on AE page in the eCRF.***

All SAEs will be recorded and reported to the Sponsor ~~or designee~~ within 24 hours of learning of the event, as indicated in Section 10.3 Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting. The investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

8.4.8. Medical Device Deficiency

Medical devices are being provided for use in this study as the study intervention. To fulfill regulatory reporting obligations worldwide, the investigator is responsible for the

detection and documentation of events meeting the definitions of device deficiency that occur during the study with such devices.

If the site(s) uses non-sponsor medical devices, i.e., medical devices not provided by GSK, then the investigators are obligated to report any device deficiencies to the legal manufacturer of the devices directly.

The definition of a medical device deficiency can be found in Section 10.7.

Note: Deficiencies fulfilling the definition of an AE/SAE will follow the processes outlined in Section 10.7 of the protocol.

8.4.8.1. Time period for detecting medical device deficiencies

- ***Medical device deficiencies that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.***
- ***If the investigator learns of any device deficiency at any time after a participant has been discharged from the study, and such a deficiency is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.***

The method of documenting medical device deficiencies is provided in Section 10.7.

8.4.8.2. Follow-up of medical device deficiencies

- ***Follow-up applies to all participants, including those who discontinue study intervention.***
- ***The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.***
- ***New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.***

8.4.8.3. Prompt reporting of device deficiencies to the Sponsor

- ***Device deficiencies will be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device deficiency.***
- ***The medical device deficiency report form will be sent to the sponsor by via email (please refer to Pharmacy Manual). If email is unavailable, then fax should be utilized. This information will also be recorded in EDC and alternative method is a paper form faxed to sponsor.***
- ***The sponsor will be the contact for the receipt of device deficiency reports.***

8.4.8.4. Regulatory reporting requirements for device deficiencies

- ***The investigator will promptly report all device deficiencies occurring with any medical device provided for use in the study in order for the sponsor to fulfill the legal responsibility to notify appropriate regulatory authorities and other entities***

about certain safety information relating to medical devices being used in clinical studies.

- ***The investigator, or responsible person according to local requirements (e.g., the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.***

8.6. Total Amount of Blood

~~The approximate total amount of blood to be collected ***per participant*** for laboratory assessment (is presented in ***Table 8*** .Blood should be drawn at Visits 3, 5, 6 and 7 (see schedule of assessments (Table 1). Repeat and additional blood samples may be taken if required; however, the maximum blood volume drawn from the participant will not exceed 3 mL per kg in an 8-week period. Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.~~

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Blood should be drawn at visits mentioned in Table 1 and Table 2.

The approximate total amount of blood to be collected per participant for laboratory assessment ***and/or for assay development, validation, and maintenance*** is presented in ***Table 8***.

Table 8 Blood Sampling

Study Part	***Component or additional intend to use blood samples, per priority ranking∞***	***Maximum* Number of Samples *[including optional]***	***Approx.* Sample Volume (mL) *[including optional]***	***Approx.* Total Volume (mL) *[including optional]***
Part 1*	1. Pneumococcal serotype-specific anti-capsular PS IgG 2. Pneumococcal ***serotype-specific*** OPA ***titers***	4	4.0	16.0*
	~~Total~~			~~16.0*~~
Part 2	~~***Pneumococcal serotype-specific anti-capsular PS IgG, Pneumococcal OPA and anti-CP1 IgG Assays development***~~	4 ***[or 5]***	***Visit 1: [Up to 2.5]*** ***Visit 3: 5.0 [up to 7.5]***	20.0 ***[up to 32.5]***

	1. Pneumococcal serotype-specific anti-capsular PS IgG ***2. Assay validation and/or maintenance*** 3. Pneumococcal ***serotype-specific*** OPA ***titers*** 4. Anti-CP1 IgG		***Visit 5: 5.0 [up to 7.5]*** ***Visit 6: 5.0*** ***Visit 7: 5.0 [up to 10.0]***	
	~~***Total***~~			~~20.0~~

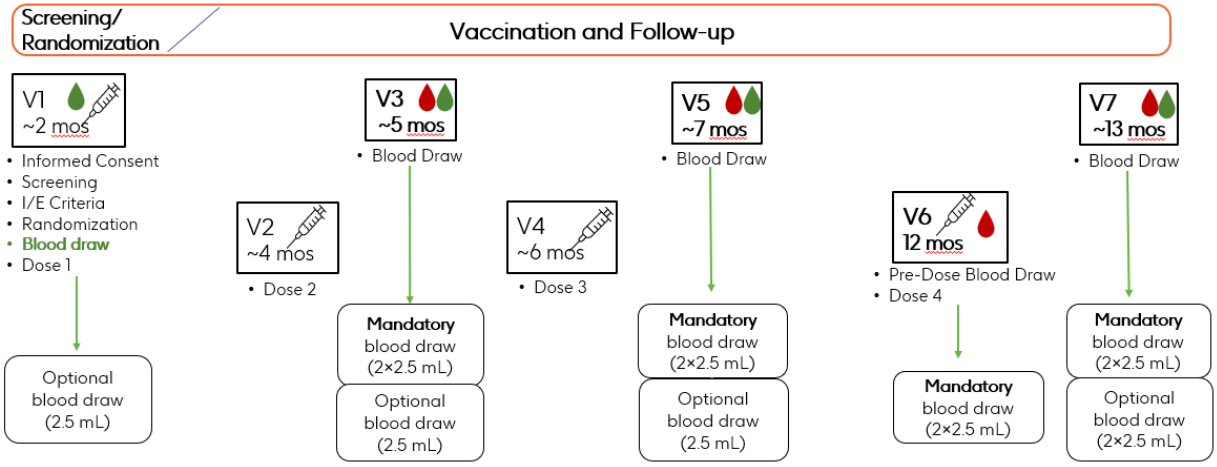
CP1: fusion protein; IgG: immunoglobulin G; OPA: opsonophagocytic activity

Note: the maximum blood volume drawn from the participant will not exceed ***9.5*** mL per kg in an 8-week period ***for the first year of life***.

°***In case of insufficient blood sample volume to perform assays for all endpoints or for assay development/validation, the samples will be used according to the described priority ranking, 1 being the highest priority.***

* The total volume of blood collected from Part 1 participants who complete the primary series including post Dose 2 and post Dose 3 sampling, will be 8.0 mL, while for those who receive SoC pneumococcal vaccines as Dose 2 will be 4.0 mL. Note that for all Part 1 participants, pre and post booster blood sampling has been cancelled.

Figure 5: Blood Sampling Schedule for Part 2b



Optional additional blood sampling at Visits 1, 3, 5, and 7 (see Figure 5 for timepoints). Visit 1 blood sampling is for the purpose of assay development only. These optional blood sampling will be done in Part 2b.

Repeat and additional blood samples may be taken if required. ***Maximum blood drawing is calculated based on the 5th percentile of infant girl body weight in the US population, such as that no more than 5 mL/kg body weight could be collected from children for research purposes in a single day and no more than 9.5 mL/kg over any 8-week period.***

9.2. Analysis Sets

Population	Description
Randomized	All participants who are randomized to receive either AFX3772 or PCV*.

Population	Description
PPS	Participants who receive rescue ***vaccination*** (an intercurrent event) will be discontinued from study intervention and not included in subsequent immunogenicity assessments.

9.3.2.1. Primary Vaccination Series Assessments

~~The~~***In Part 1 participants, t***he final assessment of safety and immunogenicity will be performed after the last participant completes the study i.e., approximately 6 months after the last participant has completed the primary series ***and the results will be unblinded at individual-level***.

9.3.2.3. Final Safety Assessment

Safety data used for dose escalation decisions will be provided for each dose cohort. ~~Safety data from both phases of the study will be combined in the safety summaries.~~

~~No analysis will be performed for Part 1 participants for the booster dose.~~

9.3.3. Secondary Endpoint(s)/Estimand(s) Analysis

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9.3.5.3. Reactogenicity

See Section 9.3.2 Primary Analysis. Immediate reactions will be collected by the site staff for 30 minutes after each vaccination and recorded on the appropriate eCRF. The LARs will complete the eDiary for 7 days following each vaccine administration. After the 7-day eDiary has been completed, the site personnel will review the data with the participant's LAR. ***Reactogenicity ongoing beyond 7 days will be recorded as a solicited AE in the e-Dairy by the LAR on a daily basis until resolution.*** Reactogenicity data will be summarized.

9.4. Interim Analyses

A total of ~~*5*~~*7* analyses will be performed in the study:

In Part 2 participants, ~~*1*~~*3* primary vaccination series assessment***s,*** 1 booster vaccination assessment and a final safety assessment.

9.4.1. Primary Vaccination Series Assessments

In Part 1 participants, the first analysis, an interim analysis of the primary vaccination series will be performed. This will include the analysis of all available safety data for all three cohorts post study intervention and available immunogenicity data for the 1 µg, 2 µg and 5 µg cohorts. A second analysis, i.e., the final assessment will be performed after the last participant in Part 1 completes the study, approximately 6 months after the last participant has received the primary series. ***The results of this analysis will be unblinded at individual-level.***

The third ~~analysis, an~~***, fourth and fifth*** interim ~~analysis~~ analyses of the primary vaccination series of Part 2 will be performed when ~~the *360*~~***at least 50%, 75% and 100% of participants, respectively,*** have completed 30 days after the primary series and the immunogenicity results become available.

9.4.2. Booster Vaccination Assessments

The ~~*fourth*~~***sixth*** analysis, an interim analysis of the booster vaccination of Part 2 ***and***, will be performed after the ~~*360* participants~~***last*** participant have completed 30 days after the booster vaccination and the immunogenicity results become available.

9.4.3. Final Safety Assessment

The ~~*fifth*~~ ***seventh*** analysis will be performed to report the final safety assessment (SAEs) of Part 2**,** after the last participant completes the study, approximately 6 months after the booster vaccination in the last participant.

9.5. Sample Size Determination

~~Approximately~~***Up to*** 480 healthy infants are planned to be enrolled in this study with an initial 120 participants enrolled (Part 1) followed by ~~the remaining~~ 360 participants (Part 2).

Further, ***all*** the ~~***two***~~ AFX23372 formulations will be compared to the PCV20 in terms of pneumococcal serotype specific IgG concentrations PD2, PD3 and PD4 using a one- ***way ANOVA model.*** ~~***The sample size of 102 evaluable subjects per group achieves approximately 85% power to detect a difference of at least 2.12-fold (Log10 SD=0.5) to 3.87 fold (Log10 SD=0.9) for each serotype (2-sided alpha=0.05, PASS 2022 Multiple comparisons Analysis using Dunnett's adjustment for multiplicity).***~~

10.1.3. Informed Consent Process

Sample testing will be done in accordance with the recorded consent of the individual participant's parent(s)/LAR(s).

By default, collected samples for the study will be stored for a maximum of 20 years. This storage period begins when the last participant completes the last study visit. This timeline can be adapted based on local laws, regulations or guidelines requiring different timeframes or procedures. In all cases, the storage period should be aligned with participant's parent(s)/LAR(s) consent. These additional requirements must be formally communicated to, discussed and agreed with GSK.

10.1.4. Data Protection

The participant' LAR(s) must be informed that their child's medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

The contract between sponsor and study sites specifies responsibilities of the parties related data protection, including handling of data security breaches and respective communication and cooperation of the parties.

Information technology systems used to collect, process, and store study-related data are secured by technical and organizational security measures designed to protect such data against accidental or unlawful loss, alteration, or unauthorized disclosure or access. GSK, third parties working on behalf of GSK, and/or institutions working with GSK for the purposes of this study are contractually bound to protect participant coded data. GSK will protect participant coded data and will only share it as described in the ICF.

GSK has a global, internal policy that requires all GSK staff and complementary workers to report data incidents or breaches immediately, using dedicated tools. Clear procedures are defined for assessing and investigating data breaches to identify and to

take appropriate remediation steps, to contain and to mitigate any risks for individuals resulting from a breach, in compliance with applicable laws.

10.1.5.1. Data Monitoring Committee ~~Structure~~

10.1.5.2. Internal Data Review Committee

The internal data review committee (iDRC) is a multidisciplinary committee including immunogenicity, safety, clinical and statistics experts from GSK. The role of iDRC is to oversee interim analyses) and to determine the composition of the investigational study intervention formulation (selection of dose levels 2 µg or 5 µg and aluminum content or any combination of those) to be assessed in Phase 3. Internal DRC will review data for making a dose escalation and dose selection recommendation. The iDRC charter will describe the required data review and documentation for making a dose escalation and dose selection recommendation.

10.2.1. Assessment of Intensity for Local AEs

The participant's LAR will observe reactogenicity and tolerability from Day 1 through Day 7 and record observed events daily in the eDiary. Study site personnel will instruct the participant's LAR on how to observe reactogenicity and tolerability and how to record observed events daily in the eDiary. A measurement tool will be provided to the participants to measure the diameter of erythema/redness and swelling if it occurs. ***Any treatment of reactogenicity will be recorded in the e-Diary.*** Injection site redness and swelling will be evaluated by maximum size and then categorized during analysis as mild, moderate, or severe based on the grading scale in Table 11 below.

10.2.2. Grading of Signs of Systemic Reactogenicity

Grading for intensity will be performed within 7 days for solicited events and within 30 days for unsolicited events post ***vaccination*** by PI in addition to LAR in appendix 2, for any intensity including ***gG***rade 4.

10.3.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording
An eDiary will be used in this study to capture solicited administration site or systemic events. The participant's LAR(s) should be trained on how and when to complete the eDiary.
Anyone who measures administration site or systemic events and who will record the event in the eDiary should be trained on using the eDiary. This training must be documented in the participant's source record.
For each solicited and unsolicited AE the participant experiences, the participant's LAR(s) will be asked if their child received medical attention (defined as unscheduled visit to or from medical personnel for any reason, including emergency room visits). This information will be recorded in the participant's eDiary and in the participant's eCRF as part of normal AE reporting (for solicited and unsolicited AEs). If the collection of solicited AEs was not possible for any reasons via the eDiary and solicited

AEs were reported to the investigator by the participant's LAR(s), then it would be possible for it to be reported directly to the site staff and submitted (e.g., via the eCRF). Medical attention received for SAEs/AESIs will have to be reported using the normal AE reporting process in the eCRF.

If any individual other than the participant's LAR(s) is making entries in the eDiary, their identity must be documented in the participant's source record.

All solicited events that occur during the 7 days following administration of each dose of study intervention (Day 1 to Day 7 post-Dose) must be recorded into the eDiary, irrespective of intensity. An automatic reminder to complete the eDiary will be sent to the participants during this time frame. Daily eDiary compliance will be checked by the investigator or delegate on the eDiary portal. In case of non-compliance, the investigator should contact the participant to remind the importance of daily entries. All other AEs occurring within this time frame should be recorded into the appropriate section of the eCRF, irrespective of their intensity or whether or not they are considered related to the study intervention.

After review and verbal discussion of the e-Diary entries with the participant's LAR(s), if there are eDiary errors, the investigator will complete his/her own assessment in the relevant sections of the eCRF.

10.3.1. Definition of AE

Events Meeting the AE Definition
3. Reactogenicity events that extend beyond the 7-day period ***will be recorded in the eDiary.***

10.3.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording
An eDiary will be used in this study to capture solicited administration site or systemic events. The participant's LAR(s) should be trained on how and when to complete the eDiary. **Anyone who measures administration site or systemic events and who will record the event in the eDiary should be trained on using the eDiary. This training must be documented in the participant's source record.** **For each solicited and unsolicited AE the participant experiences, the participant's LAR(s) will be asked if their child received medical attention (defined as unscheduled visit to or from medical personnel for any reason, including emergency room visits). This information will be recorded in the participant's eDiary and in the participant's eCRF as part of normal AE reporting (for solicited and unsolicited AEs). If the collection of solicited AEs was not possible for any reasons via the eDiary and solicited AEs were reported to the investigator by the participant's**

LAR(s), then it would be possible for it to be reported directly to the site staff and submitted (e.g., via the eCRF). Medical attention received for SAEs/AESIs will have to be reported using the normal AE reporting process in the eCRF.

If any individual other than the participant's LAR(s) is making entries in the eDiary, their identity must be documented in the participant's source record.

All solicited events that occur during the 7 days following administration of each dose of study intervention (Day 1 to Day 7 post-Dose) must be recorded into the eDiary, irrespective of intensity. An automatic reminder to complete the eDiary will be sent to the participants during this time frame. Daily eDiary compliance will be checked by the investigator or delegate on the eDiary portal. In case of non-compliance, the investigator should contact the participant to remind the importance of daily entries. All other AEs occurring within this time frame should be recorded into the appropriate section of the eCRF, irrespective of their intensity or whether or not they are considered related to the study intervention.

After review and verbal discussion of the e-Diary entries with the participant's LAR(s), if there are eDiary errors, the investigator will complete his/her own assessment in the relevant sections of the eCRF.

10.4. Appendix 4: List of Excluded Concomitant Medications

Blood products, including immunoglobulins. **Note:** This does not include monoclonal antibodies, such as palivizumab ***and nirsevimab***.

10.5. Appendix 5: Clinical Laboratory Assessments

~~Immunogenicity of AFX3772 will be evaluated by measuring ***pneumococcal*** serotype-specific anti-***capsular polysaccharide*** IgG antibody concentrations by 2 complementary multiplex (12-plex) Luminex immunoassays (dLIAs) to cover simultaneously the 24 vaccine serotypes. ***Alternatively, an Electrochemiluminescent (ECL) multiplex assay could be used to measure this endpoint.***~~

~~Immunogenicity of AFX3772 will be also evaluated by measuring ***pneumococcal*** serotype-specific opsonophagocytic activity using multiplexed opsonophagocytic assay (MOPA) targeting the 24 AFX3772 vaccine serotypes. The MOPA measures the functional opsonic activity of antibodies against the pneumococcal serotypes that results in bacteria killing by phagocytic cells in vitro.~~

~~Immunogenicity ***against*** the CP1 fusion protein ***in terms of anti-CP1 IgG antibodies*** will be measured using ***a ligand-binding assay (e.g., ECLassay).***~~

10.7. Appendix 7: Medical Device AEs, ADEs, SAEs, SADEs, USADEs and Device Deficiencies: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting in Medical Device Studies

- ***Both the investigator and the sponsor will comply with all local reporting requirements for medical devices.***
- ***The detection and documentation procedures described in this protocol apply to all sponsor medical devices provided for use in the study. See Section 6.1.1 for the list of sponsor medical devices.***

10.7.1. Definition of medical device AE and ADE

Medical device AE and ADE definition
• ***A medical device AE is any untoward medical occurrence, in a clinical study participant, users, or other persons, temporally associated with the use of study intervention whether or not considered related to the investigational medical device. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of an investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices.***
• ***An ADE is defined as an AE related to the use of an investigational medical device. This definition includes any AE resulting from insufficient or inadequate instructions for use or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.***

10.7.2. Definition of medical device SAE, SADE, and USADE

A medical device SAE is any serious AEs that:
a. Led to death.
b. Led to serious deterioration in the health of the participant, that either resulted in:
• ***A life-threatening illness or injury. The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.***
• ***A permanent impairment of a body structure or a body function.***
• ***Inpatient or prolonged hospitalization. Planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE.***
• ***Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.***

• ***Chronic disease (MDR 2017/745).***
c. Led to fetal distress, fetal death or a congenital abnormality or birth defect.
d. Is a suspected transmission of any infectious agent via a medicinal product.
SADE definition
• ***A SADE is defined as an ADE that has resulted in any of the consequences characteristic of an SAE.*** • ***Any device deficiency that might have led to an SAE if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate.***
Unanticipated SADE (USADE) definition
• ***An USADE (also identified as UADE in US Regulations 21 CFR 813.3), is defined as a serious ADE that by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report/IB (see Section 2.3).***

10.7.3. Definition of device deficiency

Device deficiency definition
• ***A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequacy of the information supplied by the manufacturer.***

10.7.4. Recording and follow-up of medical device AEs and/or SAEs and device deficiencies

10.7.4.1. Medical device AE, SAE, and device deficiency recording

- ***When an AE/SAE/device deficiency occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.***
- ***The investigator will then record all relevant AE/SAE/device deficiency information in the participant's medical records, in accordance with the investigator's normal clinical practice and on the appropriate form.***
- ***It is not acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the Sponsor AE/SAE/device deficiency form.***
- ***There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the***

exception of the participant number, will be redacted on the copies of the medical records before submission to the Sponsor.

