Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals SA
Rue de l'Institut 89,
1330 Rixensart, Belgium

Primary Study vaccine (and number)	• GlaxoSmithKline (GSK) Biologicals' meningococcal group-B vaccine *Bexsero* (GSK3536829A). • Pfizer's pneumococcal polysaccharide conjugate vaccine (13-valent, adsorbed, *Prevnar13*). • ***Pfizer's pneumococcal polysaccharide conjugate vaccine (20-valent, adsorbed, Prevnar20).***
Other Study vaccines/products	• GSK Biologicals' diphtheria and tetanus toxoids and acellular pertussis adsorbed, hepatitis B (recombinant) and inactivated poliovirus vaccine (*Pediarix*, GSK217744). • GSK Biologicals' oral live attenuated human rotavirus (HRV) vaccine (*Rotarix*, GSK444563). • GSK Biologicals' Haemophilus influenzae type b (Hib) conjugate vaccine (*Hiberix*, GSK208108). • Merck's measles, mumps and rubella (MMR) virus live vaccine (*M-M-R II*). • Merck's varicella virus live vaccine (*Varivax*). • Saline placebo.
eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN-023 [V72_57])
Investigational New Drug (IND) number	IND-011561
EudraCT number	2016-003268-37
Date of protocol	Final Version 1: 16 December 2014
Date of protocol amendment	Amendment 1 Final: 02 August 2016 Amendment 2 Final: 11 December 2017 Amendment 3 Final: 25 April 2018 Amendment 4 Final: 02 November 2018 Amendment 5 Final: 10 April 2020 Amendment 6 Final: 9 December 2021 Amendment 7 Final: 24 October 2022 ***Amendment 8 Final:*** 19 Dec 2023
Title	Safety and Immunogenicity of GSK Meningococcal Group B Vaccine and 13-valent Pneumococcal Vaccine administered Concomitantly with Routine Infant Vaccines to Healthy Infants

Detailed Title	A Phase IIIB, Observer-Blind, Randomized, Placebo-Controlled, Multi-Center Study to Assess the Safety and Immunogenicity of GSK Meningococcal Group B Vaccine and 13-valent Pneumococcal Vaccine when Administered Concomitantly with Routine Vaccines to Healthy Infants

Protocol Amendment *8* Sponsor Signatory Approval

eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN-023 [V72_57])
IND number	IND-011561.
EudraCT number	2016-003268-37
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Sponsor signatory	***Alessandro Ble***, ***Director, Associate Clinical Project Lead***
Signature	
Date	

Note: Not applicable if an alternative signature process (e.g. electronic signature or email approval) is used to get the sponsor approval.

Protocol Amendment *8* Rationale

Amendment number: Amendment ***8***
Rationale/background for changes: The purpose of the amendment 8 is to align the protocol with the recent update of CDC's ACIP for the US NIP (National Immunization Program). According to this ACIP update, the 20-valent pneumococcal conjugate vaccine (PCV20) is listed as one of the recommended vaccines for the immunization of pneumococcal disease in children in U.S while PCV13 is no longer recommended for full series of pneumococcal vaccination. Children who received 3 PCV13 doses before 12 months but have not received their fourth booster dose, have now the option to receive PCV20 or PCV13. Therefore, to incorporate the current ACIP recommendations, subjects who have not reached their visit 5 at the time when this protocol amendment becomes effective have the option to receive either PCV13 or PCV20 based on the investigator judgment and/or parent's preference. Amended text is indicated in ***bold italics*** in the body of the protocol.

Protocol Amendment *8* Investigator Agreement

I agree:

- **To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.**
- **To assume responsibility for the proper conduct of the study at this site.**
- **That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.**
- **To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' study vaccine and other study-related duties and functions as described in the protocol.**
- **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 onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.**
- **To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).**
- **To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.**
- **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 investigational vaccines, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.**

Hence I:

- **Agree to supply GSK Biologicals 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 course of the study and for one year following completion of the study.**
- **Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.**
- **Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.**

eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN-023 [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Date of protocol amendment	***Amendment 8 Final:*** 19 Dec 2023
Detailed Title	A Phase IIIB, Observer-Blind, Randomized, Placebo-Controlled, Multi-Center Study to Assess the Safety and Immunogenicity of GSK Meningococcal Group B Vaccine and 13-valent Pneumococcal Vaccine when Administered Concomitantly with Routine Vaccines to Healthy Infants.
Investigator name	_____
Signature	_____
Date	_____

SPONSOR INFORMATION

1. **Sponsor**

GlaxoSmithKline Biologicals
Rue de l'Institut 89, 1330, Rixensart, Belgium

2. **Sponsor Medical Expert for the Study**

Refer to the local study contact information document.

3. **Sponsor Study Monitor**

Refer to the local study contact information document.

4. **Sponsor Study Contact for Reporting of a Serious Adverse Event**

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 7.3.2.

5. **GSK Biologicals' Central Safety Physician On-Call Contact information for Emergency Unblinding**

GSK Biologicals Central Safety Physician and Back-up Phone contact: refer to protocol Section 7.3.2.

SYNOPSIS

Detailed Title	A Phase IIIB, Observer-Blind, Randomized, Placebo-Controlled, Multi-Center Study to Assess the Safety and Immunogenicity of GSK Meningococcal Group B Vaccine and 13-valent Pneumococcal Vaccine when Administered Concomitantly with Routine Vaccines to Healthy Infants
Indication	Active immunization against invasive disease caused by *Neisseria meningitidis* serogroup B strains. Although the meningococcal group B vaccine was developed for individuals aged 2 months and older, the actual age range for which this recommendation extends varies depending on the approval from different health authorities. In the US, the current indication is for individuals 10 through 25 years of age.
Rationale for the study and study design	• Rationale for the study As part of the US Food and Drug Administration's approval of a biologics license application for GSK meningococcal group B vaccine (rMenB+OMV NZ, *Bexsero*), the present study V72_57 (205239) is a required pediatric study under the Pediatric Research Equity Act to evaluate the safety and immunogenicity of *Bexsero* in North American infants 6 weeks through 12 months of age. Previous clinical studies conducted in Europe and other countries worldwide have shown that rMenB+OMV NZ demonstrated a robust immune response in all age groups against indicator test strains, H44/76 (fHbp), 5/99 (NadA), NZ98/254 (PorA P1.4) and M10713 (NHBA) and that the concomitant administration of rMenB+OMV NZ does not clinically interfere with response to the vaccine antigens present in routinely administered infant vaccines: diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type b, inactivated poliomyelitis, hepatitis B, measles, mumps, rubella, varicella and 7-valent pneumococcal conjugate vaccine. However, no data on the concomitant use of rMenB+OMV NZ with PCV13 and routine infant vaccines in North American infants is currently available. The 3 strains M14459 (fHbp), 96217 (NadA), NZ98/254 (PorA P1.4) in addition to M13520 (NHBA) strain are considered by the CBER to better evaluate the protective response of the vaccine. The purpose of this study therefore is to assess the immune response against those strains and the safety of rMenB+OMV NZ vaccine when administered concomitantly with the PCV13 and other US Advisory Committee on Immunization Practices

(ACIP) recommended RIV. The study will also assess the safety and the immunogenicity of the PCV13 vaccine when concomitantly administered with rMenB+OMV NZ vaccine and RIV, compared to PCV13 administered with a placebo and RIV. In addition, the safety and immunogenicity of RIV (*Pediarix*, *Hiberix*, *Rotarix*, *M-M-R II*, *Varivax*) will be assessed following their concomitant administration with rMenB+OMV NZ and/or PCV13.

- Rationale for the study design

The parallel group study design was selected to allow an assessment of the immune responses and safety of the rMenB+OMV NZ and PCV13 vaccines when co-administered, along with other RIV.

- Rationale for the use of placebo

For this study, a placebo (saline solution) will be administered concomitantly with the PCV13 vaccine to subjects in group Placebo+PCV. As all other vaccines recommended for this age group have been included as study vaccines, a placebo is the only available option to minimize possible introduction of reporting bias in collecting information about AEs and to ensure the same number of vaccinations are administered to subjects assigned to either of the 2 study groups.

- Rationale for the use of non-study vaccine

In order to ease the disruption to the standard infant vaccine schedule caused by participating to the study, all subjects will also receive the following standardized non-study vaccines at 13 months of age (Visit 6):

- Diphtheria, tetanus toxoids and acellular pertussis adsorbed vaccine (DTaP, *Infanrix*).
- *Haemophilus influenzae* type b Conjugate Vaccine (Hib, *Hiberix*).

Study design

- Experimental design: Phase IIIB, observer-blind, randomized, placebo-controlled, multi-centric, study with 2 parallel groups.
- Duration of the study:
  - Epoch 001 Primary starting at Visit 1 (Day 1) and ending at Visit 5 (Day 301).
  - Epoch 002: Secondary starting at Visit 5 (Day 301) and ending at Visit 6 (Day 331).

  - Epoch 003: Safety follow-up period starting at Visit 6 (Day 331) and ending at Visit 7 (Day 481 or Day 661). For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will take place on Day 481.

- Primary completion date (PCD): Visit 7 (Day 481 or Day 661).
- End of Study (EoS): Date of the last testing/reading released of the Human Biological Samples (HBS), related to primary and secondary endpoints. Study completion must be achieved no later than 8 months after LSLV.
- Study groups:
  - Group MenB+PCV: rMenB+OMV NZ given concomitantly with PCV13 at 2, 4, 6, and 12 months of age. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment 8 becomes effective***. Subjects will also receive routine infant vaccines (DTPa-HBV-IPV, HRV, Hib, MMR, VV) at applicable timepoints.
  - Group Placebo+PCV: Placebo and PCV13 at 2, 4, 6, and 12 months of age. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment 8 becomes effective***. Subjects will also receive routine infant vaccines (DTPa-HBV-IPV, HRV, Hib, MMR, VV) at applicable timepoints.

Objectives	Endpoints
Primary Safety	
• To assess the safety and tolerability of rMenB+OMV NZ, PCV13 and other RIV when administered concomitantly to healthy infants at 2, 4, 6 and 12 months of age, throughout the study duration.	• The percentages of subjects with solicited local Adverse events (AEs) ***(administration site event)*** and systemic AEs during the 7 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with solicited systemic AEs of parotid/salivary gland swelling, fever and rash during the 30 days (including the day of vaccination) after the 4th vaccination (Visit 5). • The percentages of subjects with all unsolicited AEs (including serious adverse events [SAEs], AEs leading to withdrawal, adverse event of special interest [AESIs], and medically attended AEs) during 30 days (including the day of

	vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with SAEs, AEs leading to withdrawal, AESIs and medically attended AEs from study Day 1 (Visit 1) until study end (6 months or 12 months after last study vaccination, Visit 7¥).
Co-Primary Immunogenicity	
• To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4 and 6 months of age, at one month after the 3rd vaccination. *Criteria: The sufficiency of the immune response to rMenB+OMV NZ* at one month *after the 3rd vaccination will be demonstrated if the adjusted lower confidence limit for the percentage of subjects achieving serum bactericidal assay using human complement (hSBA) titers ≥ Lower Limit of Quantitation (LLOQ) is ≥ 60% for the N. meningitidis serogroup B test strains* M14459, 96217, NZ98/254, M13520 (individually); and is ≥ 50% for all strains combined (composite endpoint).	• At 1 month after the 3rd vaccination (Day 151): – the percentages of subjects with hSBA titers ≥LLOQ; for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the percentages of subjects with hSBA titers ≥LLOQ for all strains combined (composite endpoint).
• To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 4th vaccination. *Criteria: The sufficiency of the immune response to rMenB+OMV NZ will be demonstrated if the adjusted lower confidence limit for the percentage of subjects* with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) is ≥75% for the individual N. meningitidis serogroup B test strains and is ≥65% for all strains combined (composite endpoint).	• At 1 month after the 4th vaccination (Day 331): – the percentages of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) for each of the test strains. – the percentages of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) for all strains combined (composite endpoint).
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants 2, 4 and 6 months of age, compared to PCV13 without rMenB+OMV NZ, at one month after the 3rd vaccination. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the adjusted lower confidence limit for the between-group ratio of electrochemiluminescence (ECL) assay GMCs is >0.5 for each of the 13 PCV13 antigens.*	• At 1 month after the 3rd vaccination (Day 151): – the ECL GMCs for each of the 13 PCV13 antigens.
Secondary Immunogenicity	
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants 2, 4, 6 and 12 months of age, compared to PCV13 and other RIV alone, at one month after the 4th vaccination.	• At one month after the 4th vaccination (Day 331): – The ECL GMCs for each of the 13 PCV13 antigens.

Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of 2-sided 95% CI for the between-group-ratio of ECL assay GMCs is >0.5 for each of the 13 PCV13 antigens.	
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants at 2, 4, 6 and 12 months of age compared to PCV13 and other RIV alone, at both one month after the 3rd and the 4th vaccinations. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of the 2-sided 95% CI for the group differences in percentage of subjects with IgG ≥ 0.35 µg/mL is >-10% for each of the 13 PCV13 antigens at one month after both 3rd and 4th vaccination.*	• At 1 month after the 3rd (Day 151) and the 4th vaccination (Day 331): – Percentages of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 µg/mL for each of the 13 PCV13 antigens.
• To demonstrate the immunological non-inferiority of DTaP-HBV-IPV and Hib vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy infants at 2, 4, and 6 months compared to DTaP-HBV-IPV and Hib vaccines concomitantly administered with PCV13 without rMenB+OMV NZ, in terms of D, T, PT, FHA, PRN, Hep B and Hib, at one month after the 3rd vaccination. *Criterion: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group differences is greater than the pre-specified margin† for each antigen at one month after 3rd vaccination.*	• At 1 month after the 3rd vaccination (Day 151): – GMCs against the 3 pertussis antigens (Pertussis toxin [PT], pertactin [PRN], filamentous hemagglutinin [FHA]). – Percentages of subjects with anti-HBs antibody concentrations ≥10 mIU/mL. – Percentages of subjects with anti-diphtheria and anti-tetanus concentrations ≥0.1 IU/mL. – Percentages of subjects with anti-PRP concentration ≥0.15 µg/mL and ≥1 µg/mL.
• To demonstrate the immunological non-inferiority of MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy subjects at 12 months compared to MMR and VV vaccines concomitantly administered with PCV13, without rMenB+OMV NZ, at one month after vaccination. *Criterion: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group ratio of GMCs is >0.67 at one month after the MMR and VV vaccinations.*	• At 1 month after the 4th vaccination (Day 331): – GMCs against measles, mumps, rubella and VZV antigens.
• To evaluate the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 3rd vaccination, at 6 months after the 3rd vaccination (immediately before the 4th vaccination), and at one month after the 4th vaccination against the M14459, 96217, NZ98/254 and M13520 test strains.	• At 1 month after the 3rd vaccination (Day 151) – the percentages of subjects with hSBA titers ≥5, ≥8 and ≥16 for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the hSBA geometric mean titers (GMTs) against each strain. • At 6 months after the 3rd vaccination (Day 301, immediately before the 4th vaccination):

	– the percentages of subjects with hSBA titers ≥LLOQ for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the percentages of subjects with hSBA titers ≥5 and ≥8 for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the hSBA GMTs against each strain. • At 1 month after the 4th vaccination (Day 331): – the percentages of subjects with hSBA titers ≥LLOQ for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the percentages of subjects with hSBA titers ≥5 for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the hSBA GMTs and geometric mean ratios (GMRs) over pre-4th vaccination against each strain. – the percentages of subjects with 4-fold rise* in hSBA titers (from pre-4th vaccination) for each of the M14459, 96217, NZ98/254 and M13520 test strains.
• To evaluate immune responses to routine infant vaccines DTaP-HBV-IPV, Hib, MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 3rd vaccination, at 1 month after the 4th vaccination.	• At 1 month after the 3rd vaccination (Day 151): – Percentages of subjects with anti-HBs antibody concentrations ≥100 mIU/mL. – Anti-HBsAg GMCs. – Percentages of subjects with anti-diphtheria and anti-tetanus concentrations ≥1 IU/mL. – Anti-diphtheria and anti-tetanus antibody GMCs. – Percentage of subjects with anti-polio type 1, 2 and 3 neutralization antibody titers ≥8. • At 1 month after the 4th vaccination (Day 331): – Seroresponse, defined as post-vaccination anti-VZV virus, anti-measles virus, anti-mumps virus and anti-rubella virus antibody concentration^ ≥ a protective threshold among subjects who were seronegative (antibody concentration <assay cut-off) before vaccination. ^ A suitable ELISA assay for analysis of anti-VZV, anti-measles virus, anti-mumps virus and anti-rubella virus antibody concentrations is yet to be selected and/or developed.

* A 4-fold rise in hSBA titers is defined as
- if pre-vaccination titer <Limit of Detection (LOD), then a post-vaccination titer ≥ 4 times the LOD or ≥ LLOQ, whichever is greater;
- if pre-vaccination titer is ≥LOD but <LLOQ, then a post-vaccination titer ≥4 times the LLOQ;
- if pre-vaccination titer is ≥LLOQ, then a post-vaccination titer ≥4 times the pre-vaccination titer,

where pre-vaccination titer=pre-4th dose titers (Day 301)
† The endpoints and thresholds used for evaluation of the non-inferiority criteria for DTaP-HBV-IPV and Hib vaccines can be found in Table 21 and the thresholds and endpoint for evaluating the non-inferiority criteria for MMR and VV vaccines can be found in Table 22.
¥ Visit 7 will occur either on Day 481 (for subjects who have not yet reached the 6-month follow-up after the last dose at the time protocol amendment 7 takes effect) or on Day 661 (for all other subjects).
Note 1: To control the risk of erroneously concluding, a hierarchical procedure will be used for the multiple confirmatory study objectives, with the possibility to conclude on the study success based on the results of the co-primary objectives alone (refer to Section 9.5.3 for details).
Note 2: In the context of this study, the following are considered as RIVs at different visits:
Visit 1 and Visit 2: DTPa-HBV-IPV, HRV, Hib;
Visit 3: DTPa-HBV-IPV, Hib;
Visit 5: MMR and VV.

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min – Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
MenB+PCV	800	6 weeks – 12 weeks	x	x	x
Placebo+PCV	400	6 weeks – 12 weeks	x	x	x

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine name	Study Groups	
		MenB+PCV	Placebo+PCV
Bexsero	rMenB+OMV NZ	X	-
Prevnar13	PCV13*	X	X
Prevnar20	***PCV20****	***X***	***X***
Placebo	NaCl	-	X
Pediarix	DTPa-HBV-IPV	X	X
Rotarix	HRV	X	X
Hiberix	Hib	X	X
M-M-R II	MMR	X	X
Varivax	VV	X	X

Note: Subjects in both groups will receive a fourth dose of Hib vaccine (*Hiberix*) and a single dose of DTPa vaccine (*Infanrix*) as non-study vaccines at Visit 6.
**** Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective***.

- Control: placebo control.
- Vaccination schedule: Visit 1 (Day 1), Visit 2 (Day 61) Visit 3 (Day 121), Visit 5 (Day 301).
- Treatment allocation: Subjects to be randomized in a 2:1 ratio at Visit 1 (Day 1) to Group MenB+PCV and Group Placebo+PCV, respectively.
- Blinding: observer-blind study.

Synopsis Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	observer-blind
Epoch 002	observer-blind
Epoch 003	observer-blind

	• Sampling schedule: The sampling schedule for both study groups is the same. Three blood samples of approximately 5 mL each are to be taken at: – Visit 4 (Day 151), i.e., 30 (-9 to +30) days after the 3rd vaccination. – Visit 5 (Day 301), i.e., 180 (-7 to +91) days after the 3rd vaccination (pre-4th vaccination). – Visit 6 (Day 331), i.e., 30 (-9 to +30) days after the 4th vaccination. • Type of study: self-contained. • Data collection: electronic case report form (eCRF). Solicited symptoms will be collected using a subject electronic Diary (eDiary).
Number of subjects	Target enrolment is 1200 subjects who will be randomly assigned to the 2 study groups in a 2:1 ratio.

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LIST OF ABBREVIATIONS

ACIP:	Advisory Committee on Immunization Practices
ADEM:	Acute disseminated encephalomyelitis
AE:	Adverse Event
AESI:	Adverse Event of Special Interest
ANOVA:	Analysis of Variance
CBER:	Center for Biologics Evaluation and Research
CDC:	Centers for Disease Control
CFS	Chronic fatigue syndrome
CI:	Confidence Interval
CLIA:	ChemiLuminescence ImmunoAssay
CLS:	Clinical Laboratory Sciences
COVID-19	Coronavirus Disease 2019
CRO:	Contract Research Organization
DTPa-HBV-IPV:	Diphtheria, Tetanus, acellular Pertussis, Haemophilus influenzae type b, inactivated Poliovirus combined vaccine
ECDC:	European Center for Disease Prevention and Control
ECL:	Electrochemiluminescence
eCRF:	electronic Case Report Form
EoS:	End of Study
FAS:	Full Analysis Set
FDA:	Food and Drug Administration, United States of America
FHA:	Filamentous hemagglutinin
fHbp:	Factor H binding protein
GBS:	Guillain-Barre Syndrome
GCP:	Good Clinical Practice
GDS:	Global Data Sheet
GMC:	Geometric Mean Concentration
GMR:	Geometric Mean Ratio
GMT:	Geometric Mean Titer
GSK:	GlaxoSmithKline
HBV:	Hepatitis B (recombinant)vaccine
HBS:	Human Biological Samples
Hib:	Haemophilus influenzae type b conjugate vaccine
HIPAA:	Health Insurance Portability and Accountability Act
HRV:	Human rotavirus vaccine (2-dose regimen)
hSBA:	Human Serum Bactericidal Assay
IB:	Investigator Brochure
iPSP	initial Pediatric Study Plan
ICF:	Informed Consent Form
ICH:	International Conference on Harmonization
ID:	Identification (Subject ID), Intradermal
IEC:	Independent Ethics Committee
IM:	Intramuscular

IMD:	Invasive meningococcal disease
IMP:	Investigational Medicinal Product
IND:	Investigational New Drug
IPV:	Inactivated poliovirus vaccine
IRB:	Institutional Review Board
IU:	International Unit
JIA:	Juvenile Idiopathic Arthritis
LAR:	Legally Acceptable Representative
LLOQ:	Lower Limit of Quantitation
LOD:	Lower Limit of Detection
LSLV:	Last Subject Last Visit
MedDRA:	Medical Dictionary for Regulatory Activities
mL:	Milliliter
MMR:	Measles, Mumps, Rubella vaccine
NadA:	*Neisseria* adhesin A
NHBA:	Neisserial Heparin Binding Antigen
NIMP:	Non-investigational medicinal product
OMV:	Outer Membrane Vesicles
PCD:	Primary Completion Date
PCV13:	13-valent Pneumococcal Conjugate Vaccine
PCV20:	***20-valent Pneumococcal Conjugate Vaccine***
pIMD:	Potential Immune-Mediated Disease
PorA:	Porin Protein A
PPS:	Per Protocol Set
PRN:	Pertactin
PT:	Pertussis toxin
RIV:	Routine Infant Vaccines
rMenB+ OMV NZ:	Recombinant Meningococcal B with Outer Membrane Vesicle derived from *Neisseria meningitidis* serogroup B strain NZ98/254 (New Zealand strain) Vaccine
SAE:	Serious Adverse Event
SBIR:	Source Database for Internet Randomization
SD:	Standard Deviation
SDA:	Source Documentation Agreement Form
SDV:	Source Document Verification
SmPC:	Summary of Product Characteristics
SMQ	Standard MedDRA Query
SOC:	System Organ Class
SPM:	Study Procedures Manual
SRT:	Safety Review Team
VV:	Varicella vaccine
WHO:	World Health Organization

GLOSSARY OF TERMS

Adverse Event of Special Interest:	Adverse events of special interest (AESIs): are predefined (serious or non-serious) adverse events of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate, because such an event might warrant further investigation in order to characterize and understand it.
Adverse event:	Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An adverse event (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 a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.
Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event. In an observer-blind study, the subject and the site and sponsor personnel involved in the clinical evaluation of the subjects are blinded while other study personnel may be aware of the treatment assignment (see Section 5.2.3 for details on observer-blinded studies).
Child in care:	A child who has been placed under the control or protection of an agency, organization, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation. The definition of a child in care can include a child cared for by foster parents or living in a care home or institution, provided that the arrangement falls within the definition above. The definition of a child in care does not include a child who is adopted or has an appointed legal guardian.

Co-administered (concomitant) products	***A product given to clinical trial participants as required in the protocol as part of their standard care for a condition which is not the indication for which the IMP is being tested and is therefore not part of the objective of the study.***
Combination product:	Combination product comprises any combination of – drug – device – biological product Each drug, device and biological product included in a combination product is a constituent part.
Comparator	***Any product used as a reference (including placebo, marketed product, GSK or non-GSK) for an investigational product being tested in a clinical trial. This is any product that is being used to assess the safety, efficacy, or other measurable value against the test product (IMP).***
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study: **(Synonym of End of Trial)**	For studies without collection of human biologicals samples or imaging data EoS is the Last Subject Last Visit (LSLV). For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after LSLV.
Enrolled:	All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments and are randomized and have received a Subject ID, regardless of the subject's treatment status in the study**.**

Epoch:	An epoch is a set of consecutive time points or a single time point from a single protocol. Epochs are defined to support a main purpose which is either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the time points included in an epoch must be sufficient to fulfill the purpose of the epoch. Typical examples of epochs are screening, primary vaccinations, boosters, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety.
Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.
Investigational vaccine: **(Synonym of Investigational Medicinal Product)**	***A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form.***
Legally acceptable representative: **(The terms legal representative or legally authorized representative are used in some settings)**	An individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.
Medically attended adverse events:	Symptoms or illnesses requiring hospitalization, or emergency room visit, or tele visit or visit to/by a health care provider.
Medical device deficiency*:*	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 information supplied by the manufacturer.

Non-study Vaccine:	A vaccine which will be given to subjects during the study, but does not have associated study objectives. Non-study vaccines are Non-Investigational Medicinal Products (NIMPs).
Observer-blind:	The subject, site and Sponsor personnel involved in the evaluation of the subjects are blinded while other study personnel (unblinded staff) may be aware of the treatment allocation.
Placebo	***An inactive substance or treatment that looks the same as, and is given in the same way as, an active drug or intervention/treatment being studied.***
Potential Immune-Mediated Disease:	Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune aetiology.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical trial was concluded according to the pre-specified protocol or was terminated.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study.
Protocol amendment:	The International Conference on Harmonization (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Protocol violation (deviation):	A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. In general, subjects associated with protocol deviations may remain in the study unless continuation in the study jeopardizes the subject's health, safety, or rights.
Qualified health care professional:	Any licensed or certified health care professional who is permitted by institutional policy to perform protocol required procedures, and who is identified within the Study Staff Signature Log.