- ***The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.***
- ***For device deficiencies, it is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the deficiency.***

A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of a device deficiency. This includes any amendment to the device design to prevent recurrence.

- ***If the site during the course of the study becomes aware of any serious, nonserious incident (including device deficiencies and malfunctions) related to any GSK product that is not part of the study design, they will report these events to GSK or to the concerned CA via the national spontaneous reporting system. These will be classified as spontaneous ICSRs.***

10.7.4.2. Assessment of intensity

See Section 10.3.3.

10.7.4.3. Assessment of causality

See Section 10.3.3.

10.7.4.4. Follow-up of medical device AE/SAE and device deficiency

- ***The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE/SAE/device deficiency as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.***
- ***If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the Sponsor with a copy of any post-mortem findings including histopathology.***
- ***New or updated information will be recorded in the originally completed form.***
- ***The investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.***

10.7.4.5. Reporting of medical device SAEs

Medical Device SAE Reporting to the Sponsor via an Electronic Data Collection Tool

- ***The primary mechanism for reporting an SAE to the Sponsor will be the electronic data collection tool.***
- ***If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next table) to report the event within 24 hours.***
- ***The site will enter the SAE data into the electronic system as soon as it becomes available.***
- ***After the study is completed at a given site, the electronic data collection tool will be taken offline to prevent the entry of new data or changes to existing data.***
- ***If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken offline, then the site can report this information on a paper SAE form (see next table) or to the medical monitor by telephone.***
- ***If the site during the course of the study or poststudy becomes aware of any serious, nonserious AEs, pregnancy exposure, related to any GSK device they will report these events to GSK or to the concerned CA via the national spontaneous reporting system. These will be classified as spontaneous ICSRs.***

Medical device SAE reporting to the Sponsor via paper data collection tool

- ***Investigator/sites are to enter all initial medical device SAE information and follow-up information into the Electronic Data Collection (EDC) within 24 hours of awareness. The paper-based method is a secondary method of reporting that is to be used only when the EDC is unavailable.***
- ***The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the Sponsor (or designee) by email immediately (within 24 hours of awareness) if the EDC is not accessible.***
- ***The SAE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE data, as well as the investigator causality assessment including the explanation for the causality assessment.***
- ***Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE paper data collection tool within the designated reporting time frames.***
- ***Please see Section 10.3.4 for contact details.***

10.7.6. Reporting of SADEs

SADE Reporting to the Sponsor

Note: There are additional reporting obligations for medical device deficiencies that are potentially related to SAEs that must fulfill the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

- ***Any device deficiency that is associated with an SAE must be reported to the sponsor within 24 hours after the investigator determines that the event meets the definition of a device deficiency.***
- ***The sponsor will review all device deficiencies and determine and document in writing whether they could have led to an SAE. These device deficiencies will be reported to the regulatory authorities and IRBs/IECs as required by national regulations.***

10.7.7. Reporting of medical device deficiencies for associated person

• ***Reporting to GSK***
If an Associated Person (e.g., spouse, caregiver, site staff) experiences a device deficiency, the medical device deficiency information, and any associated AE/SAE information will be reported to GSK. The associated person will be provided with the authorization to contact physician letter. ***If follow-up information is required, authorization to contact physician (or other licensed medical practitioner) must be signed to obtain consent.*** • ***Medical device deficiencies that are not related to an AE or SAE should be reported via email to gsk-rd.complaints@gsk.com, using the medical device deficiency report form.*** • ***If the medical device deficiency is related to a nonserious AE and not linked to an SAE, please send the medical device deficiency report form with details of the associated AE via email to gsk-rd.complaints@gsk.com only.*** • ***If the device incident is linked to an SAE, please email the medical device deficiency report form, within 24 hours.*** • ***GSK will review all device deficiencies and determine and document in writing whether they could have led to an SAE. These device deficiencies will be reported to the regulatory authorities and IRBs/IECs as required by national regulations.***

Amendment 3 ***(09 October 2023)***

On 24 February 2023, GSK made the decision to halt study vaccinations in Part 1 of the study and pause start of recruitment for Part 2. The purpose of this protocol

amendment is to introduce the changes prior restart of the study i.e., prior start of recruitment to Part 2.

The purpose of this amendment is to (i) update the study design for Part 2 to include a dose escalation safety lead in Part 2; (ii) update the study design to reduce the number of AFX3772 dose levels from 3 (1, 2 and 5 µg) to 2 (2 and 5 µg); (iii) change the comparator vaccine from PCV13 to PCV20 following the ACIP recommendation of the 20-valent pneumococcal conjugate vaccine for routine infant immunization (iv) update the sample size of treatment arms (v) change the presentation of the investigational product from vial to pre-filled syringe (vi) add a safety follow-up telephone call at 1-month post-Dose 1 to the schedule of assessment; and (vii) make minor editorial changes throughout the document for consistency and clarity.

Detailed description of the current Protocol Amendment 3

Amended text has been included in bold italics and deleted text in strikethrough in the following sections:

Title page

Protocol Title: A Phase 2, Randomized, Double-blind, Multi-dose, Dose Finding Study to Evaluate the Safety, Tolerability and Immunogenicity of AFX3772 Compared with PCV***s*** ~~13~~in Healthy Infants

Compound: AFX3772 ***(also referred to as Pn-MAPS24v)***

Note: PCV13 was used as the comparator in Part 1 of the study and PCV20 will be used as the comparator for Part 2 of the study. Unless specified, PCV is synonymous to PCV13 (Part 1) and PCV20 (Part 2) throughout this Protocol.

1. Protocol summary

1.1 Synopsis

Protocol Title: A Phase 2, Randomized, Double-blind, Multi-dose, Dose Finding Study to Evaluate the Safety, Tolerability and Immunogenicity of AFX3772 Compared with PCV***s*** ~~13~~in Healthy Infants

Rationale: The purpose of this study is to confirm the safety and tolerability of a 3+1 regimen of AFX3772 in healthy infants and to evaluate the immunogenicity of ~~3~~ different dose levels of AFX3772 as compared with ***PCVs.***

Objectives, Endpoints, and Estimands

Objectives	Endpoints
Primary	
• To describe the safety and tolerability profiles of ~~3~~ ***different*** dose levels (1**, 2 and 5µg) of AFX3772	Following any vaccination with AFX3772 or PCV*~~13~~:

compared with PCV*~~13~~ with respect to the proportion of participants with AEs	• Solicited injection site AEs (tenderness, redness/erythema and swelling) from Day 1 through Day 7 postvaccination • Solicited systemic AEs (irritability, ***fever,*** decrease of appetite, increased sleep, and decrease in sleep) from Day 1 through Day 7 • AEs Day 1 through Day 30 • SAEs through completion of study participation (6 months post last study vaccination)
Secondary	
• To evaluate the immunogenicity of ~~3~~ ***different*** dose levels of AFX3772 (1**, 2 and 5 µg) compared with PCV*	• The proportion of participants with a pneumococcal serotype-specific IgG concentration ≥ 0.35 µg/mL at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4 • Serotype-specific IgG GMC at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4
AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; PCV13: Prevnar 13 ***;PCV20: Prevnar 20;*** PD: post-dose; SAE: serious adverse event ***:PCV13 [Part 1] and PCV20 [Part 2].*** **** in Part 1 only***	
Estimands	
Primary Estimand description: Percentage of pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age who receive at least 1 vaccination with either AFX3772, or PCV*~~13~~***(*PCV13 [Part 1] and PCV20 [Part 2])*** and develop a solicited local or systemic AE within 7 days of each vaccination, AEs through Day 30 after each vaccination, and SAEs through completion of study participation. • A treatment policy strategy is used for assessing safety irrespective of missed vaccination or taking of prohibited medication/vaccination.	

Brief Summary: This is a Phase 2 clinical study to support the use of AFX3772 in healthy infants for the prevention of pneumococcal disease. The purpose of this study is to determine the safety, tolerability, and immunogenicity of different dose levels of AFX3772 compared with PCV~~13~~.

Approximately 480~~760~~ healthy infants approximately 2 months of age ***are planned*** ~~will~~***to*** be enrolled and receive 4 doses of study vaccine over 8 protocol-defined visits spanning a duration of approximately 18 to 21 months. Part 1 is the dose escalation, lead-in portion of the study in which 40 infants at each dose level ***were***~~ill be~~ randomized 3:1 in sequential cohorts of increasing doses of AFX3772 (Cohort 1: 1 µg; Cohort 2: 2 µg and Cohort 3: 5 µg) or PCV13. Enrollment in Cohorts 2 and 3 ~~will~~ proceed***ed*** following Data Monitoring Committee (DMC) review of cumulative safety and tolerability data through Day 7 from preceding cohorts. ***Prior to the completion of vaccination series in Part 1, the study vaccination was halted and urgent safety measures, i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines, were implemented for participants of Part 1 and the start of recruitment in Part 2 was paused at the same time.***

Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 1, ***Part 2 will start with target recruitment of a total 360 healthy infants. In the dose escalation lead-in the step of Part 2 (Part 2a) i.e., 45 infants at each dose level will be randomized 2:1 into 2 cohorts of increasing dose levels of AFX3772 (Cohort 4: 2 µg and Cohort 5: 5 µg) or PCV20.***

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Remaining 270 infants will be enrolled ***in Part 2b*** and randomized equally to receive either ~~PCV13~~ AFX3772 at dose levels ~~(1,~~2 or 5 µg) or ***PCV20***. . ***Enrollment in Cohorts 5 and Part 2b will proceed following Data Monitoring Committee (DMC) review of cumulative safety and tolerability data through Day 7 from the preceding cohort.***

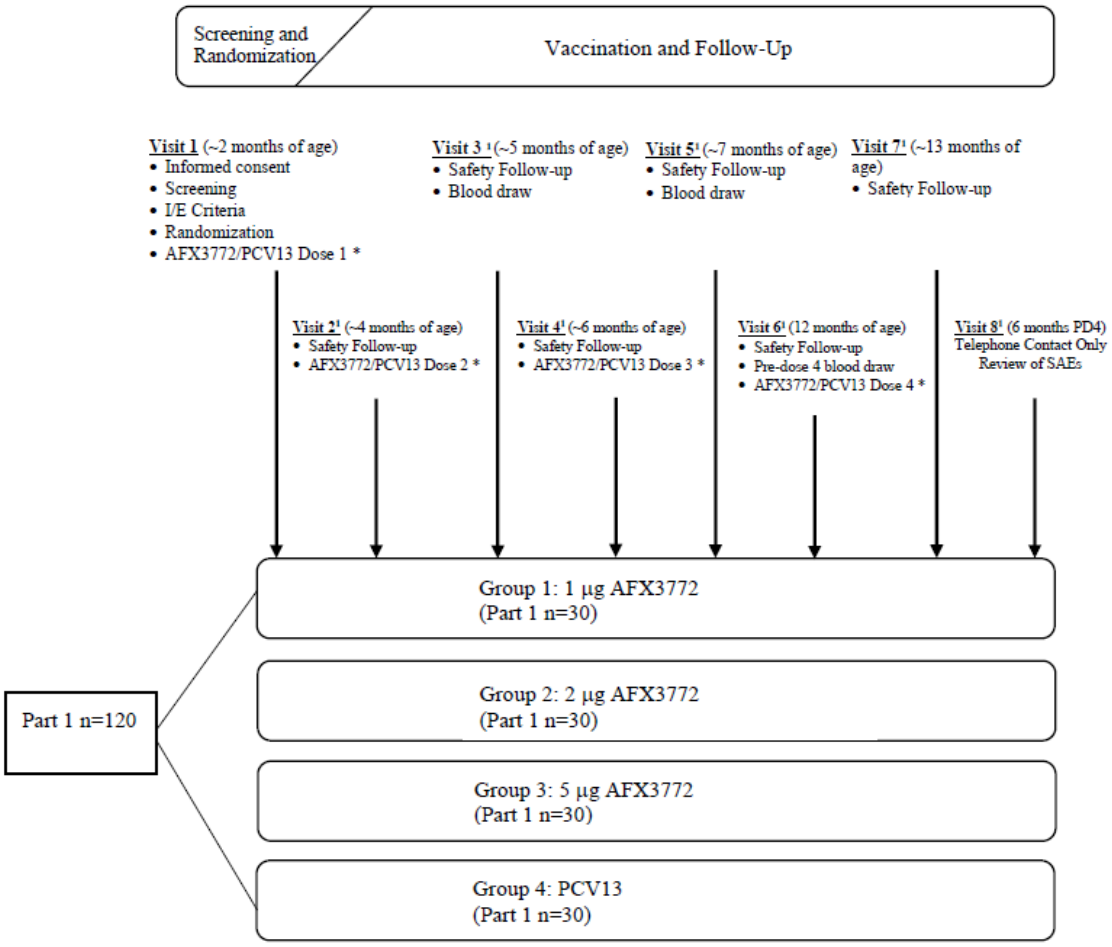
Vaccinations are scheduled at approximately 2, 4, and 6 months of age for the primary series and 12 months ***of age*** for the booster dose. Subsequent immunogenicity assessments are scheduled at 30 days PD2, 30 days PD3, pre-dose 4 and 30 days PD4. Interim analyses of safety and immunogenicity 30 days PD3, 30 days PD4 and a final analysis of safety and immunogenicity are planned for the study.

Number of Participants: Approximately ~~760~~ ***480*** healthy infants are planned to be enrolled in this study with a subset of 120 initially enrolled (Part 1) followed by the remaining ***360***~~640~~ participants (Part 2). Accounting for an 85% evaluable rate, such a sample size predicts approximately ~~***161***~~ ***102*** evaluable participants ***will be available for analysis*** in each treatment arm ***in Part 2***.

An independent DMC has been appointed for this study. This external group of experts will conduct periodic reviews of blinded and, if they request it, unblinded safety and tolerability data for each cohort. The DMC provides recommendations to ensure participant safety and ethical integrity throughout the study. The decision to progress to a higher AFX3772 dose level ~~in Part 1~~ will be determined by the Sponsor, based on the safety and tolerability data available at the time and the DMC's recommendation. In addition, the DMC will review all available data at 6-month intervals to ensure the benefit/risk profile continues to support the continued dosing of AFX3772 in infants. If the DMC does not recommend moving all AFX3772 dose levels into Part 2, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to ~~Part 2b~~ the ***next step.***

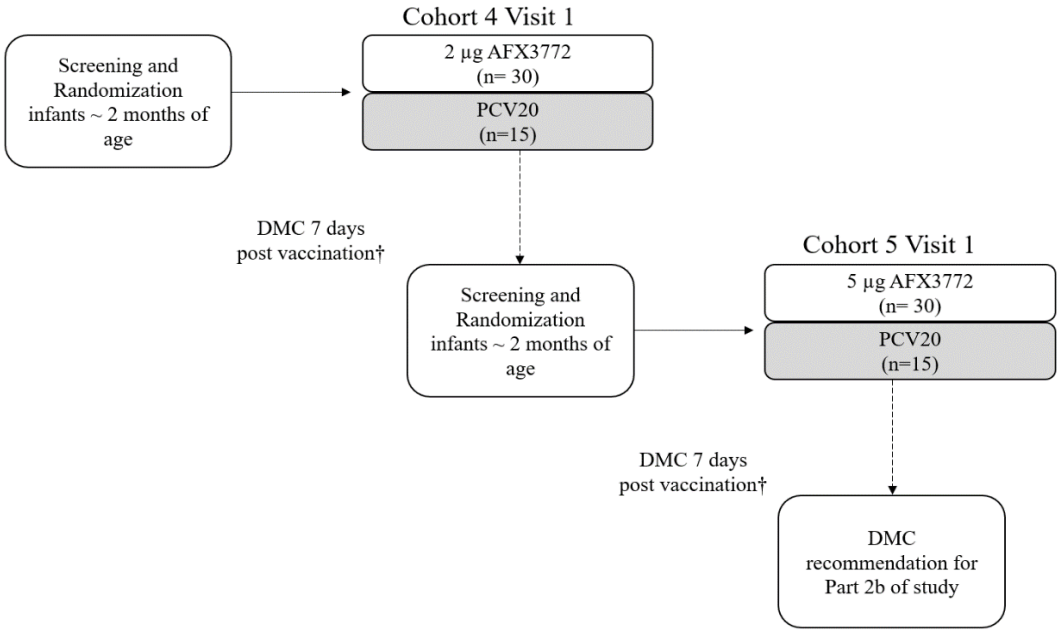
1.2 Schema

Figure 2 Study Schema Part 1 ~~& Part 2~~



~~All study-related procedures pertaining to Part 2 remain unchanged.~~

Figure 3 Study Schema Part 2a – Dose escalation



DMC: Data Monitoring Committee; PCV20: Prevnar 20

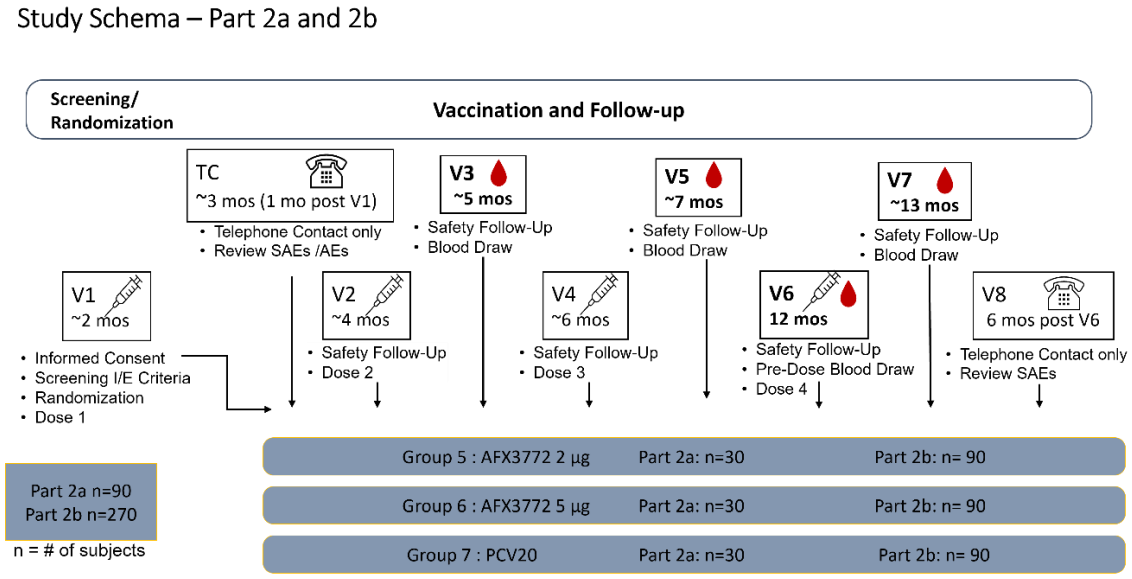
†DMC reviews safety data through Day 7 for each cohort (approximately 45 participants) after all participants have completed eDiary safety assessments through Day 7 postvaccination.