Randomization:	Process of random attribution of treatment/schedule to subjects in order to reduce bias of selection.
Routine infant vaccines:	Certain routine infant vaccines that are part of the recommended schedule. In the context of this study, the following are considered as RIVs, based on the recommended infant childhood vaccination schedule by the Advisory Committee on Immunization Practices (ACIP) and on the different study visits: • Visit 1 and Visit 2: DTPa-HBV-IPV, HRV, Hib. • Visit 3: DTPa-HBV-IPV, Hib. • Visit 5: MMR, VV.
Self-contained study:	Study with objectives not linked to the data of another study.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Solicited adverse event:	AEs to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
Study vaccine/product:	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/ product /placebo as a reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product. Study vaccines are Investigational Medicinal Products (IMPs).
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccines or as a control.
Subset (Synonym of Immunosubset):	Selection of blood samples among all blood sample collected at given time point(s) for testing by specific assay
Treatment number:	A unique number identifying a treatment to a subject, according to treatment allocation.

Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.
Unblinded site staff:	Study personnel aware of the subject treatment allocation, delegated for administering study vaccines to subjects but not involved in the safety or immunogenicity evaluation of the subjects. All study vaccines will be administered only by unblinded personnel who are qualified to perform that function under applicable laws and regulations for the specific study site.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also any 'solicited' symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

TRADEMARKS

The following trademarks are used in the present protocol.

Note: In the body of the protocol (including the synopsis), the names of the vaccines will be written without the superscript symbol ™ or ® and in *italics*.

Trademarks of the GSK group of companies	Generic description
Bexsero	Meningococcal group B vaccine (rDNA, component, adsorbed)
Hiberix	*Haemophilus influenzae* type b (Hib) conjugate vaccine (tetanus toxoid, conjugate)
Infanrix	Diphtheria, Tetanus Toxoids and Acellular Pertussis Adsorbed Vaccine
Pediarix	Diphtheria, Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B and Inactivated Poliovirus Vaccine
Rotarix	Rotavirus vaccine, live, oral

Trademarks not owned by the GSK group of companies	Generic description
M-M-R II (Merck & Co., Inc.)	Measles, Mumps and Rubella Virus Vaccine Live
Prevnar 13 (Wyeth Pharmaceuticals Inc./Pfizer Inc.)	Pneumococcal polysaccharide conjugate vaccine (13-valent, adsorbed)
Prevnar 20 (Wyeth Pharmaceuticals Inc./Pfizer Inc.)	***Pneumococcal polysaccharide conjugate vaccine (20-valent, adsorbed)***
Varivax (Merck & Co., Inc.)	Varicella Virus Vaccine Live

1. INTRODUCTION

1.1. Background

Invasive meningococcal disease occurs when the normally asymptomatically carried bacterium *Neisseria meningitidis* enters the bloodstream, multiplies and causes sepsis. If the bacteria cross the blood–brain barrier, meningitis occurs [Khatami, 2010].

Invasive meningococcal disease occurs worldwide. Although incidence varies in different regions of the world, infants, children and adolescents are the most vulnerable to developing invasive disease. Symptoms of the disease occur rapidly and often result in severe outcomes within a few hours; otherwise healthy individuals can be permanently disabled or disfigured or die of the disease. Despite the availability of medical treatment and effective antibiotics for invasive meningococcal disease, approximately 9% of European patients die, with case-fatality rates generally increasing with age [ECDC, 2016]. In the United States (US), the overall case-fatality ratio remains at 10%–15%, and 11%–19% of survivors have long-term sequelae [Cohn, 2013].

No reliable estimates of the global burden of disease are currently available as case definitions differ and surveillance data from many regions are incomplete [WHO, 2011]. The overall incidence of confirmed invasive meningococcal diseases (IMDs) in European countries in 2012 ranged from 0.11 to 1.77 cases per 100,000 populations [ECDC, 2016]. In the US, the incidence was 0.18 cases per 100 000 population in 2014 [Adams, 2016].

Currently, 13 serogroups of pathogenic *N. meningitidis* [Harrison, 2013] are known to exist; however, virtually all meningococcal meningitis and septicemia are caused by only six serogroups: (i.e. A, B, C, W, Y and X). The introduction of conjugate serogroup C meningococcal vaccines has dramatically changed the epidemiology of the disease in industrialized nations, showing potential for broader control with A, C, Y and W conjugates, and leaving serogroup B as the predominant cause of disease. Development of vaccines for prevention of serogroup B disease in industrialized nations and serogroup A conjugate vaccines for Africa could lead to global control of meningococcal disease [Khatami, 2010].

For the US, the 2015 Centers for Disease Control (CDC) data indicate a total of 370 cases and 60 deaths attributable to meningococcal disease, with the majority of these caused by serogroup B, C and Y infections [Cohn, 2013; CDC, 2015a]. In Europe, the incidence of disease due to serogroup B is particularly high in Ireland, the United Kingdom (UK), Denmark and Spain. These same countries had early introduction of Meningococcal serogroup C vaccination after high incidences of serogroup C infections in the late 1990s [ECDC, 2016].

In Europe, the majority of disease occurs in infants under 1 year of age, followed by children from 1 through 5 years of life. A second peak occurs in adolescents 15 to 19 years of age. In the US, incidence of meningococcal disease peaks in three age groups: infants and children aged <5 years, adolescents and young adults aged 16 through 21 years, and adults aged ≥65 years. Approximately 60% of disease in the first year of life is caused by serogroup B [Cohn, 2013].

Capsular polysaccharide vaccines have been used successfully in preventing disease and limiting epidemics and outbreaks caused by meningococcal serogroups A, C, W and Y. However the capsular polysaccharide of meningococcal serogroup B is poorly immunogenic in humans, possibly due to similarities in serogroup B carbohydrate moieties to carbohydrates widely distributed in the human body. As a result, research has focused on proteins in the outer membrane of meningococci as potential antigens for candidate vaccines. Serogroup B vaccines based on protein-containing outer membrane vesicles (OMV) have been safe and effective in controlling epidemic disease caused by strains homologous to the vaccine strain in Cuba, Brazil, Chile, Norway, and New Zealand. The use of these OMV vaccines to combat serogroup B meningococcal disease has been limited, however, due to the strain-specific nature of the protection and the lack of consistent efficacy in young children.

The knowledge gained during vaccine development for the Norwegian (MenBvac) and New Zealand (MeNZB) epidemics, together with the identification of the *N. meningitidis* serogroup B genome sequence, was used to develop GSK serogroup B meningococcal vaccine (rMenB+OMV NZ). The availability of the bacterial genome sequence allowed identification of conserved surface-exposed outer membrane proteins of serogroup B strains that were targets for bactericidal antibodies [Pizza, 2000].

The current vaccine formulation (referred to as rMenB+OMV NZ, i.e., GSK recombinant Meningococcal B vaccine with the OMV derived from New Zealand strain) is based on three proteins: i) factor H binding protein (fHbp), ii) Neisseria adhesin A (NadA) and iii) Neisserial Heparin Binding Antigen (NHBA) or 287. The fHbp protein has been combined with the accessory protein GNA2091 (936), and the 287 protein has been combined with GNA1030 (953), to create two fusion proteins. In addition the vaccine also contains OMV derived from the New Zealand epidemic strain.

On 23 January 2015, the US Food and Drug Administration (FDA) approved a biologics licence application for GSK meningococcal group B vaccine according to the regulations for accelerated approval; the vaccine was approved for use in individuals 10 through 25 years of age. As part of the approval, the present study V72_57 (205239) meets the requirement for a pediatric study under the Pediatric Research Equity Act to evaluate the safety and immunogenicity of GSK meningococcal group B vaccine in North American infants 6 weeks through 12 months of age for the prevention of invasive group B meningococcal disease.

Previous data from clinical studies conducted largely in Europe and Latin America in several age groups showed that rMenB+OMV NZ is generally well tolerated and immunogenic. These studies formed the basis of the marketing authorizations in the European Union (EU), Canada and Australia and other countries in individuals over 2 months of age.

The rMenB+OMV vaccine has shown to generate robust antibody responses in pediatric populations, even when administered concomitantly with other routine infant vaccines. Concomitant administration of rMenB+OMV NZ was shown not to interfere with the immune response to the following vaccine antigens, either as monovalent or as combination vaccines: diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae*

type b, inactivated poliomyelitis, hepatitis B, measles, mumps, rubella, varicella and 7-valent pneumococcal conjugate vaccine. Overall, data from these studies demonstrated that the immune responses of the co-administered routine vaccines were unaffected by concomitant administration of rMenB+OMV NZ, based on non-inferior antibody response rates to the routine vaccines given alone.

The use of rMenB+OMV NZ in infants was associated with frequent reports of reactogenicity and especially of local reactions, although most were of mild or moderate severity and transient. Consistent with the extensive safety data collected in the vaccination campaigns with other OMV-based vaccines, increased rates of fever were associated with rMenB+OMV NZ vaccination in the infant population, especially when given concomitantly with routine vaccines. As the fever resolved soon after vaccination and these higher rates were not associated with an increase in medically attended adverse events (AEs) or an increase in febrile seizure risk, these data overall supported giving rMenB+OMV NZ either concomitantly with or without routine pediatric vaccines. Additionally, the use of prophylactic acetaminophen (paracetamol) given at the time of vaccination and/or closely thereafter has shown to decrease fever and reactogenicity in infants, without interfering with the antibody response to rMenB+OMV NZ, nor to the concomitantly administered routine vaccines (DTPa-HBV-IPV/Hib and PCV7) [Prymula, 2014].

Please refer to the current Investigator Brochure for information regarding the pre-clinical and clinical studies and the epidemiological information of rMenB+OMV NZ (*Bexsero*).

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

As part of the US FDA's approval of a biologics license application for GSK meningococcal group B vaccine (rMenB+OMV NZ, *Bexsero*), a pediatric study under the Pediatric Research Equity Act was required to evaluate the safety and immunogenicity of GSK meningococcal group B vaccine, *Bexsero* in North American infants 6 weeks through 12 months of age.

Previous clinical studies conducted in Europe and other countries worldwide have shown that rMenB+OMV NZ demonstrated a robust immune response in all age groups against strains H44/76 (fHbp), 5/99 (NadA), NZ98/254 (PorA P1.4) and M10713 (NHBA) and that the concomitant administration of rMenB+OMV NZ did not interfere with response to the vaccine antigens present in routinely administered infant vaccines. However, no data on the concomitant use of rMenB+OMV NZ with PCV13 and routine infant vaccines in North American infants is currently available.

The 3 strains M14459 (fHbp), 96217 (NadA), NZ98/254 (PorA P1.4) in addition to M13520 (NHBA) strain are considered by the Center for Biologics Evaluation and Research (CBER) to better evaluate the protective response of the vaccine.

The purpose of this study therefore is to assess the immune responses against those strains and the safety of the rMenB+OMV NZ vaccine when administered concomitantly with the 13-valent pneumococcal conjugate vaccine (PCV13) and with other US Advisory Committee on Immunization Practices (ACIP) recommended routine infant vaccines (RIV). The study will also assess the safety and the immunogenicity of the PCV13 vaccine when concomitantly administered with rMenB+OMV NZ and RIV, compared to PCV13 administered with a placebo and RIV. In addition, the safety and immunogenicity of RIV (*Pediarix*, *Hiberix*, *Rotarix*, *M-M-R II*, *Varivax*) will be assessed following administration with rMenB+OMV NZ and/or PCV13.

1.2.2. Rationale for the study design

The parallel group study design was selected to allow an assessment of the immune responses and safety of the rMenB+OMV NZ and PCV13 vaccines when co-administered, along with other RIV.

1.2.3. Rationale for the use of placebo

For this study, a placebo (saline solution) will be administered concomitantly with the PCV13 vaccine to subjects in Group Placebo+PCV. As all other vaccines recommended for this age group have been included as study vaccines, a placebo is the only available option to minimize possible introduction of reporting bias in collecting information about AEs and to ensure the same number of vaccinations are administered to subjects assigned to either of the 2 study groups.

1.2.4. Rationale for the use of non-study vaccines

In order to ease the disruption to the standard infant vaccine schedule caused by participating to the study, GSK will provide all subjects the following standardized non-study vaccines at 13 months of age (Visit 6):

- Diphtheria, tetanus toxoids and acellular pertussis adsorbed vaccine (DTPa, *Infanrix*)
- *Haemophilus influenzae* type b Conjugate vaccine (Hib, *Hiberix*).

1.3. Benefit : Risk Assessment

Please refer to the Prescribing Information **[*BEXSERO*; 2023]** for information regarding the summary potential risks and benefits of *Bexsero*.

The following section outlines the risk assessment and mitigation strategy for this study protocol:

1.3.1. Risk Assessment

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
IMP: *Bexsero*		
Important identified risk: **Fever**	Fever may occur following vaccination. Fever is listed in the Bexsero US Product Information (PI).	Any febrile illness constitutes a contraindication to administration of the vaccine at the time scheduled for vaccination (see Section 6.5). Prophylactic use of analgesic/antipyretic medications during the first 7 days after vaccination is allowed on a voluntary basis, but must be recorded in the source document and the eCRF (see Section 6.7.1).
Important potential risks : **Guillain-Barre Syndrome (GBS)**	GBS has been observed with other vaccines. No cases have been observed during the Bexsero clinical development program.	The potential risk of events of possible autoimmune aetiology that might occur is mentioned in the Informed Consent Form (ICF). GBS will be monitored through the potential immune-mediated diseases (pIMDs) collection.
Important potential risk: **Acute disseminated encephalomyelitis (ADEM)**	ADEM has been observed with other vaccines. No cases have been reported during the Bexsero clinical development program.	The potential risk of events of possible autoimmune aetiology that might occur is mentioned in the ICF. ADEM will be monitored through the pIMDs collection.
Important potential risk: **Anaphylaxis and anaphylactic shock**	No cases of anaphylaxis related to Bexsero have been reported in the Bexsero clinical development program. However, one related case of anaphylaxis within 30 minutes post-vaccination was reported in a third party expanded access program (V72_70TP). Allergic reactions (including anaphylactic reactions) are listed in the Bexsero US PI.	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).
Important potential risk: **Vasculitis/Kawasaki Disease (KD)**	There were some cases of suspected KD reported in subjects receiving Bexsero in four clinical studies, all recovered completely. The occurrence of the cases in Bexsero studies is consistent with the known epidemiology of KD in terms of geographic and temporal clustering, and seasonal variation. As the exposure in clinical trial of subject was limited considering the incidence of the disease, KD is considered as a potential risk.	The potential risk of events of possible autoimmune aetiology that might occur is mentioned in the ICF. KD and vasculitis will be monitored through the pIMDs collection.
Important potential risk: **Seizure including Febrile Seizure**	Cases of seizure, including febrile seizure, have been reported during the Bexsero clinical development program and from the post-marketing experience. Seizures (including febrile seizures) are listed (see US PI).	Close monitoring of all seizures, as reported in Section 7.1.6.1. Seizures will be monitored through the adverse event of special interest (AESI) collection.

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Important potential risk: **Arthritis**	Attenuated live virus vaccines (rubella) were reported to be associated with joint-related diseases [Tingle, 1986]. Among inactivated vaccines, different manifestations of arthritis following hepatitis B vaccination (psoriatic arthritis, reactive arthritis, etc.) were described [IOM, 2011]; however no association with meningococcal vaccines has been reported in the literature. One clinical case of juvenile idiopathic arthritis (JIA) possibly related to rMenB+OMV NZ and 6 spontaneous reports considered to have a possible causal relationship have been observed.	Arthritis will be monitored through the AESI collection.
IMP: *Prevnar13 and *Prevnar20****		
Hypersensitivity and anaphylaxis	Hypersensitivity including anaphylaxis to one or several components of the vaccine can rarely occur. Hypersensitivity reaction including face oedema, dyspnea, bronchospasm is listed in the clinical trial section of *Prevnar13* US PI as well as anaphylactic /anaphylactoid reaction including shock is a listed adverse reaction from the post-marketing experience.	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).
Seizures (including febrile seizures)	The febrile convulsion is a risk for the infant and toddler groups. Seizures (including febrile seizures) are listed adverse reactions in the clinical trial section of *Prevnar13* US PI.	Close monitoring of all seizures, as reported in Section 7.1.6.1. Seizure will be monitored through the AESI collection.
Fever	Fever may occur following vaccination. Fever is listed in the *Prevnar13* and ***Prevnar20*** US PI.	Any febrile illness constitutes a contraindication to administration of the vaccine at the time scheduled for vaccination e study (see Section 6.5). Prophylactic use of analgesic/antipyretic medications during the first 7 days after vaccination is allowed on a voluntary basis, but must be recorded in the source document and in the eCRF (see Section 6.7.1).
Erythema multiforme	Erythema multiforme is listed in the post-marketing section of the *Prevnar13* US PI.	No specific mitigation in this study: SAE collection is part of the study protocol.
IMP: *Pediarix*		
Important identified risk: **Hypersensitivity**	People may develop allergic reactions to any type of vaccine and/or its components. Hypersensitivity reactions to vaccines highly vary in severity, ranging from mild to potentially life-threatening hypersensitivity reactions. Severe allergic reactions to vaccines are rare but tend to occur within hours after vaccination.	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Important identified risk: **Temperature of ≥ 40.0° C**	In rare occasion, hyperpyrexia above 40.0°C may occur after vaccination, particularly associated with booster vaccination.	Any febrile illness constitutes a contraindication to administration of the vaccine at the time scheduled for vaccination (see Section 6.5). Prophylactic use of analgesic/antipyretic medications during the first 7 days after vaccination is allowed on a voluntary basis, but must be recorded in the source document and in the eCRF (see Section 6.7.1).
Important identified risk: Hypotonic-hyporesponsive episode (HHE)	HHE occurs more commonly in early childhood after primary vaccination and particularly after the first dose of vaccine. Most HHE events have been associated with whole cell pertussis-containing vaccines (36-250 episodes per 100,000 doses). A much lower rate of HHE has been observed following vaccination with acellular pertussis vaccine (4-140 episodes per 100,000 doses).	Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours of vaccination is a contraindication to further administration of *Pediarix* (see US PI).
Important identified risk: **Apnoea in infants born prematurely**	Apnoea of prematurity is due to immaturity of the neurological and respiratory system in premature infants. The risk appears to be related to the degree of prematurity of the infant.	Only subjects born full-term (i.e. after a gestation period of ≥ 38 weeks) will be enrolled (see Section 4.2).
Important identified risk: **Convulsions with or without fever, occurring within 3 days**	Seizures occurring soon after immunization are mostly triggered by fever induced by the vaccine or are not vaccine related. Febrile convulsions usually occur in individuals aged between 3 months and 6 years with a peak incidence at 18 months.	Close monitoring of all seizures, as reported in Section 7.1.6.1. Seizure will be monitored through the AESI collection.
Important potential risk: **Encephalopathy**	Reports of encephalopathy were received with previous whole cell pertussis vaccines.	Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of administration of a previous dose of a pertussis - containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including *Pediarix* (see US PI).
IMP: *Hiberix*		
Important identified risk: **Apnoea in premature infants**	No case observed in the *Hiberix* clinical development programme. The risk is listed in the US PI. The apnoea is due to the immaturity of the neurological and respiratory systems in premature infants and appears to be related to the degree of prematurity. The mechanism by which immunization may result in apnoea in premature infants remains unknown.	Only subjects born full-term (i.e. after a gestation period of ≥ 38 weeks) will be enrolled (see Section 4.2).

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Important identified risk: **Allergic reactions (including anaphylactic and anaphylactoid reactions)**	No case of anaphylaxis was observed in the *Hiberix* clinical development programme. The risk is listed in the US PI.	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).
Important identified risk: **Angioedema**	See allergic reactions above	See allergic reactions above
Important identified risk: **Hypotonic-Hyporesponsive Episodes (HHE)**	No case observed in the Hiberix clinical development programme. The risk is listed in the post-marketing section of the core safety information. The mechanism by which immunization may result in HHE remains unknown; however, most reported episodes followed the administration of a pertussis-containing vaccine and have been associated with whole-cell vaccines more often than acellular.	HHE is unpredictable and although it may be frightening for parents/caregivers, it usually resolves spontaneously without any intervention being required or any sequelae. No specific mitigation in this study: SAE collection is part of the study protocol (see Section 5.6.15).
Important identified risk: **Convulsion (with or without fever)**	Cases of convulsion, including febrile convulsion, have been reported during the *Hiberix* clinical development program, and also from post-marketing experience. Convulsions (including febrile convulsions) are listed in the US PI.	Close monitoring of all seizures, as reported in Section 7.1.6.1. Seizure will be monitored through the AESI collection.
IMP: *Rotarix*		
Important identified risk: **Intussusception (IS)**	Following administration of a previously licensed oral live rhesus rotavirus-based vaccine, an increased risk of intussusception was observed. In a postmarketing, observational study conducted in Mexico, cases of intussusception were observed in temporal association within 31 days following the first dose of *Rotarix*, with a clustering of cases in the first 7 days. Other postmarketing observational studies conducted in Brazil and Australia also suggest an increased risk of intussusception within the first 7 days following the second dose of *Rotarix*. Intussusception (including death) is listed (see US PI).	Infants with a history of uncorrected congenital malformation of the gastrointestinal tract (such as Meckel's diverticulum) that would predispose the infant for intussusception or with a history of intussusception should not receive *Rotarix* (see US PI).
Important identified risk: **Hematochezia**	Hematochezia has been observed during the post-marketing experience with *Rotarix* and it is listed in the US PI.	No specific mitigation in this study: SAE collection is part of the study protocol.
Important identified risk: **Gastroenteritis with vaccine viral shedding in infants with Severe Combined Immunodeficiency (SCID)**	Cases of SCID has been reported during the post-marketing experience with *Rotarix*. This risk is listed (see US PI).	Subjects with SCID will be excluded from participating in this study (see Section 4.3).

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Important potential risk: **Kawasaki Disease (KD)**	Based on signal observed for *RotaTeq* vaccine. KD is listed in the US PI.	The potential risk of events of possible autoimmune aetiology that might occur is mentioned in the ICF. KD will be monitored through the pIMDs collection.
IMP: *M-M-R II*		
Important identified risk: **Exposure to immunocompromized individuals**	In severely immunocompromized individuals who have been inadvertently vaccinated with measles-containing vaccine; measles inclusion body encephalitis, pneumonitis, and fatal outcome as a direct consequence of disseminated measles vaccine virus infection have been reported. In this population, disseminated mumps and rubella vaccine virus infection have also been reported (see *M-M-R II* US PI).	Abnormal function of the immune system including immunodeficiency is an exclusion criterion in this study (see Section 4.3).
Important potential risk: **Hypersensitivity including Anaphylaxis**	Hypersensitivity including anaphylaxis to one or several components of the vaccine can rarely occur. Anaphylaxis and anaphylactoid reactions are listed in the *M-M-R II* US PI.	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).
Important potential risk: **Thrombocytopenia**	The risk of an exacerbation of thrombocytopenia following vaccination and the risk to develop thrombocytopenia with repeat doses in individuals who experienced thrombocytopenia with the first dose constitute a warning in the *M-M-R II* US PI. Moreover, thrombocytopenia is an adverse reaction listed in the US PI.	Thrombocytopenia will be monitored through the SAE collection (see Section 5.6.15).
Important potential risk: **Fever**	Fever may occur following vaccination. Fever is listed in the *M-M-R II* US PI.	Any febrile illness constitutes a contraindication to administration of the vaccine at the time scheduled for vaccination (see Section 6.5). Prophylactic use of analgesic/antipyretic medications during the first 7 days after vaccination is allowed on a voluntary basis, but must be recorded in the source document and in the eCRF (see Section 6.7.1).
IMP: *Varivax*		
Important identified risk: **Hypersensitivity reactions**	Hypersensitivity to one or several components of the vaccine can rarely occur. Anaphylaxis (including anaphylactic shock) and related phenomena such as angioneurotic edema, facial edema, and peripheral edema are listed (see *Varivax* US PI).	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Important identified risk: **Exposure to immunocompromized individuals**	*Varivax* is a live, attenuated varicella-zoster vaccine (VZV) and may cause an extensive vaccine-associated rash or disseminated disease in individuals who are immunosuppressed or immunodeficient (see US PI).	Abnormal function of the immune system including immunodeficiency is an exclusion criterion in this study (see Section 4.3).
Important identified risk: **Vaccine Virus Transmission**	Post-marketing experience suggests that transmission of vaccine virus may occur rarely between healthy vaccinees who develop a varicella-like rash and healthy susceptible contacts.	Vaccinees should attempt to avoid whenever possible close association with susceptible high-risk individuals (e.g. immunocompromized individuals) for up to 6 weeks following vaccination with *Varivax* (see US PI).
Non-IMP: Infanrix		
Important identified risk: **Hypersensitivity**	People may develop allergic reactions to any type of vaccine and/or its components. Hypersensitivity reactions to vaccines highly vary in severity, ranging from mild to potentially life-threatening hypersensitivity reactions. Severe allergic reactions to vaccines are rare but tend to occur within hours after vaccination.	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).
Important identified risk: **Temperature of ≥ 40.0° C**	In rare occasion, hyperpyrexia above 40.0°C may occur after vaccination, particularly associated with booster vaccination.	Any febrile illness constitutes a contraindication to administration of the vaccine at the time scheduled for vaccination (see Section 6.5). Prophylactic use of analgesic/antipyretic medications during the first 7 days after vaccination is allowed on a voluntary basis, but must be recorded in the source document and in the eCRF (see Section 6.7.1).
Important identified risk: Hypotonic-hyporesponsive episode (HHE)	HHE occurs more commonly in early childhood after primary vaccination and particularly after the first dose of vaccine. Most HHE events have been associated with whole cell pertussis-containing vaccines (36-250 episodes per 100,000 doses). A much lower rate of HHE has been observed following vaccination with acellular pertussis vaccine (4-140 episodes per 100,000 doses).	Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours of vaccination is a contraindication to further administration of *Infanrix* (see US PI).
Important identified risk: **Apnoea in infants born prematurely**	Apnoea of prematurity is due to immaturity of the neurological and respiratory system in premature infants. The risk appears to be related to the degree of prematurity of the infant.	Only subjects born full-term (i.e. after a gestation period of ≥ 38 weeks) will be enrolled (see Section 4.2).
Important identified risk: **Convulsions with or without fever, occurring within 3 days**	Seizures occurring soon after immunization are mostly triggered by fever induced by the vaccine or are not vaccine related. Febrile convulsions usually occur in individuals aged between 3 months and 6 years with a peak incidence at 18 months.	Close monitoring of all seizures, as reported in Section 7.1.6.1 Seizure will be monitored through the AESI collection.

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Important potential risk: **Encephalopathy**	Reports of encephalopathy were received with previous whole cell pertussis vaccines.	Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of administration of a previous dose of a pertussis - containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including *Infanrix* (see US PI).
Study procedures		
Risk of blood sampling	Blood sampling is associated with a risk of local reactions and infection after or during venipuncture.	Blood samples will be obtained by a trained professional and medical assistance will be available. The potential risk of experiencing mild local pain, bruising, irritation or redness at the site where blood was taken, is mentioned in the ICF. The amount of blood to be taken for sampling is within the allowed range for this age and will not be harmful to the health of the subject.

* information derived from the publicly available data.

Note 1: According to the label, events from clinical trials and post marketing experience of Prevnar and Prevnar 13 are applicable also to Prevnar 20.