Part 2b starts after DMC review of Part 2a Cohort 5 safety data through Day 7 plus cumulative safety data of Part 2a and agreement to proceed to full enrollment.

Subsequent cohort starts after DMC recommendation to dose escalate.

Infants enrolled in Part 2a will follow the same visit schedule as depicted in Figure 4.

Figure 4 Study Schema Part 2



PCV20: Prevnar 20

****Telephone contact occurs Day 8 post-vaccination for safety follow-up and eDiary review,***

*****TC = Safety follow-up telephone contact at 1-month post-Dose 1***

Part 2b starts after DMC review of Part 2a Cohort 2 safety data through Day 7 and agreement to proceed to full enrollment.

Concomitant vaccines are permitted as per local requirements.

1.3 Schedule of Assessments

Table 1 Schedule of Assessments (SoA) for Part 1

Prior/Concomitant Medication	X†	X	X†	X	X	X†	X	X	X†	X	X	X	All ~~clinically significant~~ concomitant medications or therapies during the eDiary reporting period or associated with SAEs through Visit 8 will be collected. This also includes concomitant vaccinations ***Refer to Section 6.8.1.***
~~Serum~~ ***Blood*** Sample ~~for Immunogenicity~~(***~~54~~ mL***)				X				X[3]	X†		X		
AFX3772 or PCV13 Administration (Blinded)	X		X			X			X				Before vaccine administration, the investigator (or designee) must review medical history to ensure that the participant has no new contraindication to the vaccine. This information ~~should~~ must be documented in the participant's chart. Any concomitant vaccinations ~~should~~ must not be administered in the same limb as the study vaccine.

CP1: ~~carrier~~ ***fusion*** protein 1

Note: Visit 5 (1-month post Dose 3) includes blood sampling and is maintained for all Part 1 participants (including those who received SoC pneumococcal vaccine) to assess a need for rescue vaccination. ~~All study-related procedures pertaining to Part 2 remain unchanged.~~

6 All unscheduled visits and telephone contacts must be reported in the eCRF.

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Table 2 Schedule of Assessments (SoA) for Part 2

Table 2 included to SoA for assessments in Part 2

2. Introduction

Affinivax has developed an investigational 24-valent pneumococcal vaccine (referred to as AFX3772) for the prevention of pneumococcal disease caused by the serotypes contained in the vaccine. AFX3772 contains 24 serotypes of *Streptococcus pneumoniae* and a unique fusion protein (CP1) created by the fusion of 2 pneumococcal proteins (sp1500 and sp0785), which has the potential to induce T- and B-cell responses and provide extended protection. This novel investigational pneumococcal vaccine uses the CCI (MAPS) platform [Zhang, 2013] containing ~~each of the 13~~ pneumococcal capsular polysaccharides (PS) ***individually biotinylated and complexed with CP1. It contains 11*** additional PS (serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F) compared to the pneumococcal conjugate vaccine Prevnar13 (PCV13) (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) ~~additional PS (serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F), individually biotinylated and complexed with CP1~~***or 4 additional PS (serotypes 2, 9N, 17F and 20B) compared the Prevnar 20/Apexxnar (PCV20) (serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F).***

This clinical trial, to be conducted in healthy infants approximately 2 months of age (42 to 90 days), is a Phase 2, proof-of-concept study to support advancement of AFX3772 into Phase 3 pediatric clinical development. AFX3772-003 will evaluate the safety, tolerability, and immunogenicity of ***different*** ~~3~~ AFX3772 dose levels (1, 2 and 5 µg***, with 1 µg used only in Part 1***) administered as a 3+1 regimen (at 2, 4 and 6 months of age for the primary series and 12 months of age for the booster dose). Data in adults and toddlers demonstrated that AFX3772 has an acceptable safety and tolerability profile and generates antibody responses to all 24 serotypes contained in the vaccine [Chichili, 2022].

2.2. Background

Prevnar 20 has been approved for children 6 weeks of age and older for the prevention of IPD caused by the 20 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F) contained in the vaccine and for the prevention of otitis media in children 6 weeks to 5 years of age for the 7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) in Prevnar.

AFX3772 is a 24-valent pneumococcal vaccine (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F) being developed for the prevention of ~~IPD~~ ***pneumococcal disease*** in children 6 weeks to 17 years of age and adults 18 years of age and older. AFX3772 uses a novel MAPS platform in which high-affinity, non-covalent binding between biotin and rhizavidin links pneumococcal PS to CP-1 made up of the fusion of 2 conserved pneumococcal proteins (SP1500 and SP0785) [AFX3772 Investigator's Brochure, ~~2022~~].

2.2.1. Information on Other Study-related Therapy

Refer to the approved labelling for detailed background information on Prevnar 13.

Prevnar 13 (PCV13) contains 13 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) and is approved in the United States (US) and elsewhere for use in infants 6 weeks of age and older ***[Prevnar 13 Product information, 2019]*** . The US Advisory Committee on Immunization Practices (ACIP) recommends PCV13 be given to infants as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a booster dose given at 12 months of age [Nuorti, 2010]. Prevnar 13 can be given concomitantly with other recommended childhood vaccines.

Prevnar 20 (PCV20) contains 20 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F) and is approved in the United States (US) for the prevention of pneumococcal disease in individuals 6 weeks of age and older [Prevnar 20 Product information, 2023].

2.3. Benefit/Risk Assessment

It is unknown if AFX3772, as an investigational vaccine, will have the same benefit/risk profile as PCV13 ***or PCV20***. Because clinical trials are designed to provide information about the safety and efficacy/immunogenicity of an investigational medicine or vaccine, participants in a clinical trial may not directly benefit from study participation.

Approximately 25% ***(Part 1) and 33% (Part 2)*** of participants will receive ***either*** PCV13 ***in Part 1 or PCV20 in Part 2)***, ~~the standard of care~~, as the comparator in this trial and 75% will receive 1 of 3 different dose levels of AFX3772 (1, 2 or 5 µg) ***in Part 1 and .67%. 2 different dose levels of AFX3772 (2 or 5 µg) in Part 2.*** Safety data from previous studies in adults and toddlers demonstrate to be evaluated in this study (1, 2 and 5 µg), and no clinically significant findings following administration of AFX3772 have been observed. Based on data in adults and toddlers, AFX3772 is predicted to have a safety and immunogenicity profile comparable to ***PCV*** ~~PCV13~~ for the ~~13~~ shared serotypes while providing additional coverage for the ~~11~~ unique serotypes in AFX3772.unique serotypes in AFX3772.

At the time this study is being conducted, there is a COVID-19 global pandemic. The sites are expected to implement risk mitigation strategies for the participant and his or her legally acceptable representative (LAR). Some data can be collected remotely via telephone. A summary of clinically significant risks and proposed mitigations strategies is presented in ***Table 3***~~Table 2.~~

Table 3 Summary of Clinically Significant Risks and Mitigation Strategies

Mitigation strategy
• To mitigate the impact of potential lack of immunogenicity in AFX3772 recipients, immunogenicity data will be evaluated for all participants following completion of the primary series. Rescue vaccination with ~~PCV13~~***PCV**** will be offered within 3 weeks of site notification. Summary of participants of Part 1 meeting criteria for rescue vaccination will be evaluated by DMC.

PCV20: Prevnar 20
****PCV13 (Part 1) and PCV20 (Part 2)***

3. Objectives, Endpoints, and Estimands

Objectives	Endpoints
Primary	
• To describe the safety and tolerability profiles of ~~3~~ ***different*** dose levels (1**, 2 and 5µg) of AFX3772 compared with PCV***~~13~~** with respect to the proportion of participants with AEs	Following any vaccination with AFX3772 or PCV***~~13~~**: • Solicited injection site AEs (tenderness, redness/erythema and swelling) from Day 1 through Day 7 postvaccination • Solicited systemic AEs (irritability, ***fever,*** decrease of appetite, increased sleep, and decrease in sleep) from Day 1 through Day 7 • AEs Day 1 through Day 30 • SAEs through completion of study participation (6 months post last study vaccination)
Secondary	
• To evaluate the immunogenicity of ~~3~~ ***different*** dose levels of AFX3772 (1**, 2 and 5 µg) compared with PCV*	• The proportion of participants with a pneumococcal serotype-specific IgG concentration ≥ 0.35 µg/mL at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4 • Serotype-specific IgG GMC at 30 days PD2, 30 days PD3, pre-Dose 4 and 30 days PD4
AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; PCV13: Prevnar 13***; PCV20: Prevnar 20;*** PD: post-dose; SAE: serious adverse event ****:PCV13 [Part 1] and PCV20 [Part 2].*** ****** ***in Part 1 only***	
Estimands	
Primary Estimand description: Percentage of pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age who receive at least 1 vaccination with either AFX3772, or PCV***~~13~~****(**PCV13 [Part 1] and PCV20 [Part 2])*** and develop a solicited local or systemic AE within 7 days of each vaccination, AEs through Day 30 after each vaccination, and SAEs through completion of study participation. • A treatment policy strategy is used for assessing safety irrespective of missed vaccination or taking of prohibited medication/vaccination.	

Primary Estimand

Treatment: A total of 4 vaccinations 0.5 mL each of either AFX3772 (1, 2 or 5µg) or PCV~~13~~ administered as 4 separate vaccinations with a 3-dose primary series at approximately 2 (no earlier than 6 weeks of age), 4 and 6 months of age + 1 booster vaccination at 12 months of age.

Population: Pneumococcal vaccine-naïve healthy infants approximately 2 months (42 to 90 days) of age at enrollment defined by the inclusion/exclusion criteria for the study who will receive at least 1 vaccination with either AFX3772 or PCV~~13~~ during this study.

Secondary Estimand(s)

Treatment:

A total of 4 vaccinations 0.5 mL each of either AFX3772 (1, 2 or 5µg) or PCV~~13~~ administered as 4 separate vaccinations with a 3-dose primary series at approximately 2 (no earlier than 6 weeks of age), 4 and 6 months of age + 1 booster vaccination at 12 months of age.

Intercurrent events *(ICE)* and their corresponding strategies: ~~A principal stratum strategy will be used for the immunogenicity endpoints.~~***The immunogenicity data after the following~~corresponding~~ICEs are considered not relevant for assessing the vaccine effect and data post ICE will not be considered for the analysis of the outcome measure.***

Population level summary:

The percentage of participants with an IgG concentration ≥ 0.35 µg/mL for each serotype, the 95% confidence interval (CI) and the difference (95% CI) between each AFX3772 dose level and PCV~~13~~, the geometric mean concentration (GMC) (95% CI) for each serotype-specific IgG concentration and the ratio of the GMCs (95% CI) (each AFX3772 dose level/PCV~~13~~). The antibody GMCs and the corresponding 2-sided 95% CIs will be calculated using logarithmically transformed values and the Student t distribution for the CIs.

4.1. Overall Design

This is a Phase 2, randomized, double-blind, multi-dose, dose finding study in infants approximately 2 months of age at the time of first vaccination who are naïve to licensed or investigational pneumococcal vaccines. The safety, tolerability and immunogenicity of different dose levels of AFX3772 will be studied and compared to PCV~~13~~, (active control)

This study will be conducted at approximately 65-75 study sites in the US including Puerto Rico. Approximately ~~760~~***480*** healthy infants are planned to be enrolled in this study with an initial enrollment of 120 infants in Part 1, followed by up to ~~640~~ ***360*** infants in Part 2.

Vaccinations with either AFX3772 (1, 2 or 5 µg***, with 1 µg in Part 1 only***) or PCV are scheduled at approximately 2, 4, and 6 months of age for the primary series and 12 months for the booster dose. Subsequent immunogenicity assessments are scheduled at 30 days PD2, 30 days PD3, pre-dose 4 and 30 days PD4. In rare cases, if the participant is unable to return to the site, study data may be collected by telephone and blood collected

at a remote location, following study-specific procedures that have been approved by the Sponsor (refer to Section 10.6 Appendix 6 Clinical Study Continuity for alternative visit procedures in the case of a crisis). The follow-up visit will occur 6 months following the booster dose.

Vaccinations with either AFX3772 (1, 2 or 5 µg***, with 1 µg in Part 1 only***) or PCV~~13~~are scheduled at approximately 2, ~~4~~, and 6 months of age for the primary series and 12 months for the booster dose.

On Day 1 to Day 7 following each vaccination, the participant's LAR will record ***body*** temperature and solicited local and systemic AEs in an eDiary.

Immunogenicity data from the primary series (after the third dose of study vaccine) will be used to determine if any participant meets criteria for rescue vaccination, defined as a serotype-specific IgG concentration below 0.35 µg/mL for ≥ 3 of the 13 serotypes contained in PCV13***[Part 1] and 5 out of 20 gserotypes in PCV20 [Part 2]***. These assessments will be performed by the Sponsor in a timely manner, and the investigator or sub-investigator(s) will be informed of any participants meeting rescue criteria.

Participants will be offered a rescue vaccination with PCV13 ***(Part 1) or PCV20 (Part 2)*** within 3 weeks of site notification.

Additional blood volumes will be collected from study participants for the development and validation of assays measuring responses to the pneumococcal vaccine needed for the vaccine clinical development program.

Part 1: Dose escalation

Cohorts 1, 2 and 3 consist of 40 participants per cohort to be randomized 3:1 to receive either AFX3772 (1, 2 or 5 µg) or PCV13 as noted in ***Table 4*** and Figure 1. Following a review of the most recent cohort safety data through Day 7 postvaccination and any additional safety data from earlier cohorts, the ~~Data Monitoring Committee (~~DMC~~)~~ will determine whether to initiate enrollment in the next cohort. The DMC will review all available safety data from Cohorts 1, 2 and 3 to determine if the study will proceed into Part 2 as planned. Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 1, approximately ***1260*** participants per dose level (approximately ~~640~~ ***360*** infants if all dose levels are evaluated in Part 2) will be enrolled and randomized equally to receive either PCV***20***~~13~~ or AFX3772 at dose levels (~~1,~~ 2 or 5 µg) approved for Part 2. If the DMC does not recommend evaluating all ~~***3***~~ AFX3772 dose levels in Part 2, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to Part 2.

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Part 2

Since the halt of recruitment for Part 2 of the study, the FDA has indicated to GSK that the newly manufactured AFX3772 drug product (DP) planned for use in Part 2 may not be sufficiently similar to the DP used in Part 1 of the study. Secondly, the DMC has reviewed the data from Part 1 of the study, and GSK has decided to drop the 1 µg dose level of AFX3772 from Part 2. In addition, the ACIP has recommended the use of 20-valent pneumococcal conjugate vaccine, PCV20, for routine vaccination to help protect infants and children from IPD. As such, the dose escalation portion of the study will be repeated in Part 2, referred to as Part 2a, with the newly manufactured AFX3772 DP at 2 µg and 5 µg using PCV20 as comparator.

Part 2a – Dose escalation-repeat

Part 2a Cohorts 1 and 2 consist of 45 participants per cohort to be randomized 2:1 to receive either AFX3772 (2 or 5 µg) or PCV20 as noted in Table 4 and Figure 3. Following a review of the most recent cohort safety data through Day 7 postvaccination and any additional safety data from earlier cohorts, the Data Monitoring Committee (DMC) will determine whether to initiate enrollment in the next cohort. The DMC will review all available safety data from Cohorts 1 and 2 to determine if the study will proceed into Part 2b as planned. Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 2a, approximately 90 participants per dose level (approximately 270 infants if both dose levels are evaluated in Part 2b) will be enrolled and randomized equally to receive either PCV20 or AFX3772 at dose levels (2 or 5 µg) approved for Part 2b. If the DMC does not recommend evaluating 2 AFX3772 dose levels in Part 2b, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to Part 2b.

A description of the structure, function, and guidelines for decision-making by the DMC, along with the timing and content of the safety data collection is provided in Section 10.1.5.

Part 2b Full Enrollment

Following DMC review and agreement to proceed to Part 2***b***, up to ***270***~~640~~ participants (~***90*** ~~***160***~~per dose level) will be randomized equally to receive approved AFX3772 dose levels (2 µg or 5 µg) or ~~PCV13~~ PCV20 (Figure 4)~~(Figure 2). Dose levels to be evaluated in Part 2 will be chosen based on DMC review of the safety data and recommendations.~~ The DMC will continue to review safety data as outlined in the DMC charter throughout the study. If the number of dose groups is reduced, the number of participants enrolled in the study overall will be reduced, with the number of participants in each dose group remaining at approximately ***90***~~160~~

Table 5 Number of Participants by Vaccination Group for Part 2a

Cohort	AFX3772	Number of Participants	Comparator	Number of Participants
4	2 µg	30	PCV20	15
5	5 µg	30		15

PCV20: Prevnar 20

Table 6 Number of Participants by Vaccination Group for Part 2b

~~AFX3772 1 µg~~	AFX3772 2 µg	AFX3772 5 µg	Comparator ~~PCV13~~PCV20
~~160~~	~~160~~ 90	~~160~~ 90	~~160~~ 90

PCV20~~13~~: Prevnar ~~13~~20

4.1. Dose Escalation and Stopping Criteria

After all vaccinated participants in a ~~Part 1~~ ***dose escalation*** cohort have completed study safety procedures through Day 7 postvaccination, the DMC will recommend whether to initiate enrollment in the next cohort based on the review of all available safety data from the current and preceding cohorts. Once the Day 7 safety assessments in the final cohort (Cohort 3) of Part 1 are complete, the DMC will review all available safety data from ***the*** Cohorts 1, 2 and 3 to recommend if the study should proceed into Part 2 and, if so, which AFX3772 dose level(s) will be recommended for continued evaluation in Part 2.

***Once the Day 7 safety assessments in the final cohort (Cohort 5) of Part 2a are complete, the DMC will review all available safety data from the Cohorts 4 and 5 to recommend if the study should proceed into Part 2b and, if so, which AFX3772 dose level(s) will be recommended for continued evaluation in Part 2b (Refer to Figure 3 and Figure 4*).**

Depending on the safety data observed in the study, the DMC will recommend whether to proceed with or stop dose escalation. The Sponsor will make the final decision based on all the data available and the DMC's recommendation(s). A stopping rule is met if any of the following ~~rules~~ ***situations*** occur after administration of AFX3772. Reactogenicity eDiary data confirmed by the investigator as being entered by the LAR in error will not contribute to the stopping rule. Dose escalation may proceed unless the following applies to participant(s) receiving AFX3772:

The DMC will review the event(s) and may decide to unblind the participant(s) treatment assignment. After review of all information, the DMC will recommend whether to resume the study and/or dose escalation. If the DMC does not recommend evaluating all AFX3772 dose levels, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. The Sponsor will take the DMC recommendation into account when deciding how to proceed to ***the next step***.

4.2 Scientific Rationale for Study Design

The study will implement a 3+1 pneumococcal vaccination schedule for infants as recommended by the US ACIP. In order to evaluate other vaccination schedules (i.e., 2 + 1 schedule used in Europe and elsewhere), immunogenicity will also be assessed after the second vaccination. A dose escalation approach is being implemented in ***this trial*** ~~Part 1~~ to ensure adequate safety review following the first administration of each dose level prior to initiating enrollment into the next dose level. Once dose escalation has been completed ~~in Part 1~~ and DMC recommendation has been given and followed by the Sponsor's decision to continue the study, enrollment in Part 2 will be initiated.