1.3.2. Benefit Assessment

Benefits considerations include:

- Receiving GSK's meningococcal vaccine (rMenB+OMV NZ, *Bexsero*) during study duration may protect against meningococcal B disease. *Bexsero* is currently licensed in the US for use in individuals 10 through 25 years of age.
- Receiving *Prevnar 13* during the study may prevent occurrence of pneumococcal pneumonia and invasive disease caused by 13 *Streptococcus pneumoniae* strains (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F). ***For subjects who received PCV20 at visit 5, PCV20 may prevent occurrence of pneumococcal pneumonia and invasive disease caused by 20 Streptococcus pneumoniae strains (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F).***
- Receiving ACIP recommended vaccines (*Pediarix*, *Rotarix*, *Hiberix*, *M-M-R II, Varivax, Infanrix*) may protect against the diseases for which these vaccines are indicated for. Medical evaluations/assessments associated with this study (i.e. physical examination).

1.3.3. Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to subjects participating in this study, the potential or identified risks in association with the rMenB+OMV NZ, PCV13 ***(or PCV20),*** DTPa-HBV-IPV, HRV, Hib, MMR and VV vaccines are justified by the potential benefits (prevention) that may be afforded to subject(s) receiving these vaccines.

2. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary Safety	
• To assess the safety and tolerability of rMenB+OMV NZ, PCV13 and other RIV when administered concomitantly to healthy infants at 2, 4, 6 and 12 months of age, throughout the study duration.	• The percentages of subjects with solicited local Adverse events (AEs) ***(administration site event)*** and systemic AEs during the 7 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with solicited systemic AEs of parotid/salivary gland swelling, fever and rash during the 30 days (including the day of vaccination) after the 4th vaccination (Visit 5). • The percentages of subjects with all unsolicited AEs (including SAEs, AEs leading to withdrawal, AESIs, and medically attended AEs) during 30 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with SAEs, AEs leading to withdrawal, AESIs and medically attended AEs from study Day 1 (Visit 1) until study end (6 months or 12 months after last study vaccination, Visit 7¥).
Co-Primary Immunogenicity	
• To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4 and 6 months of age, at one month after the 3rd vaccination. *Criteria: The sufficiency of the immune response to rMenB+OMV NZ* at one month *after the 3rd vaccination will be demonstrated if the adjusted lower confidence limit for the percentage of subjects achieving serum bactericidal assay using human complement (hSBA) titers ≥ Lower Limit of Quantitation (LLOQ) is ≥ 60% for the N. meningitidis serogroup B test strains* M14459, 96217, NZ98/254, M13520 (individually); and is ≥ 50% for all strains combined (composite endpoint).	• At 1 month after the 3rd vaccination (Day 151): – the percentages of subjects with hSBA titers ≥LLOQ; for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the percentages of subjects with hSBA titers ≥LLOQ for all strains combined (composite endpoint).
• To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 4th vaccination. *Criteria: The sufficiency of the immune response to rMenB+OMV NZ will be demonstrated if the adjusted lower confidence limit for the percentage of subjects* with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) is ≥75% for the individual N. meningitidis serogroup B test strains and is ≥65% for all strains combined (composite endpoint).	• At 1 month after the 4th vaccination (Day 331): – the percentages of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) for each of the test strains. – the percentages of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) for all strains combined (composite endpoint).

Objectives	Endpoints
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants 2, 4 and 6 months of age, compared to PCV13 without rMenB+OMV NZ, at one month after the 3rd vaccination. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the adjusted lower confidence limit for the between-group ratio of electrochemiluminescence (ECL) assay GMCs is >0.5 for each of the 13 PCV13 antigens.*	• At 1 month after the 3rd vaccination (Day 151): – the ECL GMCs for each of the 13 PCV13 antigens.
Secondary Immunogenicity	
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants 2, 4, 6 and 12 months of age, compared to PCV13 and other RIV alone, at one month after the 4th vaccination. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of 2-sided 95% CI for the between-group-ratio of ECL assay GMCs is >0.5 for each of the 13 PCV13 antigens.*	• At one month after the 4th vaccination (Day 331): – The ECL GMCs for each of the 13 PCV13 antigens.
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants at 2, 4, 6 and 12 months of age compared to PCV13 and other RIV alone, at both one month after the 3rd and the 4th vaccinations. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of the 2-sided 95% CI for the group differences in percentage of subjects with IgG ≥ 0.35 µg/mL is >-10% for each of the 13 PCV13 antigens at one month after both 3rd and 4th vaccination.*	• At 1 month after the 3rd (Day 151) and the 4th vaccination (Day 331): – Percentages of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 µg/mL for each of the 13 PCV13 antigens.
• To demonstrate the immunological non-inferiority of DTaP-HBV-IPV and Hib vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy infants at 2, 4, and 6 months compared to DTaP-HBV-IPV and Hib vaccines concomitantly administered with PCV13 without rMenB+OMV NZ, in terms of D, T, PT, FHA, PRN, Hep B and Hib, at one month after the 3rd vaccination. *Criterion: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group differences is greater than the pre-specified margin† for each antigen at one month after 3rd vaccination.*	• At 1 month after the 3rd vaccination (Day 151): – GMCs against the 3 pertussis antigens (Pertussis toxin [PT], pertactin [PRN], filamentous hemagglutinin [FHA]). – Percentages of subjects with anti-HBs antibody concentrations ≥10 mIU/mL. – Percentages of subjects with anti-diphtheria and anti-tetanus concentrations ≥0.1 IU/mL. – Percentages of subjects with anti-PRP concentration ≥0.15 µg/mL and ≥1 µg/mL.
• To demonstrate the immunological non-inferiority of MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy subjects at 12 months compared to	• At 1 month after the 4th vaccination (Day 331): – GMCs against measles, mumps, rubella and VZV antigens.

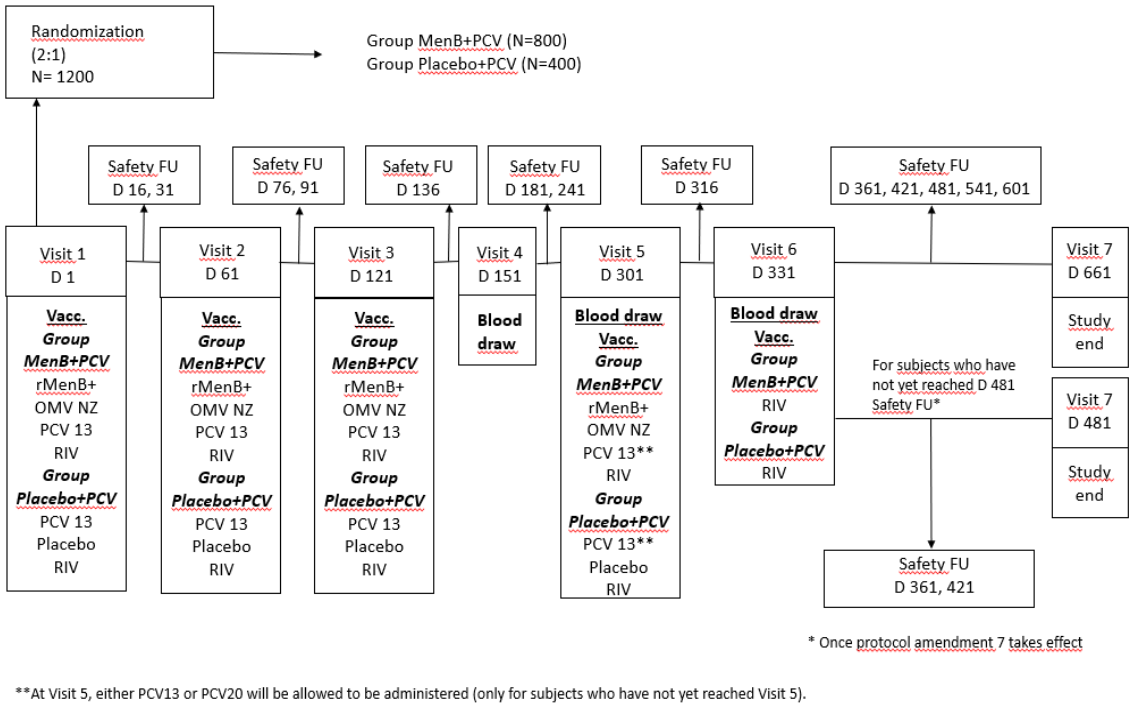
Objectives	Endpoints
MMR and VV vaccines concomitantly administered with PCV13, without rMenB+OMV NZ, at one month after vaccination. *Criterion: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group ratio of GMCs is >0.67 at one month after the MMR and VV vaccinations.*	
• To evaluate the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 3rd vaccination, at 6 months after the 3rd vaccination (immediately before the 4th vaccination), and at one month after the 4th vaccination against the M14459, 96217, NZ98/254 and M13520 test strains.	• At 1 month after the 3rd vaccination (Day 151) – the percentages of subjects with hSBA titers ≥5, ≥8 and ≥16 for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the hSBA geometric mean titers (GMTs) against each strain. • At 6 months after the 3rd vaccination (Day 301, immediately before the 4th vaccination): – the percentages of subjects with hSBA titers ≥LLOQ for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the percentages of subjects with hSBA titers ≥5 and ≥8 for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the hSBA GMTs against each strain. • At 1 month after the 4th vaccination (Day 331): – the percentages of subjects with hSBA titers ≥LLOQ for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the percentages of subjects with hSBA titers ≥5 for each of the M14459, 96217, NZ98/254 and M13520 test strains. – the hSBA GMTs and geometric mean ratios (GMRs) over pre-4th vaccination against each strain. – the percentages of subjects with 4-fold rise* in hSBA titers (from pre-4th vaccination) for each of the M14459, 96217, NZ98/254 and M13520 test strains.
• To evaluate immune responses to routine infant vaccines DTaP-HBV-IPV, Hib, MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 3rd vaccination, at 1 month after the 4th vaccination.	• At 1 month after the 3rd vaccination (Day 151): – Percentages of subjects with anti-HBs antibody concentrations ≥100 mIU/mL. – Anti-HBsAg GMCs. – Percentages of subjects with anti-diphtheria and anti-tetanus concentrations ≥1 IU/mL. – Anti-diphtheria and anti-tetanus antibody GMCs.

Objectives	Endpoints
	– Percentage of subjects with anti-polio type 1, 2 and 3 neutralization antibody titers ≥8. • At 1 month after the 4th vaccination (Day 331): – Seroresponse, defined as post-vaccination anti-VZV virus, anti-measles virus, anti-mumps virus and anti-rubella virus antibody concentration^ ≥ a protective threshold among subjects who were seronegative (antibody concentration <assay cut-off) before vaccination. ^ A suitable ELISA assay for analysis of anti-VZV, anti-measles virus, anti-mumps virus and anti-rubella virus antibody concentrations is yet to be selected and/or developed.

* A 4-fold rise in hSBA titers is defined as
- if pre-vaccination titer <Limit of Detection (LOD), then a post-vaccination titer ≥ 4 times the LOD or ≥ LLOQ, whichever is greater;
- if pre-vaccination titer is ≥LOD but <LLOQ, then a post-vaccination titer ≥4 times the LLOQ;
- if pre-vaccination titer is ≥LLOQ, then a post-vaccination titer ≥4 times the pre-vaccination titer,
where pre-vaccination titer=pre-4th dose titers (Day 301)
† The endpoints and thresholds used for evaluation of the non-inferiority criteria for DTaP-HBV-IPV and Hib vaccines can be found in Table 21 and the thresholds and endpoint for evaluating the non-inferiority criteria for MMR and VV vaccines can be found in Table 22.
¥ Visit 7 will occur either on Day 481 (for subjects who have not yet reached the 6-month follow-up after the last dose at the time protocol amendment 7 takes effect) or on Day 661 (for all other subjects).
Note 1: To control the risk of erroneously concluding, a hierarchical procedure will be used for the multiple confirmatory study objectives, with the possibility to conclude on the study success based on the results of the co-primary objectives alone (refer to Section 9.5.3 for details).
Note 2: In the context of this study, the following are considered as RIVs at different visits:
Visit 1 and Visit 2: DTPa-HBV-IPV, HRV, Hib;
Visit 3: DTPa-HBV-IPV, Hib;
Visit 5: MMR and VV.

3. STUDY DESIGN OVERVIEW

Figure 1 Overview of Study Design – V72_57



D: day; Vacc: vaccination, FU: follow-up call, RIV: routine infant vaccines (Visit 1 and Visit 2: DTPa-HBV-IPV, HRV, Hib; Visit 3: DTPa-HBV-IPV, Hib; Visit 5: MMR, VV)
Refer to Section 7.2.4 for study procedures to be considered during special circumstances.

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 5.5), are essential and required for study conduct. Refer to Section 7.2.4 for study procedures to be considered during special circumstances.

- **Experimental design**: Phase IIIB , observer-blind, randomized, placebo-controlled, multi-centric study with 2 parallel groups.
- **Duration of the study**:
  - Epoch 001 Primary starting at Visit 1 (Day 1) and ending at Visit 5 (Day 301).
  - Epoch 002: Secondary starting at Visit 5 (Day 301) and ending at Visit 6 (Day 331).
  - Epoch 003: Safety follow-up period starting at Visit 6 (Day 331) and ending at Visit 7 (Day 481 or Day 661). For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will take place on Day 481.
- **Primary completion Date (PCD)**: Visit 7 (Day 481 or Day 661).

Refer to glossary of terms for the definition of PCD.

- **End of Study (EoS)**: Date of the last testing/reading released of the Human Biological Samples (HBS) related to primary and secondary endpoints. Study completion must be achieved no later than 8 months after LSLV.

Refer to glossary of terms for the definition of EoS.

- **Study groups**:
  - Group MenB+PCV: rMenB+OMV NZ vaccine given concomitantly with PCV13 at 2, 4, 6, and 12 months of age. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective***. Subjects should also receive routine infant vaccines (DtaP-HBV-IPV**,** HRV, Hib, MMR, VV) at applicable timepoints.
  - Group Placebo+PCV: PCV 13 vaccine given concomitantly with Placebo at 2, 4, 6 and 12 months of age. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.*** Subjects should also receive routine infant vaccines (DtaP-HBV-IPV**,** HRV, Hib, MMR, VV) at applicable timepoints.

Table 1 Study Groups and Epochs Foreseen in the Study

Study Groups	Number of subjects	Age (Min – Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
MenB+PCV	800	6 weeks – 12 weeks	x	x	x
Placebo+PCV	400	6 weeks – 12 weeks	x	x	x

Table 2 Study Groups and Treatment Foreseen in the Study

Treatment name	Vaccine name	Study Groups	
		MenB+PCV	Placebo+PCV
Bexsero	rMenB+OMV NZ	X	-
Prevnar13	PCV13*	X	X
Prevnar 20	***PCV20****	***X***	***X***
Placebo	NaCl	-	X
Pediarix	DTPa-HBV-IPV	X	X
Rotarix	HRV	X	X
Hiberix	Hib	X	X
M-M-R II	MMR	X	X
Varivax	VV	X	X

Note: Subjects in both groups will receive a fourth dose of Hib vaccine (*Hiberix*) and a single dose of DTPa vaccine (*Infanrix*) as non-study vaccines at Visit 6.

**** Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.***

Table 3 Overview of the Study Design: Blood Draws and Study vaccines

Clinic Visits (Study Day)		Visit 1 (Day 1)	Visit 2 (Day 61)	Visit 3 (Day 121)	Visit 4 (Day 151)	Visit 5 (Day 301)	Visit 6 (Day 331)
Months of Age (MoA)		**~2 MoA[1]**	**4 MoA**	**6 MoA**	**7 MoA**	**12 MoA**	**13 MoA[3]**
Study Vaccines	Group MenB+PCV N=800	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	*Blood Draw[2]*	*Blood Draw[2]* rMenB+ OMV NZ PCV13[4]	*Blood Draw[2, 3]*
	Group Placebo+PCV N=400	Placebo PCV13	Placebo PCV13	Placebo PCV13		*Blood Draw[2]* Placebo PCV13[4]	
Routine Study Vaccines	**Both groups** N=1200	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV Hib		MMR VV	

MOA, months of age.
[1] Age at enrollment can be between 6 weeks to 12 weeks of age.
[2] Blood draw to be performed prior to any vaccination (study or non-study).
[3] Subjects in both groups will receive a fourth dose of Hib vaccine (*Hiberix*) and a single dose of DTPa vaccine (*Infanrix*) at 13 months of age, i.e. Visit 6 at Day 331. No immunogenicity or safety assessments will be performed following these Visit 6 vaccinations.
[4] Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.

Note: Study visits do not include safety follow-up calls. Although not conducted face-to-face, safety calls are intended for safety data collection. For details, refer to Table 5.

- **Control**: placebo control.
- **Vaccination schedule**: Visit 1 (Day 1), Visit 2 (Day 61) Visit 3 (Day 121), Visit 5 (Day 301).
- **Treatment allocation**: Subjects to be randomized in a 2:1 ratio at Visit 1 (Day 1) to Groups MenB+PCV and Group Placebo+PCV, respectively.

Refer to Section 5.2 for a detailed description of the randomization method.

- **Blinding**: observer-blind study.

Table 4 Blinding of Study Epochs

Study Epochs	Blinding
Epoch 001	Observer-blind
Epoch 002	Observer-blind
Epoch 003	Observer-blind

- **Sampling schedule**: The sampling schedule for both study groups is the same. Three blood samples of approximately 5 mL each are to be taken at:
  - Visit 4 (Day 151), i.e., 30 (-9 to +30) days after the 3rd vaccination.
  - Visit 5 (Day 301), i.e., 180 (-7 to 91)days after the 3rd vaccination (pre-4th vaccination).

  - Visit 6 (Day 331), i.e., 30 (-9 to +30) days after the 4th vaccination.

- **Type of study**: self-contained.
- **Data collection**: Standardized Electronic Case Report Form (eCRF). Solicited symptoms will be collected using a subject Diary (electronic Diary [eDiary]).

4. STUDY COHORT

4.1. Number of subjects/centers

A total of 1200 healthy subjects, 42 through 84 days of age (i.e. 6 through 12 weeks) will be enrolled in a 2:1 ratio. A detailed description of the criteria used in the determination of the sample size is provided in Section 9.1. The enrolment rate will be monitored using a study-specific central Randomization System on Internet (SBIR). The randomization algorithm will use a minimization procedure accounting for center.

For the analysis of the immune responses to the RIVs, subjects in each of the two study groups will be randomly assigned to different immunogenicity subsets. Further details are found in Section 5.2.3 and Section 5.7.

4.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Subjects' parent(s)/Legally Acceptable Representative(s) [LAR(s)] who, in the opinion of the investigator, can and will comply, with the requirements of the protocol (e.g. completion of the eDiary, return for follow-up visits).
- Written informed consent obtained from the parent(s)/LAR(s) of the subject prior to performing any study specific procedure.
- A male or female between, and including, 42 and 84 days of age (i.e., 6 through 12 weeks) at the time of the first vaccination.
- Healthy subjects as established by medical history and clinical examination before entering into the study.
- Born full-term (i.e. after a gestation period of ≥38 weeks).

4.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Child in care

  Please refer to the glossary of terms for the definition of child in care.

Each subject must not have:

- Progressive, unstable or uncontrolled clinical conditions.
- Hypersensitivity, including allergy to any component of vaccines, medicinal product or medical equipment whose use is foreseen in this study.
- Hypersensitivity to latex.
- Clinical conditions representing a contraindication to intramuscular vaccination and blood draws.
- Abnormal function of the immune system resulting from:
  - Clinical conditions.
  - Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days from birth.
  - Administration of antineoplastic and immunomodulating agents or radiotherapy for any duration from birth.
  - autoimmune disorders (including, but not limited to: blood, endocrine, hepatic, muscular, nervous system or skin autoimmune disorders; lupus erythematosus and associated conditions; rheumatoid arthritis and associated conditions; scleroderma and associated disorders) or immunodeficiency syndromes (including, but not limited to: acquired immunodeficiency syndromes and primary immunodeficiency syndromes).
- Received immunoglobulins or any blood products from birth.
- Received an investigational or non-registered medicinal product from birth.
- Any other clinical condition that, in the opinion of the investigator, might pose additional risk to the subject due to participation in the study.
- Neuroinflammatory disorders (including but not limited to: demyelinating disorders, encephalitis or myelitis of any origin), congenital and peripartum neurological conditions, encephalopathies, seizures (including all subtypes such as: absence seizures, generalized tonic-clonic seizures, partial complex seizures, partial simple seizures or febrile convulsions).
- Congenital or peripartum disorders resulting in a chronic condition (including but not limited to: chromosomal abnormalities, cerebral palsy, metabolism or synthesis disorders, cardiac disorders).
- Study personnel as an immediate family or household member.
- Current or previous, confirmed or suspected disease caused by *N. meningitidis*

- Household contact with and/or intimate exposure from birth to an individual with laboratory confirmed *N. meningitidis* and/or *Streptococcus pneumoniae* infection or colonization.
- Previous administration of meningococcal B or pneumococcal vaccine at any time prior to informed consent.
- Received a dose of DTPa-HBV-IPV , HRV, MMR, VV and/or Hib at any time prior to informed consent. Receipt of one dose of HBV up to 4 weeks prior to informed consent is allowed.
- Serious chronic illness.
- Uncorrected congenital malformation (such as Meckel's diverticulum) of the gastrointestinal tract that would predispose for Intussusception (IS).

Prior to receipt of each study vaccination, subjects must be evaluated to confirm that they are eligible for subsequent vaccination. If subjects meet any of the original exclusion criteria listed above (with the exception of the criterion regarding previous administration of meningococcal B, pneumococcal or any of the other RIV vaccines), they should not receive additional study vaccinations. Similarly, certain events may lead to a delay in vaccination (see Section 6.5) or blood sampling (see Section 5.6.11.1). Eligibility to each study vaccination should be documented in the source documents.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for GCP, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

The study has been designed and will be conducted in accordance with the ICH Harmonized Tripartite Guideline for clinical investigation of medicinal products in the paediatric population (ICH E11) and all other applicable ethical guidelines.

GSK will obtain favorable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favorable opinion/approval of study protocol and any subsequent amendments.
- Subject's parent(s)/LAR(s) informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Properly constituted IRB/EC is defined in ICH Guideline for GCP E6 (R2), Section 3 [ICH, 2018].

Freely given and written or witnessed/ thumb printed informed consent must be obtained from each subject's parent(s)/LAR(s), as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC. A copy of the approved version must be provided to the GSK monitor after IRB/EC approval.

This review and approval will be documented and stored with other study documents. The investigator or designee must fully inform the subject's parent(s)/LAR(s) of all pertinent aspects of the study. A copy of the written informed consent will be given to the subjects' parent(s)/LAR(s) or the designee. The subject/designee must be allowed ample time to ask about the details of the study and to make a decision as to whether or not to participate in the study. The subject's parent(s)/LAR(s) must sign the consent form indicating their agreement to participate in the study before any study-related procedures are conducted. The informed consent process may be conducted up to 5 days prior to vaccination on Day 1. If the subject's parent(s)/ legal guardian(s) is unable to read and write, a witness must be present during the informed consent discussion and at the time of informed consent signature.

Potential subjects' parent(s)/LAR(s) or the designee must be informed that their participation is voluntary. They will be required to physically or digitally sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, HIPAA requirements, privacy and data protection requirements, where applicable, and the IRB/IEC or study center.

5.1.1. Responsibilities of the Investigator

Prior to study start and at any time the protocol is amended during study conduct, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to GSK monitors, auditors, GSK Clinical Quality Assurance representatives, designated agents of GSK, IRBs/Ecs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform GSK immediately that this request has been made.

In addition to what is stated in the Investigator Agreement, the investigator is also responsible for the following:

- Maintaining a list of appropriately qualified persons to whom the investigator has delegated significant study-related duties.
- Demonstrating the capability of recruiting the required number of suitable subjects within the recruitment period.
- Demonstrating sufficient time and staffing to properly conduct and complete the study within the agreed study period.
- Ensuring that qualified trained health care professionals (see glossary of terms) are responsible for all study-related medical decisions and for ensuring appropriate medical care of subjects experiencing any adverse event related to the study.
- If permission to do so is given by the subject's parent(s)/LAR(s), ensuring that the subject's primary healthcare provider is informed of the subject's participation in the study.

The investigator should not implement any deviation from, or changes of the protocol without agreement by the Sponsor and prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects, or when the change(s) involves only logistical or administrative aspects of the study (e.g., change in monitor(s), change of telephone number(s)). In addition, the investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to study subjects without prior IRB/IEC approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted within 10 working days:

1. to the IRB/IEC for review and approval/favorable opinion,
2. to the Sponsor for agreement and, if required,
3. to the regulatory authority(ies).

5.1.2. Protocol Amendments

An amendment is a written description of change(s) to or formal clarification of a study protocol which may impact on the conduct of the clinical study, potential benefit of the clinical study, or may affect subject safety, including changes of study objectives, study design, subject population, sample sizes, study procedures, or significant administrative aspects. An administrative change of a study protocol is a minor correction or clarification that has no significant impact on the way the clinical study is to be conducted and no effect on subject safety (e.g., change of telephone number(s), logistical changes). Protocol amendments must be approved by GSK, health authorities where required, and the IRB/EC.

5.2. Subject identification and randomization

5.2.1. Subject identification

Subject identification numbers will be assigned sequentially to the subjects who have consented to participate in the study, according to the range of subject identification numbers allocated to each study center. The Subject ID will be documented in the Screening and Enrolment log. The eligibility of the subject will be determined based on the inclusion and exclusion criteria listed in Section 4 and evaluated during this screening procedure. The Subject ID will be the subject's unique identification number for all eCRFs and associated study documentation that will be used for duration of the study.

5.2.2. Randomization of treatment

5.2.2.1. Randomization of supplies

The randomization of supplies within blocks will be performed at GSK Biologicals, using MATerial Excellence (MATEX), a program developed for use in SAS (Cary, NC, USA) by GSK (Rixensart, Belgium).

To allow GSK Biologicals to take advantage of greater rates of recruitment than anticipated at individual centers in this multi-center study and to thus reduce the overall study recruitment period, an over-randomization of supplies will be prepared. Further details are provided in the Study Procedures Manual (SPM).

5.2.2.2. Treatment allocation to the subject

The treatment numbers will be allocated by dose for Bexsero and placebo vaccines. Treatment allocation will be by components for all other products.

5.2.2.2.1. Study group and treatment number allocation

The target will be to enroll approximately 1200 eligible subjects who will be randomly assigned to 2 study groups in a 2:1 ratio.

Allocation of the subject to a study group at the investigator site will be performed using the Source Data Base for Internet Randomization system (SBIR). The randomization algorithm will use a minimization procedure accounting for center.

After obtaining the signed and dated ICF from the subject's parent/ LAR and having checked the eligibility of the subject, the site staff will access SBIR. Upon providing the subject identification number, the randomization system will determine the study group and will provide the treatment number to be used for each dose.