4.2.1.1.Rationale for Immunogenicity Endpoints

Sera collected from participants will be used to measure vaccine-induced serotype-specific IgG ***concentrations*** for the ***24 serotypes i.e.,*** 13 serotypes shared with PCV13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) as well as the 11 additional serotypes in AFX3772 (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F, and 33F) ***in Part 1, and the 20 serotypes shared with PCV20 (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F) and the 4 unique serotypes (2, 9N, 17F and 20B) in AFX3772 in Part 2,*** at ***post Dose 2 (***PD2***)***, PD3, pre-dose 4 and PD4. Blood sampling at pre-dose 4 and ***post-dose 4 (***PD4***)*** will be used to evaluate the persistence of protective immunity and the anamnestic antibody responses.

4.2.2. Rationale for Use of a Comparator

Prevnar 13 ***(Part 1) and Prevnar 20 (Part 2)*** will be the active comparator***s*** for use in this trial and ~~is~~ ***are*** ~~currently~~ the most widely recommended vaccine***s*** for the prevention of pneumococcal disease in infants in many countries worldwide, including the US.

Refer to approved labeling for more details on Prevnar 13 ***and Prevnar 20***.

4.3. Justification of Dose

AFX3772 has been evaluated in adults in a dose escalation, dose finding study (3772-CL-1001). AFX3772 was immunogenic and induced both ***pneumococcal*** serotype-specific IgG responses and ~~OPA~~ antibody***-mediated serotype-specific opsonophagocytic activity (OPA)*** titers to all 24 serotypes included in the vaccine, and at all 3 dose levels (1, 2 and 5 µg) tested in healthy adults aged 18 to 64 years and 65 to 85 years (3772-CL-1001).

In Part 1 of this study, 3 dose levels (1, 2 and 5 µg) were assessed and preliminary safety and immunogenicity data became available prior start of Part 2. AFX3772 vaccine formulations at 1 µg, 2 µg, and 5 µg dose levels elicited immune responses in terms of serotype-specific IgG and OPA titers for both the 13 common and 11 unique serotypes in infants for whom a 3-dose primary series was initiated at the age of approximately 2 months. No safety signals were identified for any of the 3 dose levels of AFX3772 administered in infants aged approximately 2 months at the time the 3-dose primary vaccination series was initiated. Following DMC review of data from Part

1, GSK decided to discontinue further investigation of the 1 µg dose level. due to observation of a trend for lower immunogenicity following vaccination with 1 µg dose level versus other dose levels.

5.2. Exclusion Criteria

Participant has a known or suspected hypersensitivity to AFX3772, PCV13, ***PCV20*** or any components of the formulations used.

Table 7 Study Intervention(s) Administered

Intervention Label	AFX3772	Prevnar 13*	***Prevnar 20****
Intervention Name	AFX3772	PCV13	***PCV20***
Intervention Description	AFX3772 is administered as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a booster dose given at 12 months of age.	PCV13 is administered as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a booster dose given at 12 months of age.	***PCV20 is administered as a 4-dose regimen, with 3 doses given at 2, 4, 6 months of age as part of a primary series and a booster dose given at 12 months of age.***
Type	Biologic	Biologic	***Biologic***
Dose Formulation	Vial ***(Part 1)#*** ***Prefilled syringe (Part 2)***#	Prefilled syringe ***(Part 1)***	***Prefilled syringe (Part 2)***
Unit Dose Strength(s)	0.5 mL each administered as 4 separate vaccinations	0.5 mL each administered as 4 separate vaccinations	***0.5 mL each administered as 4 separate vaccinations***
Dosage Level(s)	1 µg ***(Part 1 only)*** 2 µg 5 µg	See PCV13 label for details	***See PCV20 label for details***
Route of Administration	Intramuscular injection	Intramuscular injection	***Intramuscular injection***
Use	Investigational vaccine	Comparator vaccine	***Comparator vaccine***
IMP and NIMP/AxMP	IMP	IMP	***IMP***
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor	***Provided centrally by the Sponsor***
Packaging and Labeling	AFX3772 ~~will be~~provided as a sterile suspension in single-dose vials ***(Part 1) / Pre-filled syringe (Part 2)***# and appears as a grey/white suspension. Each vial ***(Part 1)/ Pre-filled syringe (Part 2)*** ~~will be~~ ***is*** labeled as required per country requirement.	PCV13 ~~will be~~ provided in a single- dose 0.5 mL prefilled syringe along with instructions for storage, handling and use. Each syringe ***is*** ~~will be~~ labeled as required per country requirement.	***PCV20*** ~~will be~~ ***provided in a single- dose 0.5*** mL ***prefilled syringe along with instructions for storage, handling and use. Each syringe is*** ~~will be~~ ***labeled as required per country requirement.***

PCV20: Prevnar 20.
#Vials of AFX3772 used in Part 1 and Pre-filled syringes containing AFX3772 used in Part 2.
****Refer to Figure 2 for Part 1, Figure 3 and Figure 4 for Part 2***

6.3.1. Intervention Assignment

In Part 1, 40 participants per cohort w***ere*~~ill be~~ enrolled and randomized 3:1 to receive AFX3772 (1, 2 or 5 µg) or PCV13. ***In part 2a, 45 participants per cohort will enrolled and randomized 2:1 to receive AFX3772 (2 or 5 µg) or PCV20.*** If continuation into Part 2***b*** is recommended approximately ~~640~~ ***270*** additional participants will be randomized 1:1:1~~:1 (160~~ ***90*** per group) ***are*** to receive either 1 of up to ***2*** AFX3772 dose levels (~~1,~~2 or 5 µg), or ~~or PCV13~~***PCV20*** as agreed to for evaluation in Part 2***b***. At Visit 1, after the ICF signature, study site personnel will access the electronic data collection (EDC) system and enter the participant's required information as described in the eCRF Completion Guidelines. Upon completion of the Randomization ***e***CRF page, the EDC system will assign a unique Subject Randomization Number to each randomized participant. Upon completion of the Vaccine Administration ***e***CRF page, the IRT system will assign a unique kit to each randomized participant. An unblinded pharmacist will access the IRT to find out which investigational product kit was assigned to a randomized participant.

Specific procedures for randomization through the IRT are contained in the IRT~~/~~ ***and*** pharmacy manual.

6.3.3. Blinding

Because AFX3772 and Prevnar 13/ ***Prevnar 20*** have different appearances, a member of the study staff (and back- up) will be unblinded for purposes of receiving, maintaining, preparing, and administering study vaccines.

6.8.1. Permitted Concomitant Therapy

Concomitant vaccines (oral or injectable) should be administered on the same day as trial vaccine. Concomitant oral vaccines ~~should~~ ***must*** be administered prior ***to other injectable concomitant vaccines followed by*** trial vaccine ~~and other injectable concomitant vaccines~~ to prevent choking. To avoid any confounding local reactogenicity results, non-trial injectable vaccines must not be administered in the same limb as trial vaccine.

Documentation of limb used for the administration of trial vaccine ~~should~~ ***must*** be recorded in the participant's medical record, and the LAR should be instructed on the appropriate limb to monitor for AEs. Injection site AEs for concomitantly administered non-study vaccines will not be collected.

6.8.3. Rescue Vaccination

Investigators will be informed if a participant met criteria for rescue vaccination (defined as the pneumococcal serotype-specific IgG concentration below 0.35 µg/mL for 3 or more of the PCV13 ***[Part 1] and 5 or more of the PCV20 [Part 2]*** serotypes after

completion of the primary series), so that a rescue dose of ~~PCV13~~ ***PCV*** can be administered within 3 weeks following site notification.

7.3. Lost to Follow-Up

Every reasonable effort should be made to contact any participant lost to follow-up during the course of the study to complete study-related assessments and record outstanding data (~~eg~~***e.g.***, telephone calls and/or a certified letter to the LAR's last known mailing address).

8.1. Administrative and General Procedures

A participant also can be seen for an unscheduled visit at any time during the study. An unscheduled visit may be prompted by reactogenicity issues, new or ongoing AEs, or other reasons per investigator discretion. The site also has the discretion to make reminder telephone calls or send text messages to inform the participant about visits, review eDiary requirements, or follow-up on ongoing or outstanding issues. ***All unscheduled visits and telephone contacts must be reported in the eCRF.***

8.2. Immunogenicity Assessments

The following measures will be used to characterize the immunological response in infants following administration of either AFX3772 or ~~PCV13~~***PCV***.

Considering the mechanism of action, AFX3772 is expected to induce an immune response to ***serotype-specific*** pneumococcal ***polysaccharides*** and to the ***fusion*** protein, CP1. Blood samples to assess immunogenicity will be collected as indicated in the schedule of assessments (Table 1).

Immunogenicity of AFX3772 ***and PCV*** will be evaluated by measuring ***pneumococcal*** serotype-specific anti-***capsular polysaccharide*** IgG antibody concentrations by multiplex direct Luminex immunoassays (dLIAs) ***or alternative assay format (e.g. Electrochemiluminescent (ECL) multiplexed assay).***

Immunogenicity of AFX3772 or PCV vaccines will be also evaluated by measuring the antibody-mediated pneumococcal serotype-specific opsonophagocytic activity (OPA***)*** titers by multiplexed opsonophagocytic assay (MOPA) targeting the 24 AFX3772 vaccine serotypes. ***The M***OPA measures the functional opsonic activity of antibodies against the pneumococcal serotypes that results in bacteria killing by phagocytic cells in vitro.

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Additional blood volumes will be collected from study participants for the development and validation of assays measuring responses to the pneumococcal vaccine needed for the vaccine clinical development program.

8.3.3. Reactogenicity

Solicited injection site AEs (tenderness, redness/erythema and swelling) Day 1 to Day 7
Note: Only injection-site AEs from AFX3772 or ***PCV*** ~~PCV13~~ and not from concomitant injectable vaccines will be collected. The location of AFX3772 or ***PCV*** ~~PCV13~~ administration can be used by the participant's LAR to monitor the appropriate limb for injection-site AEs related to AFX3772 or ***PCV*** ~~PCV13~~

Solicited systemic AEs (irritability, ***fever (body temperature)***, decrease of appetite, increased sleep and decrease in sleep) Day 1 to Day 7

8.6. Total Amount of Blood

The approximate total amount of blood to be collected ***per participant*** for ~~immunogenicity and anti-CP1 IgG~~ laboratory assessment (is presented in ***Table 8) ~~Table 6.~~***

Table 8 Blood Sampling

Study Part	**Assay Tested**	**Number of Samples**	**Sample Volume (mL)**	**Total Volume (mL)**
Part 1	Pneumococcal ***serotype-specific*** anti-capsular PS IgG, Pneumococcal OPA and anti-CP1 IgG ~~Assays development~~	4	4.0	16.0*
	Total			16.0*
Part 2	***Pneumococcal serotype-specific anti-capsular PS IgG, Pneumococcal OPA and anti-CP1 IgG Assays development***	***4***	***5.0***	***20.0***
	Total			***20***

9. STATISTICAL CONSIDERATION

Estimates of a treatment difference between AFX3772 treatment groups and ***PCV*** ~~PCV13~~ will be conducted for some immunogenicity measures.

All analyses will be performed and summarized separately for Part 1 and Part 2.

9.1. Statistical Hypotheses

There is no ***formal*** hypothesis testing planned in this Phase 2 study for the primary endpoint. For the secondary endpoints, a 1-way analysis of variance (ANOVA) will be used to test for overall treatment differences in the geometric means. A t-test will be used for paired comparisons between each AFX3772 dose and ***PCV*** ~~PCV13~~ There will be no multiplicity adjustment. These comparisons will be used to aid in dose selection.

The study will provide estimates of safety and immunogenicity for 3 dose levels of AFX3772 and PCV13 ***in Part 1 and 2 dose levels of AFX3772 and PCV20 in Part 2***. Estimates of differences in response following AFX3772 relative to response following ***PCV*** ~~PCV13~~ administration will be used to determine sample size for hypothesis testing for future studies.

9.2. Analysis Sets

For each AFX3772 or ~~PCV13~~ ***PCV*** immunization group, the number and percentage of participants will be summarized for all randomized participants (AFX3772 or ~~PCV13~~***PCV***), the SAF and the PPS.

Population	Description
Randomized	All participants who are randomized to receive either AFX3772 or ~~PCV13~~ ***PCV**.
PPS	Pneumococcal vaccine-naïve healthy infants 42 to 90 days of age defined by the inclusion/exclusion criteria for the study who receive at least 2 vaccinations with either AFX3772 or ~~PCV13~~ ***PCV**** during this study and do not have exclusionary intercurrent events defined in Section 3. The following participants will have their data excluded from assessments of immunogenicity for the PPS, the primary analysis set for immunogenicity. 1. Participants who have their 30-day blood draw ***not earlier than 28 days*** and ~~0 or more days after the 30-day~~ ***no more than 50 days*** postimmunization scheduled blood collection for that immunogenicity assessment only 2. Participants who receive systemic corticosteroids at the doses, duration and timing noted in Section 10.4 Appendix 4: List of Excluded Concomitant Medications and for that immunogenicity assessment only. 3. Participants who have newly diagnosed immunologic abnormalities within the first 2 weeks after receiving the study vaccine will be excluded from all immunogenicity assessments. Participants who receive rescue (an intercurrent event) will be discontinued from study intervention and not included in subsequent immunogenicity assessments.
SAF	All participants who are randomized and receive at least 1 vaccination with either AFX3772 or ~~PCV13~~ ***PCV****. Participants will be analyzed according to the vaccine received.

PPS: Per Protocol Set; PCV13: Prevnar 13; ***PCV20: Prevnar 20***; SAF: Safety Analysis Set
PCV13 [Part 1] and PCV20 [Part 2]

9.3.1. General Considerations

Demographics and baseline characteristics (age, sex, race, ethnicity, and weight) will be summarized by treatment group for AFX3772 (1 µg, 2 µg or 5 µg) and immunization group (AFX3772 or ~~PCV13~~ ***PCV***)

The number and percentage of participants who receive rescue vaccination will be provided by immunization group (AFX3772 [1 µg, 2 µg and 5 µg] or ~~PCV13~~ ***PCV***).

9.3.2.1. Primary Vaccination Series Assessments

In Part 1 participants, an interim analysis of safety and immunogenicity following primary vaccination series will be performed. This will include the analysis of all available safety ***and immunogenicity*** data for all three cohorts post study intervention.~~and available immunogenicity data for 1 µg and 2 µg cohorts~~.. Section 9.4.1 for full details of analyses.

9.3.3. Secondary Endpoint(s)/Estimand(s) Analysis

Key secondary endpoints for this Phase 2 study are assessments of immunogenicity 30 days after the second and third dose of the primary infant 3-dose series ***with either AFX3772 or PCV13 for Part 1, and 30 days after the second and third dose of the primary infant 3-dose series*** and before and 30 days after the booster vaccination with either AFX3772 or ***PCV20 for Part 2***.

CCI [redacted]

9.3.3.1. IgG concentration

Geometric means for the IgG concentration will be provided for each dose level of AFX3772 (1, 2 and 5 µg) and the combined ~~PCV13~~***PCV*** group after each concentration has been logarithmically transformed. Two-sided 95% CIs for the GMC will be constructed after back transforming the CIs for the mean of the logarithmically transformed assays computed using Student t distribution. The ratio of the IgG GMC (each AFX3772 dose level/~~PCV13~~***PCV***) and the 95% CI will be provided.

CCI [redacted]

CCI

[redacted]

9.3.5.2. Body Temperature

Proportion of participants with maximum temperature measurements meeting the Brighton Collaboration cut points Day 1 through Day 7 following any vaccination with AFX3772 or ~~PCV13~~ ***PCV*** will be listed.

9.4. Interim Analyses

A total of ~~7~~ ***5*** analyses will be performed in the study:

In Part 1 participants, 1 primary vaccination series assessment, and a final assessment.

In Part 2, 1 primary vaccination series ***assessment, 1*** booster vaccination assessment and a final safety assessment.

~~In Part 1 participants only, 1 primary vaccination series assessment, and a final assessment~~

9.4.1. Primary Vaccination Series Assessments

In Part 1 participants, the first analysis, an interim analysis of the primary vaccination series will be performed. This will include the analysis of all available safety data for all three cohorts post study intervention and available immunogenicity data for the 1 µg, ~~and~~2 µg ***and 5ug*** cohorts.

The third analysis, an interim analysis of the primary vaccination series ***of Part 2***, will be performed when the ~~first 472~~***360*** participants have completed 30 days after the primary series and the immunogenicity results become available. ~~The fourth analysis, i.e., final analysis of the primary vaccination series, will be performed when all 760480 participants have completed 30 days after the primary vaccination series and the immunogenicity data become available.~~

9.4.2. Booster Vaccination Assessments

The ~~fifth~~ ***fourth*** analysis, an interim analysis of the booster vaccination ***of Part 2***, will be performed after the ~~first 472~~***360*** participants have completed 30 days after the booster vaccination and the immunogenicity results become available. ~~The sixth analysis, i.e., final analysis of the booster vaccination, will be performed when all 760480 participants~~

~~have completed 30 days after the booster vaccination and the immunogenicity data become available.~~

9.4.3. Final Safety Assessment

The fifth ~~An~~ analysis will be performed to report the final safety assessment (SAEs) ***of Part 2,*** after the last participant completes the study, approximately 6 months after the booster vaccination in the last participant.

9.5. Sample Size Determination

Approximately ~~760~~***480*** healthy infants are planned to be enrolled in this study with an initial 120 participants enrolled ***(Part 1)*** followed by the remaining ~~640~~ ***360*** participants ***(Part 2)***. Of the initial 120 participants enrolled, 40 infants will be randomized (3:1) to receive either AFX3772 ***(1, 2 or 5 µg)*** or ***PCV13*** within each of ***the*** 3 dose cohorts. Once safety has been evaluated for additional dose levels of AFX3772, up to ***360*** participants will be ***enrolled in Part 2, of which 90 in Part 2a and 270 in Part 2b. In Part 2a, 45 infants at each dose level will be randomized 2:1 into 2 cohorts of 2 dose levels of AFX3772 (Cohort 4: 2 µg and Cohort 5: 5 µg) or PCV20***. ~~randomized 1:1:1:1 to receive AFX3772 (1, 2 or 5 µg) or PCV13.~~

~~Accounting for an 85% evaluable rate, such a sample size is predicted to result in approximately 161 34 evaluable participants in each treatment arm~~

The sample size is not based on ***formal*** statistical hypothesis testing.

The probability of observing at least 1 AE in this study depends on the number of participants vaccinated and the underlying incidence of participants with an AE in the study population. Calculations below assume that 100% of the randomized participants will be evaluable for safety analyses.

In Part 1 and 2a, there is at least 95% chance of observing at least 1 AE among 30 participants in AFX3772 group of each cohort, if the underlying incidence of an AE is 10%. The chance would be 26% for an AE incidence of 1% and 14% for an AE incidence of 0.5%.

In addition, if no AEs are observed among the 30 participants in a AFX3772 group, we will be able to rule out the hypothesis that the incidence of AE in that AFX3772 dose level group is above 11.57% with 97.5% confidence (11.57% = upper limit of two-sided 95% confidence interval [CI]) (See Table 9 below).

In Part 2, there is a 100% chance of observing at least 1 AE among 120 participants in each AFX3772 group, if the underlying incidence of an AE is 10%. The chance of observing at least 1 AE would be at least 70% for an incidence of 1% and at least 45% for an incidence of 0.5%. In addition, if no AEs are observed among the 120 participants in a AFX3772 group, we will be able to rule out the hypothesis that the incidence of AE in that AFX3772 dose level group is above 3.03% with 97.5%

confidence (3.03% = upper limit of two-sided 95% confidence interval [CI]) (See Table 9 below).

Table 9 Exact two-sided 95% CI for a range of incidence of AE , considering 30 and 120 participants.

Incidence of AE(%)	30 participants (Part 1 and 2a)		120 participants (Part 2)	
	Lower Limit (%)	Upper Limit (%)	Lower Limit (%)	Upper Limit (%)
0	0.00	11.57	0.00	3.03
0.1	0.00	11.76	0.00	3.23
0.5	0.00	12.51	0.00	3.98
1.0	0.00	13.40	0.04	4.83
5.0	0.36	19.71	1.86	10.57
10.0	2.11	26.53	5.28	16.82
15.0	4.67	32.74	9.14	22.67
20.0	7.71	38.57	13.26	28.28
25.0	11.09	44.10	17.55	33.73
30.0	14.74	49.40	21.98	39.04

Exact (Clopper-Pearson) Confidence Interval using PASS2022: Confidence Intervals for One Proportion.

~~Data from both phases of the study will be combined by treatment group in the immunogenicity and safety summaries.~~

The ~~secondary~~ immunogenicity objective is to estimate the percentage of participants with an IgG antibody level ≥ 0.35 µg/mL after the primary series. Accounting for an 85% evaluable rate, such a sample size is predicted to result in approximately 26 evaluable participants in each treatment arm ***in Part 1 and 102 evaluable participants in each treatment arm in Part 2.***

A sample size of ~~~~161~~***102*** evaluable participants per treatment group in Part 2 provides reasonable estimates in this Phase 2 study of the immune response rate when the response rate ranges from 60% to 95% (See Table 10 below).

Table 10 Exact two-sided 95% CI around percentage of participant with IgG concentration ≥ 0.35 µg/mL, considering 102 evaluable participants.

True response (%)	Lower Limit (%)	Upper Limit (%)
60	49.83	69.58
65	54.92	74.18
70	60.13	78.68
75	65.45	83.05
80	70.92	87.27
85	76.57	91.30
90	82.47	95.06
95	88.80	98.33

Exact (Clopper-Pearson) Confidence Interval using PASS2022: Confidence Intervals for One Proportion.

Further, the two AFX23372 formulations will be compared to the PCV20 in terms of Pneumococcal serotype specific IgG concentrations PD2, PD3 and PD4 using a one way ANOVA model. The sample size of 102 evaluable subjects per group achieves approximately 85% power to detect a difference of at least 2.12-fold (Log10 SD=0.5) to 3.87 fold (Log10 SD=0.9) for each serotype (2-sided alpha=0.05, PASS 2022 Multiple comparisons Analysis using Dunnett's adjustment for multiplicity)

10.1.3. Informed Consent Process

A ***signed*** copy of the ICF(s) must be provided to the participant's LAR.

10.1.5.1. Data Monitoring Committee Structure

Responsibilities of the DMC will include:

Making recommendations on all cohort dose escalation decisions during Part 1 and ***Part 2a***.

10.1.9. Quality Assurance

The Sponsor or Sponsor's designee may arrange to audit the study at any or all study sites and facilities. The audit may include on-site review of regulatory documents, ***e***CRFs and source documents. Direct access to these documents will be required by the auditors.

10.2.1. Assessment of Intensity for Local AEs

Injection site redness and swelling will be evaluated by maximum size and then categorized during analysis as mild, moderate, or severe based on the grading scale in ***Table 11*** ~~Table 7~~ below.

Table 11 Local Adverse Event Grading Scale

Local Reaction to Injectable Product	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Grade 4[b]
Pain/Tenderness	Hurts if gently touched (~~eg~~***e.g.,*** whimpers, winces, protests, or withdraws)	Hurts if gently touched, with crying	Interferes with limb movement	Emergency room visit or hospitalization for severe pain (tenderness) at injection site
Induration/Swelling	0.5 to 2.0 cm	> 2.0 to 7.0 cm	>7 cm[a]	Necrosis
Erythema/redness	0.5 to 2.0 cm	> 2.0 to 7.0 cm	>7 cm[a]	Necrosis

a. Legally acceptable representatives of the participants experiencing local reactions >7.0 cm are to be contacted by the study site. An unscheduled visit may be required ***for targeted physical examination and symptom driven assessment based on PI's judgement.***.

Table 13 Fever Ranges for Grouped Analysis

Grading for intensity will be performed within 7 days for solicited events and within 30 days for unsolicited events post IMP by PI in addition to LAR in appendix 2, for any intensity including grade 4.

Table 14 Clinical Laboratory Tests

System	Component	Method	Testing Lab	Unit
Serum	Pneumococcal serotype-specific ***anti-capsular polysaccharide*** IgG*	dLIA ***or ECL***	~~PPD~~***GSK or GSK designated laboratory*** ~~PPD~~ ~~CMIC~~	ng/mL ***µg/mL***
Serum	Anti-pneumococcal serotype-specific opsonophagocytic activity*	MOPA		dil^{-1}
Serum	Anti-CP1 IgG~~Ab~~	ECL~~ELISA~~		***AU***~~µg~~/mL

Ab: antibody; CP1: fusion protein; ***dLIA: direct Luminex immunoassay;*** IgG: immunoglobulin G; MOPA: multiplexed opsonophagocytic assay; dil^{-1}: reciprocal of the dilution; µg = microgram; mL = milliliter; ng: nanogram; ~~ELISA, enzyme-linked immunosorbent assay~~***ECL: electrochemiluminescent assay.***

* Testing for each of the 24 pneumococcal AFX3772 vaccine serotypes (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F).~~** Affinivax/GSK delegates testing to other external laboratories designated by the Sponsor~~.

Immunogenicity of AFX3772 will be evaluated by measuring ***pneumococcal*** serotype-specific anti-***capsular polysaccharide***~~pneumococcal~~ IgG antibody concentrations by 2 complementary multiplex (12-plex) Luminex immunoassays (dLIAs) to cover simultaneously the 24 vaccine serotypes. ***Alternatively, an Electrochemiluminescent (ECL) multiplex assay could be used to measure this endpoint.***

Immunogenicity of AFX3772 will be also evaluated by measuring ***pneumococcal*** serotype-specific opsonophagocytic activity using multiplexed opsonophagocytic assay (MOPA) targeting the 24 AFX3772 vaccine serotypes.

~~. The assay has been developed for all 24 serotypes covered by AFX3772 vaccine.~~

Immunogenicity ~~of AFX3772~~ ***against*** ~~in terms of antibodies to~~ the CP1 fusion protein ***in terms of anti-CP1 IgG antibodies*** will be measured using ***a ligand-binding assay (e.g. ECL assay).***

Amendment 2 ***(22 March 2023)***

Overall rationale for the amendment

On 24 February 2023, GSK made the decision to halt study vaccinations in Part 1 of the study and pause the start of recruitment in Part 2. During a GSK audit at a manufacturing third party, observations were made regarding the manufacturing process introducing a potential risk on sterility assurance of the investigational vaccine. As a consequence, and as a precautionary measure, GSK has decided to revoke the release of the investigational vaccine.

The purpose of this amendment is to (i) reflect implementation of Urgent Safety Measures (USM) in Part 1 of the study, i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines following the decision to halt study vaccinations and the Dear-Investigator-Letter (DIL) sent on 24 February 2023; (ii) change the safety assessment following the administration of SoC pneumococcal vaccines; (iii) modify the statistical analysis plan; and (iv) make minor editorial changes throughout the document for consistency and clarity

Detailed description of the current Protocol Amendment 2

Amended text has been included in bold italics and deleted text in strikethrough in the following sections:

Title page

Registry	**ID**
IND	18657
EudraCT	~~2019-004503-12~~
	2023-000423-36
Pediatric Investigational Plan	~~EMA-002641~~
	EMEA-002641-PIP01-19

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Note on the PI agreement page

Note: ***On 24 February 2023, GSK made the decision to halt study vaccinations in Part 1 of the study and pause recruitment for Part 2. This protocol was amended to reflect Urgent Safety Measures consisting of replacement of the study intervention with the standard-of-care pneumococcal vaccines and changes to the study procedures and analyses related to the use of the standard-of-care pneumococcal vaccines to complete Part 1.***

1. Protocol summary

1.1 Synopsis

Number of Participants: Approximately 760 healthy infants are planned to be enrolled in this study with a subset of 120 initially enrolled (Part 1) followed by the remaining 640 participants (Part 2). Accounting for an 85% evaluable rate, such a sample size predicts approximately ~~181~~***161*** evaluable participants in each treatment arm.

Intervention Groups and Duration:

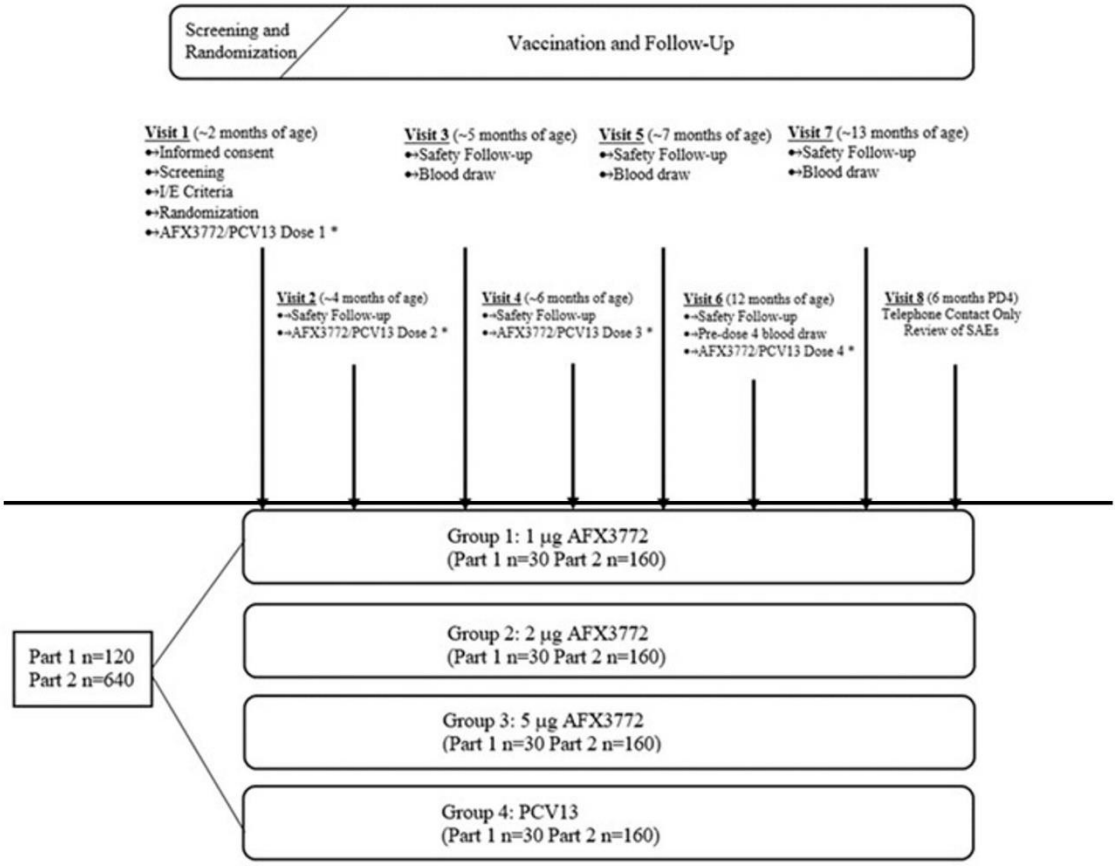
~~The Sponsor estimates the study will require approximately 23 months from the time the first participant's LAR signs the informed consent until the last participant's last study-related telephone call or visit.~~

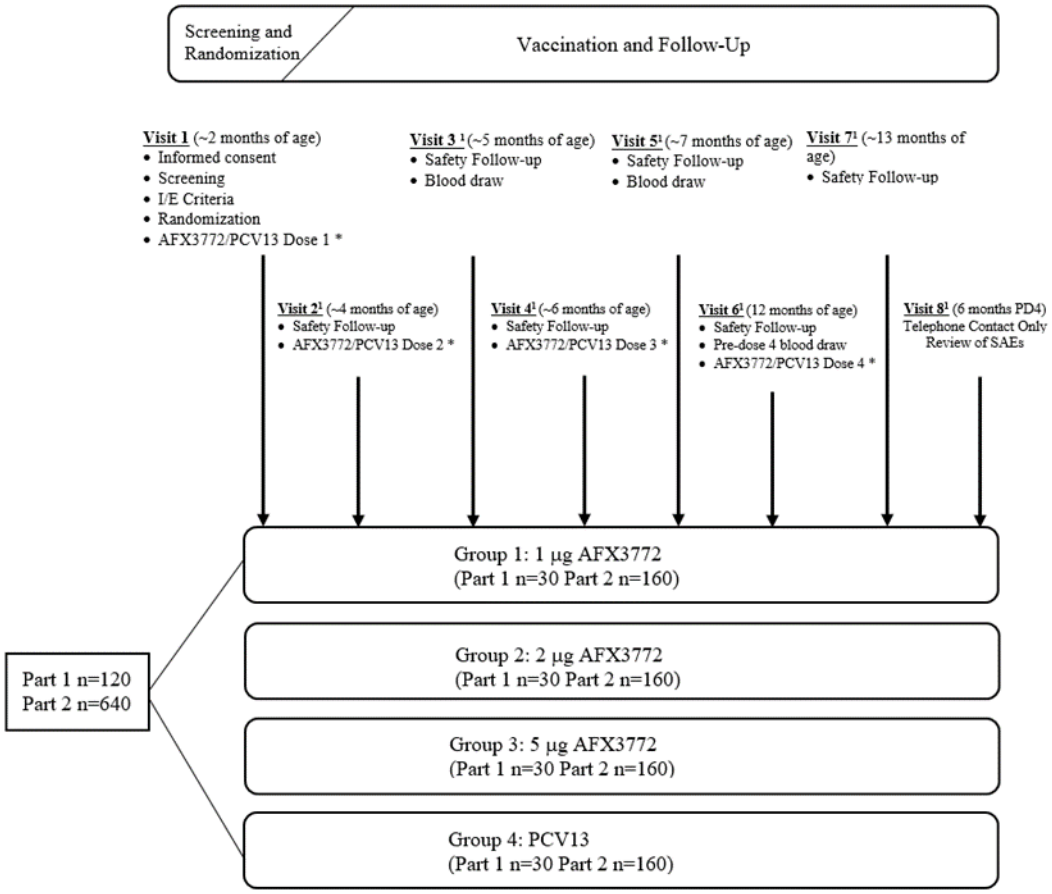
For Part 1 participants, ~~The~~ the Sponsor estimates the study will require approximately ***10*** months from the time the first participant's LAR signs the informed consent until the last participant's last study-related telephone call or visit, ***and for Part 2 participants, the study duration will be approximately 23 months.***

1.2 Schema

Figure 2 Study Schema Part 1 & Part 2

Figure 2 was adapted with footnotes added:





PCV13: Prevnar 13

*Telephone contact occurs Day 8 postvaccination for safety follow-up and eDiary review

Part 2 starts after DMC review of Cohort 3 safety data through Day 7, plus cumulative safety data from prior cohorts of Part 1 and agreement to proceed to full enrolment.

Concomitant vaccines are permitted as per local requirements.

[1] ***Applicable only for Part 1 participants affected by Urgent Safety Measure i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines following the decision to halt study vaccinations and the Dear-Investigator-Letter (DIL) sent on 24 February 2023:***
Timepoints impacted by protocol amendment 2:

- ***Visit 2 – SoC pneumococcal vaccine to be administered, if not received prior 24 February 2023***
- ***Visit 3 – not applicable for participants who received SoC pneumococcal vaccine as Dose 2.***
- ***Visit 4 – SoC pneumococcal vaccine to be administered, if not received prior 24 February 2023***
- ***Visit 6 –is 6-months post Dose 3 safety follow-up and timepoint for administration of SoC pneumococcal vaccine booster dose for all Part 1 participants (first subject's booster dose expected in April 2023). Visit 6 is to be performed at study site if this option is exercised by participants' LARs or via TC if participants' LARs opt to receive the booster dose at their primary care center.***
- ***Visit 7 and 8 are not applicable for participants who receive SoC pneumococcal vaccine as booster dose, no specific safety and immunogenicity assessment will be performed following the booster dose with SoC***

Note: Visit 5 (1-month post Dose 3) includes blood sampling and is maintained for all Part 1 participants (including those who received SoC pneumococcal vaccine) to assess a need for rescue vaccination. All study-related procedures pertaining to Part 2 remain unchanged. **1.3 Schedule of Assessments**

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Table 1 was updated with footnotes to reflect timepoints impacted and observation period adapted to standard medical practice following replacement of study vaccine with SoC in part 1 of the study.

Footnotes to Table 1 Schedule of Assessments (SoA) for Part 1 and Part 2

4 Applicable only for Part 1 participants affected by Urgent Safety Measure i.e., the replacement of study vaccines with local standard-of-care (SoC) pneumococcal conjugate vaccines following the decision to halt study vaccinations and the Dear-Investigator-Letter (DIL) sent on 24 February 2023: Study timepoints impacted by amendment 2:

- ***Visit 2 – SoC pneumococcal vaccine is administered, if not received prior 24 February 2023***
- ***Visit 3 –not applicable for participants who received SoC pneumococcal vaccine as Dose 2***
- ***Visit 4 – SoC pneumococcal vaccine is administered, , if not received prior 24 February 2023***
- ***TC2, TC3 and TC4 are not applicable for participants who receive the SoC pneumococcal vaccine.***
- ***Visit 6 – is 6-months post Dose 3 safety follow-up and timepoint for administration of SoC pneumococcal vaccine booster dose for all Part 1 participants (first subject's booster dose expected in April 2023). Visit 6 is to be performed at study site if this option is exercised by participants' LARs or via TC if participants' LARs opt to receive the booster dose at their primary care center.***
-
- ***Visit 7 and Visit 8 – not applicable for participants who receive SoC pneumococcal vaccine as booster dose. no specific safety and immunogenicity assessment will be performed following the booster dose with SoC***

Note: Visit 5 (1-month post Dose 3) includes blood sampling and is maintained for all Part 1 participants (including those who received SoC pneumococcal vaccine) to assess a need for rescue vaccination. All study-related procedures pertaining to Part 2 remain unchanged.

5 Applicable following administration of SoC pneumococcal conjugate vaccines: Duration of observation period as required by standard medical practice [CDC., 2023].

2. Introduction

Affinivax has developed an investigational 24-valent pneumococcal vaccine (referred to as AFX3772) for the prevention of pneumococcal disease caused by the serotypes contained in the vaccine. AFX3772 contains 24 serotypes of *Streptococcus pneumoniae* and a unique ***fusion*** ~~carrier~~ protein (CP1) created by the fusion of 2 pneumococcal proteins (sp1500 and sp0785), which has the potential to induce T- and B-cell responses and provide extended protection.

2.2 Background

AFX3772 is a 24-valent pneumococcal vaccine (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F) being developed for the prevention of IPD in children 6 weeks to 17 years of age and adults 18 years of age and older. AFX3772 uses a novel MAPS platform in which high-affinity, non-covalent ***binding*** between biotin and rhizavidin links pneumococcal PS to CP-1 made up of the fusion of 2 conserved pneumococcal proteins (SP1500 and SP0785) [AFX3772 Investigator***'s*** Brochure, 2022].

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Refer to the AFX3772 Investigator***'s*** Brochure (IB) for detailed background, including pneumococcal epidemiology and completed preclinical and clinical trials conducted with AFX3772.