Subjects will be randomized in the SBIR system to one of the two groups in a 2:1 ratio to receive:

- Group MenB+PCV: rMenB+OMV NZ given concomitantly with PCV13 at 2, 4, 6 and 12 months of age, along with other RIV. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.***
- Group Placebo+PCV: PCV13 and Placebo given concomitantly at 2, 4, 6 and 12 months of age, along with other RIV. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.***

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

When SBIR is not available, please refer to the SBIR user guide or the Study Procedures Manual (SPM) for specific instructions.

Note: In case of supplies shortage for the next assigned vaccine according to the randomization schedule at the clinical site, the SBIR system will use the forced randomization procedure in order to continue to enroll and vaccinate subjects. The system moves seamlessly to the next treatment in the minimization algorithm arm for which the vaccine supplies are available. The site will not be aware of the forced randomization event.

5.2.2.2.2. Treatment number allocation for subsequent doses

For each dose subsequent to the first dose, the study staff will access SBIR, provide the subject identification number, and the system will provide a treatment number consistent with the allocated study group.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

5.2.3. Allocation of subjects to assay subsets

For the analysis of the immune responses to the RIV antigens, subjects in each of the 2 study groups will be randomly assigned to different immunogenicity subsets. The assignment of the immunogenicity subsets will be performed by a second randomization, whereas the first randomization will be performed at the enrolment to assign subjects to Group MenB+PCV or Group Placebo+PCV.

Group MenB+PCV: All 800 subjects will be randomly assigned into one of the 3 immunogenicity subsets:

- Subset A1: 400 subjects
- Subset A2: 200 subjects
- Subset A3: 200 subjects

Group Placebo+PCV: A total of 400 subjects will be randomly assigned into 2 immunogenicity subsets:

- Subset B1: 200 subjects
- Subset B2: 200 subjects

The different subsets against which the different antigens will be tested are described in Table 9. Testing of diphtheria, tetanus, pertussis, polio, HepB and Hib antigens will be performed at Visit 4 (post-3rd vaccination). Testing of MMR and V*V* will be done at Visit 5 (pre-4th vaccination) and Visit 6 (post-4th vaccination). In addition, subjects from subset B1 (200 subjects) will also be tested against the 4 serogroup B strains at all 3 timepoints in order to maintain the observer-blindness of the study during the blood sample testing.

5.3. Method of blinding

This trial is designed as an observer-blind study.

Data will be collected in an observer-blind manner. By observer-blind, it is meant that during the course of the study, the vaccines recipient and those responsible for the evaluation of any study endpoint (e.g. safety and reactogenicity) will all be unaware of which vaccine was administered. To do so, vaccine preparation and administration will be done by authorized medical personnel who will not participate in any of the study clinical evaluation assays or procedures.

Each study site is responsible for having a blinding plan. To work in an observer-blind manner, vaccine preparation and administration will be done by authorized medical personnel who will not participate in any of the study clinical evaluation assays or procedures. See the glossary of terms for definition of unblinded study staff. Two teams of study personnel will hence be set up:

- A team of unblinded personnel (responsible for the preparation and the administration of the vaccines).

- A team of blinded personnel (responsible for the clinical evaluation of the subjects including blood sampling).

Refer to the SPM for guidance on vaccine preparation and administration while maintaining the blind. The serological data, which would lead to the unblinding of the study groups, will not be available during the course of the study to any investigator or any person involved in the clinical conduct of the study (including data cleaning).

The laboratory in charge of the laboratory testing will be blinded to the treatment as well as to the subject number. In addition, a different subject code will be used for each timepoint tested. This subject coding will prevent the laboratory from linking the consecutive visits to a specific subject.

The serological data, which would lead to the unblinding of the study groups, will not be available during the course of the study to any investigator or any person involved in the clinical conduct of the study (including data cleaning).

5.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

The staff participating in this study conducted as multicenter trial will be trained in a uniform fashion and sites will be monitored to ensure consistency in study execution across all centers.

No Data Monitoring Committee will be convened for this trial.

5.5. Outline of study procedures

An outline of the different study procedures to be performed are listed in Table 5.

Table 5 List of Study Procedures

Age (approximate)	~2 moa			4 moa			6 moa		7 moa			12 moa		13 moa						24 moa
Epoch	Epoch 001											Epoch 002			Epoch 003					
Type of contact	Visit 1	SFC 1	SFC 2	Visit 2	SFC 3	SFC 4	Visit 3	SFC 5	Visit 4	SFC 6	SFC 7	Visit 5	SFC 8	Visit 6	SFC 9	SFC 10	SFC 11	*Visit 7*[10]	SFC 12	SFC 13	Visit 7[11]
Timepoints	Day 1	Day 16	Day 31	Day 61	Day 76	Day 91	Day 121	Day 136	Day 151	Day 181	Day 241	Day 301	Day 316	Day 331	Day 361	Day 421	Day 481		Day 541	Day 601	Day 661
Days post-vaccination [1]	0	15 days post-vacc 1	30 days post-vacc 1	60 days post-vac 1	15 days post vacc 2	30 days post vacc 2	60 days post-vacc 2	15 days post-vacc 3	30 days post-vacc 3	60 days post-vacc 3	120 days post-vacc 3	180 days post-vacc 3	15 days post-vacc 4	30 days post-vacc 4	60 days post-vacc 4	120 days post-vacc 4	180 days post-vacc 4		240 days post-vacc 4	300 days post-vacc 4	360 days post-vacc 4
Visit window (days) [2]	-5	-3/+3	-3/+3	-31/+21	-3/+3	-3/+3	-31/+21	-3/+3	-9/+30	-3/+3	-3/+3	-7/+91	-3/+3	-9/+30	-7/+21	-7/+21	-7/+21		-7/+21	-7/+21	-7/+21
Sampling timepoints									Post-vacc 3			Pre-vacc 4		Post-vacc 4				-			-
Informed consent [3]	●																				
Check inclusion/exclusion criteria	●			O			O					O									
Medical history	●																				
History directed physical examination	O																				
Symptom directed physical examination				O			O		O			O		O				O			O
Check contraindications and warnings and precautions to vaccination	O			O			O					O									
Pre-vaccination body temperature	●			●			●					●									
Study group and/or treatment number allocation	●																				

Age (approximate)	**~2 moa**			**4 moa**			**6 moa**		**7 moa**			**12 moa**		**13 moa**							**24 moa**
Epoch	**Epoch 001**											**Epoch 002**			**Epoch 003**						
Type of contact	**Visit 1**	**SFC 1**	**SFC 2**	**Visit 2**	**SFC 3**	**SFC 4**	**Visit 3**	**SFC 5**	**Visit 4**	**SFC 6**	**SFC 7**	**Visit 5**	**SFC 8**	**Visit 6**	**SFC 9**	**SFC 10**	**SFC 11**	***Visit 7[10]***	**SFC 12**	**SFC 13**	**Visit 7[11]**
Timepoints	**Day 1**	**Day 16**	**Day 31**	**Day 61**	**Day 76**	**Day 91**	**Day 121**	**Day 136**	**Day 151**	**Day 181**	**Day 241**	**Day 301**	**Day 316**	**Day 331**	**Day 361**	**Day 421**	**Day 481**		**Day 541**	**Day 601**	**Day 661**
Days post-vaccination [1]	**0**	**15 days post-vacc 1**	**30 days post-vacc 1**	**60 days post-vac 1**	**15 days post vacc 2**	**30 days post vacc 2**	**60 days post-vacc 2**	**15 days post-vacc 3**	**30 days post-vacc 3**	**60 days post-vacc 3**	**120 days post-vacc 3**	**180 days post-vacc 3**	**15 days post-vacc 4**	**30 days post-vacc 4**	**60 days post-vacc 4**	**120 days post-vacc 4**	**180 days post-vacc 4**		**240 days post-vacc 4**	**300 days post-vacc 4**	**360 days post-vacc 4**
Visit window (days) [2]	**-5**	**-3/+3**	**-3/+3**	**-31/+21**	**-3/+3**	**-3/+3**	**-31/+21**	**-3/+3**	**-9/+30**	**-3/+3**	**-3/+3**	**-7/+91**	**-3/+3**	**-9/+30**	**-7/+21**	**-7/+21**	**-7/+21**		**-7/+21**	**-7/+21**	**-7/+21**
Sampling timepoints									**Post-vacc 3**			**Pre-vacc 4**		**Post-vacc 4**				**-**			**-**
Treatment number allocation for subsequent doses				●			●					●									
Vaccines administration [4]	●			●			●					●*[13]*		● [5]							
Blood sampling (~5 mL) [6]									●			●		●							
Record any concomitant medications/vaccinations administered for treatment of an AE	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Training of eDiary and distribution	●			O			O					●									
Review of eDiary				O			O		O					O							
Return of eDiary									O					O							
30 Minutes post-injection assessment (including body temperature measurement)	●			●			●					●									

Age (approximate)	**~2 moa**			**4 moa**			**6 moa**		**7 moa**			**12 moa**		**13 moa**							**24 moa**
Epoch	**Epoch 001**											**Epoch 002**			**Epoch 003**						
Type of contact	**Visit 1**	**SFC 1**	**SFC 2**	**Visit 2**	**SFC 3**	**SFC 4**	**Visit 3**	**SFC 5**	**Visit 4**	**SFC 6**	**SFC 7**	**Visit 5**	**SFC 8**	**Visit 6**	**SFC 9**	**SFC 10**	**SFC 11**	***Visit 7*[10]**	**SFC 12**	**SFC 13**	**Visit 7[11]**
Timepoints	**Day 1**	**Day 16**	**Day 31**	**Day 61**	**Day 76**	**Day 91**	**Day 121**	**Day 136**	**Day 151**	**Day 181**	**Day 241**	**Day 301**	**Day 316**	**Day 331**	**Day 361**	**Day 421**	**Day 481**		**Day 541**	**Day 601**	**Day 661**
Days post-vaccination [1]	**0**	**15 days post-vacc 1**	**30 days post-vacc 1**	**60 days post-vac 1**	**15 days post vacc 2**	**30 days post vacc 2**	**60 days post-vacc 2**	**15 days post-vacc 3**	**30 days post-vacc 3**	**60 days post-vacc 3**	**120 days post-vacc 3**	**180 days post-vacc 3**	**15 days post-vacc 4**	**30 days post-vacc 4**	**60 days post-vacc 4**	**120 days post-vacc 4**	**180 days post-vacc 4**		**240 days post-vacc 4**	**300 days post-vacc 4**	**360 days post-vacc 4**
Visit window (days) [2]	**-5**	**-3/+3**	**-3/+3**	**-31/+21**	**-3/+3**	**-3/+3**	**-31/+21**	**-3/+3**	**-9/+30**	**-3/+3**	**-3/+3**	**-7/+91**	**-3/+3**	**-9/+30**	**-7/+21**	**-7/+21**	**-7/+21**		**-7/+21**	**-7/+21**	**-7/+21**
Sampling timepoints									**Post-vacc 3**			**Pre-vacc 4**		**Post-vacc 4**				**-**			**-**
Recording of solicited local ***(administration site)*** and systemic AEs (Days 1–7 post vaccination) in eDiary [9]	O			O			O					O									
Recording of selected solicited systemic AEs of parotid/salivary gland swelling, fever and rash (Days 1–30 post vaccination) in eDiary												O	O	O							
Recording of all unsolicited AEs within 30 days post-vaccination[12]	●	●	●	●	●	●	●	●	●			●	●	●							
Recording of SAEs, medically attended AEs, AEs leading to withdrawal and AESIs [7, 12]	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Study Conclusion [8]																		●			●

AE: adverse event, Moa: months of age, Pre-vacc: pre-vaccination, Post-vacc: post-vaccination, SFC: safety follow-up call, SAE: serious adverse event.
● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
Note: Procedures to be performed prior to any vaccination.
Although not conducted face-to-face, safety follow-up calls are intended for safety data collection and considered equivalent to clinic study visits.
The double line after Visit 6 at Day 331 indicates the potential interim analysis time period.
[1]. Days post injection are included to keep track of the days intercurring between vaccinations, they do not indicate 'Study Day'.
[2]. All visits and visit windows should be planned using the day of last vaccination as the reference point.
[3]. Confirm consent form(s) signed prior to any procedures. Pre-vaccination clinic visit can occur within 5 days prior to up to the time of the vaccination Clinic Visit on Day 1 (inclusive). Procedures performed include obtaining informed consent from subject's parent(s)/legal guardian(s), screening, enrolment and randomization.
[4]. Subjects will receive study and concomitant vaccinations according to the study group they are randomized. See Section 6 for further details.
[5]. Administration of non-IMP vaccines DTPa and fourth dose of Hib to all subjects at 13 months of age (Visit 6 Day 331). See Section 6.1.2 for further details.
[6]. Blood sampling at all timepoints should be done prior to administration of any study or non-study vaccines.
[7]. Includes recording of all SAEs related to study participation, or to a concurrent GSK medication/vaccine.
[8]. Subjects who terminate the study early are recommended to complete certain study-related procedures. See Section 5.6.15 for further details.
[9]. Solicited AEs that are ongoing after the 7-day reporting period will continue to be recorded in the eDiary until resolution or day 30, whichever occurs first.
[10] For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481.
[11]. Visit 7 may be a telephone contact during special circumstances. Refer to Section 5.6.15 for study procedures to be considered during special circumstances.
[12].COVID-19 infection related AEs and SAEs should be recorded in a separate eCRF.
[13] Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time the protocol amendment 8 becomes effective.

The investigator should arrange study visits within the interval described in Table 6.

Table 6 Intervals Between Study Visits

Interval	Optimal length of interval	Allowed interval[1, 2]
Visit 1 →Visit 2	60 days	*29* days – 81 days
Visit 2 → Visit 3	60 days	*29* days – 81 days
Visit 3 →Visit 4	30 days	*21* days – *60* days
Visit 3 → Visit 5	180 days	173 days – ***201*** days
Visit 5 → Visit 6	30 days	*21* days – *60* days
Visit 5 → Visit 7 (Study termination)[3]	180 days	173 days – 201 days
Visit 5→ Visit 7 (Study termination)	360 days	353 days – 381 days

[1]The investigator should arrange study visits within this interval as subjects may not be eligible for inclusion in the PPS cohorts if they make the study visit outside this interval. All visits and windows should be planned using the day of last vaccination as the reference point

[2]The allowed interval have been modified in keeping with ACIP guidelines to facilitate the safe scheduling of study visits during the COVID-19 pandemic situation.

[3] For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481.

Refer to Table 6 for intervals between study visits that determine subjects' eligibility for inclusion in the per-protocol analysis.

5.6. Detailed description of study procedures

The sections that follow provide an overview of the procedures that are to be followed in enrolling, evaluating, and following subjects who participate in this clinical study. An outline is provided in Table 5. An overview of the data collected during the course of the study is given in Section 5.6.1.

During special circumstances, exemplified by the COVID-19 pandemic, certain study procedures may be adapted to protect the subject and promote data integrity. Refer to Section 5.6.15 for further details.

5.6.1. Data collected from subjects

The following data will be collected from each subject over the duration of their study participation:

- Demographic information
- Medical history
- Vaccination history
- Prior and concomitant medication, as defined in Section 6.7.
- Pre-vaccination body temperature
- 30 minutes post-vaccination immediate reactions: signs or symptoms of anaphylaxis, allergic phenomena (such as rashes, itching, or other allergic manifestations). Solicited Local ***(administration site)*** and systemic data (including e.g., use of medication to treat or prevent fever and/or pain, body temperature measurement).

- Adverse Events (AEs):
  - Post-vaccination solicited local ***(administration site)*** and systemic AEs (including related information such as use of medication to treat or prevent fever), collected at home by subjects' parent(s)/LAR(s), and recorded on Subject eDiary for 7 days following each vaccination visit (including the day of vaccination) or until they resolve or Day 30 (whichever occurs first). Specific systemic AEs (parotid/salivary gland swelling, fever and rash) will be collected for 30 days (including the day of vaccination) following the MMR and VV vaccinations at Visit 5.
  - Unsolicited AEs occurring within 30 days after each vaccination will be collected by interviewing the subject's parent(s)/legal guardian(s) and by review of available medical records at the next visit.
  - SAEs will be collected from study Day 1 until study end, inclusive.
  - Medically attended AEs (including any medical visit) will be collected from study Day 1 until study end, inclusive.
  - AEs leading to premature withdrawal from the study will be collected from study Day 1 until study end, inclusive.
  - AESIs (pIMDs, seizures, and arthritis) will be collected from study Day 1 until study end, inclusive.

All data collected must only be identified using the GSK Subject ID, as described in Section 5.2.

5.6.2. Pre-vaccination procedures (Visit 1)

A pre-vaccination clinic visit may occur within 5 days prior and up to the time of the vaccination Visit 1 on Day 1 (inclusive). The following procedures will be performed for each potential subject prior to the first vaccination at Visit 1:

- Obtaining informed consent from subject's parent(s)/LAR(s).
- Subject ID assignment
- Screening the subject for eligibility based in the inclusion and exclusion criteria listed in Section 4. This includes all the following procedures: collecting demography and medical history, review of systems, collecting prior and concomitant medications (including vaccination history), and general physical examination by a qualified health care practitioner
- Subject enrolment.
- Subject randomization.

If a pre-vaccination clinic visit was performed, the investigator will review and confirm subject eligibility on Day 1 (Visit 1) prior to first vaccination. Unless deemed necessary, procedures performed during pre-vaccination clinic visit should not be repeated on Day 1 (Visit 1). If a pre-vaccination clinic visit did not occur, all procedures mentioned here will be performed at Visit 1 instead.

5.6.3. Informed consent

The signed/witnessed/thumb printed informed consent of the subject's parent(s)/LAR(s) must be obtained before study participation. Refer to Section 5.1 for the requirements on how to obtain informed consent as appropriate.

5.6.4. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 4.2 and 4.3 after obtaining informed consent and before randomization.

5.6.5. Collect demographic data

At Visit 1, record demographic data such as date of birth, gender, race and ethnic origin, weight and length in the subject's eCRF.

Collection of sex, race and ethnicity data is necessary to assess and monitor the diversity of the trial participants, and to determine if the trial participants are truly representative of the impacted population.

5.6.6. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination in the eCRF.

Medical history will be collected at Visit 1 (or pre-visit 1, if applicable), including but not limited to any medical history that may be relevant to subject eligibility for study participation such as prior vaccinations, concomitant medications (refer to Section 5.6.13), and previous and ongoing illnesses or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an AE that occurs during study participation, if it represents an exacerbation of an underlying disease/pre-existing problem.

Review of systems is a structured interview that queries the subjects' parent(s)/legal guardian(s) as to any complaints the subject has experienced across each organ system. This will be performed before enrolment and used to guide physical examination. A general physical examination is to be performed by a qualified health care professional. "Qualified health care professional" refers to any licensed or certified health care professional who is permitted by institutional policy to perform physical examinations and who is identified within the Study Staff Signature Log. The physical examination will include a check of general appearance, the measurement of vital signs (rectal body temperature preferable [as subjects are <12 months of age], and heart rate), auscultation of heart and lungs, measurement of length and weight. The medical history-directed exam of other body parts and systems to assess eligibility will be performed during Visit 1.

5.6.7. Physical examination

At Visits 2 through 7, a brief symptom-directed physical examination is to be performed if necessary according to symptoms the subject's parent(s)/LAR(s) has reported. This is a physical examination that will include an examination of organ systems that are relevant to the investigator based only on review of the subject's reported adverse events and concomitant medication use. This assessment may include measurement of heart rate and temperature.

These data will be written in the source document. Should the physical assessment reveal any abnormal values or events, these must be documented in the eCRF Adverse Events Form.

Treatment of any abnormality observed during this examination should be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

5.6.8. Check contraindications, warnings and precautions to vaccination

Contraindications, warnings and precautions to vaccination must be checked at the beginning of each vaccination visit. Refer to Sections 6.5 and 6.6 for more details.

5.6.9. Assess pre-vaccination body temperature

The body temperature of each subject needs to be measured prior to any study vaccines administration. If the subject has fever [fever is defined as a body temperature ≥38.0°C/100.4°F regardless the location of measurement on the day of vaccination], the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 6 and Section 6.5).

5.6.10. Study group and treatment number allocation

Study group and treatment number allocation will be performed as described in Section 5.2.2. The number of each administered treatment must be recorded in the eCRF.

5.6.11. Sampling

The following clinical specimen is required to be collected from each subject in this study:

- Blood samples for serologic evaluation.

Refer to the Module on Biospecimen Management in the SPM and the Central Laboratory Investigator Manual for detailed instructions for the collection, handling and processing of the samples.

5.6.11.1. Blood sampling for immune response assessments

Blood samples will be obtained from each enrolled subject in both study groups at 3 study visits as follows:

- Visit 4 at Day 151, i.e., 30 (-9 to +30) days after the 3rd vaccination.
- Visit 5 at Day 301, i.e., 180 (-7 to +91) days after the 3rd vaccination (pre-4th vaccination).
- Visit 6 at Day 331 i.e., 30 (-9 to +30) days after the 4th vaccination.

In order to support all serological assay testing, a volume of approximately 5 mL of whole blood (to provide approximately 2 mL of serum) should be drawn from each subject at each pre-defined timepoint mentioned above, resulting in a total of approximately 15 mL over the entire study period. After centrifugation, serum samples should be kept at –20°C/ –4°F or below until shipment. Refer to the SPM and Central Laboratory Investigator Manual for more details on sample storage conditions.

A topical anesthetic (e.g. EMLA adhesives or cream) may be used at the site of blood sample draw, according to local practice, in order to minimize pain.

The blood will be used for immunological assays, as described in Section 5.7.4.

In clinic visits where a blood draw and a vaccination are planned, ensure all serology samples are taken **prior** to vaccination. The following are clinical circumstances that warrant delay of blood samples collection for immunogenicity assessments in this study:

- Receipt of systemic antibiotics within the previous 3 days (72 hours) before blood sample collection

In the event that a subject meets the criterion for delay of blood samples collection, blood samples collection may proceed once the appropriate window for delay has passed.

5.6.12. Study vaccines administration

- Study vaccination will be performed on Day 1 (Visit 1), Day 61 (Visit 2), Day 121 (Visit 3), and Day 301 (Visit 5).
- Prior to administration of each vaccination, confirm that the subject is eligible for additional study vaccinations and does not meet any criteria for delaying additional study vaccinations as described in Section 4 and Section 6.5.
- Prior to the first vaccination (Visit 1) perform a general physical examination. Prior to the second, third and fourth vaccination (Visit 2, Visit 3 and Visit 5) perform a brief symptom-directed physical examination if necessary according to symptoms the subject's parent(s)/LAR(s) has reported (see Section 5.6.5 and Section 5.6.7).
- After completing all prerequisite procedures prior to vaccination, one dose of study vaccines will be administered intramuscularly (IM) or subcutaneously (SC) as described in Section 6.3. If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccines administration,

the visit will be rescheduled within the allowed interval for this visit (refer to Table 6).

- Prophylactic administration of antipyretics at the time of and closely after vaccination is advised, as this can reduce post-vaccination febrile reactions. This may be initiated according to the investigator's judgment. (See Section 6.5).
- The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis.

For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available [CDC, 2015b]

5.6.13. Post-vaccination procedures

The following post-vaccination procedures for each subject will be performed on Day 1 (Visit 1), Day 61 (Visit 2), Day 121 (Visit 3), and Day 301 (Visit 5). After vaccination, the subject will be observed for at least 30 minutes including observation for solicited and unsolicited AEs, including measurement of body temperature. Record all safety data collected during this time in the subject's source document and eCRF.

The site should schedule the next study activity clinic visit or safety follow-up call with the subject's parent(s)/LAR (s). The subject's parent(s)/LAR(s) should be reminded of the next planned study activity. Instruction regarding recording of safety data will be provided by the site based on the visit script

In addition, the post-vaccination procedures related to the reporting of solicited AEs will be performed on Day 1 (Visit 1), Day 61 (Visit 2), Day 121 (Visit 3), and Day 301 (Visit 5). The eDiary will be dispensed at Day 1 (Visit1) to subjects and the subject's parent/LAR will bring the eDiary at each clinic visit. The eDiary will be collected by site staff at Day 151 (Visit 4). A new eDiary will be provided to the subjects again at Day 301 (Visit 5) and collected by the site staff at Day 331(Visit 6). A script will be provided to site staff monitoring safety to advise the subjects parent(s)/LAR(s) on collecting and reporting of solicited safety data.

At each vaccination visit, the vaccine injection sites(s) will be stored in the eDiary.

The eDiary is the only source for collection of solicited local ***(administration site)*** and systemic AEs starting after the initial 30 minutes post-vaccination; therefore**, it is critical that the subject's parent(s)/LAR(s) complete the Subject Diary correctly**. The subject's parent(s)/LAR(s) will be instructed on how and when to complete each field of the Subject Diary (see Section 5.6.16.1).

The subject's parent(s)/LAR(s) should be instructed how to perform body temperature measurement (preferably rectal for subjects <12 months of age and the axilla for subjects ≥12 months of age) using the thermometer provided by the site. If the subject is unusually hot or cold during the day, the subject's parent(s)/LAR(s) should check body temperature. If the subject has fever, the highest body temperature observed that day

should be recorded in the eDiary. Temperature measurements should be taken prior to the administration of any antipyretic medication.

The subject's parent(s)/LAR(s) will be instructed to contact study personnel if a subject experiences temperature >39.5 °C (>103° F) during the 7-day post-vaccination period, in order to assess the extent of the event.

Further details regarding the use of the eDiary are provided in Section 5.6.16.1.

5.6.14. Check and record concomitant medication/vaccination and intercurrent medical conditions

Concomitant medication/vaccination must be checked and recorded in the eCRF as described in Section 6.7.

Intercurrent medical conditions must be checked and recorded in the eCRF as described in Section 6.8.

5.6.14.1. Follow-up safety clinic visit

Follow-up clinic visits will be performed in the clinic on Day 151 (Visit 4) and on Day 331 (Visit 6).

The subject's parent(s)/legal guardian(s) will be interviewed to determine if any unsolicited AEs occurred and if any concomitant medications or vaccines were taken/ received in the time since the last clinic visit. The qualified healthcare professional reviewing these data will discuss the symptoms (if any) reported by the subject and will determine if any additional diagnoses and/or AEs are present. Adverse events reported by the subject's parent(s)/LAR(s) at this follow-up clinic visit must be recorded in the subject's source document and on an Adverse Events eCRF, as specified Section 7.

A symptom-directed physical examination will be performed if necessary according to symptoms reported by the subject's parent(s)/LAR(s). This is a physical examination that will include an examination of organ systems that are relevant to the investigator based on review of the subject's reported AEs, concomitant medication use. This assessment may include: vital signs (at a minimum body temperature [rectal for subjects <12 months of age and axilla for subjects ≥12 months of age, preferably) and a check of general appearance. The physical assessment must be performed by the investigator or designee of the investigator, who is qualified to perform a physical assessment in accordance with their institutional policy (see glossary of terms for definition of a qualified healthcare provider). Any relevant clinical finding resulting from the symptom-directed physical examination should be documented in the subject's source document and eCRF(s).