8.2 Immunogenicity Assessments

CCI

[redacted]

8.3.3 Reactogenicity

Note that as part of protocol amendment 2, following the administration of SoC, participants should remain at the study site for a period of time in line with the standard medical practice for vaccination with SoC pneumococcal vaccines. In addition, LARs are no longer asked to record reactogenicity events and body temperature in the eDiary following the vaccination with SoC pneumococcal vaccines.

8.4.1 Time Period and Frequency for Collecting AE and SAE Information

For all Part 1 participants, SAEs will be collected for 6 months after completion of the primary vaccination series, i.e., 6 months post Dose 3. No specific safety follow-up will be performed following administration of the booster dose with SoC pneumococcal vaccines. Instead, routine PV reporting should be performed in line with local medical practice.

8.6 Total amount of Blood

Table 6 Blood Sampling

Footnotes were added to Table 6.

CP1: ***fusion*** ~~carrier~~ protein

**** The total volume of blood collected from Part 1 participants who complete the primary series including post Dose 2 and post Dose 3 sampling, will be 8.0 mL, while for those who receive SoC pneumococcal vaccines as Dose 2 will be 4.0 mL. Note that for all Part 1 participants, pre and post booster blood sampling has been cancelled.***

9. Statistical considerations

9.3 Statistical Analyses

A statistical analysis plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be

finalized before the database ~~soft~~ lock. Any changes from the analyses planned in the SAP will be justified in the clinical study report.

9.3.2.1 Primary Vaccination Series Assessments

In Part 1 participants, an interim analysis of safety and immunogenicity following primary vaccination series will be performed. This will include the analysis of all available safety data for all three cohorts post study intervention and available immunogenicity data for 1 µg and 2 µg cohorts. Section 9.4.1 for full details of analyses.

The final assessment of safety and immunogenicity will be performed after the last participant completes the study i.e., approximately 6 months after the last participant has completed the primary series.

9.3.2.2 Booster Vaccination Assessments

A***n*** ~~second~~ analysis (through 30 days after the last participant has received the booster vaccination) for safety and immunogenicity will occur once all data have been collected and cleaned. See Section 9.4.2 for full details of analyses.

Since Part 1 participants should receive SoC for the booster vaccination and no safety and immunogenicity assessment is planned for SoC pneumococcal vaccines, no analysis will be performed for Part 1 participants for booster series.

9.3.2.3 Final Safety Assessment

No analysis will be performed for the Part 1 participants for the booster dose.

9.3.5.5.1 Immunoglobulin G Concentration

In Part 1 participants, the ratio of the IgG GMC between the two AFX3772 dose levels (1 µg and 2 µg), 1-month PD2 and the 95% CI will be provided.

9.4 Interim Analyses

A total of ***7*** ~~**5**~~ analyses will be performed in the study:

2 primary vaccination series assessments, 2 booster vaccination assessments and a final safety assessment.

In Part 1 participants only, 1 primary vaccination series assessment, and a final assessment

9.4.1 Primary Vaccination Series Assessments

In Part 1 participants, the first analysis, an interim analysis of the primary vaccination series will be performed. This will include the analysis of all available safety data for all three cohorts post study intervention and available immunogenicity data for the 1 µg and 2 µg cohorts. A second analysis, i.e., the final assessment will be performed after the last participant in Part 1 completes the study, approximately 6 months after the last participant has received the primary series.

The ***third*** ~~first~~ analysis, an interim analysis of the primary vaccination series, will be performed when the first 472 participants have completed 30 days after the primary series and the immunogenicity results become available. The ***fourth*** ~~second~~ analysis, i.e., final analysis of the primary vaccination series, will be performed when all 760 participants have completed 30 days after the primary vaccination series and the immunogenicity data become available.

9.4.2 Booster Vaccination Assessments

Since Part 1 participants should receive SoC for the booster vaccination and no safety and immunogenicity assessment is planned for SoC pneumococcal vaccines, no analysis will be performed for the Part 1 participants for booster series.

The ***fifth*** ~~third~~ analysis, an interim analysis of the booster vaccination, will be performed after the first 472 participants have completed 30 days after the booster vaccination and the immunogenicity results become available. The ***sixth*** ~~fourth~~ analysis, i.e., final analysis of the booster vaccination, will be performed when all 760 participants have completed 30 days after the booster vaccination and the immunogenicity data become available.

9.5 Sample Size Determination

Accounting for an 85% evaluable rate, such a sample size is predicted to result in approximately ***161*** ~~181~~ evaluable participants in each treatment arm.

The secondary objective is to estimate the percentage of participants with an IgG antibody level ≥ 0.35 µg/mL after the primary series. A sample size of ~~~181~~***161*** evaluable participants per treatment group provides reasonable estimates in this Phase 2 study of the immune response rate when the response rate ranges from 60% to 95%.

Appendix 5 Table 10

System	*Component*	*Method*	*Testing Lab***	*Unit*
Serum	Anti-pneumococcal serotype-specific IgG*	dLIA	PPD	***ng***/mL
Serum	Anti-pneumococcal serotype-specific opsonophagocytic activity*	MOPA	PPD	dil^{-1}
Serum	Anti-CP1 Ab	ELISA	CMIC	µg/mL

Ab: antibody; CP1: ~~carrier~~ ***fusion*** protein; IgG: immunoglobulin G; ~~OPA: opsonophagocytic activity;~~ MOPA: multiplexed opsonophagocytic assay; dil-1: reciprocal of the dilution; µg = microgram; mL = milliliter; ***ng: nanogram,*** ELISA, enzyme-linked immunosorbent assay.

* Testing for each of the 24 pneumococcal AFX3772 vaccine serotypes (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F).

** Affinivax/GSK delegates testing to other external laboratories designated by the Sponsor.

Immunogenicity of AFX3772 ~~against~~ ***in terms of antibodies to*** the CP1 ~~carrier~~ ***fusion*** protein will be measured using MSD format.

13 References

During the Clinic Activities. CDC. Accessed 21 March 2023. https://www.cdc.gov/vaccines/hcp/admin/mass-clinic-activities/during-clinic-activities.html

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Summary of changes table of previous amendments:

Amendment 1 *(22 November 2022)*

Section # and *Name* ~~Title~~	Description of change	Brief rationale
Title page	Update Sponsor's name and address	To reflect GSK as the trial sponsor
Investigator's Agreement	Agreement expanded to cover GSK required clauses and included a note on acquisition.	To reflect GSK's standards and the acquisition of Affinivax by GSK Plc.
Synopsis Schema 4.1 Overall design 9.5 Sample size determination	Changes related to an increase in the sample size i.e., -total number of participants in the trial from 472 to 760; number of participants included in Part 2 of the trial from 352 to 640; -number of participants in Part 2 from 88 to 160 per dose level.	To increase the precision of the immunogenicity results.
9.3.2.1 Primary Vaccination Series Assessment 9.3.2.2 Booster Vaccination Assessment 9.4.1 Primary Vaccination Series Assessment 9.4.2 Booster Vaccination Assessment 9.5 Sample Size Determination	Updated the analyses sections to include interim analyses for: - 472 participants Post dose 3 - All 760 participants Post Dose 3 - 472 participants Post Dose 4	To allow dose selection for Phase 3 clinical trial and launch of preparatory activities including material planning.
1.1 Synopsis 1.2 Schema 9.5 Sample size determination	Changed the number of evaluable participants in each treatment arm from 100 to 181	To increase the precision of the immunogenicity results
1.1 Synopsis 4.1.1 Dose escalation and stopping criteria	Changed the wording to read that the DMC makes recommendation to the Sponsor	To reflect that the DMC is an advisory body that makes recommendations to the Sponsor, and the Sponsor makes the decisions regarding continuation of the trial.
6.3.3 Blinding 6.7 Treatment of Overdose 7.1 Discontinuation of individual participants from study intervention 7.2 Discontinuation of individual participants from the study 7.3 Lost to follow-up 8.1 Study assessments and procedures	Added GSK mandatory wording to these sections.	To align with GSK standards
6.8.1 Permitted Concomitant Therapy	Added COVID-19 vaccine to list of concomitant vaccinations	To reflect recent approval of SARS-COV-2 (COVID-19) vaccines for use in infants 6 months of age and older.
8.6 Total Amount of Blood Table 6	Changed the volume of blood collected from 5 mL to 4 mL and the total volume collected from 20 mL to 16 mL.	To reflect the maximum volume (4 mL) of blood possible to collect using the tubes provided for this study.

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Overall Rationale for Amendment 1

The purpose of this amendment is to (i) change the study Sponsor following acquisition of Affinivax by GSK Plc and introduce GSK's mandatory wording, as applicable, for alignment with GSK standards; (ii) increase the size of the study population in order to increase the precision of the immunogenicity results; (iii) introduce interim analyses related to initial and new sample sizes; and (iv) make minor editorial changes throughout the document for consistency and clarity.

Detailed description of Protocol Amendment 1

Amended text has been included in ***bold italics*** and deleted text in strikethrough in the following sections:

Title page

Sponsor Name: ~~Affinivax, Inc.~~ ***GSK Biologicals SA***

~~Legal Registered~~ Address:

~~301 Binney Street~~

~~Cambridge, MA 02142 USA~~

Rue de l'Institut, 89

1330 Rixensart, Belgium

Sponsor Signatory:

~~Kara Bickham MD Chief Medical Officer Affinivax, Inc.~~

Dorota Borys, MD

Clinical Project Lead

Investigator agreement:

A note was added to notify Investigator of the acquisition of Affinivax by GSK

Note: On 15 August 2022, Affinivax Inc. was acquired by GSK Plc., making Affinivax an affiliate of the GSK group of companies. Therefore, the name of the sponsor (authorized by the FDA as IND holder) of the clinical trial referred to in this protocol has been updated to GSK Biologicals SA.

New mandatory wording (as per the GSK template) was added in the Investigator Agreement:

I agree:

To conduct the study in compliance with this protocol, any future protocol amendments, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GSK.

To assume responsibility for the proper conduct of the study at this site.

That I will comply with the terms of the site agreement.

To comply with local bio-safety legislation.

To ensure that all persons assisting me with the study are adequately informed about the GSK study intervention and other study-related duties and functions as described in the protocol.

To supervise any individual or party to whom I have delegated study-related duties and functions conducted at the study site.

To ensure that any individual or party to whom I have delegated study-related duties and functions conducted at the study site are qualified to perform those study-related duties and functions.

To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.

To ensure that no clinical samples (including serum samples) are retained on-site or elsewhere without the approval of GSK and the express physical or digital informed consent of the participant and/or the participant's LAR.

To perform no biological assays on the clinical samples other than those described in the protocol or its amendment(s).

To co-operate with representative(s) of GSK in the monitoring and data management processes of the study with respect to data entry and resolution of queries about the data.

To have control of all essential documents and records generated under my responsibility before, during, and after the study.

That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator(s)' ownership interest in the sponsor or the study intervention(s), and more generally about their financial ties with the sponsor. GSK will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence, I:

Agree to supply GSK with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).

Agree to promptly update this information if any relevant changes occur during the study and for 1 year following completion of the study.

Agree that GSK may disclose any information about such ownership interests and financial ties to regulatory authorities.

Agree to provide GSK with an updated Curriculum Vitae and all other documents required by regulatory agencies for this study.

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Section 1.1. Synopsis

Objectives, Endpoints and Estimands:

Objectives	Endpoints
Primary	
	Following any vaccination with AFX3772 or PCV13: • Solicited injection site AEs (tenderness, redness/erythema and swelling) from Day 1 through Day 7 postvaccination • Solicited systemic AEs (irritability, decrease of appetite, increased sleep, and decrease in sleep) from Day 1 through Day 7 • AEs Day 1 through Day 30 • SAEs through completion of study participation ***(6 months post last study vaccination)***
AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; PCV13: Prevnar 13® ;	

Brief Summary:

This is a Phase 2 clinical study to support the use of AFX3772 in healthy infants for the prevention of pneumococcal disease. The purpose of this study is to determine the safety, tolerability, and immunogenicity of 3 different dose levels of AFX3772 compared with PCV13. Approximately ~~472~~ ***760*** healthy infants approximately 2 months of age will be enrolled and receive 4 doses of study vaccine over 8 protocol-defined visits spanning a duration of approximately 18 to 21 months. Part 1 is the dose escalation, lead-in portion of the study in which 40 infants at each dose level will be randomized 3:1 in sequential cohorts of increasing doses of AFX3772 (Cohort 1: 1 µg; Cohort 2: 2 µg and Cohort 3:5 µg) or PCV13. Enrollment in Cohorts 2 and 3 will proceed following Data Monitoring Committee (DMC) review of cumulative safety and tolerability data through Day 7 from preceding cohorts. Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 1, up to ~~352~~ ***640*** infants will be enrolled and randomized equally to receive either PCV13 or AFX3772 at dose levels (1, 2 or 5 µg) approved for evaluation in Part 2. Vaccinations are scheduled at approximately 2, 4, and 6 months of age for the primary series and 12 months for the ~~toddler~~ ***booster*** dose. Subsequent immunogenicity assessments are scheduled at 30 days PD2, 30 days PD3, pre-dose 4 and 30 days PD4. ***Interim analyses of safety and immunogenicity 30 days PD3, 30 days PD4 and a final analysis of safety and immunogenicity are planned for the study.***

Number of Participants: Approximately ~~472~~ **760** healthy infants are planned to be enrolled in this study with a subset of 120 initially enrolled (Part 1) followed by the remaining ~~352~~ **640** participants (Part 2). Accounting for an 85% evaluable rate, such a sample size predicts approximately ~~100~~ **181** evaluable participants in each treatment arm.

Safety Review Team: Yes

Executive Oversight Committee: No

Steering Committee: No

Clinical Adjudication Committee: No

The DMC ~~can~~ provide***s*** recommendations to ensure participant safety and ethical integrity throughout the study. The decision to progress to a higher AFX3772 dose level in Part 1 will be determined by the ***Sponsor*** ~~DMC~~, based on ***the*** safety and tolerability data available at the time ***and the DMC's recommendation***.

~~Affinivax~~ ***The Sponsor*** will take the DMC recommendation into account when deciding how to proceed to Part 2.

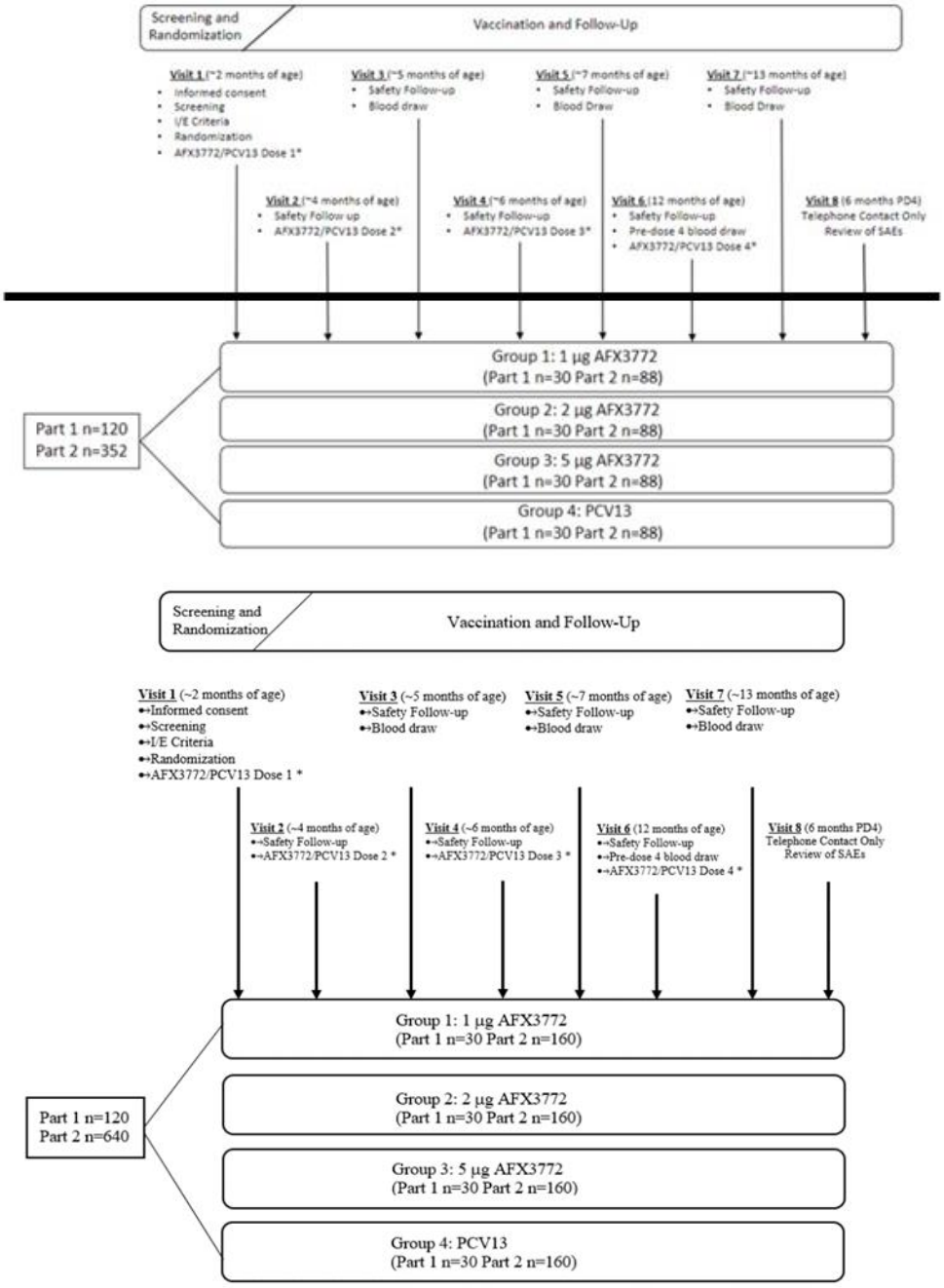
Figure 1 Footnote:

DMC: Data Monitoring Committee; PCV13: Prevnar 13®

†DMC reviews safety data through Day 7 for each cohort (approximately 40 participants) after all participants have completed eDiary safety assessments through Day 7 postvaccination. For Cohorts 2 and 3, DMC will also review all safety data available for prior cohorts.

Subsequent cohorts start after DMC ~~approval~~ ***recommendation*** to dose escalate.

Figure 2 Study Schema Part 1 & Part 2 was replaced to reflect the new number of the participants.



DMC: Data Monitoring Committee; PCV13: Prevnar 13®

Section 1.3. Schedule of Assessments

Table 1: Schedule of Assessments *(SoA)* for Part 1 and Part 2

Assessments	Intervention										Follow-up		Comments
Visit Number	1	TC1	2	TC2	3[1]	4	TC3	5[1]	6	TC4	7[1]	8	
Scheduled Time	***Dose 1*** *(*~2 months of age*)* ~~Dose 1~~	***1-week post Dose 1*** ~~Day 8 after Dose 1~~	***Dose 2*** *(*~4 months of age*)* ~~Dose 2~~	***1 week post Dose 2*** ~~Day 8 after Dose 2~~	***1 month post Dose 2*** *(*~5 months of age*)*	***Dose 3*** (~6 months of age*)* ~~Dose 3~~	***1 week post Dose 3*** (~~Day 8 after Dose 3~~	***1 month post Dose 3*** (~7 months of age)	***Dose 4*** *(*~12 months of age*)* ~~Dose 4~~	***1 week post Dose 4*** ~~Day 8 after Dose~~ 4	***1 month post Dose 4*** ~~1 month after Dose 4~~	~6-months ~~after Dose 4~~ ***post Dose 4***	
Visit Window		49 to 70 days after ~~Visit~~ ***Dose*** 1				49 to 70 days after ~~Visit~~ ***Dose*** 2		Day 28 to Day 42 after ~~Visit~~ ***Dose*** 3					

Table 1 line 7: Moved ***Randomization*** from line 7 to line 13 in the SoA table prior to AFX3772 or PCV13 Administration (Blinded)

AE: adverse event; CP1: carrier protein 1; IgG: immunoglobulin G; IRT: interactive response technology; LAR: legally acceptable representative; PCV13:
Prevnar 13®; PD: post-dose: SAE: serious adverse event; TC: telephone contact
†To be performed prior to administration of study vaccine by the investigator or a medically qualified designee.