At both visits on Day 151 (Visit 4) and Day 331 (Visit 6), the eDiary should be returned to the study site; ***the eDiary dispensed at Day 1 (Visit1) will be collected at Day 151 (Visit 4) and the eDiary dispensed at Day 301 (Visit 5) will be collected at Day 331 (Visit 6)***. For details on the eDiary see Section 5.6.16.1. A blood sample will also be collected for immunological evaluation at both visits.

The site should schedule the next study activity with the subject's parent(s)/LAR(s). The subject's parent(s)/LAR(s) will receive a written reminder of the next planned study activity. Instruction regarding recording of safety data will be provided by the site based on the visit script.

5.6.15. Study procedures during special circumstances

During special circumstances (e.g., COVID-19 pandemic), the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied. For the duration of such special circumstances, the following measures may be implemented for enrolled participants:

- Visit 7 (Day 481 or Day 661): the study conclusion visit has been revised to allow for either a site visit or concluding telephone call. The telephone call is intended for safety data collection and will be considered equivalent to study conclusion visit.
- The existing protocol-specified windows have been modified in keeping with ACIP guidelines to facilitate the safe scheduling of study visits (See allowed interval in Table 6).
- If despite best efforts it is not possible to collect blood samples within the allowed interval between visits (see Table 6), then the interval may be extended as per the clinical judgement of the investigator.
- If despite best efforts it is not possible to give the vaccine dose within the allowed interval between visits (see Table 6), then the interval may be extended as per the clinical judgement of the investigator and in keeping with ACIP guidelines.

Impact on the per protocol set for immunogenicity will be determined on a case by case basis.

5.6.16. Recording of AEs, SAEs and AESIs

- Refer to Section 7.2 for procedures for the investigator to record AEs, SAEs, and AESIs. Refer to Section 7.3 for guidelines and how to report SAE and AESI reports to GSK Biologicals. Refer to Section 7.2.4 for recording of COVID-19 infection related AEs.
- The subjects' parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.

5.6.16.1. Subject Diary

A electronic Diary (eDiary) hereafter referred to as Subject Diary will be used in this study to capture solicited adverse events. The subject's parent/LAR should be trained on how and when to complete each field of the Subject Diary.

Subject Diary training should be directed at the individual(s) who will perform the measurements of adverse events and who will enter the information into the Subject Diary. This individual may not be the subject's parent/LAR, but if a person other than the subject's parent/LAR enters information into the Subject Diary, this person's identity must be documented in the subject's source record. Any individual that makes entries into the Subject Diary must receive training on completion of the Subject Diary at the time of the visit when Subject Diary is dispensed. This training must be documented in the subject's source record.

The same individual should preferably complete the Subject Diary throughout the course of the study.

The subject's parent(s)/LAR(s) should be trained on how to self-measure local solicited adverse events and body temperature.

The measurement of solicited local AEs ***(administration site event)*** is to be performed using the ruler provided by the site.

Subject's parent(s)/LAR(s) will be instructed to measure and record the rectal (subjects <12 months of age) or axillary (subjects ≥12 months of age) body temperature in the evening. Should additional temperature measurements be performed at other times of day, subject's parent(s)/LAR(s) will be instructed to record the highest temperature in the Subject Diary."

Subject Diary assignment and use (at Visit 1 and Visit 5):

- Each subject's parent/LAR will be assigned a Subject Diary and shown how to use the device – this will include how to access the diary, performing test data entry on sample questions, and how to charge and store the device.
- The subject's parent/LAR will self-select a numeric access code secret to themselves. The same individual should preferably make the assessments and complete the Subject Diary throughout the relevant reporting period.
- The subject's parent(s)/LAR(s) will select an alarm time that suits their daily routines whilst ensuring compliance with protocol requirements.

Subject Diary instructions must ensure that the subject's parent(s)/LAR(s) understands the following:

- Timely completion of the Subject Diary on a daily basis is a critical component to study participation.
- The Subject Diary will allow certain time windows for completion of each day's observations.
- The Subject Diary employs the use of audio-visual alarms to ensure timely completion of data entry.
- The trained and assigned user of the Subject Diary must not share access codes with anyone.

- A helpdesk will be provisioned for users of subject diary in case of technical issues, though it must be stressed that the Helpdesk is not a replacement for normal medical care and no medical issues can be discussed with the agents.
- The Subject Diary itself must never be considered a substitute for direct medical care and any concerns must be communicated to site staff as soon as possible.

5.6.16.1.1. Subject diary alerts

The subject's parent(s)/LAR(s) will receive daily reminders via the Subject Diary device's in-built audio-visual alarms to alert the user to complete the diary during the post vaccination period, which is from Day 1 to Day 7 after Visits 1, 2 and 3 (or until the solicited AE resolves) and from Day 1 to Day 30 after Visit 5.

The Subject Diary system will also allow for regular alerts to be issued via email to site staff indicating where subjects may need to be contacted due to:

- Non-compliance (i.e. failing to enter or transmit diary data),
- Reporting of any severe solicited reactions,
- Subject experienced any AE that required hospitalization or visit to the emergency room or medically attended events that were of concern to the parents/LAR(s).

Sites must assess these alerts when received and contact subjects as necessary. Please refer to Section 8.2 on the premature withdrawals from the study and Section 7.2.3 on the evaluation of AEs for guidance on necessary action in the event of one of these alerts.

5.6.16.2. Safety follow-up calls

During the course of the trial, a total of 10 or 13 safety follow-up calls will be performed. Safety follow-up calls will be performed on the following time points:

Treatment period:

- Day 16 and Day 31 (15 and 30 days post 1st vaccination, respectively),
- Day 76 and Day 91 (15 and 30 days post 2nd vaccination, respectively),
- Day 136, Day 181 and Day 241 (15, 60 and 120 days post 3rd vaccination, respectively),
- Day 316 (15 days post 4th vaccination).

Follow-up period:

- Day 361, Day 421, Day 481, Day 541 and Day 601 (60, 120, 180, 240, and 300 days post 4th vaccination, respectively). For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481 instead of the follow-up call, and follow-up calls at Day 541 and Day 601 will not be performed.

Safety follow-up calls are calls made to the subject's parent(s)/LAR(s) by a qualified healthcare professional designated on the site log. These calls will follow a script which will facilitate the collection of relevant safety information. The subject's parent(s)/LAR(s) will be interviewed according to the script, and information relating to unsolicited AEs [including serious adverse events (SAEs), AESIs, medically attended AEs, and AEs leading to vaccine/study withdrawal] and concomitant medications or vaccinations associated with those events. All safety information described by the subject's parent(s)/LAR(s) must be written down in a designated location within the source document and not written on the script used for the telephone call.

The site should schedule the next study activity (clinic visit or safety call) with the subject's parent(s)/LAR(s).

The subject's parent(s)/LAR(s) will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

5.6.17. Study conclusion

- The study termination visit will occur on Day 481 or Day 661 (Visit 7). The termination visit will be a clinic visit.
- If Visit 7 is performed by telephone for subjects who are impacted by protocol amendment 7, reconsenting should be performed as per site and IRB guidelines.
- The date of termination is the date of the last contact (clinic visit or telephone call) in which the subject's health status was assessed or, in cases where the subject does not agree to any further safety follow-up, it is the date consent is withdrawn. This date should be recorded on the termination eCRF page. For visit procedures to be performed for a subject whose planned study participation ends prematurely, please see below.
- At this clinic visit or telephone call, subject's parent(s)/LAR(s) will be interviewed for any SAEs, medically attended AEs, AESIs (pIMDs, seizures, and/or arthritis) experienced by their child. Any concomitant medications associated with those events will also be collected and recorded in the subject's records and on the eCRF.
- The site will review with the subject's parent(s)/legal guardian(s) the plan of when information relating to the subject's participation in the study may be available (e.g., study results). It will also be discussed how information relating to the subject's participation in the study will be shared with the subject's healthcare provider, if the subject's parent(s)/LAR(s) choose to share this information or if it is required by local regulations.
- At this visit or telephone call, subject's parent(s)/LAR(s) will be asked if they can be contacted to participate in any future related study. If the subject's parent(s)/LAR(s) are not interested in participating in future studies the reason for refusal will be documented in the subject's eCRF.

- The site will complete the termination eCRF page and this will mark the completion of the subject's participation in the study.

Early termination:

When a subject is withdrawn from treatment or withdraws from the study, the investigator will notify the Sponsor and, when possible, will perform the procedures listed below. The reason(s) for the early termination will be included in the subject's source documentation and in the eCRF.

If the Early Termination is at a clinic visit, the following procedures will be performed: review of Subject Diary (if applicable), review of systems, interview of subject's parent(s)/LAR(s) to collect: unsolicited AEs, AESI, SAEs, medically attended AEs, AEs leading to vaccine/study withdrawal, interview of subject's parent(s)/legal guardian(s) to collect concomitant medications/ vaccinations, symptom-directed physical assessment including vital signs (at a minimum rectal (subjects <12 months of age) or axillary (≥12 months of age) body temperature preferably) and a check of general appearance, and blood sampling for immunogenicity (if applicable at the visit).

If the Early Termination Visit is a telephone call, collect as much information as possible. Early Termination Visits also include subjects who were enrolled and or/randomized, but may or may not have been treated.

The site will review with the subject's parent(s)/LAR(s) the plan of when information relating to the subject's participation in the study may be available (e.g., study results). It will also be discussed how information relating to the subject's participation in the study will be shared with the subject's healthcare provider, if the subject's parent(s)/LAR(s) choose to share this information or if it is required by local regulations.

The site will complete the termination eCRF page and this will mark the completion of the subject's participation in the study.

5.7. Biological sample handling and analysis

Please refer to the SPM and Central Laboratory Investigator Manual for details on biospecimen management (handling, storage and shipment). Refer to Section 5.6.15 for changes in biological samples collection that may be implemented during special circumstances.

- 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. Therefore, all subjects parent(s)/LAR(s) will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in the US and will only be performed once an independent Ethics Committee or Review Board has approved this research.

Although not planned, additional serologic testing may be performed in the future to further characterize the antibody response to the antigens included in the study vaccines or concomitantly administered vaccines (e.g. Rotavirus [HRV], hSBA against other meningococcal strains).

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject's parent(s)/LAR(s).

Refer also to Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

If additional testing is performed, the marker priority ranking given in Section 5.7.4 may be changed.

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

5.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the per-protocol analysis (See Section 9.2.5 for the definition of analysis sets to be analyzed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

The above applies to any external partners designated by GSK Biologicals as well.

5.7.2. Biological samples

The quantities of blood samples required and the corresponding timepoints are described in Table 8. Analysis of immune responses to the RIV antigens will be performed in immunogenicity subsets as described in Section 5.2.3 and Table 9.

5.7.3. Laboratory assays

The measures of immunogenicity used in this study are standard, i.e., widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response).

The immunogenicity of rMenB+OMV NZ is assessed in this study by measuring the serum bactericidal activity, which is a functional measure of the ability of antibodies, in conjunction with human complement, to kill meningococci, and is widely accepted and generally recognized as the serological surrogate of protection.

Serological testing for immunogenicity to rMenB+OMV NZ, PCV13 ***(same pneumococcal serotypes panel (PCV13)will be used for subjects who received PCV20)*** and the other routine vaccines will be performed at a GSK Biologicals' laboratory(ies) or in a laboratory(ies) designated by GSK Biologicals using standardized and validated procedures (refer to Table 7).

Please refer to Appendix A for a detailed description of the assays performed in the study. Please refer to Appendix B for the address(es) of the clinical laboratories used for sample analysis.

Table 7 Humoral Immunity (Antibody determination)

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
Serum	N Men B M13520 (NHBA) Ab*,∞	hSBA	In house	1/dil	4	***Laboratory*** designated by GSK Biologicals	1
	N Men B NZ98/254 (PorA) Ab				4		
	N Men B M14459 (fHbp) Ab				3		
	N Men B 96217 (NadA) Ab				6		
Serum	Streptococcus pneumoniae.Polysaccharide 01 Ab.IgG	ECL	In house	µg/mL	0.08	GSK Biologicals ***	2
	Streptococcus pneumoniae.Polysaccharide 03 Ab.IgG				0.075		
	Streptococcus pneumoniae.Polysaccharide 04 Ab.IgG				0.061		

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
	Streptococcus pneumoniae.Polysaccharide 05 Ab.IgG				0.198		
	Streptococcus pneumoniae.Polysaccharide 06A Ab.IgG				0.111		
	Streptococcus pneumoniae.Polysaccharide 06B Ab.IgG				0.102		
	Streptococcus pneumoniae.Polysaccharide 07F Ab.IgG				0.063		
	Streptococcus pneumoniae.Polysaccharide 09V Ab.IgG				0.066		
	Streptococcus pneumoniae.Polysaccharide 14 Ab.IgG				0.16		
	Streptococcus pneumoniae.Polysaccharide 18C Ab.IgG				0.111		
	Streptococcus pneumoniae.Polysaccharide 19A Ab.IgG				0.199		
	Streptococcus pneumoniae.Polysaccharide 19F Ab.IgG				0.163		
	Streptococcus pneumoniae.Polysaccharide 23F Ab.IgG				0.073		
Serum	Corynebacterium diphtheriae.Diphtheria Toxoid Ab.IgG	ELI	In-house	IU/mL	0.030	GSK Biologicals ***	10
	Clostridium tetani.Tetanus Toxoid Ab.IgG	ELI	In-house	IU/mL	0.037		11
	Bordetella pertussis.Filamentous Hemaglutinin Ab.IgG	ELI	In-house	IU/mL	2.046		4
	Bordetella pertussis.Pertussis Toxin Ab.IgG	ELI	In-house	IU/mL	2.693		4
	Bordetella pertussis.Pertactin Ab.IgG	ELI	In-house	IU/mL	2.187		4
	Poliovirus Sabin Type 1 Ab	NEU	In-house	ED50	8		12
	Poliovirus Sabin Type 2 Ab	NEU	In-house	ED50	8		14
	Poliovirus Sabin Type 3 Ab	NEU	In-house	ED50	8		13

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
	Hepatitis B Virus.Surface Ab	CLIA	CCI	mIU/mL	6.2		5
Serum	Haemophilus influenzae type b.Polyribosyl Ribitol Phosphate Ab	ELI*	In-house	µg/mL	0.066	GSK Biologicals ***	3
Serum	Measles Virus Ab.IgG	***Luminex***	***In-house***	***mIU/mL***	TBD	***Laboratory*** designated by GSK Biologicals	8
	Rubella Virus Ab.IgG	***Luminex***	***In-house***	***IU/mL***	TBD	***Laboratory*** designated by GSK Biologicals	9
	Mumps Virus Ab IgG	***Luminex***	***In-house***	***AU/mL***	TBD	***Laboratory*** designated by GSK Biologicals	6
Serum	Varicella Zoster Virus Ab.IgG	ELI*	***In-house***	***mIU/mL***	97.0	GSK Biologicals***	7

Ab: Antibody, CLIA: Chemiluminescence immunoassay, IgG: Immunoglobin G, µg: Microgram, mL: Milliliter, 1/dilution: reciprocal of the dilution; ECL: Electrochemiluminescent Assay, ELI: enzyme-linked immunosorbent assay (ELISA), MSBA: manual human serum bactericidal assay (hSBA), NEU: neutralization assay, TBD: to be determined.
*Type of assays, the validated assay cut-offs and units might be subject to change during the course of the study (e.g. in case of requalification, revalidation or standardization, availability of assays). In case testing is done at another GSK designated laboratory, the validated assay cut-off may also need to be adapted accordingly. Any of these changes will be documented in a protocol amendment or clinical study report. The LLOQ for the meningococcal B strains are provided in Table 20.
**Refer to Appendix B for the laboratory addresses.
GSK Biologicals laboratory refers to the ***Vaccines Clinical Laboratory and Assay Portfolio (Vx CL&AP) in Rixensart, Belgium; Wavre, Belgium.
Additional exploratory testing on the vaccine and/or on the disease under study may be performed if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

5.7.4. Biological samples evaluation

5.7.4.1. Immunological read-outs

In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analyzed according to priority ranking provided in Table 8. Testing against RIV antigens will be performed in immunogenicity subsets as defined in Section 5.2.3 and in Table 9.

Table 8 Immunological Read-outs

Blood sampling timepoint		Group Name	Max No. subjects[a]	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Visit 4 (Day 151)	Post-Vacc 3	Group MenB+PCV, Placebo+PCV[b]	1000	hSBA-M13520 hSBA-NZ98/254 hSBA-M14459 hSBA-96217	1
		Groups MenB+PCV, Placebo+PCV	800	Anti-PCV13 (1,3, 4, 5, 6°, 6B, 7F, 9V, 14, 18C, 19°, 19F, 23F)	2
		Groups MenB+PCV, Placebo+ PCV	1200	DT,TT, PT, FHA, PRN, HBs, Polio, Hib[a]	See Table 9 [a]
Visit 5 (Day 301)	Pre-Vacc 4	Groups MenB+PCV Placebo+PCV[b]	1000	hSBA-M13520 hSBA-NZ98/254 hSBA-96217 hSBA-M14459	1
		Groups MenB+PCV and Placebo+PCV	1200	M,M,R, V	See Table 9 [a]
Visit 6 (Day 331)	Post-Vacc 4	Groups MenB+PCV Placebo+PCV[b]	1000	hSBA-M13520 hSBA-NZ98/254 hSBA-96217 hSBA-M14459	1
		Groups MenB+PCV Placebo+PCV	800	Anti-PCV13 (1,3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)	2
		Groups MenB+PCV and Placebo+PCV	1200	M,M,R, V	See Table 9 [a]

DT: diphtheria toxoid, TT: tetanus toxoid, FHA: filamentous hemagglutinin, PT: pertussis toxin, PRN: pertactin, HBs: Hepatitis B surface antigen, Hib: *Haemophilus influenzae* type b antigen, MMR: measles, mumps, rubella virus antigens, PCV; pneumococcal serotype antigens; V: varicella virus antigens, NA, not applicable.
* Laboratory assays
Note: As testing against pneumococcal serotypes will be performed using a multiplex assay, no ranking is applicable.
[a] Testing at Visit 4, Visit 5 and Visit 6 will be performed based on immunogenicity subsets, defined in Section 5.2.3 and in Table 9.
[b] A subset (B1) of 200 subjects (120 evaluable) from Group Placebo+PCV will be tested against the 4 serogroup B strains in order to maintain the observer-blindness of the study during blood sample testing.

Table 9 Immunogenicity Subsets

Blood sampling timepoint		Group Name	Subset	Max No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 4 (Day 151)	Post-Vacc 3	Group MenB+PCV	A1	400	MenB PCV13 Hib Pertussis Diphtheria Tetanus	1 2 3 4 5 5
			A2	200	MenB Hib Pertussis HepB	1 2 3 4
			A3	200	MenB Pertussis Polio	1 2 3
		Group Placebo+PCV	B1	200	MenB PCV13 Hib Pertussis	1 2 3 4
			B2	200	PCV13 Hib Pertussis HepB Diphtheria Tetanus Polio	1 2 3 4 5 5 6
Visit 5 (Day 301)	Pre-Vacc 4	Group MenB+PCV	A1	400	MenB Measles Rubella Mumps Varicella	1 2 2 2 3
			A2	200	MenB Varicella Measles Rubella Mumps	1 2 3 3 3
			A3	200	MenB Varicella Measles Rubella Mumps	1 2 3 3 3
		Group Placebo+PCV	B1	200	MenB Measles Rubella Mumps Varicella	1 2 2 2 3
			B2	200	Varicella Measles Rubella Mumps	1 2 2 2

Blood sampling timepoint		Group Name	Subset	Max No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
Visit 6 (Day 331)	Post-Vacc 4	Group MenB+PCV	A1	400	MenB PCV13 Measles Rubella Mumps Varicella	1 2 3 3 3 4
			A2	200	MenB Varicella Measles Rubella Mumps	1 2 3 3 3
			A3	200	MenB Varicella Measles Rubella Mumps	1 2 3 3 3
		Group Placebo+PCV	B1	200	MenB PCV13 Measles Rubella Mumps Varicella	1 2 3 3 3 4
			B2	200	PCV13 Varicella Measles Rubella Mumps	1 2 3 3 3

Note: The individual components and the order of testing is given below:
MenB: hSBA-M13520, hSBA-NZ98/254, hSBA-M14459, hSBA-96217.
PCV13: 1,3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F – no ranking is applicable for pneumococcal serotypes as a multiplex assay will be used.
Pertussis : Pertactin, Pertussis toxin, Filamentous Hemagglutinin.
Polio: I, III, II.

5.7.5. Immunological correlates of protection

For pneumococcal conjugate vaccines for use in infants, pertussis, and rotavirus antigens no generally accepted immunological correlate of protection has been clearly identified yet.

For the following antigens in the DTPa-HBV-IPV, Hib, MMR and VV vaccines, an immunological correlate of protection has been established:

- Anti-D and anti-T concentrations ≥0.1 IU/mL (ELISA),
- Anti-HBS concentration ≥10 mIU/mL (ELISA),
- Polio ≥8 ED50 for types 1, 2 and 3 (neutralizing antibody assay),
- Anti-PRP concentrations ≥0.15 µg/mL (ELISA),
- Anti-measles ≥threshold to be defined
- Anti-mumps ≥threshold to be defined

- Anti-rubella ≥threshold to be defined
- The immunological assay results will be communicated to the investigator as soon as they become available. For antigens for which an immunological correlate of protection has been established, the following applies.

The investigator is encouraged to share the immunological assay results for non-responders with the study subjects/subjects' parent(s)/LAR(s).

For the subjects identified as non-responders, it remains the responsibility of the investigator in charge of the subject's clinical management to determine the medical need for re-vaccination and to re-vaccinate the subjects as per local/regional practices.

6. STUDY VACCINES AND ADMINISTRATION

6.1. Description of study vaccines

6.1.1. Study vaccines

The study vaccines' (see glossary of terms) will be provided by the Sponsor. All study participants are expected to receive these vaccines as specified by the study schedule. The study vaccines specific to this study are described below. ***In exceptional circumstances, in order to avoid any inconvenience for the participant, a commercially-acquired vaccine with identical formulation and brand of that reported in the study protocol can be administered to the participant. In this case this subject will still be eligible for the Per Protocol Set.***

- GSK Biologicals' Meningococcal group-B vaccine, (rMenB+OMV NZ, *Bexsero*);
- Pneumococcal polysaccharide conjugate vaccine, (13-valent Pneumococcal Vaccine) (PCV13, *Prevnar13*) ***(In order to comply with the change in the US NIP, 20-valent Pneumococcal Vaccine (PCV20, Prevnar20) will also be allowed as specified in the protocol)***;
- Saline placebo vaccine.

Standardized routine infant immunizations included in this study are described below.

- GSK Biologicals' Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine (DTPa-HBV-IPV, *Pediarix*): a 3-dose vaccine given by IM injection at 2, 4, and 6 months of age. A single-dose prefilled syringes contains a 0.5-mL suspension for injection.
- GSK Biologicals' Rotavirus Vaccine, live, oral (HRV, *Rotarix*): a vaccine containing attenuated human strain P1AG1. HRV is a 2-dose vaccine typically given at 2 months and 4 months of age; it is available in single-dose vials of lyophilized vaccine, accompanied by a prefilled oral applicator of liquid diluent.
- GSK Biologicals' *Haemophilus influenzae* type b vaccine (Hib, *Hiberix*): a vaccine indicated for active immunization for the prevention of invasive disease caused by *Haemophilus influenzae* type b. Hib is given as a 4-dose series by IM injection; the primary series consists of one dose each at 2, 4, and 6 months of age, followed by a booster dose at 12 through 15 months of age.

- Merck's measles, mumps and rubella virus vaccine live (MMR, *M-M-R II*): a vaccine indicated for active immunization for simultaneous vaccination against measles, mumps, and rubella in individuals 12 months of age or older. Each lyophilized dose is approximately 0.5 mL after reconstitution and is administered by subcutaneous injection.
- Merck's varicella zoster virus vaccine live (VV, *Varivax*): a vaccine indicated for active immunization for the prevention of varicella in individuals 12 months of age or older. Each dose is approximately 0.5 mL after reconstitution and is administered by subcutaneous injection.

The characteristics of the study vaccines are presented in Table 10.

The Quality Control Standards and Requirements for the candidate vaccine are described in separate Quality Assurance documents (e.g. release protocols, certificate of analysis) and the required approvals have been obtained.

The vaccines are labelled and packed according to applicable regulatory requirements.

Commercial vaccines are assumed to comply with the specifications given in the manufacturer's Summary of Product Characteristics.

Table 10 Study Vaccines

Treatment name	Vaccine	Formulation	Presentation	Volume to be administered*	Number of doses
Bexsero	rMenB+OMV NZ	rp936-741=50µg; rp287-953=50µg; rp961c=50µg; OMV NZ98/254=25µg; $Al(OH)_3$=1.5mg; Histidine=776µg; NaCl=3.125mg; Sucrose=10mg; water=0.5ml	Prefilled syringe (liquid)	0.5 mL	4
Prevnar13	PCV13	PS1=2.2µg CRM197; PS3=2.2µg CRM197; PS4=2.2µg CRM197; PS5=2.2µg CRM197; PS6A=2.2µg CRM197; PS6B=4.4µg CRM197; PS7F=2.2µg CRM197; PS9V=2.2µg CRM197; PS14=2.2µg CRM197; PS18C=2.2µg CRM197; PS19A=2.2µg CRM197; PS19F=2.2µg CRM197; PS23F=2.2µg CRM197; $AlPO_4$=125µg Al3+	Homogenous white suspension after shaking (in pre-filled syringe)	0.5 mL	4**

Treatment name	Vaccine	Formulation	Presentation	Volume to be administered*	Number of doses
Prevnar20	***PCV20***	***PS1(2.2 µg)[1,2]; PS3(2.2 µg)[1,2]; PS4(2.2 µg)[1,2]; PS5(2.2 µg)[1,2]; PS6A(2.2 µg)[1,2]; PS6B(4.4 µg)[1,2]; PS7F(2.2 µg)[1,2]; PS8(2.2 µg)[1,2]; PS9V(2.2 µg)[1,2]; PS10A(2.2 µg)[1,2]; PS11A(2.2 µg)[1,2]; PS12F(2.2 µg)[1,2]; PS14(2.2 µg)[1,2]; PS15B(2.2 µg)[1,2]; PS18C(2.2 µg)[1,2]; PS19A(2.2 µg)[1,2]; PS19F(2.2 µg)[1,2]; PS22F(2.2 µg)[1,2]; PS23F(2.2 µg)[1,2]; PS33F(2.2 µg)[1,2]***	***Suspension for injection (in pre-filled-syringe)***	***0.5 mL***	***1***
Placebo	Placebo (NaCl)	NaCl=150mM	Prefilled syringe (liquid)	0.65 mL*	4
Pediarix	DTPa-HBV-IPV	DT>=30IU; TT>=40IU; PT=25µg; FHA=25µg; PRN=8µg; HbsAg=10µg; Inactivated Poliovirus type 1 (Mahoney strain)=40DU; Inactivated Poliovirus type 2 (MEF-1 strain)=8DU; Inactivated Poliovirus type 3 (Saukett strain)=32DU; Aluminium=700µg Al3+	Prefilled syringe (liquid)		3
Hiberix	Hib	PRP=10µg; TT~=25µg; Aluminium=120µg Al3+	Lyophilized cake to be reconstituted with saline diluent	0.5 mL	3
	Hib diluent (NaCl)	NaCl=150mM	Vial (liquid)		
Rotarix	HRV Lyo	HRV RIX4414 live attenuated >=$10^{6.0}$$CCID_{50}$	Lyophilized vaccine in a monodose glass vial.	1 mL	2
	HRV Diluent	$CaCO_3$=60mg	Diluent for lyophilized vaccine (calcium carbonate liquid antacid) supplied separately in a prefilled oral applicator		

Treatment name	Vaccine	Formulation	Presentation	Volume to be administered*	Number of doses
M-M-R II	MMR-II	Measles>=1.000TCID50; Mumps=12.500TCID50; Rubella=1.000TCID50	Lyophilized pellet in a vial for reconstitution with water for injections	0.5 mL	1
	MMR-II diluent (Water for injection)	Water=0.5ml	vial		
Varivax	Varivax	Oka=1350pfu	Monodose vial of lyophilized vaccine	0.5 mL	1
	Varivax Diluent (Water for injection)	Water=0.5ml	vial		

Note: Refer to the SPM for the volume after reconstitution. The fourth dose of Hib vaccine will be administered as an non-study vaccine at 13 months of age.
$CCID_{50}$:=median Cell Culture Infective Dose (quantity of virus causing infection in 50% of exposed cells)
$CaCO_3$: Calcium carbonate, HRV: Human Rotavirus, Hib: Haemophilus influenzae type b vaccine, DT: diphtheria toxoid, TT: tetanus Toxoid, PT: pertactin, FHA: filamentous hemagglutinin, PRN: Pertactin, HbsAg: Hepatitis B surface antigen, PRP: Polyribosyl-ribitol phosphate, MMR: measles, mumps, rubella.
* The volume of the saline syringe may be between 0.6mL and 0.8 mL. The full volume is to be injected.
***** For subjects who receive PCV20 at Visit 5, only 3 doses of PCV13 will be administered.***
[1]conjugated to CRM_{197} (51 µg);
[2]adsorbed on $AlPO_4$ (0.125 mg Al^{3+}); Water for injections

6.1.2. Non-study vaccines

The term 'non-study vaccine' refers to those vaccines which will be given to subjects during the study but do not have associated study objectives. Non-study vaccines are Non-Investigational Medicinal Products (NIMPs) and must be licensed in the country where they are administered. The Investigator is responsible for reporting AEs associated to the use of a non-study vaccine according to local laws and regulations in addition to the safety reporting procedures foreseen in this study. The Sponsor is responsible for the ongoing safety evaluation of the clinical trial subjects including events related to non-study vaccines.