1. Refer to Section 10.6 Appendix 6: Clinical Study Continuity for alternative visit procedures allowed at the noted visits in the case of a crisis.
2. For calculating the visit windows, the day of vaccination is considered Day 1. To calculate visit windows for subsequent vaccinations, confirm ~~subject~~ ***participant*** date of birth and ensure the age of the ~~subject~~ ***participant*** will fall within the appropriate age range for each study visit.

Section 2. Introduction (Amended 22 November 2022)

Affinivax ~~is developing~~ ***has developed*** an investigational 24-valent pneumococcal vaccine (referred to as AFX3772) for the prevention of pneumococcal disease caused by the serotypes contained in the vaccine. AFX3772 contains 24 serotypes of Streptococcus pneumoniae and a unique carrier protein (CP1) created by the fusion of 2 pneumococcal proteins (sp1500 and sp0785), which has the potential to induce T- and B-cell responses and provide extended protection. This novel ***investigational*** pneumococcal vaccine uses the CCI [redacted] (MAPS) platform **[*Zhang, 2013*]** containing each of the 13 pneumococcal capsular polysaccharides (PS) in the pneumococcal conjugate vaccine, Prevnar13® (PCV13).

Section 2.1 Study Rationale

AFX3772-003 will evaluate the safety, tolerability and immunogenicity of 3 AFX3772 dose levels (1, 2 and 5 µg) administered as a 3+1 regimen (at 2, 4 and 6 months ***of age*** for the primary series and 12 months ***of age*** for the ~~toddler~~ ***booster*** dose). Data in adults and toddlers demonstrate***d*** that AFX3772 has an acceptable safety and tolerability profile and generates antibody responses to all 24 serotypes contained in the vaccine **[*Chichili, 2022*]**.

Section 2.2 Background

Prevnar 13® has been approved for children 6 weeks to 17 years of age for the prevention of IPD caused by the 13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) contained in the vaccine and for the prevention of acute otitis media in children up to 6 years of age for the 7 serotypes in Prevnar® (4, 6B, 9V, 14, 18C, 19F, 23F).

Widespread use of PCVs has reduced the burden of pneumococcal disease due to the serotypes contained in the vaccines in vaccinated children, as well as unvaccinated individuals through herd protection [Pilishvili et al. 2010; Feikin et al. 2013; CDC, 2008; Moore et al. 2015]. However, an increase in the burden of IPD caused by serotypes not contained in currently available vaccines has been observed ***in settings where PCV vaccines were routinely implemented***.

AFX3772 is a 24-valent pneumococcal vaccine (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F) being developed for the prevention of IPD in children 6 weeks to 17 years of age and adults 18 years of age and older. AFX3772 uses a novel MAPS platform in which high-affinity, non-covalent bonding between biotin and rhizavidin links pneumococcal PS to CP-1 made up of ***the fusion of*** 2 conserved pneumococcal proteins (SP1500 and SP0785) ***[AFX3772 Investigator Brochure, 2022]***.

AFX3772 aims to provide broad and durable protection by enabling both B- and T-cell responses to pneumococcal PS and proteins ***[Lu, 2018].***

Section 2.2.1. Information on Other Study-related Therapy

Refer to the approved labeling for detailed background information on Prevnar 13®

Prevnar 13® (PCV13) contains 13 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) and is approved in the United States (US) and elsewhere for use in infants 6 weeks of age and older. The US Advisory Committee on Immunization Practices (ACIP) recommends PCV13 be given to infants as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a ~~toddler~~ ***booster*** dose given at 12 months of age. Prevnar 13® can be given concomitantly with other recommended childhood vaccines.

Table 2 Summary of Clinically Significant Risks and Mitigation Strategies

Clinically Significant Risks	Mitigation Strategies
Study Intervention	
• No clinically significant findings following receipt of AFX3772 (1, 2, or 5 µg) have been observed in previous studies in adults and toddlers. • Based on data in adults and toddlers, AFX3772 is predicted to have a safety and immunogenicity profile comparable to PCV13 for the 13 shared serotypes while providing additional coverage for the 11 unique serotypes in AFX3772	• An independent DMC will review all available data at least every 6 months throughout the study to ensure the benefit/risk profile continues to support the study of AFX3772 in infants. • 30-minute observation period following each dose, plus daily safety/tolerability monitoring for 7 days and collection of adverse events for 30 days following each vaccination. • To mitigate the impact of potential lack of immunogenicity in AFX3772 recipients, immunogenicity data will be evaluated for all participants following completion of the primary series. Rescue vaccination with PCV13 will be offered within 3 weeks of site notification. ***Summary of participants of Part 1 meeting criteria for rescue vaccination will be evaluated by DMC.*** • Potential for broader serotype coverage from pneumococcal disease.

PCV13: Prevnar 13 ®

Section 3. Objectives, Endpoints, and Estimands

Objectives	Endpoints
Primary	
	Following any vaccination with AFX3772 or PCV13: • Solicited injection site AEs (tenderness, redness/erythema and swelling) from Day 1 through Day 7 postvaccination • Solicited systemic AEs (irritability, decrease of appetite, increased sleep, and decrease in sleep) from Day 1 through Day 7 • AEs Day 1 through Day 30 • SAEs through completion of study participation ***(6 months post last study vaccination)***
AE: adverse event; GMC: geometric mean concentration; IgG: immunoglobulin G; PCV13: Prevnar 13®	

Primary Estimand

Treatment: A total of 4 vaccinations 0.5 mL each of either AFX3772 (1, 2 or 5µg) or PCV13 administered as 4 separate vaccinations with a 3-dose primary series at approximately 2 (no earlier than 6 weeks of age), 4 and 6 months of age + 1 ~~toddler~~ ***booster*** vaccination at 12 months of age.

Secondary Estimand(s)

Treatment:

A total of 4 vaccinations 0.5 mL each of either AFX3772 (1, 2 or 5µg) or PCV13 administered as 4 separate vaccinations with a 3-dose primary series at approximately 2 (no earlier than 6 weeks of age), 4 and 6 months of age + 1 ~~toddler~~ ***booster*** vaccination at 12 months of age**.**

Population level summary: Summaries will be provided after the second vaccination, after the third vaccination and pre- and post-***booster*** ~~toddler~~ vaccination. By-treatment summaries for each of the endpoints listed above are:

The ***antibody*** GMCs and the corresponding 2-sided 95% CIs will be calculated using logarithmically transformed values and the Student t distribution for the CIs.

Section 4.1. Overall Design

This study will be conducted at approximately ~~35 40~~ ***65-75*** study sites in the US ***including Puerto Rico.*** Approximately ***760*** ~~472~~ healthy infants are planned to be enrolled in this study with an initial enrollment of 120 infants in Part 1, followed by up to ***640*** ~~352~~ infants in Part 2.

Vaccinations with either AFX3772 (1, 2 or 5 µg) or PCV13 are scheduled at approximately 2, 4, and 6 months of age for the primary series and 12 months for the ~~toddler~~ ***booster*** dose.

The follow-up visit will occur 6 months following the ~~toddler~~ ***booster*** dose.

Part 1 Dose Escalation

Following completion of DMC review of safety and tolerability data for the cohorts enrolled in Part 1, approximately ***160*** ~~88~~ participants per dose level (approximately ***640*** ~~352~~ infants if all dose levels are evaluated in Part 2) will be enrolled and randomized equally to receive either PCV13 or AFX3772 at dose levels (1, 2 or 5 µg) approved for Part 2.

~~Affinivax~~ ***The Sponsor*** will take the DMC recommendation into account when deciding how to proceed to Part 2.

Part 2 Full Enrollment

Following DMC review and agreement to proceed to Part 2, up to ***640*** ~~352~~ participants (~***160*** ~~88~~ per dose level) will be randomized equally to receive approved AFX3772 dose levels (1 µg, 2 µg or 5 µg) or PCV13 (Figure 2).

If the number of dose groups is reduced, the number of participants enrolled in the study overall will be reduced, with the number of participants in each dose group remaining at approximately ***160*** ~~88~~.

Table 4. Number of Participants by Vaccination Group for Part 2

AFX3772 1 µg	AFX3772 2 µg	AFX3772 5 µg	Comparator: PCV13
160 ~~88~~	***160*** ~~88~~	***160*** ~~88~~	***160*** ~~88~~

PCV13: Prevnar 13®;

Section 4.1.1. Dose Escalation and Stopping Criteria

After all vaccinated participants in a Part 1 cohort have completed study safety procedures through Day 7 postvaccination, the DMC will ~~determine~~ ***recommend*** whether to initiate enrollment in the next cohort based on the review of all available safety data from the current and preceding cohorts. Once the Day 7 safety assessments in the final cohort ***(Cohort 3)*** of Part 1 are complete, the DMC will review all available safety data from Cohorts 1, 2 and 3 to ~~determine~~ ***recommend*** if the study ~~will~~ ***should*** proceed into Part 2 and, if so, which AFX3772 dose level(s) will be recommended for continued evaluation in Part 2.

Depending on the safety data observed in the study, the DMC will ~~decide~~ ***recommend*** whether to proceed with or stop dose escalation. ***The Sponsor will make the final decision based on all the data available and the DMC's recommendation(s).*** A stopping rule is met if any of the following rules occur after administration of AFX3772.

Dose escalation may proceed unless the following applies to participant(s) receiving AFX3772:

~~Death in any participant vaccinated with AFX3772 that is possibly related to AFX3772 or for which there is no alternative, plausible, attributable cause.~~

≥ 1 participant(s) experience an SAE, ***including death***, possibly related to AFX3772, or for which there is no alternative, plausible, attributable cause.

> Note: Any events that have been previously reported and deemed related to licensed ~~PCV~~ PCV***s*** or other vaccines administered concomitantly to participants will not be considered to meet the halting criteria.

The DMC will review the event(s) and may decide to unblind the participant(s) treatment assignment. After review of all information, the DMC will ~~determine~~ ***recommend*** whether to resume the study and/or dose escalation. If the DMC does not recommend evaluating all 3 AFX3772 dose levels in Part 2, an alternative approach, using a reduced number of AFX3772 dose levels, can be used. ~~Affinivax~~ ***The Sponsor*** will take the DMC recommendation into account when deciding how to proceed to Part 2.

Section 4.2. Scientific Rationale for Study Design

The proposed safety, tolerability (including reactogenicity) and immunological assessments (i.e., immunological endpoints [0.35 µg/mL threshold value, IgG GMCs and opsonophagocytic activity (OPA) geometric mean titers~~])~~ ***(GMTs)***]) are also consistent with standard PCV pediatric clinical development endpoints

The study will implement a 3+1 pneumococcal vaccination schedule for infants ~~and toddlers~~ as recommended by the US ACIP. In order to evaluate other vaccination schedules (i.e., 2 + 1 schedule used in Europe and elsewhere), immunogenicity will also be assessed after the second vaccination. A dose escalation approach is being implemented in Part 1 to ensure adequate safety review following the first administration of each dose level prior to initiating enrollment ~~at~~ ***into*** the next dose level. Once dose escalation has been completed ***in Part 1*** and DMC ~~agreement obtained~~ ***recommendation has been given and followed by the Sponsor's decision to continue the study***, enrollment in Part 2 will be initiated.

Section 4.2.1.1. Rationale for Immunogenicity Endpoints

Blood sampling at pre-dose 4 and PD4 will be used to evaluate the ~~anamnestic antibody responses and the persistence of protective immunity.~~ ***persistence of protective immunity and the anamnestic antibody responses.***

Section 4.2.2. Rationale for Use of a Comparator

Prevnar 13® will be the active comparator for use in this trial and is currently the most widely recommended vaccine for the prevention of pneumococcal disease in infants in many countries worldwide, including the US.

Refer to approved labeling for ~~detailed background information~~ ***more details*** on Prevnar 13®.

Section 4.3. Justification for Dose

AFX3772 has been evaluated in adults in a dose escalation, dose finding study (3772-CL-1001). AFX3772 was ~~highly~~ immunogenic and induced both serotype-specific IgG responses and OPA antibody titers to all 24 serotypes included in the vaccine, and at all 3 dose levels (1, 2 and 5 mg) tested in healthy adults aged 18 to 64 years and 65 to 85 years ***(3772-CL-1001).***

The same doses (1, 2 and 5 µg) were evaluated in toddlers aged 12 to 15 months of age (study 3772-CL- 2001) and were demonstrated to have an acceptable safety/tolerability profile and were immunogenic for all 24 serotypes contained in the vaccine (***3772-CL-2001).***

Section 4.4. End of Study Definition

A participant is considered to have completed the study if the participant has completed all periods of the study including the last study-related telephone call or visit or the sponsor receives the last laboratory result.

For purposes of analysis and reporting, the end of study occurs when the Sponsor receives the last laboratory result ~~or at the time of final contact with the last participant. whichever comes last.~~

Section 5. Study Population

Sentence moved from end of paragraph to beginning of the paragraph:

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

~~Prospective approval of protocol deviations to eligibility criteria (also known as protocol waivers or exemptions) is not permitted.~~

Section 5.3 Screen Failures

Screen failures are defined as participants whose LAR provides consent to participate in the clinical study but who are not subsequently randomized ***and vaccinated***.

For each study participant, a ~~A~~ unique ~~subject~~ identifier (Subject ID) is assigned at Screening when the site completes the Enrollment CRF page. The eCRF Completion Guidelines will describe what data points need to be entered on the Enrollment CRF page. If screening criteria ~~is~~ ***are*** not met, the participant will be considered a screen failure.

Section 6.1 Study Intervention(s) Administered

Table 5 Study Intervention(s) Administered

Intervention Label	AFX3772	Prevnar 13®
Intervention Description	AFX3772 is administered as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and ~~toddler~~ *a* ***booster*** dose given at 12 months of age.	PCV13 is administered as a 4-dose regimen, with 3 doses given at 2, 4 and 6 months of age as part of a primary series and a ~~toddler~~ ***booster*** dose given at 12 months of age.
Use	~~Experimental~~ ***Investigational*** vaccine	Comparator vaccine

PCV13: Prevnar 13®;

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Section 6.3.1. Intervention Assignment

In Part 1, 40 participants per cohort will be enrolled and randomized 3:1 to receive AFX3772 (1, 2 or 5 µg) or PCV13. If continuation into Part 2 is recommended, approximately ~~352~~ ***640*** additional participants will be randomized 1:1:1:1 (~~88~~ ***160*** per group) to receive ***either*** 1 of up to 3 AFX3772 dose levels (1, 2 or 5 µg) or PCV13 as agreed to for evaluation in Part 2.

Section 6.3.3. Blinding

A double-blinded technique will be used. The participant's LAR, the investigator***,*** and all site personnel responsible for the clinical evaluation of participants will remain blinded until study completion.

The laboratory in charge of the sample testing will be blinded to the intervention assignment. Codes will be used to link the participant and study (without any link to the intervention attributed to the participant) to each sample.

Because AFX3772 and Prevnar 13® have different appearances, a member of the study staff (and back- up) will be unblinded for purposes of receiving, maintaining, preparing***,*** and administering study vaccines.

Breaking the Treatment Code by the Sponsor

The Sponsor and DMC may break the study vaccine assignment code for participants who experience a suspected unexpected serious adverse reaction (SUSAR), in order to determine if the individual case or a group of cases requires expedited regulatory reporting. ***In addition, the Sponsor's safety staff may unblind the intervention assignment for any participant with an SAE that is fatal or life-threatening***.

Section 6.7. Treatment of Overdose

There is no specific information available on the treatment of overdose. ~~In the event of suspected AFX3772 overdose (2 doses within 24 hours), the participant should be monitored and receive supportive care as needed. Decisions regarding dose interruption or modification will be made by the investigator in consultation with the Sponsor.~~

In the event of an overdose, the investigator/treating physician should:

Evaluate the participant to determine, in consultation with the medical monitor, if possible, whether study intervention should be interrupted or whether the next dose should be reduced.

Closely monitor the participant for any AE/SAE.

Document the quantity of the excess dose.

Section 6.8.1 Permitted Concomitant Therapy

Routine infant vaccinations licensed for use in the US are permitted in this study with the exception of PCVs. The schedule for routine childhood vaccines given outside the protocol is at the investigator's discretion. During influenza season, it is anticipated that participants 6 months of age and older may be given an influenza vaccine. Influenza vaccine should be administered either 7 days prior to or 15 days after the administration of the study vaccine. ***SARS-CoV-2 (COVID-19) vaccine is permitted if administered either 7 days prior to or 15 days after administration of the study vaccine.*** ~~Other non-live~~ ***Non-live*** vaccines not given concomitantly (i.e., on the same day as study vaccine) should be given at least 7 days prior or at least 15 days after administration of study vaccine.

To avoid any confounding local reactogenicity results, non-trial injectable vaccines ~~should~~ ***must*** not be administered in the same limb as trial vaccine.

Documentation of limb used for the administration of trial vaccine should be recorded ~~on the appropriate eCRF,~~ ***in the participant's medical record*** and the LAR should be instructed on the appropriate limb to monitor for AEs. Injection site AEs for concomitantly administered non-study vaccines will not be collected.

Section 6.8.3. Rescue Vaccination

The discontinuation ***of study intervention*** date should be the date the LAR was notified of the participant meeting rescue criteria. Adverse events or changes to medical history occurring during the AE and SAE reporting period following Dose 3 will be collected ***for up to 6 months*** **for** all infants meeting rescue criteria.

Section 7.1. Discontinuation of Individual Participant(s) From Study Intervention

GSK mandatory wording added to Section 7.1

If rescue vaccination is needed, the participants will be discontinued from study intervention but will be asked to continue to participate in safety follow-up assessments ***for up to 6 months PD3.***

The primary reason for premature discontinuation of the study intervention will be documented in the eCRF based on the list below:

- ***Adverse Event***
- ***Rescue Medication***
- ***Protocol Deviation Affecting Efficacy and/or Safety***
- ***Investigator Decision***
- ***Lost to Follow-up***

- ***Withdrawal of Consent by Parent/Guardian***
- ***Death***
- ***Other (specify)***

Section 7.2. Discontinuation of Individual Participant(s) from Study

GSK mandatory wording added to Section 7.2

Investigators will attempt to contact participants who do not return for scheduled visits or follow-up.

The participant will be permanently discontinued from the study intervention and the study at that time.

All data and samples collected up to and including the date of withdrawal of/last contact with the participant will be included in the study analyses. If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, the participant may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

The primary reason for participant discontinuation/ withdrawal from the study will be documented in the eCRF based on the list below:

- ***Adverse Event***
- ***Rescue Medication***
- ***Protocol Deviation Affecting Efficacy and/or Safety***
- ***Investigator Decision***
- ***Withdrawal of Consent by Parent/Guardian***
- ***Death***
- ***Other (specify)***

Participants who are withdrawn from the study because of AEs/SAEs must be clearly distinguished from participants who are withdrawn for other reasons. Investigator will follow participants who are withdrawn from the study due to an AE/SAE until the event is resolved (see Section 8.4.3).