The following non-study vaccine will be administered to all subjects in both groups at 13 months of age, at Visit 6 (Day 331).

- GSK Biologicals' GSK Biologicals' Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed Vaccine (DTPa, *Infanrix*): DTPa is a 5-dose vaccine given by IM injection at 2, 4, and 6 months of age, with a booster dose at 15 to 20 months of age and another booster dose at 4 to 6 years of age. A single-dose prefilled syringes contains a 0.5-mL suspension for injection.

In order to complete the dosage scheme for DTPa, a single dose of DTPa (*Infanrix*) will be administered IM as a booster dose at Visit 6 (as subjects would have already received 3 doses of *Pediarix* as an IMP study-vaccine).

In addition, all subjects will receive the fourth dose of Hib (*Hiberix*) as a non-study vaccine during Visit 6. Details on the vaccine are provided in Section 6.1.1.

Non-study vaccines will be supplied by the Sponsor to ease the potential disruption to the standard vaccine schedule caused by participating to the study. They will be recorded in the subjects' eCRF.

Note: Other non-study vaccines whose use, depending on local requirements, may be foreseen in this trial includes, but are not limited to: monovalent Hepatitis B, Hepatitis A and influenza. If any of these vaccines are administered at Visit 4 or Visit 6, the administration should occur after the study blood draw at these visits. Any other vaccine not foreseen in the protocol should be administered outside a period of 14 days (or 28 days for live vaccines and 7 days for influenza vaccines) pre- or post-administration of the study vaccines (forbidden window). For any vaccine administered outside the forbidden window, the Investigators will be allowed to choose the type, the timing and the number of doses of intercalated non-study vaccines to be administered, in accordance with local regulations and practices.

6.2. Storage and handling of study and non-study vaccines

Detailed vaccine supply, labeling, storage and tracking instructions will be provided to investigators in the Clinical Trial Supplies module in the SPM prior to study start. The below mentioned details on vaccine storage and handling are applicable to all vaccines used in the study, including NIMPs.

All vaccines associated with this study must be checked for expiration date prior to use. Expired vaccines must not be administered to subjects. All vaccines associated with this study are to be stored separately from other vaccines and medications in a secure location under appropriate storage conditions with temperature monitoring.

The study vaccines must be stored at the respective label storage temperature conditions in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded. Refer to the Module on Clinical Trial Supplies in the SPM for more details on storage of the study vaccines.

For the *Varivax* vaccine, the antigen is to be stored between -25 C/-13 F to -15 C/5 F and the diluent at +4°C/+39.2 F.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of 2.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) or below -25 C/-13 or above -15.0°C/5 F (for -20°C/-4°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form (Advanced Temperature Excursion Analysis and Management [ATEAM]). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor. Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines.

The Sponsor will ensure the following:

- Supply the study vaccines and above mentioned non-study vaccines (single dose of DTPa and 4th dose of Hib).
- Appropriate labelling of all study and non-study vaccines provided that complies with the legal requirements of each country where the study is to be performed.

The investigator must ensure the following:

- Acknowledge receipt of the supplied vaccines by a designated staff member at the site, including confirmation that the vaccines were received in good condition
- SBIR system encoding in case of temperature excursion
- Proper storage of the supplied vaccines, including:
  - Storage in a secure, locked, temperature-controlled location.
  - Proper storage according to the instructions specified on the labels.
  - Appropriate record keeping and inventory of the study vaccines, including regular documentation of adequate storage temperature.
- Appropriate use of the supplied vaccines, including

  Use only in accordance with the approved protocol.

  Appropriate documentation of administration of vaccines to study subjects by unblinded study staff including:
  - Date, dosage, batch/lot numbers, expiration dates, unique identifying numbers assigned to subjects and study vaccines, and time of vaccine administration. This information will be maintained in an accountability log that will be reviewed by the site monitor.
  - Reconciliation of all vaccines received from the Sponsor. Reconciliation is defined as maintaining records of which and how many vaccines were received, which vaccines were administered to subjects, which vaccines were destroyed at the site, and which vaccines were returned to the Sponsor, as applicable.
- Proper adherence to the local institutional policy with respect to destruction of study vaccines.
- Complete record keeping of vaccine use, wastage, return or destruction, including documentation of:
  - Copy of the site's procedure for destruction of hazardous material.
  - Copy of Destruction Certificate

Vaccines that have been stored differently from the manufacturer's indications must not be used unless the Sponsor provides authorization for use. In the event that the use cannot be authorized, the Sponsor will make every effort to replace the vaccine supply. All vaccines used in conjunction with this protocol must be stored separately from normal hospital/practice stocks to prevent unintentional use of study vaccines outside of the clinical study setting.

Monitoring of the supplied study and non-study vaccines' accountability will be performed by the study monitor during site visits and at the completion of the study.

At the conclusion of the study, and as appropriate during the course of the study, the investigator must ensure that all unused study and non-study vaccines, packaging and supplementary labels are destroyed locally (upon approval from Sponsor) or returned to the Sponsor.

6.3. Dosage and administration of study vaccines

The investigator or designee will be responsible for oversight of the administration of vaccine to subjects enrolled in the study according to the procedures stipulated in this study protocol. All vaccines will be administered only by personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site. It is required that the vaccine administrator and the health care professional collecting safety information are different. The vaccine administrator for all the study vaccines would be an unblinded study staff (see glossary of terms).

Refer to Section 5.6.15 in case of a change in the allowed interval to receive the vaccine dose between visits during special circumstances.

Study vaccines should be administered using the preferred site locations shown in Table 11 below. The rMenB+OMV NZ/Placebo vaccine should be administered at least 2.5 cm above the injection site of Hib, when concomitantly administered on the same thigh. Similarly, PCV13 ***(or PCV20)*** should be administered at least 2.5 cm above the injection site of DTPa-HBV-IPV, when concomitantly administered on the same thigh. The HRV vaccine will be administered orally. The MMR and VV vaccines should be given subcutaneously in the upper arm (deltoid region) by inserting the needle in a pinched-up fold of skin and subcutaneous tissue to prevent injection into muscle.

Standard immunization practices are to be observed and care should be taken to administer the injection by the route described in Table 11 below. Before administering vaccine, the vaccination site is to be disinfected with a skin disinfectant as applicable (e.g., 70% alcohol). Allow the skin to dry. DO NOT inject intravascularly.

Table 11 Dosage and Administration

Study group	Type of contact and timepoint	Treatment name	Vol. administered	Route[1]	Site[3]		
					Location	Directionality[2]	Laterality
MenB+PCV	Visit 1 (Day 1)	*Bexsero*	0.5 mL	IM	Thigh	Upper	Right
		Prevnar13	0.5 mL	IM	Thigh	Upper	Left
	Visit 2 (Day 61)	*Pediarix*	0.5 mL	IM	Thigh	Lower	Left
		Hiberix	0.5 mL	IM	Thigh	Lower	Right
	Visit 3 Day 121)	*Rotarix*[4]	1 mL	O	-	-	-
	Visit 5 (Day 301)	*Bexsero*	0.5 mL	IM	Thigh	-	Right
		Prevnar13[5]	0.5 mL	IM	Thigh	-	Left
		M-M-R II	0.5 mL	SC	Arm	Upper	Right
		Varivax	0.5 mL	SC	Arm	Upper	Left
Placebo+PCV	Visit 1 (Day 1)	*Placebo*	0.5 mL	IM	Thigh	Upper	Right
		Prevnar13	0.5 mL	IM	Thigh	Upper	Left
	Visit 2 (Day 61)	*Pediarix*	0.5 mL	IM	Thigh	Lower	Left
		Hiberix	0.5 mL	IM	Thigh	Lower	Right
	Visit 3 (Day 121)	*Rotarix*[4]	1 mL	O	-	-	-
	Visit 5 (Day 301)	*Placebo*	0.5 mL	IM	Thigh	-	Right
		Prevnar13[5]	0.5 mL	IM	Thigh	-	Left
		M-M-R II	0.5 mL	SC	Arm	Upper	Right
		Varivax	0.5 mL	SC	Arm	Upper	Left

[1]Oral (O)/ Intramuscular (IM)/Subcutaneous (SC).
[2]Directionality is a qualifier for further detailing the location of the vaccine administration location (e.g. Upper, Lower).
[3] Site mentioned is the preferred location for injection. The site should be recorded in the eDiary. Please note however that the rMenB+OMV NZ vaccine and the PCV13 vaccine should not be administered in the same thigh.
[4] Only at Visit1 and Visit 2.
[5] Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.

The exact anatomic location of each injection must be carefully recorded in the Medical Chart and in the eCRF, as well as the Subject eDiary.

The vaccines should be visually inspected for particulate matter and discoloration prior to administration. In the event of any foreign particulate matter and/or variation of physical aspect being observed, do not administer the vaccine. For all GSK vaccines, the issue should be reported to GSK as described in the SPM. The vaccine should not be discarded until authorized by GSK

Detailed vaccine preparation and administration instructions will be provided to investigators in the Clinical Trial Supplies module in the SPM prior to study start.

The other non-study vaccines (fourth dose of Hib and DTPa) are licensed pediatric vaccines and therefore must be prepared and administered according what is described in their approved product label/package insert. It is the responsibility of the investigator to maintain proper storage and use according to the instructions in the SMP. The investigator will also be responsible for documenting vaccine administration on the eCRF and keeping records of vaccines administered to subjects.

Vaccine administration error is defined as receiving a dose of study vaccine that was not reconstituted as instructed or administered by a different route from the intended route of administration. An overdose of study vaccine (whether accidental or intentional) is defined when a dosage higher than the recommended dosage is administered in one dose of any of the study vaccines. Administration of a lower dosage should be recorded in the eCRF as well.

An overdose would also occur if two doses of the study vaccine are administered within half the time of the recommended interval between doses, as defined in the protocol.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

6.4. Replacement of unusable vaccine doses

In addition to the vaccine doses provided for the planned number of subjects (including over-randomization when applicable), at least 10% additional vaccine doses will be supplied to replace those that are unusable.

The investigator will use SBIR to obtain the replacement vaccine number. The replacement numbers will be allocated by dose for Bexsero and placebo vaccines. Replacement numbers will be allocated by components for all other products. The system will ensure, in a blinded manner, that the replacement treatment matches the formulation the subject was assigned to by randomization.

6.5. Contraindications to subsequent vaccination

The following events constitute absolute contraindications to further administration of ***vaccines specified in the protocol***. If any of these events occur during the study, the subject must not receive additional doses of vaccines but may continue other study procedures at the discretion of the investigator (see Section 7.4).

- Anaphylaxis following the administration of vaccines.
- Occurrence of a new pIMD or the exacerbation of an existing pIMD. Refer to Section 7.1.6.2 for the definition of pIMDs.
- Any occurrence of an event listed in the exclusion criteria which must be always re-assessed by the investigator before administration of the next dose of study vaccine. Refer to Section 4.2.
- Subjects who experience any SAE judged to be possibly or probably related to study vaccine, including hypersensitivity reactions.

- Subjects who develop any new condition which, in the opinion of the investigator, may pose additional risk to the subject if he/she continues to participate in the study.

The following events constitute contraindications to administration of rMenB+OMV NZ at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 5.5).

- Body temperature elevation [≥ 100.4° F (≥38.0° C)] within the previous 3 days.
- Significant acute illness within the previous 7 days.
- Use of antipyretics and/or analgesic medications within the previous 6 hours.
- Receipt of, or plan to receive, any other vaccine(s) than those listed as study or non-study vaccines, within 14 days (or 28 days for live attenuated vaccines and 7 days for influenza vaccines) of study vaccination.

For contraindications to administered other study vaccines, refer to their approved product label/package insert.

6.6. Warnings and precautions

Refer to the IB for rMenB+OMV NZ and to the approved product label/package insert for all vaccines administered during the study.

6.7. Concomitant medications/products and concomitant vaccinations

At each study visit, the investigator or delegate should question the subject's parent(s)/LAR(s) about any medications/products taken and vaccinations received by the subject.

6.7.1. Recording of concomitant medications/products and concomitant vaccinations

The following concomitant medication(s)/product(s)/vaccine(s) must be recorded in the eCRF.

- All concomitant medications/products, except vitamins and dietary supplements, administered during the 30 days post- vaccination period.
- Any concomitant medications/products administered for treatment of an AE to be recorded as per the protocol-specified reporting period (see Table 13).
- Any concomitant vaccination administered in the period starting from birth and ending at the last study visit (Day of birth to Day 481 or Day 661).
- Any non-study concomitant vaccination (see Section 6.1.2).
- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

E.g. an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring [fever is defined as temperature ≥38.0°C/100.4°F regardless the location of measurement]. The preferred location for measuring temperature in this study will be the rectum for subjects < 12 months of age and the axilla for subjects ≥1 year of age.

- Any concomitant medications/products/vaccines listed in Section 6.7.2.
- Any concomitant medications/products/vaccines relevant to a SAE/AESI to be reported as per protocol or administered at any time during the study period for the treatment of a SAE /AESI. In addition, concomitant medications relevant to SAEs and AESI need to be recorded on the expedited Adverse Event report.
- The use of antipyretic and/or other medications to prevent (prophylactic use) and/or treat fever during the first 7 days after vaccination to be recorded in the eCRF as well.

6.7.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from per-protocol analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the per-protocol analysis. See Section 9.2 for analyses sets to be analyzed.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccines used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e. more than 15 days consecutive days in total) during the study period. Inhaled and topical steroids are allowed.
- Long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab).
- Immunoglobulins and/or any blood products administered during the study period.
- A vaccine not foreseen by the study protocol administered during the period of 14 days (28 days for live vaccines, 7 days for influenza vaccines) pre- or post-administration of the study vaccines (forbidden window) at Visit 1, Visit 2, Visit 3 and Visit 5*.

*In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organized by the public health authorities, outside the routine immunization program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Prescribing Information and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.

6.8. Intercurrent medical conditions that may lead to elimination of a subject from per-protocol analyses

At each study visit subsequent to the first vaccination/the vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

7. SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol.

Each subject's parent(s)/LAR(s) will be instructed to contact the investigator immediately should they/the subject manifest any signs or symptoms they perceive as serious.

7.1. Safety definitions

7.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

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 a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study vaccines administration even though they may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms temporally associated with study vaccines administration.
- Significant failure of expected pharmacological or biological action.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

Adverse Events to be recorded as solicited AEs are described in Section 7.1.3. All other AEs will be recorded as UNSOLICITED AEs.

Solicited AEs are derived from organized data collection systems, such as Subject Diaries.

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- 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.
- Pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination. These events will be recorded in the medical history section of the eCRF.

Since non-study vaccines described in this protocol will not be evaluated as part of the study objectives, no study specific safety assessment is foreseen for these vaccines.

7.1.2. Definition of a serious adverse event

A SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: The term ‘life-threatening’ in the definition of ‘serious’ refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalization or prolongation of existing hospitalization,

   Note: In general, hospitalization signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or in an out-patient setting. Complications that occur during hospitalization are also considered AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether ‘hospitalization’ occurred or was necessary, the AE should be considered serious.

   Hospitalization for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.

d. Results in disability/incapacity,

   Note: The term disability means a substantial disruption of a person’s ability to conduct normal life functions. 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) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

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

All AEs which do not fall into these categories are defined as non-serious. It should be noted that a severe AE need not be serious in nature and that an SAE need not, by definition, be severe. All SAEs will be evaluated by the investigator for relationship of the event to study vaccine.

7.1.3. Solicited adverse events

The term "reactogenicity" refers to solicited signs and symptoms ("solicited adverse events") occurring in the hours and days following a vaccination, to be collected by the parent(s)/LAR(s) for 7 consecutive days (with ongoing solicited AEs collected until resolution or day 30, whichever occurs first), using a pre-defined Subject Diary. Reactogenicity will be collected from all subjects after each vaccination visit (study Days 1, 61, 121 and 301). In addition, specific solicited system AEs (parotid/salivary gland swelling, fever and rash) will be collected for 30 days following the MMR and VV vaccine administration at Visit 5.

The following solicited adverse events are included in the Subject Diary. Each adverse event is to be assessed using the scoring system reported in the tables below.

The collected data will be entered into the Subject Diary. Please see Section 5.6.16.1 for more detail.

7.1.3.1. Solicited local adverse events *(administration site event)*

Solicited local AEs ***(administration site event)*** will be collected following each study vaccine, by site of administration.

- Hardness of skin at injection site (*Induration at injection site*),
- Swelling of skin at injection site (*Swelling at injection site*),
- Redness at injection site (*Erythema at injection site*),
- Tenderness or discomfort to the touch at injection site (*Tenderness at injection site*).

In the eCRF, solicited local AEs ***(administration site event)*** occurring within 30 minutes after vaccination are described as mentioned within brackets.

7.1.3.2. Solicited systemic adverse events

- Decreased eating (*Change in eating habits*),

- Increased sleepiness (*Sleepiness*),
- Vomiting/throwing up (*Vomiting*),
- Loose stools/diarrhea (*Diarrhea*),
- Irritability (*Irritability*),
- Persistent/continuous crying (*Persistent crying*),
- Rash (only after MMR and VV administration at Visit 5),
- Parotid/salivary gland swelling (only after MMR and VV administration at Visit 5),
- Fever, defined as body temperature ≥38.0°C/100.4°F*.

*Body temperature measurement; temperature should be taken preferably via the rectal route for subjects < 12 months of age and the axilla for subjects >1 year of age, ideally at the same time each day. Other routes of temperature measurement include axillary, oral and tympanic. Temperature measurements should be taken prior to administration of antipyretic medication.

In the eCRF, solicited systemic AEs occurring within 30 minutes after vaccination are described as mentioned within brackets.

The study staff must review the data entered into the Subject Diary as described in Section 10.2.

7.1.3.3. Other solicited adverse events

Solicited local ***(administration site)*** and systemic AEs that continued beyond day 7 after vaccination will not need to be entered as an AE in the AE eCRF or the subject's source document. They will be collected via the eDiary.

Note: Any solicited adverse event that meets any of the following criteria must be entered into subjects' source document (see Section 10.2) and also as an adverse event on the Adverse Event eCRF:

- Solicited local or systemic adverse event that leads to a visit to a healthcare provider (medically attended adverse event, see Section 7.2.3.4).
- Solicited local or systemic adverse event leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator (adverse event leading to withdrawal).

Solicited local or systemic adverse event that otherwise meets the definition of a serious adverse event (see Section 7.1.2).

7.1.4. Unsolicited Adverse Events

An unsolicited adverse event is an adverse event that was not solicited using a Subject Diary and that was spontaneously communicated by a parent(s)/LAR(s) who has signed the informed consent.

Unsolicited AEs will be collected during interview with the parent(s)/LAR(s) and by review of available medical records at the next visit. Unsolicited AEs will be reviewed at the safety follow-up calls as well.

Parent(s)/LAR(s) will be instructed to contact the site as soon as possible to report potential unsolicited AEs that required hospitalization, or emergency room visit, or visit to/by a health care provider that were of concern to the parent(s)/LAR(s). The detailed information about the reported unsolicited AEs will be collected by the qualified site personnel during the interview and will be documented in the subject's records.

All subjects' parent(s)/ legal guardian(s) will be instructed to contact the site in case their child experiences unsolicited AEs which persist beyond 30 days after study vaccination.

7.1.5. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g. clinical chemistry, hematology, urinalysis) or other abnormal assessments (e.g. x-ray imaging studies) that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to Sections 7.1.1 and 7.1.2). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs. The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

7.1.6. Adverse events of special interest

Adverse events of special interest (AESIs) are predefined (serious or non-serious) AEs of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate, because such an event might warrant further investigation in order to characterize and understand it.

7.1.6.1. Febrile seizures

Seizures are defined according to the Brighton Collaboration Working Group (BCWG) case definition of generalized convulsive seizure as an adverse event following immunization [Bonhoeffer, 2004].

The list of Preferred Terms (PTs) corresponding to the diagnosis of seizure, are those included in the Medical Dictionary for Regulatory Activities (MedDRA) Standard MedDRA Query (SMQ) Narrow "Generalized convulsive seizures following immunization".

For any new diagnosis of generalized convulsive seizure (including febrile seizure), irrespective of seriousness, the investigator (or designate) must complete, an electronic Expedited Adverse Events Report and an ad-hoc eCRF page on seizures to further characterize this AESI.

7.1.6.2. Arthritis

Cases of arthritis are defined according to the following ad-hoc definition:

- Presence of a physical exam findings of swelling, redness, heat, or limitation in range of motion and/or
- Presence of a diagnostic imaging studies interpreted by a health care provider as demonstrating evidence of joint inflammation and/or arthrocentesis results evidencing inflammation.

Due to the heterogeneity of the presentation of arthritis which can be either acute or chronic, the threshold of duration of symptoms of 6 weeks is to be considered.

The list of PTs corresponding to the diagnosis of arthritis, are those included in the MedDRA SMQ Narrow "Arthritis". For any new diagnosis of arthritis (serious or non-serious) in a study subject, the investigator (or designate) must complete, an electronic Expedited Adverse Events Report and an ad-hoc eCRF page on arthritis to further characterize this AESI.

7.1.6.3. Potential immune-mediated diseases

Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune aetiology. The AEs that need to be recorded and reported as pIMDs include those listed in Table 12.

However, the investigator will exercise his/her medical and scientific judgement in deciding whether other diseases have an autoimmune origin (i.e. pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

Table 12 List of Potential Immune-Mediated Diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve neuropathy, including paralysis and paresis (e.g. Bell's palsy). • Optic neuritis. • Multiple sclerosis. • Transverse myelitis. • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants.	• Systemic lupus erythematosus and associated conditions • Systemic scleroderma (Systemic sclerosis), including: - Diffuse Scleroderma - CREST syndrome • Idiopathic inflammatory myopathies, including: - Dermatomyositis - Polymyositis	• Psoriasis. • Vitiligo. • Erythema nodosum. • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis). • Lichen planus. • Sweet's syndrome.

• **Acute disseminated encephalomyelitis, including site specific variants e.g.: non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis.** • **Myasthenia gravis, including Lambert-Eaton myasthenic syndrome.** • **Demyelinating peripheral neuropathies including**: - **Chronic inflammatory demyelinating polyneuropathy,** - **Multifocal motor neuropathy** - **Polyneuropathies associated with monoclonal gammopathy.** • **Narcolepsy.**	• **Anti-synthetase syndrome.** • **Rheumatoid Arthritis and associated conditions including:** - **Juvenile Idiopathic Arthritis** - **Still's disease.** • **Polymyalgia rheumatica.** • **Spondyloarthropathies, including:** - **Ankylosing Spondylitis,** - **Reactive Arthritis (Reiter's Syndrome),** - **Undifferentiated Spondyloarthritis,** - **Psoriatic Arthritis,** - **Enteropathic arthritis.** • **Relapsing Polychondritis.** • **Mixed Connective Tissue disorder.** • **Gout.**	• **Localized Scleroderma (Morphoea).**
Vasculitis	**Blood disorders**	**Others**
• **Large vessels vasculitis including:** - **Giant Cell Arteritis (Temporal Arteritis),** - **Takayasu's Arteritis.** • **Medium sized and/or small vessels vasculitis including:** - **Polyarteritis nodosa,** - **Kawasaki's disease,** - **Microscopic Polyangiitis,** - **Wegener's Granulomatosis (granulomatosis with polyangiitis),**	• **Autoimmune hemolytic anemia.** • **Autoimmune thrombocytopenia.** • **Antiphospholipid syndrome.** • **Pernicious anemia.** • **Autoimmune aplastic anemia.** • **Autoimmune neutropenia.** • **Autoimmune pancytopenia.**	• **Autoimmune glomerulonephritis including:** - **IgA nephropathy,** - **Glomerulonephritis rapidly progressive,** - **Membranous glomerulonephritis,** - **Membranoproliferative glomerulonephritis,** - **Mesangioproliferative glomerulonephritis.** - **Tubulointerstitial nephritis and uveitis syndrome**.
- **Churg–Strauss syndrome (allergic granulomatous angiitis or eosinophilic granulomatosis with polyangiitis),** - **Buerger's disease (thromboangiitis obliterans),** - **Necrotizing vasculitis**		• **Ocular autoimmune diseases including:** - **Autoimmune uveitis** - **Autoimmune retinitis.** • **Autoimmune myocarditis.** • **Sarcoidosis.** • **Stevens-Johnson syndrome.** • **Sjögren's syndrome.**

(cutaneous or systemic), - anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), - Henoch-Schonlein purpura (IgA vasculitis), - Behcet's syndrome, - Leukocytoclastic vasculitis.		• Alopecia areata. • Idiopathic pulmonary fibrosis. • Goodpasture syndrome. • Raynaud's phenomenon.
Liver disorders	**Gastrointestinal disorders**	**Endocrine disorders**
• Autoimmune hepatitis. • Primary biliary cirrhosis. • Primary sclerosing cholangitis. • Autoimmune cholangitis.	• Inflammatory Bowel disease, including: - Crohn's disease, - Ulcerative colitis, - Microscopic colitis, - Ulcerative proctitis. • Celiac disease. • Autoimmune pancreatitis.	• Autoimmune thyroiditis (Hashimoto thyroiditis). • Grave's or Basedow's disease. • Diabetes mellitus type I. • Addison's disease. • Polyglandular autoimmune syndrome. • Autoimmune hypophysitis.

When there is enough evidence to make any of the above diagnoses, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as pIMDs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

In order to facilitate the documentation of pIMDs in the eCRF, a pIMD standard questionnaire and a list of MedDRA preferred terms (PTs) and PT codes corresponding to the above diagnoses will be available to investigators at study start.

7.2. Detecting and recording adverse events and serious adverse events

7.2.1. Time period for detecting and recording adverse events and serious adverse events

All AEs starting within 30 days following administration of each dose of study vaccines (Day 1 to Day 30 postvaccination) must be recorded onto/into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

The time period for collecting and recording SAEs will begin at Day 1 (ie, the first receipt of study vaccines) and will end 6 months or 12 months following administration of the last dose of study vaccine for each subject (ie, study end). See Section 7.3 for instructions on reporting of SAEs.

All medically attended AEs will be collected and recorded from Day 1 (ie, the time of the first receipt of study vaccines) until 6 months or 12 months following administration of the last dose of study vaccine for each subject (ie, study end).

All AEs/SAEs leading to withdrawal from the study will be collected and recorded from Day 1 (ie, the time of the first receipt of study vaccines) until 6 months or 12 months following administration of the last dose of study vaccine for each subject (ie, study end).

In addition to the above-mentioned reporting requirements and in order to fulfil international reporting obligations, SAEs that are related to study participation (i.e. protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged from the study.

The time period for collecting and recording of AESIs will begin at Day 1 (ie, the first receipt of study vaccines) and will end 6 months or 12 months following administration of the last dose of study vaccines (ie, study end). See section 7.3 for instructions on reporting of AESIs. Details regarding follow-up of AEs, SAEs and AESIs are given in Section 7.4.

An overview of the protocol-required reporting periods for AEs, SAEs, medically attended AEs and AESIs is given in Table 13.

Table 13 Reporting Periods for Collecting Safety Information

Event	Pre-V1[1]	V1 D1	D16	D31	V2 D61 (2 M post-vacc 1)	D76	D91	V3 D121 (2 M post-vacc 2)	D136	V4 D151 (1 M post-vacc 3)	D181	D241	V5 D301 (6 M post-vacc 3)	D 316	V6 D331 (1 M post-vacc 4)	D361, 421, 481, 541, 601[2]	V7 *D481 or* D661 (*6 M or* 12 M post-vacc 4)[3]
Solicited local and systemic AEs[4]																	
All Unsolicited AEs																	
AEs/SAEs leading to withdrawal from the study																	
SAEs (including SAEs related to study vaccine(s))																	
SAEs related to study participation or concurrent GSK medication/vaccine																	
AESIs																	
Medically attended AEs																	
COVID-19 infection related AEs																	

M: month, V: Visit; D: Day, AE: adverse event, SAE: serious adverse event, AESI: adverse event of special interest, COVID-19: Coronavirus Disease 2019.

Note: All concomitant medications/products, except vitamins and dietary supplements, administered during the 30-days postvaccination period as well as those used to treat an AE or a SAE (throughout the study period), should be recorded in the eCRF and subject medical records.

[1] After informed consent is obtained.

[2]For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, follow-up calls on Day 481, Day 541 and Day 601 will not be performed.

[3]For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481.

[4] Solicited AEs to be collected for 7 days following each vaccination, with ongoing solicited AEs collected until resolution or day 30, whichever occurs first. Specific solicited systemic AEs (parotid gland swelling, fever, rash) will be collected for 30 days following MMR/V vaccination a Visit 5 (Day 301).

7.2.2. Post-Study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 13. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study vaccines, the investigator will promptly notify the Study Contact for Reporting SAEs.

For further details, please see Section 7.4.1.2.

7.2.3. Evaluation of adverse events and serious adverse events

7.2.3.1. Active questioning to detect adverse events and serious adverse events

As a consistent method of collecting AEs, the subject's parent(s)/LAR(s) should be asked a non-leading question such as:

'*Has your child acted differently or felt different in any way since receiving the vaccine(s) or since the last visit?*'

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms**.**

Pre-defined scripts will be used during the clinic visits and safety follow-up calls for instructing parents in recording safety data.

7.2.3.2. Assessment of adverse events

7.2.3.2.1. Assessment of intensity

The intensity of the following solicited AEs will be assessed as described:

Table 14 Grading of Solicited Local Adverse Events *(Administration Site Event)* for All Subjects

Adverse Events	Grading of Severity			
	Grade 0	Mild	Moderate	Severe
Tenderness or discomfort to touch at injection site	None	Minor reaction to touch	Cried or protested to touch	Cried when injected limb was moved
Hardness of skin at the injection site	0 mm	1 – 25 mm	26 - <50 mm	>50 mm
Swelling of skin at the injection site				
Redness at injection site				

Table 15 Grading of Solicited Systemic Adverse Events for All Subjects

Adverse Events	Grading of Severity			
	Grade 0	Mild	Moderate	Severe
Decreased eating	None	Eating less than normal for 1 – 2 feeds/ meals	Missed 1 or 2 feeds / meals	Missed more than 2 feeds/ meals
Increased sleepiness	None	Shows an increased drowsiness	Sleeps through feeds/ meals	Sleeps most of the time and it is hard to arouse him/her
Vomiting / throwing up	None	1-2 times in 24 hours	3 – 5 times in 24 hours	6 or more times in 24 hours or requires intravenous hydration
Loose stools / Diarrhea	Fewer than 2 loose stools in 24 hours	2 – 3 loose stools in 24 hours	4 – 5 loose stools in 24 hours	6 or more loose stools in 24 hours or requires intravenous hydration
Irritability	None	Requires more cuddling and is less playful than usual	More difficult to settle	Unable to console
Persistent / continuous crying	None	Crying less than 1 hour	Crying for 1 up to 3 hours	Crying for 3 or more hours
Rash[1]	0	1-50 lesions	51-150 lesions	>150 lesions
Parotid/salivary gland swelling[1]	None	Swelling without difficulty moving the jaw	Swelling with difficulty moving the jaw	Swelling with accompanying general symptoms
Fever[2]	<38.0°C (<100.4°F)	≥38.0 – 38.9°C (≥100.4 – 102.1°F)	≥39.0 – 39.9°C (≥102.2 – 103.9°F)	≥40.0°C (≥ 104.0°F)

[1].Rash and parotid/salivary gland swelling will be recorded only after receiving the MMR and VV vaccines at 12 months of age at Visit 5 (Day 301). These specific systemic AEs, along with fever, will be recorded for 30 days following the MMR and VV vaccine administration.

[2] Preferred location for measuring temperature is the rectum for subjects <12 months of age and the axilla for subjects ≥12 months of age. Body temperature is to be recorded daily, ideally at the same time each day.

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgement.

Every effort should be made by the investigator to evaluate safety information reported by a subject's parent(s)/LAR(s) for an underlying diagnosis and to capture this diagnosis as the event in the AE page. In other words, the practice of reporting only symptoms (e.g., "cough" or "ear pain") are better reported according to the underlying cause (e.g., "asthma exacerbation" or "otitis media").

The severity of events reported on the Adverse Events eCRF will be determined by the investigator as:

Mild: transient with no limitation in normal daily activity.
Moderate: some limitation in normal daily activity.
Severe: unable to perform normal daily activity.

7.2.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between study vaccine(s) and the occurrence of each AE/SAE using clinical judgement. In case of concomitant administration of multiple vaccines/products, if possible, the investigator should specify if the AE could be causally related to a specific vaccine/product administered (i.e. investigational, control/placebo or co-administered vaccine). When causal relationship to a specific vaccine(s)/product(s) cannot be determined, the investigator should indicate the AE to be related to all products.

Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study vaccine(s) will be considered and investigated. The investigator will also consult the IB and/or SmPC and/or Prescribing Information for marketed products to determine his/her assessment.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the Expedited Adverse Events Report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

All solicited AEs will be considered causally related to vaccination. Causality of all other AEs should be assessed by the investigator using the following question:

Is there a reasonable possibility that the AE may have been caused by the study vaccine?

YES	:	There is a reasonable possibility that the study vaccine(s) contributed to the AE.
NO	:	There is no reasonable possibility that the AE is causally related to the administration of the study vaccine(s). There are other, more likely causes and administration of the study vaccine(s) is not suspected to have contributed to the AE.

If an event meets the criteria to be determined as ‘serious’ (see Section 7.1.2), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine(s), if applicable.
- Erroneous administration.
- Other cause (specify).

All AEs, regardless of severity, will be monitored by a qualified healthcare professional until resolution or until the investigator assesses them as chronic or stable, and recorded in the subject's records. All subjects experiencing AEs – whether considered associated with the use of the study vaccine(s) or not – must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. The investigator's assessment of ongoing AEs at the time of each subject's last visit should be documented in the subject's medical chart and eCRF.

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

7.2.3.3. Assessment of outcomes

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

7.2.3.4. Medically attended visits

For each solicited and unsolicited symptom the subject experiences, the subject's parent(s)/LAR(s) will be asked if the subject received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will be recorded in the eCRF and source documents.

7.2.4. Recording of AEs related to COVID-19

For COVID-19 infection related AEs, sites should follow routing AE/SAE processes as outlined in the protocol using the following terms per WHO defined case definitions:

- Suspected COVID-19 infection
- Probable COVID-19 infection
- Confirmed COVID-19 infection.

7.2.4.1. WHO Case Definition

- Suspected case

  A. A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), AND a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset;

  OR

  B. A patient with any acute respiratory illness AND having been in contact (see definition of "contact" below) with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset;

  OR

  C. A patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; AND requiring hospitalization) AND in the absence of an alternative diagnosis that fully explains the clinical presentation.

- Probable case

  A. A suspect case for whom testing for the COVID-19 virus is inconclusive (Inconclusive being the result of the test reported by the laboratory).

  OR

  B. A suspect case for whom testing could not be performed for any reason.

- Confirmed case

  A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms.

- A contact is a person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case:
  - Face-to-face contact with a probable or confirmed case within 1 meter and for more than 15 minutes;
  - Direct physical contact with a probable or confirmed case;

– Direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment; 2OR

– Other situations as indicated by local risk assessments.

Note: for confirmed asymptomatic cases, the period of contact is measured as the 2 days before through the 14 days after the date on which the sample was taken which led to confirmation.

7.3. Reporting of serious adverse events and other events

7.3.1. Prompt reporting of serious adverse events and other events to GSK Biologicals

SAEs that occur in the time period defined in Section 7.2 will be reported promptly to GSK within the timeframes described in Table 16, once the investigator determines that the event meets the protocol definition of a SAE.

AESIs that occur in the time period defined in Section 7.2 will be reported promptly to GSK within the timeframes described in Table 16, once the investigator determines that the event meets the protocol definition of an AESI.

Table 16 Timeframes for submitting serious adverse event and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours* ‡	Electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report
AESIs	24 hours** ‡	Electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report

* Timeframe allowed after receipt or awareness of the information.
**Timeframe allowed once the investigator determines that the event meets the protocol definition of a AESI.
‡ The investigator will be required to confirm review of the SAE/AESI causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/AESI.

7.3.2. Contact information for reporting serious adverse events and AESIs

After receipt of the initial report, representatives of GSK or its designee will contact the investigator if it is necessary to obtain further information for assessment of the event.

Study Contact for Reporting SAEs and AESIs
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs and AESIs
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** US sites only: Fax: 1-610-787-7053 ***Email: RIX.CT-Safety-Vac@gsk.com***

7.3.3. Completion and transmission of SAE reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

He investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

7.3.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designate) must complete, then date and sign a paper Expedited Adverse Events Report and fax it to the Study Contact for Reporting SAEs (refer to the SPONSOR INFORMATION) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designate) must complete the electronic Expedited Adverse Events Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

7.3.4. Reporting of AESIs to GSK Biologicals

Once an AESI is diagnosed (serious or non-serious) in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS after he/she becomes aware of the diagnosis (Table 16). The report allows to specify that the event is an AESI and whether it is serious or non-serious. The report will always be completed as thoroughly as possible with all available details of the event. For pIMDs, the report will always be completed in accordance with the pIMD standard questionnaire provided. Even if the investigator does not have all information regarding an AESI, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the AESI causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the AESI.

Refer to Section 7.3.3.1 for back-up system in case the electronic reporting system does not work.

7.3.5. Updating of SAE and AESI information after removal of write access to the subject's eCRF

When additional SAE, or AESI information is received after removal of the write access to the subject's eCRF, new or updated information should be recorded on the appropriate paper report, with all changes signed and dated by the investigator. The updated report should be faxed to the Study Contact for Reporting SAEs (refer to the SPONSOR INFORMATION) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within the designated reporting time frames specified in Table 16.

7.3.6. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 7.3.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the study vaccine(s) and unexpected. The purpose of the report is to fulfil specific regulatory and GCP requirements, regarding the product under investigation.

All SAEs must be reported by the investigator to his/her corresponding EC/IRB and/ or applicable regulatory authorities in accordance with institutional policy/regulatory requirements and adequate documentation of this notification must be provided to the Sponsor.

7.4. Follow-up of adverse events and serious adverse events

7.4.1. Follow-up of adverse events and serious adverse events

7.4.1.1. Follow-up during the study

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs; refer to Table 16).

All SAEs and AESIs (serious or non-serious) documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the last visit of the subject.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last vaccination.

7.4.1.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- with SAEs, AESIs (serious or non-serious), or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper/ electronic Expedited Adverse Events Report as applicable.

GSK Biologicals may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognized follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

7.5. Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of a SAE / AESIs should be recorded in Expedited Adverse Event Report of the subject's eCRF (refer to Section 6.7).

7.6. Unblinding

GSK Biologicals' policy (which incorporates ICH E2A guidance, EU Clinical Trial Directive and US Federal Regulations) is to unblind the report of any SAE which is unexpected and attributable/suspected to be attributable to the study vaccines, prior to regulatory reporting. The GSK Biologicals' Central Safety Physician is responsible for unblinding the treatment assignment in accordance with the specified timeframes for expedited reporting of SAEs (refer to Section 7.3.1).

7.7. Emergency unblinding

Unblinding of a subject's individual treatment code should occur only in the case of a medical emergency, or in the event of a serious medical condition, when knowledge of the treatment is essential for the clinical management or welfare of the subject, as judged by the investigator.

The emergency unblinding process consists of the automated system SBIR that allows the investigator to have unrestricted, immediate and direct access to the subject's individual study treatment.

The investigator has the option of contacting a GSK Biologicals' On-call Central Safety Physician (or Backup) if he/she needs medical advice or needs the support of GSK to perform the unblinding (i.e. he/she cannot access the automated Internet-based system).

GSK Biologicals' Contact information for Emergency Unblinding **24/24 hour and 7/7 day availability**
GSK Biologicals' Central Safety Physician: For US only: +1 844 446 3133 (GSK Biologicals Central Safety Physician on-call) **GSK Biologicals' Central Safety Physician Back-up:** For US only: ***GSK Helpdesk: 1-877-528-7677 or 1 877-221-2913*** ***Email: GSKClinicalSupportHD@gsk.com***

7.8. Subject card

Study subjects / subjects' parent(s)/LAR(s) must be provided with the address and telephone number of the main contact for information about the clinical study.

The investigator (or designate) must therefore provide a "subject card" to each subject/ subject's parent(s)/LAR(s). In an emergency situation this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator.

Subjects' parent(s)/LAR(s) must be instructed to keep subject cards in their possession at all times during the study duration.

7.9. Medical device deficiencies

The study intervention is a combination product constituted of a device and biologic product (e.g. pre-filled syringes). Refer to the GLOSSARY OF TERMS for the definition of combination product and medical device deficiency.

7.9.1. Detection, follow-up and prompt reporting of medical device deficiency

The investigator is responsible for the detection, documentation and prompt reporting of any medical device deficiency occurring during the study to GSK. This applies to any medical device provided for the conduct of the study.

Device deficiencies will be reported to GSK within 24 hours after the investigator determines that the event meets the protocol definition of a device deficiency. Refer to Section 10.6 for definitions and details on recording and reporting of these events.

The investigator will ensure that follow-up includes any additional investigations to elucidate the nature and/or causality of the deficiency to the incident. Follow-up applies to all participants, including those who discontinue study intervention or the study.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator and reported to GSK within 24 hours.

7.9.2. Regulatory reporting of medical device deficiency when used as combination product

The investigator will promptly report all device deficiencies occurring with any medical device provided for use in the study to GSK. GSK has a legal responsibility to notify appropriate regulatory authorities and other entities about safety information linked to medical devices being used in clinical studies. Refer to section 10.6.3 for details of reporting.

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. SUBJECT COMPLETION AND WITHDRAWAL

8.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol is considered to have completed the study.

8.2. Subject withdrawal

Withdrawals will not be replaced.

8.2.1. Subject withdrawal from the study

From an analysis perspective, a ‘withdrawal’ from the study refers to any subject who did not come back for the concluding visit foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a ‘withdrawal’ from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject's parent(s)/LAR(s), or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Unsolicited non-serious adverse event.
- Solicited adverse event
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event*.
- Moved from the study area.
- Lost to follow-up.
- Other (specify).

*In case a subject is withdrawn from the study because the subject's parent(s)/LAR(s) has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject/subject's parent(s)/LAR(s), in the eCRF.

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of a SAE/AE until resolution of the event (see Section 7.4.1.2).

Adverse Event: For any subject withdrawn from study participation prior to the planned Study Termination Visit, it is important to determine if an AE was associated with the reason for discontinuing the study. This AE must be identified on the AE eCRF page by indicating "Withdrawn from study due to AE". Any ongoing AEs at the time of study withdrawal must be followed until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up. Subjects who develop an SAE judged to be possibly or probably related to the study vaccine, including hypersensitivity reactions, should not receive subsequent vaccination.

Death: For any subject withdrawn from study participation due to death, this should be noted on the Study Termination eCRF page and the associated SAE that led to the death must be reported.

Withdrawal of consent: The subject's parent(s)/LAR(s) can withdraw consent for participation in the study at any time without penalty or loss of benefit to which the subject is otherwise entitled. Reason for early termination should be deemed as "withdrawal of consent" if the subject withdraws from participation due to a non-medical reason (i.e., reason other than AE).

If the subject's parent(s)/LAR(s) intend(s) to withdraw consent from the study, the investigator should clarify if the subject will withdraw completely from the study or if the subject will continue study participation for safety, or a subset of other study procedures.

If the subject's parent(s)/LAR(s) requests complete withdrawal from the study, no further study interventions will be performed with the subject.

Where applicable, if a subject's parent(s)/LAR(s) withdraw(s) consent but does not revoke the HIPAA authorization, the Sponsor will have full access to the subject's medical records, including termination visit information. If a subject's parent(s)/LAR(s) revokes only the HIPAA authorization, the Sponsor will have full access to all of the subject's medical records prior to the date and time of written revocation.

Lost to follow-up: Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up. For subjects who fail to show up for final visits (clinic or telephone contacts), or for 3 consecutive visits, study staff are encouraged to make at least 3 documented attempts to contact the subject by telephone and at least 1 documented written attempt to contact the subjects' parent(s)/LAR(s) to encourage the completion of study termination procedures. These efforts to contact the subject should be recorded in the source document. The termination date for the subject to be captured on the Study Termination eCRF page is the date of the last successful contact (clinic visit or telephone) with the subject.

Administrative Reason: Examples for subjects withdrawn from the study due to administrative reason can include: Sponsor decision to terminate the study, subject meeting a pre-specified withdrawal criterion, subject discontinuation for insurance issues, moving, no time, etc. This reason should be noted in the Study Termination eCRF page and any ongoing AEs at the time of study withdrawal must be followed as described above.

If the clinical study is prematurely terminated by the Sponsor, the investigator is to promptly inform the study subjects and local EC/IRB and should assure appropriate therapy and follow up for the subjects. All procedures and requirements pertaining to the archiving of study documents should be followed. All other study materials (study medication/vaccines, etc.) must be returned to the Sponsor.

For subjects who are withdrawn from the study due to receipt of an excluded medication/vaccination or due to significant protocol non-compliance, this reason should be noted in the Study Termination eCRF page.

Protocol violation (deviation): Investigators will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact GSK or its agents, if any, monitoring the study to request approval of a protocol deviation, as no authorized deviations are permitted. If the investigator feels a change to the protocol would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by GSK and approved by the IRB/EC and health authorities it cannot be implemented (see Section 5.1.1 and Investigator Agreement).

8.2.2. Subject withdrawal from study vaccine(s)

A 'withdrawal' from the study vaccine(s) refers to any subject who does not receive the complete treatment, i.e. when no further planned dose is administered from the date of withdrawal. A subject withdrawn from the study vaccine(s) may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information relative to premature discontinuation of the study vaccine(s) will be documented on the Vaccine Administration page/screen of the eCRF. The investigator will document whether the decision to discontinue further vaccination/treatment was made by the subject's parent(s)/LAR(s), or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Unsolicited non-serious adverse event.
- Solicited adverse event
- Not willing to be vaccinated
- Other (specify).

8.3. Screen and baseline failures

In the event that the individual is determined ineligible for study participation after signing the ICF, he/she is considered a screen failure. The reason for screen failure must be documented in the Screening and Enrolment log.

9. STATISTICAL METHODS

9.1. Determination of sample size

The study sample size was determined by the revised study design while maintaining an adequate study power to achieve all co-primary objectives previously discussed with CBER. The multiplicity issue was dealt with by controlling the global α at the level of 0.05 for all associated statistical hypotheses. This reduced number will also allow for enrolment of the minimum number of subjects exposed to rMenB+OMV NZ to fulfill initial Pediatric Study Plan (iPSP) commitment. The sample sizes were also calculated for the secondary objectives to evaluate the non-inferiority of RIV antigens. The evaluation will be performed by calculating the between-group differences/ratios and their 2-sided 95% CI. Therefore, no multiplicity was adjusted for the secondary objectives.

Study Sample Size Determination by Co-Primary Objectives

For the immune response to PCV13, sample size calculations were based on data from the PCV13 package insert and for the immune response to rMenB+OMV NZ sample size calculations were based on data from the previous GSK (legacy Novartis) V72P13 and V72P13E1, and from V72_56 study.

Using the procedure described in this section, the power for the study is presented in Table 17. In case of 40% drop-out rate and 1200 subjects to be enrolled in a 2:1 ratio, it's assumed that 480 evaluable subjects from Group MenB+PCV and 240 evaluable subjects from Group Placebo+PCV could participate with data to the analyses. For PCV13 antigens a sample size of 240 will have approximately 99% power to show non-inferiority for all antigens. For MenB strains it's assumed that 480 evaluable samples from Group MenB+PCV will be evaluated in the analyses. With 240 per group for PCV13 antigen tests and 480 evaluable subjects from Group MenB+PCV for rMenB+OMV NZ strain tests, there is approximately 99% probability to reject all 23 null hypotheses, and to claim that the primary objectives of the study will be met according to the predefined success criteria.

Table 17 Power to Show Non-Inferiority PCV13 Co-Primary Objective and Sufficiency rMenB+OMV NZ Co-Primary Objectives

Co-Primary Objective	Power
Non-inferiority for GMCs for 13 antigens after the 3rd vaccination	99.0%
Sufficiency for % of subjects with titers ≥ LLOQ per each strain and all 4 strains combined (composite response) after the 3rd vaccination	99.8%
Sufficiency for % of subjects with hSBA titers≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) individually and all 4 strains combined (composite response) after the 4th vaccination	>99.999%
Probability of rejection of all 23 null hypotheses.	98.8%

The power was estimated using simulations: 10.000 simulations per case. SAS 9.4 was used for the calculations.

A short summary on the steps of simulation is presented below, in the example of 40% drop-out. The definition for families is given in Section 9.5.3.1.

In total 10.000 simulations were performed, each simulation consisted of:

a. For testing Family 1, 240 normal distributed data were drawn for two groups and hence two times thirteen antigens, according to the ratio of GMC and the SD, as described below Table 18. For PCV13, a GMC ratio of 0.8 was assumed. Correlation structure was made for between-antigen correlation of 0%.

b. For testing Family 2 and 3, 480 normal distributed data were drawn for one single group and for the four strains, according to the GMTs (for post 3rd dose) and GMTs (for post 4th dose), as shown in Table 19, and the SDs as described below Table 18. Correlation structure was made for between-antigen correlation of 0%.

c. Testing of Family 1: Shifted one-sided t-test was used to test the hypotheses and output the p-values (for the power calculation no center effect was assumed).

d. P-values were compared with the Family Wise Error Rate (FWER) to conclude on whether the null hypotheses were rejected or not. FWER for Family 1 is initially set to be 1/3 of the full alpha, which is rounded to 0.017. The FWER for Family 2 is 0.033 and the initial FWER is 0 for Family 3. No multiplicity adjustment was performed within a family. Within family 1 all thirteen antigens were tested with the full FWER for family 1. If one or more test fail to reject the hypothesis for the antigen, family 1 fails. Consequently the alpha of family 1 will not be propagated (see bullet h. below).

e. Preparing for testing Family 2 and 3, percentages were obtained by dichotomizing the normal random sets that were generated at step b, by applying the cut-off of LLOQ value for post 3rd or a cut-off of 8 (for strains M14459, NZ98/254, M13520) or 16 (for strain 96217) at post 4th. Per time point, composite endpoint was derived by looking into each subject if the titer is above the cut-off for all 4 individual strains and if it is then this subject is counted as "1". Otherwise the subject is counted as "0".

f. For each of the ten percentages for rMenB+OMV NZ (4 individual strains and 1 composite endpoint at post-3rd and post-4th) the lower confidence limit was generated by fitting the binomial distribution with the percentage obtained from the previous step and alpha that has been assigned or propagated to the family. If the lower confidence limit is greater or equal to the sufficiency margin, then the null hypothesis is rejected for the single test. For Family 2 and 3, it is required to reject the null hypothesis for each and all of strain M14459, 96217, NZ98/254, M13520, and the composite endpoint to be able to claim the success of the family.

g. Testing of Family 2: with the given FWER, if the LCL is greater or equal to 0.75 for each of strain M14459, 96217 and NZ98/254, M13520, and greater or equal to 0.65 for the composite endpoint, then Family 2 is claimed for success. The FWER for Family 2 is set up as 2/3 of the full alpha, which is rounded to 0.033.

h. Alpha propagation between Family 1, 2 and 3 was calculated for 4 scenarios:

   1) If Family 1 had succeeded and Family 2 not, then propagate ½ FWER from Family 1 to Family 3 and the other ½ FWER to Family 2, and re-test Family 2 with the new FWER which is the sum of the original FWER from Family 2 plus the ½ of FWER from Family 1. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 2 and propagate full FWER to Family 3; otherwise Family 2 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 1.