Section 7.3. Lost to Follow-up

GSK mandatory wording added to Section 7.3

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

The site must attempt to contact the participant and reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study.

Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant. These contact attempts should be documented in the participant's medical record.

Section 8. Study Assessments and Procedures

GSK mandatory wording added to Section 8

Participants who have signed informed consent but are not eligible to proceed should be recorded in the eCRF with a status of 'screen failure'

Procedures conducted as part of the participant's routine clinical management and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.

In the event of a significant study-continuity issue (e.g., caused by a pandemic), alternate strategies for participant visits, assessments, study intervention distribution and monitoring may be implemented by the sponsor or the investigator, as per local health authority/ethics requirements.

Section 8.1. Administrative and general Procedures

Immunogenicity results that could unblind the study will not be reported to ***study*** ~~investigative~~ sites or other blinded personnel until the study has been unblinded.

Study procedures and their timing are summarized in the schedule of assessments ~~(Table 1Table 5 Study Intervention(s) Administered)~~ (Table 1).

All screening evaluations must be completed and reviewed by the investigator or qualified delegate to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable. ***Participants who have signed informed consent but are not eligible to proceed should be recorded in the eCRF with a status of 'screen failure'.***

Procedures conducted as part of the participant's routine clinical management and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.

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In the event of a significant study-continuity issue (e.g., caused by a pandemic), alternate strategies for participant visits, assessments, study intervention distribution and monitoring may be implemented by the sponsor or the investigator, as per local health authority/ethics requirements.

Section 8.2. Immunogenicity Assessments

Planned timepoints for all immunogenicity assessments are provided in the SoA.

The following measures will be used to characterize the immunological response in infants following administration of either AFX3772 or PCV13. ~~The immunogenicity assessments described below are accepted surrogates of efficacy for the development of next generation pneumococcal vaccines.~~

~~Summaries will be provided for each dose level and compared to PCV13.~~

~~The immunogenicity assessments described below are accepted surrogates of efficacy for the development of next generation pneumococcal vaccines.~~

~~Summaries will be provided for each dose level and compared to PCV13.~~

Considering the mechanism of action, AFX3772 is expected to induce an immune response to pneumococcal PS and ***to*** the carrier protein, CP1.Blood samples to assess immunogenicity will be collected as indicated in the schedule of assessments ~~(Table 1Table 5Study Intervention(s) Administered)~~ (Table 1).

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Section 8.3. Safety Assessments

~~Safety procedures and their timing are summarized in the schedule of assessments (Table 1)~~. ***Planned timepoints for all safety assessments are provided in the SoA (Table 1).***

Section 8.4. Adverse Events, Serious Adverse Events and Other Safety Reporting

The investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study vaccine and comparator, or that caused the participant to discontinue the study vaccine or comparator and/or study (see Appendix 3: AEs and SAEs: Definitions and Procedures for Recording,

Evaluating, Follow-up, and Reporting). ***This includes events reported by the participant's legally authorized representative.***

Section 8.4.1. Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from the ICF consent/Dose 1 until Visit 8 (6 months Post dose 4) at the time points specified in the SoA.

Section 8.4.3. Follow-up of AEs and SAEs

~~The investigator should proactively follow all reported AEs/SAEs~~ ***After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and AEs will be followed*** until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3).

8.4.4. Regulatory Reporting Requirements for SAEs

3. ***For SAEs, the investigator must always provide an assessment of causality at the time of the initial report, as defined in the Section 10.3.4.***
4. ***An investigator who receives an investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.***

~~3.~~ ***5.*** Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and Sponsor policy and forwarded to investigators as necessary.

~~4.~~ ***6.*** An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and file it along with the IB and will notify the IRB, if appropriate according to local requirements.

Section 8.6. Total Amount of Blood

Blood should be drawn at Visits 3, 5, 6 and 7 (see schedule of assessments [~~Table 1 Table 5 Study Intervention(s) Administered~~] ***Table 1***]). Repeat and additional blood samples may be taken if required; however, the maximum blood volume drawn from the participant will not exceed 3 mL per kg in an 8-week period. ***Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.***

It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol.

Table 6 Blood Sampling

Assay Tested	**Number** of Samples	**Sample** Volume (mL)	**Total** Volume (mL)
Pneumococcal anti-capsular PS IgG, ***Pneumococcal*** OPA and anti-CP1 IgG	4	~~5~~ ***4.0***	~~20~~ ***16.0***
Total			~~20~~ ***16.0***

CP1: carrier protein; IgG: immunoglobulin G; OPA: opsonophagocytic activity
Note: the maximum blood volume drawn from the participant will not exceed 3 mL per kg in an 8-week period

Section 9.2. Analysis Set

Table footnote updated:

PPS: Per Protocol Set; PCV13: Prevnar 13®; SAF: Safety Analysis Set

Section 9.3.1. General Considerations

Demographics and baseline characteristics (age, sex, race, ethnicity, ***and*** weight ~~and height~~) will be summarized by treatment group for AFX3772 (1 µg, 2 µg or 5 µg) and immunization group (AFX3772 or PCV13).

Participants who are administered rescue vaccination will be encouraged to remain in the study for safety follow-up.

Section 9.3.2.1. Primary Vaccination Series ~~Assessments~~ *Assessments*

~~The Sponsor will remain blinded until after the data through 30 days after the third vaccination for all participants has been collected and cleaned~~. In the event there is an AE that is of concern to the DMC, the treatment assignment may be requested for that participant. The independent statistician will provide the treatment assignment and the unblinding will be documented. All other personnel involved with the study will remain blinded to vaccination assignment until the study has completed.

The primary data analysis for safety (through 30 days after the primary series) and immunogenicity will occur after the last participant completes the primary series. ***See Section 9.4.1 for full details of analyses***. The purpose is to allow proceeding with dose selection for Phase 3 clinical trial material planning. Reassessment of the dose will be confirmed once the ~~toddler~~ ***booster*** vaccination assessment is available.

Section 9.3.2.2. ~~Toddler~~ *Booster* Vaccination ~~Assessment:~~ Assessments

A second ~~set of~~ data analysis (through 30 days after the last participant has received the ~~toddler~~ ***booster*** vaccination) for safety and immunogenicity will occur once all data have been collected and cleaned. ***See Section 9.4.2 for full details of analyses.***

Section 9.3.2.3. Final Safety Assessment

The final safety assessment (SAEs) will be after the last participant completes the study, approximately 6 months after the last participant has received the ~~toddler~~ ***booster*** vaccination. ***See Section 9.4.3 for full details of analysis.***

The primary endpoints for this Phase 2 study address safety and will be assessed in participants who have received at least 1 vaccination. Summaries by treatment for each vaccination and overall will be summarized by n and percent.

The percentage of participants with any local AE, any systemic AE, and each local or systemic AE will be summarized with n, percent, and the 95% CI. Summaries will be provided for each vaccination in the primary series, for the ~~toddler~~ ***booster*** vaccination, for the primary series, and for the 4-dose series. The solicited reactions will be summarized by intensity, onset day and duration for each treatment group.

Section 9.3.3. Secondary Endpoint(s)/Estimand(s) Analysis

Key secondary endpoints for this Phase 2 study are assessments of immunogenicity 30 days after the second and third dose of the primary, infant 3-dose series, and before and 30 days after the ~~toddler~~ ***booster*** vaccination with either AFX3772 or PCV13.

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Section 9.3.5.1. Adverse Events

AEs will be collected from Day 1 to ***Day*** 30 following each vaccination. A vaccine-related AE is defined as any AE with a causal relationship assessed as "yes" by the investigator. AEs will be coded using the latest version of MedDRA. ***SAEs, including deaths, will be collected throughout the duration of an individual's study participation.***

Section 9.3.5.2 Body Temperature

Descriptive statistics for temperature will be tabulated by treatment group and scheduled ~~time point~~ ***timepoint***. Summaries will include n, mean, median, standard deviation, minimum and maximum for the temperature collected at each scheduled time point and the change from baseline.

Section 9.3.5.5.1 Immunoglobulin G Concentration

The IgG concentration for each serotype will be analyzed for dose response. An ANOVA will be conducted on the IgG concentration with AFX3772 dose as the factor of interest. Paired comparisons between each for the 3 AFX3772 dose levels will be provided for the concentrations after the primary, infant 3-dose series and after the ~~toddler~~ ***booster*** vaccination. There will be no adjustments to control for the family-wise error rate for 3 comparisons for each of the 24 serotypes in this Phase 2 study.

Dose response ***post-booster*** will also be evaluated for the percentage of participants with at least a 4-fold rise from baseline in IgG for each serotype. A Cochran Armitage test for trend will be applied to the data.

Section 9.4. Interim ~~Analysis~~ *Analyses*

A total of 5 analyses will be performed in the study: 2 primary vaccination series assessments, 2 booster vaccination assessments and a final safety assessment.

Section 9.4.1. Primary Vaccination Series ~~Assessment~~ *Assessments*

~~The Sponsor will remain blinded until after the data through 30 days after the third vaccination for all participants has been collected and cleaned. The primary data analysis (through 30 days after the primary series) for safety and immunogenicity will occur after the last participant in the study completes the primary series.~~

The first analysis, an interim analysis of the primary vaccination series, will be performed when the first 472 participants have completed 30 days after the primary series and the immunogenicity results become available. The second analysis, i.e., final analysis of the primary vaccination series, will be performed when all 760 participants have completed 30 days after the primary vaccination series and the immunogenicity data become available.

Section 9.4.2. ~~Toddler~~ *Booster* Vaccination Assessment

A second data analysis for safety and immunogenicity will occur after the data through 30 days after the ~~toddler~~ ***booster*** vaccination for all participants has been collected and cleaned. The evaluation for potential rescue vaccination will be conducted as data are received.

The third analysis, an interim analysis of the booster vaccination, will be performed after the first 472 participants have completed 30 days after the booster vaccination and the immunogenicity results become available. The fourth analysis, i.e., final analysis of the booster vaccination, will be performed when all 760 participants have completed 30 days after the booster vaccination and the immunogenicity data become available.

Section 9.4.3. Final Safety Assessment

~~The final safety assessment (SAEs) will be after the last participant completes the study, approximately 6 months after the toddler ***booster*** vaccination in the last participant.~~

An analysis will be performed to report the final safety assessment (SAEs) after the last participant completes the study, approximately 6 months after the booster vaccination in the last participant.

~~**9.4.4. Analysis Following the Toddler Vaccination**~~

~~An analysis of the primary series by treatment groups will be conducted once all data are available and cleaned. A separate analysis will be conducted for the toddler dose to confirm dose for the Phase 3 program once all data are available and cleaned. The evaluation for potential rescue vaccination will be conducted as data are received.~~

~~**9.4.5. Analysis Following Visit 8**~~

~~The final safety assessment (SAEs) will be after the last participant completes the study, approximately 6 months after the last participant has received the toddler vaccination.~~

Section 9.5. Sample Size Determination

Approximately ***760*** ~~472~~ healthy infants are planned to be enrolled in this study with an initial 120 participants enrolled followed by the remaining ***640*** ~~352~~ participants. Of the initial 120 participants enrolled, 40 infants will be randomized (3:1) to receive either AFX3772 or PCV13 within each of 3 dose cohorts. Once safety has been evaluated for additional dose levels of AFX3772, up to ***640*** ~~352~~ participants will be randomized 1:1:1:1 to receive AFX3772 (1, 2 or 5 µg) or PCV13.

Accounting for an 85% evaluable rate, such a sample size is predicted to result in approximately ***181*** ~~00~~ evaluable participants in each treatment arm.

The secondary objective is to estimate the percentage of participants with an IgG antibody level ≥ 0.35 µg/mL after the primary series. A sample size of ~~~100~~ ***~181*** evaluable participants per treatment group provides reasonable estimates in this Phase 2 study of the immune response rate when the response rate ranges from 60% to 95%.

Section 10.1.5. Data Monitoring Committee Structure

Responsibilities of the DMC will include:

~~Determining~~ ***Making recommendations on*** all cohort dose escalation decisions during Part 1. After the last participant in a dose cohort completes Dose 1 and the 7-day eDiary reporting period and prior to initiating next dose level, the DMC will review safety data from the current cohort and the overall safety of all participants of the study

Section 10.1.8. Study and Site Start and Closure

The investigator shall promptly inform the participant's LAR and should ***ensure*** ~~assure~~ appropriate participant therapy and/or follow- up.

Section 10.2.1. Assessment of Intensity for Local AEs (Table 7 footnote)

a. Legally acceptable representatives of the ~~subjects~~ ***participants*** experiencing local reactions >7.0 cm are to be contacted by the study site. An unscheduled visit may be required.

Section 10.2.2. Grading of Signs of Systemic Reactogenicity

For the first 7 days following each vaccination, the participant's LAR will be asked to assess decreased appetite, increased sleep, irritability and decrease in sleep and to record the symptoms in the eDiary.

The symptoms will be assessed by the ~~subject's~~ ***participant's*** LAR as mild, moderate, or severe according to the grading scale in Table 8 below. The ~~subject's~~ ***participant's*** LAR will also be instructed to contact site staff if the ~~subject~~ ***participant*** experiences any possible Grade 4 prompted systemic event (~~i.e.~~ ***i.e.***, emergency room visit or hospitalization for severe decreased appetite, severe drowsiness/increased sleep, or severe irritability) within 7 days after vaccination. Study staff may also contact the ~~subject's~~ ***participant's*** LAR to obtain additional information on Grade 3 events entered into the eDiary.

Only an investigator is able to classify a ~~subject's~~ ***participant's*** systemic event as Grade 4, after physical examination of the ~~subject~~ ***participant*** or documentation from another medically qualified source ~~(e.g.~~ ***e.g.***, emergency room or hospital record) or telephone contact with the ~~subject's~~ ***participant's*** LAR. If a ~~subject~~ ***participant*** experiences a Grade 4 systemic event, the investigator must immediately notify the Sponsor.

Section 10.3.2. Definition of SAE

d. . Results in persistent or significant disability/incapacity 6.The term disability means a substantial disruption of a person's ability to conduct normal life functions. 7.This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza-like illness, and accidental trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

Section 10.3.3. Recording and Follow-Up of AE and/or SAE

Assessment of Causality
There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always ~~make~~ assesses causality for every event before the initial transmission of the SAE data to the Sponsor.

When ~~making an assessment~~ ***assessing*** causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a "reasonable possibility" that an (S)AE may have been caused by the study vaccine or comparator (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

Section 10.3.4. Reporting of SAEs

SAE Reporting via Paper Data Collection Tool
Investigator/sites are to enter all initial SAE information and follow-up information into the Electronic Data Collection (EDC) within 24 hours of awareness. The paper-based method is a secondary method of reporting that is to be used only when the EDC is unavailable. The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the Sponsor (or designee) by email immediately (within 24 hours of awareness ***if the EDC is not accessible***). The SAE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE data, as well as the investigator causality assessment including the explanation for the causality assessment. Syneos Health Inc. Email: safetyreporting@syneoshealth.com Fax: +1-877-464-7787

Section 10.3.5.3.

Occupational Exposure
If occupational exposure (e.g., inadvertent exposure to the study vaccine of study site personnel while preparing it for administration to the participant) to the study vaccine occurs, the investigator must forward the special situation worksheet to the Sponsor by fax or email immediately (within 24 hours of awareness~~)~~ ***[Section 10.3.4]).*** Any associated (S)AEs occurring to the individual associated with or resulting from the special situation are to be reported on the special situations worksheet.

Section 10.4. Appendix 4: List of Excluded Concomitant Medications

Participants must not use any of the following during the study:

SARS-CoV-2 (COVID-19) vaccine is permitted if administered either 7 days prior or 15 days after administration of the study vaccine.

10.5 Appendix 5: Clinical Laboratory Assessments

~~Laboratory tests will be performed according to the schedule of assessments (Table 1) and sent to a central laboratory for analysis.~~

~~Table 10 Clinical Laboratory Tests~~

~~Panel/Assessments~~	**~~Parameters to be Analyzed~~**
~~Immunogenicity~~	~~Anti-pneumococcal IgG and OPA Anti-CP1 IgG~~

~~CP1: carrier protein; IgG: immunoglobulin G; OPA: opsonophagocytic activity~~

Laboratory tests will be performed according to the schedule of assessments (Table 1) and sent for analysis to a laboratory designated by Affinivax.

Immunogenicity of AFX3772 will be evaluated by the different assays listed in Table 10.

Table 10 Clinical Laboratory Tests

System	*Component*	*Method*	*Testing Lab***	*Unit*
Serum	*Anti-pneumococcal AFX3772 serotype-specific IgG**	*dLIA*	*PPD*	*µg/mL*
Serum	*Anti-pneumococcal AFX3772 serotype-specific Ab**	*MOPA*	*PPD*	*dil^{-1}*
Serum	*Anti-CP1 Ab*	*ELISA*	*CMIC*	*µg/mL*

Ab: antibody; CP1: carrier protein; IgG: immunoglobulin G; OPA: opsonophagocytic activity; MOPA: multiplexed opsonophagocytic assay; dil^{-1}: reciprocal of the dilution; µg = microgram; mL = milliliter; ELISA, enzyme-linked immunosorbent assay.

*** Testing against each of the 24 pneumococcal AFX3772 vaccine serotypes (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F).**

**** Affinivax/GSK delegates testing to other external laboratories designated by the Sponsor**

Immunogenicity of AFX3772 will be evaluated by measuring serotype-specific anti-pneumococcal IgG antibody concentrations by 2 complementary multiplex (12-plex) qualified Luminex immunoassays (dLIAs) to cover simultaneously the 24 vaccine serotypes.

Immunogenicity of AFX3772 will be also evaluated by measuring serotype-specific anti-pneumococcal antibodies using multiplexed opsonophagocytic assay (MOPA) targeting the 24 AFX3772 vaccine serotypes. The MOPA measures the functional opsonic activity of antibodies against the pneumococcal serotypes that results in

bacteria killing by phagocytic cells in vitro. The assay has been developed for all 24 serotypes covered by AFX3772 vaccine.

Immunogenicity of AFX3772 against the CP1 carrier protein will be measured using MSD format.

10.9. Appendix 9: Abbreviations and Definitions

List of Abbreviations

Abbreviations	Description of abbreviations
(S)AE	serious adverse event or adverse event
ACIP	Advisory Committee on Immunization Practices
AE	adverse event
ANOVA	analysis of variance
AxMP	auxiliary medicinal product
CI	confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CP1	fusion protein 1
CRO	contract research organization
DMC	Data Monitoring Committee
eCRF	electronic case report form
EDC	electronic data collection
EoS	***End of Study***
GCP	Good Clinical Practice
GMC	Geometric Mean Concentration
HEENT	head, eyes, ears, nose and throat
HIV	human immunodeficiency virus
IB	Investigator Brochure
ICE	Intercurrent events
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IgG	immunoglobulin G

IMP	investigational medicinal product
IND	Investigational New Drug
IPD	invasive pneumococcal disease
IRB	Institutional Review Board
IRT	interactive response technology
ISN	international study number
LAR	legally acceptable representative
CCI [redacted]	
OPA	opsonophagocytic activity
PCV	pneumococcal conjugate vaccine
PCV13	Prevnar 13
PCV20	Prevnar 20
PD	post-dose
PI	Principal Investigator
PPS	Per Protocol Set
PS	polysaccharide
PV	Pharmacovigilance
QA	quality assurance
QC	quality control
QTL	quality tolerance limit
SAE	serious adverse event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SoC	Standard of Care
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reactions

TC	telephone call
US	United States
USM	urgent safety measure

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