   2) If Family 2 had succeeded and Family 1 not, then propagate ½ FWER from Family 2 to Family 3 and the other ½ FWER to Family 1, and re-test Family 1 with the new FWER which is the sum of the original FWER from Family 1 plus the ½ of FWER from Family 2. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 1 and propagate full FWER to Family 3; otherwise Family 1 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 2.

   3) If both Family 1 and Family 2 had succeeded, then the FWER from both families were propagated to Family 3, and proceed to test Family 3;

4) If both Family 1 and Family 2 had failed, then no FWER will be propagated to Family 3, and we stop the testing globally.

i. Testing of Family 3: with the given FWER, if the LCL is greater or equal to 0.6 for each of strain M14459, 96217 and NZ98/254, M13520, and greater or equal to 0.5 for the composite endpoint, then Family 3 is claimed for success.

j. Add a loop on top of the aforementioned steps for 10.000 times simulation, summarize the probability of have all hypotheses rejected for each and all families, and the probability to meet all the primary objectives.

Based on the immune response observed in infant data from V72P13E1 and the PCV13 package insert, the following assumptions were made for the calculations:

1. As estimate for the SD of the different antigens and strains was taken the upper 80% CI limits of the common standard deviations for the $\log_{10}$ titers observed in GSK (legacy Novartis) study V72P13E1 and $\log_{10}$ ELISA concentrations observed from PCV13 studies. SDs that were used in the sample size calculations are presented in the table below.

Table 18 Two-sided 80% Upper Confidence Limit of Standard Deviations

	80% UCL of SDs	
rMenB+OMV NZ strains	**Post 4th vaccination (SDs of GMT)**	**Post 3rd vaccination (SDs of GMT)**
M14459	0.45097	0.32193
96217	0.63454	0.36900
NZ98/254	0.53796	0.51670
M13520[2]	0.36477	0.45023
PCV13 antigen[1]	**Post 3rd vaccination (SDs of GMC)**	
1	0.36629	
3	0.41572	
4	0.34261	
5	0.43567	
6A	0.39552	
6B	0.44974	
7F	0.34352	
9V	0.32548	
14	0.32445	
18C	0.33550	
19A	0.24439	
19F	0.45638	
23F	0.37567	

[1] Estimated SDs for PCV13 antigens are the upper confidence limit of 2-sided 80% CI of back-calculated SDs, based on information (degree of freedom, 95% CI) provided on table 17 of *Prevnar13* Package Insert (dated on July 2016) and assuming the underlying SDs fulfill a t-distribution.
[2] The assumptions for M13520 are cited from study V72_56 (205240) ad-hoc analysis on additional NHBA strains.

2. Underlying GMC ratios for each PCV13 antigens is assumed as 0.8.

3. For the sufficiency objectives, the following mean ($\log_{10}$(GMT)) were used in simulation based on observed data from previous studies (studies V72P13 and V72P13E1 and V72_56):

Table 19 GMTs at One Month Post 3rd Dose and GMRs at One Month Post 4th rMenB+OMV NZ Vaccination

rMenB+OMV NZ strains	GMTs* Post 4th vaccination	GMTs* Post 3rd vaccination
M13520	1.41225	1.10501
NZ98/254	1.57978	1.15836
M14459	1.53148	1.26400
96217	3.06183	2.66753

*log-scale

4. The LODs and LLOQs associated with the 4-fold definition are assumed to be:

Table 20 Limits of Detection and Lower Limits of Quantitation for rMenB+OMV NZ strains

rMenB+OMV NZ strains	LOD	LLOQ
M13520	4	4
NZ98/254	4	6
M14459	3	5
96217	6	15

LOD: lower limit of detection, LLOQ: lower limit of quantitation.
Note: Other than for strain M13520, these LODs and LLOQs were cited from the latest lab assay validation report released in 2018. For strain M13520, the LOD and LLOQ are validated and cited from the latest laboratory assay validation report released in 2020.

5. That each comparison is statistically independent.

In conclusion, assuming a 40% drop-out rate in this age group, a total of 1200 subjects to be enrolled in a 2:1 ratio, which will lead to 480 subjects evaluable in Group MenB+PCV and 240 subjects evaluable in Group Placebo+PCV, there will be 99% power to conclude that:

a. 13 PCV13 antigens will be shown non-inferiority of PCV13 vaccine when co-administrated with rMenB+OMV NZ vaccine, compared to PCV13 vaccine that is administered alone, at one month post 3rd vaccination

b. The percentage of subjects with titers ≥ LLOQ is more than or equal to 60% for strain M14459, 96217, NZ98/254 and M13520, and more than or equal to 50% for all 4 rMenB+OMV NZ strains at one month post 3rd vaccination.

c. Percentage of subjects with 4-fold increase is more than or equal to 75% for strain M14459, 96217, NZ98/254 and M13520, and more than or equal to 65% for all four rMenB+OMV NZ strains, at one month post 4th vaccination.

Determination of the Immunogenicity Subsets Sizes for the Secondary Objectives:

As described in detail in Table 9, due to limited serum volume available subjects will be randomly assigned to test different sets of RIV antigens. Subjects will either be assigned to subset A1, A2, A3 in Group MenB+PCV or assigned to subset B1 and B2 in Group Placebo+PCV (see Section 5.2.3). The below tables (Table 21 and Table 22) show the likelihood to achieve non-inferiority for each of the RIV antigens (excluding polio) with the proposed sample sizes for immunogenicity subsets.

Table 21 Non-inferiority to Each Individual Antigen (excluding polio antigens) in Pediarix and Hiberix, at One Month After 3rd Dose of rMenB+OMV NZ

Component	Endpoint	Non-inferiority Margin	Reference		# Evaluable Subjects per Immunogenicity Subset (MenB group/Placebo group)*	Power
			Difference in %/ratio (Co-ad vs routine)	% in Routine group or SD		
Diphtheria	%≥0.1IU/mL	10%	Diff=0%	99.4%	240/120	>99.9%
Tetanus	%≥0.1IU/mL	5%	Diff=0%	100%	240/120	>99.9%
PT	GMC	1.5	Ratio=0.8	SD: 0.31535	480/240	88.7%
FHA	GMC	1.5	Ratio=0.84	SD: 0.3111	480/240	98.3%
PRN	GMC	1.5	Ratio=0.83 [b]	SD: 0.43427	480/240	79.1%
HepB	%≥10.0mIU/mL	10%	diff=-2%	97.7%	120/120	93.5%
Hib	%≥0.15µg/mL	5%	diff=0% [a]	96.6%	360/240	91.2%
	%≥1µg/mL	10%	diff=0%	81.2%	360/240	86.7%

Abbreviations: SD, standard deviation; GMC, geometric mean concentrations; PT, pertussis toxin; FHA, Filamentous hemagglutinin; PRN, pertactin; HepB, Hepatitis B; Hib, Haemophilus influenzae type B; diff, difference

Note: The sources are V72P13 clinical study report and the package insert of vaccines, unless otherwise indicated. SDs for Pertussis were taken from GSK study Hib-97.

[a] Source: V72P12 CSR.

[b] Source: V72P16 Table 14.2.1.14 Group B+OMV versus MenC.

* Considering an estimated drop-out rate of 40%.

Table 22 Non-inferiority to Each Individual Antigen in MMR II and Varivax, at One Month After 4th Dose of rMenB+OMV NZ

Component	Endpoint	Non-inferiority Margin	Reference		# Evaluable Subjects per Immunogenicity Subset (MenB group/Placebo group)*	Power
			Ratio (co-ad vs routine)	SD		
Measles	GMC	1.5	ratio=0.9	SD: 0.45 [a]	480/240	95.5%
Mumps	GMC	1.5	ratio=0.9	SD: 0.38 [a]	480/240	99.1%
Rubella	GMC	1.5	ratio=0.9	SD: 0.34 [a]	480/240	99.8%
Varicella	GMC	1.5	ratio=0.9	SD: 0.45 [b]	480/240	95.5%

a Reference for sample size calculation: GSK Study MMR-161.

b Reference for sample size calculation: GSK Study MMR-160.

* Considering an estimated drop-out rate of 40%.

9.2. Analysis Sets

9.2.1. Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments and are randomized and have received a Subject ID, regardless of the subject's treatment status in the study.

9.2.2. Exposed Set

All subjects in the Enrolled Set who receive a study vaccination.

9.2.3. Safety Sets

9.2.3.1. Solicited Safety Set (solicited local and systemic adverse events and other solicited adverse events)

All subjects in the Exposed Set with any solicited adverse event data.

9.2.3.2. Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set with unsolicited adverse event data.

9.2.4. Full Analysis Set (FAS) *for* Immunogenicity

All subjects in the Exposed Set and provide immunogenicity data at either one month after their 4th vaccination or one month after their 3rd vaccination for at least 1 antigen/strain.

In case of vaccination error, subjects in the FAS sets will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

9.2.5. Per Protocol (PP) Set for Immunogenicity

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subjects is randomized and at the scheduled time points).
- Have no protocol deviations leading to exclusion as defined prior to unblinding / analysis.
- Are not excluded due to other reasons defined prior to unblinding or analysis

Examples for subjects excluded due to other reasons than protocol deviations are:

- Subjects who withdrew informed consent.
- Subjects who did not receive all the study vaccines up to Dose 3 for primary 3 doses analyses or up to Dose 4 for the 4th dose analyses;
- who received a vaccine not specified or forbidden in the protocol up to Visit 4 for primary 3 doses or up to Visit 6 for the 4th dose;
- who received a medication/product leading to exclusion from a per-protocol analysis as listed in Section 6.7.2 up to Visit 4 for primary 3 doses or up to Visit 6 for the 4th dose.

- who presented a medical condition leading to exclusion from a per-protocol analysis as listed in Section 6.8 up to Visit 4 for primary 3 doses or up to Visit 6 for the 4th dose.
- who did not comply with the post Dose 3 (for primary 3 doses) or post Dose 4 (for the 4th dose) blood sample schedule (Table 6).
- who had no immunogenicity results post Dose 3 (for primary 3 doses) or post Dose 4 (for the 4th dose).

The allowed intervals between each study visit are given in Table 6. Subjects will not be eligible for inclusion in the per-protocol cohort for analysis of primary 3 doses if the study visit is performed outside this interval up to visit 4, and they will not be eligible for analysis of the 4th dose if the study visit is performed outside this interval up to Visit 6.

9.2.6. Other Analysis Sets

Not applicable.

9.2.7. Subgroups

The following descriptive analyses will be performed by gender, race, ethnic origin ***and age group (6-8 weeks or ≥8 weeks)*** for the following parameters:

- GMCs and ratios of GMCs for PVC (Group MenB+PCV vs Group Placebo+PCV) at one month after the 3rd and 4th vaccination.
- Percentages of subjects with hSBA titers ≥LLOQ; for each of the 4 rMenB+OMV NZ strains and for the combined strains at one month after the 4th vaccination in Group MenB+PCV and Group Placebo+PCV.
- Percentages of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217); for each of the 4 rMenB+OMV NZ strains and for the combined strains at one month after the 4th vaccination in Group MenB+PCV and Group Placebo+PCV.

Analyses will be performed on the FAS.

Additional subgroup analyses may be performed to assess the impact of COVID-19. More details will be provided in the Statistical Analysis Plan.

9.3. Derived and transformed data

Immunogenicity:

- The GMTs/GMCs calculations are performed by taking the anti-log of the mean of the log titer transformations. Values to be used for the antibody concentrations/titres below the assay cut-off will be described in the Statistical Analysis Plan (SAP).
- Handling of missing data: Missing immunogenicity values are assumed to be missing completely at random (MCAR) and therefore may not contain information that impact the results of the analysis (i.e. not informative). Imputation methods will not be used.

Reactogenicity and Safety:

- First-line analyses will be performed without missing values. To minimize the effect of dropouts and missing safety data the study period will be divided into time intervals for statistical analysis of safety data.
- *Unsolicited AEs*: Unsolicited AEs are collected for 30 days post vaccination, and are analyzed as such without division of the time interval.
- AEs/SAEs leading to withdrawal from the study, SAEs (including SAEs related to study vaccine(s)), SAEs related to study participation or concurrent GSK medication/vaccine, AESIs, and Medically attended AEs: The entire study period will be divided into the following disjoint intervals: Day 1 through Day 30 post vaccination, Day 31 post vaccination until either the next vaccination or study termination.
- *Solicited adverse events:* For solicited AEs the solicited study period (30 minutes through Day 7) will be divided into the following intervals: 30 minutes and 6 hours through Day 7. Analyses may be performed on 6 hours through Day 3 and Day 4 through Day 7, if needed. For MMR and VV-specific solicited systemic AEs (parotid/salivary gland swelling, rash) collected after Visit 5 (30 minutes through Day 30 post-vaccination), the study period will be divided into the following intervals: 6 hours through day 7, day 8 though day 30.

  For each of the intervals the following algorithm will be applied:

  – If less than 20% of subjects are without any solicited AE data (i.e., none of the solicited adverse events have been captured) for the respective time interval, then only the solicited safety sets pertaining to the interval will be analyzed. If 20% or more of subjects are without any solicited AE data, the missing mechanism will be analyzed by vaccine group using a newly created variable indicating whether a subject is missing the respective AE-value or not (1=AE record present; 0=AE record not present).

9.4. Analysis of demographics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age, height and weight at enrolment will be calculated overall and by vaccine group.

Distributions of subjects by sex, race, ethnic origin, geographic region ***and age group (6-8 weeks or ≥8 weeks)*** will be summarized overall and by vaccine group.

9.5. Analysis of immunogenicity

The primary population for the primary immunogenicity analyses will be the PPS. All primary and selected secondary immunogenicity analyses (NI assessments to other RIVs) will be repeated on the FAS.

9.5.1. Within groups assessment

For each study group, at each timepoint that a blood sample result is available, the following endpoints will be assessed related to MenB strains and PCV13 strains:

- the percentages of subjects with hSBA titers ≥ LLOQ for each of the M14459, 96217, NZ98/254 and M13520 test strains
- the percentages of subjects with hSBA titers ≥ LLOQ for all strains combined (composite endpoint) at one month after the 3rd vaccination
- the percentages of subjects with hSBA titers ≥5, ≥8 and ≥16 for each of the M14459, 96217, NZ98/254 and M13520 test strains
- the percentages of subjects with hSBA titers ≥8 for each of the test strains (M14459, NZ98/254 and M13520), ≥16 for test strain 96217, and for all strains combined (composite endpoint) at one month after the 4th vaccination
- the percentages of subjects with 4-fold increase at post-4th (from pre-4th) for each of the M14459, 96217, NZ98/254 and M13520 test strains
- GMCs/GMTs and within group GMRs (for post-4th versus pre-4th titers) will be tabulated for antibodies for each antigen
- Percentage of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 μg/mL at one month after the 3rd and 4th vaccination in Group MenB+PCV and Group Placebo+PCV.

The CIs will be calculated as 2-sided 95%, and will be calculated at given alpha at the testing step for the primary objectives that are associated with statistical hypothesis testing.

Endpoints associated with the other RIV will be assessed within-group as specified in Section 2. The 2-sided 95% CIs will be calculated.

9.5.2. Between groups assessment

For PCV antigens, at each blood sampling timepoint and for each antibody for which results are available, for Diphtheria, Tetanus, Pertussis and Hepatitis B antigens, at blood sampling timepoint one month after the 3rd vaccination, for VV and MMR, at blood sampling timepoint one month after the 4th vaccination and for each antibody for which results are available:

- The CIs of the between-group GMC ratios (i.e. study group minus control group) will be computed using an analysis of variance (ANOVA) model on the logarithm10 transformation of the concentrations.

For Diphtheria, Tetanus, Hib, Polio and Hepatitis B antigens, at blood sampling timepoint one month after the 3rd vaccination, for VV and MMR, at blood sampling timepoint one month after the 4th vaccination and for each antibody for which results are available:

- The CIs of the between-group percentage differences (i.e. study group minus control group) will be computed.

9.5.3. Analysis of Primary Immunogenicity Objectives

9.5.3.1. Statistical Hypotheses

In total 23 hypotheses will be tested for the primary objectives. A step-wise statistical approach will be used for the 23 statistical tests. The hypotheses will be grouped into three families, based on the different immunogenicity objectives:

Family 1:13 statistical hypotheses related to non-inferiority of PCV13 with rMenB+OMV NZ as measured by the GMCs of the 13 PCV13 antigens, after the 3rd vaccination.

Family 2: 5 statistical hypotheses related to sufficiency objective on percentages of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) at one month post 4th dose, and the composite endpoint

Family 3: 5 statistical hypotheses related to sufficiency objective on percentages of subjects with hSBA titers ≥ LLOQ at one month post 3rd dose for each of the four strains M14459, 96217, NZ98/254, and M13520, and the composite endpoint.

Each family of hypotheses is linked to an objective, as follows:

- Family 1 is related to the "*immunological non-inferiority of rMenB+OMV NZ + PCV 13 compared to PCV13 after the 3rd vaccination*" objective.
- Families 2 and 3 are related to the *"sufficiency of the immune response to rMenB+OMV NZ"* objective at one month post 4th dose and one month post 3rd dose, respectively.

The statistical hypotheses for each and all families are formulated as below:

Family1: Non-inferiority of rMenB+OMV NZ + PCV13 to PCV13 with respect to GMCs at one month post 3rd vaccination

Non-inferiority will be claimed if the following null hypothesis will be rejected for 13 PCV13 antigens:

H_{01j}: $\mu_{Aj} - \mu_{Bj} \leq \delta$ vs. H_{11j} : $\mu_{Aj} - \mu_{Bj} > \delta$, j=1,..13,

where δ denotes the non-inferiority margin (log10(0.5)), μA, and μB denote the means of log 10-transformed concentrations at one month after the 3rd vaccination from Group MenB+PCV and Group Placebo+PCV, respectively, and j refers to the 13 antigens.

Family 2: Percentage of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) and the composite endpoint, at one month post-4th vaccination

Sufficiency will be claimed for each of the 4 strains and the composite endpoint, separately, if the following null hypothesis will be rejected:

H_{02j}: $P_{Aj} < A_j$ vs. H_{12j} : $P_{Aj} \geq A_j$,

where P_{Aj} denotes the percentage of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) at one month after the 4th vaccination for strain j, (j=1, 2, 3, 4, and composite). Aj represents the level of sufficient immune response for each of the four strains and composite endpoint. The immune response at one month following the 4th vaccination, will be sufficient for rMenB+OMV against each individual strain if the lower limit of the adjusted CI for the percentage of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) is ≥75% for the individual N. meningitidis serogroup B test strains, and is ≥65% for the composite endpoint.

Family 3: Percentage of subjects with hSBA≥LLOQ for each of the four strains (M14459, 96217, NZ98/254, M13520) and the composite endpoint, one month post post-3rd. vaccination

Sufficiency will be claimed for each of the 4 strains, separately, if the following null hypothesis will be rejected:

H_{03j} : $P_{Aj} < A_j$ vs. H_{13j} : $P_{Aj} \geq A_j$,

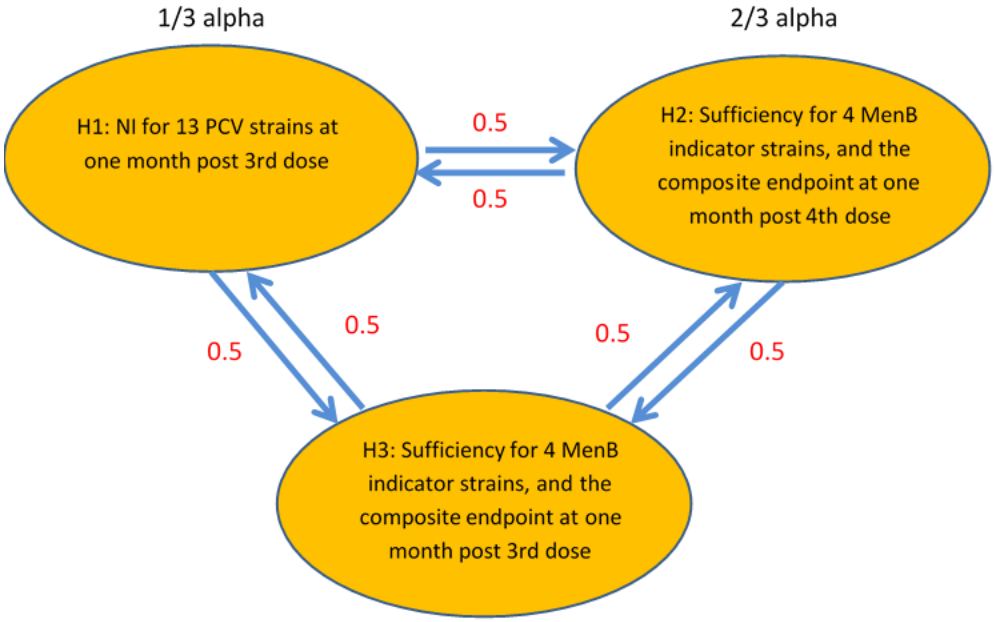
where P_{Aj} denotes the percentage of subjects hSBA≥LLOQ at one month after the 3rd vaccination for strain j, j=1, 2, 3,4, and composite. Aj represents the level of sufficient immune response for each of the four strains and composite endpoint. The immune response at one month following the 3rd vaccination, will be sufficient for rMenB+OMV NZ against each individual strain if the lower limit of the adjusted CI for the percentage of subjects with hSBA titer ≥LLOQ is ≥60% for the N. meningitidis serogroup B test strains, and ≥50% for the composite endpoint.

9.5.3.2. Statistical Methods

For statistical analyses of the concentration/titer data, the concentrations/titers will be logarithmically transformed (base10), to fulfill Gaussian distribution assumption.

The Global Type I error rate will be controlled globally at a 5%/2.5% level (2-/1-sided, respectively). If applicable, the α-level will be propagated as described below [Hommel, 2007]. The sequence of testing the different families is presented in Figure 2 and described in more detail thereafter.

Figure 2 Testing Strategy for Three Families with Corresponding α Propagation



The initial α is 0.0167 (2-sided) for Family 1 and 0.033 (2-sided) for Family 2, while it is zero for Family 3.

Step 1: Family 1 and Family 2

i. Start test Family 1 and Family 2 as the first step.

- For testing Family 1 the hypotheses per antigen the p-values will be obtained from an ANOVA model, with vaccination group and center as independent variables and log transformed titers/concentrations as response variable. The null hypotheses will be rejected if the p-values < FWER for all thirteen antigens.
- For testing Family 2, if the lower limits of the 2-sided 96.67% CIs are greater or equal to the sufficiency margins for each of the four strains and composite endpoint, then Family 2 is successful. Otherwise, Family 2 fails.

ii. If Family 1 had succeeded and Family 2 not, then propagate ½ FWER from Family 1 to Family 3 and the other ½ FWER to Family 2, and re-test Family 2 with the new FWER which is the sum of the original FWER from Family 2 plus the ½ of FWER from Family 1. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 2 and propagate full FWER to Family 3; otherwise Family 2 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 1.

iii. If Family 2 had succeeded and Family 1 not, then propagate ½ FWER from Family 2 to Family 3 and the other ½ FWER to Family 1, and re-test Family 1 with the new FWER which is the sum of the original FWER from Family 1 plus the ½ of FWER from Family 2. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 1 and propagate full FWER to Family 3; otherwise Family 1 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 2.

iv. If both Family 1 and Family 2 had succeeded, then the FWER from both families were propagated to Family 3, and proceed to test Family 3;

v. If both Family 1 and Family 2 had failed, then no FWER will be propagated to Family 3, and we stop the testing globally and success can be claimed on none of the three families.

Step 2: Family 3:

If all null hypotheses are rejected, then propagate 1/2 FWER to Family 1 and Family 2 respectively, and success can be claimed for Family 3, as well as for Family 1 and/or Family 2 whichever was successful in Step 1; otherwise stop testing globally, and success can be claimed for Family 1 and/or Family 2 whichever was successful in Step 1.

Step 3: Family 1 or Family 2

In case one of Family 1 and Family 2 failed in step 1, with the alpha passed down by Family 3, it is possible to re-test the family that failed once and again test it with ½ FWER from Family 3. If all null hypotheses are rejected, then success can be claimed for this family as well.

9.5.4. Analysis of Secondary Immunogenicity Objectives

The statistical hypotheses associated with the non-inferiority tests in the secondary immunogenicity objectives are described as below:

$H_0 : \mu_A - \mu_B \leq \delta$ vs. $H_{1j} : \mu_A - \mu_B > \delta$

Where δ denotes the non-inferiority margin, and μ_A, and μ_B denote either the means of log 10-transformed concentrations/titers or the percentage of subjects achieving the threshold response for binary variables.

Note that statistical hypotheses testing is not applicable for the seroresponse rates of antigens tested for Polio, MMR and VV vaccines. Instead, descriptive analysis will be performed. For each antigen tested, the percentage of subjects with anti-polio type 1, 2 and 3 neutralization antibody titers ≥8, or the percentage of subjects with post-vaccination anti-measles, anti-rubella, anti-mumps and anti-VZV virus antibody concentration ≥ a protective threshold will be presented as point estimates along with the associated 2-sided 95% Clopper-Pearson CIs.

9.5.4.1. Statistical Methods

Concentration and titer data will be summarized by vaccine group, strain and time point. Additionally, within-subject GMRs will be computed for GMTs at one month after 4th vaccination versus pre-4th vaccination. As for GMTs, the GMRs and 95% CIs will be computed by exponentiating (base 10) the corresponding means and 95% CIs from an ANOVA model.

For the binary variables, for example, percentage of subjects with hSBA titer ≥ LLOQ, percentage of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 µg/mL, the number and percentage of subjects and associated 2-sided 95% Clopper-Pearson CIs will be computed for each antigen/strain by vaccine group and by time-point. Between group-differences with 95% CIs will be derived using the Miettinen and Nurminen method [Miettinen, 1985].

9.6. Analysis of safety

Distribution of subjects by vaccinations will be summarized by vaccine group for the ***Exposed*** Set. The primary analysis will be performed on the ***Solicited/Unsolicited Safety Set.***

9.6.1. Within groups assessment

9.6.1.1. Analysis of Solicited Adverse events

All solicited AEs will be summarized according to defined severity grading scales, see Section 7.2.3.2.1.

Frequencies and percentages of subjects (with 95% CI) experiencing each AE will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic AE overall and at each time point will also be presented.

Post-vaccination solicited AE reported from Day 1 to Day 7 will be summarized for the interval Day 1-7 (and Day 1-3, Day 4-7 if needed) by maximal severity and by vaccine group, excluding the 30-minute measurement, which will be summarized separately. For MMR and VV-specific solicited systemic AEs (parotid/salivary gland swelling, fever, rash) collected after Visit 5 (30 minutes through Day 30 post-vaccination), the study period will be divided into the following intervals: 6 hours through ***D***ay 7, ***D***ay 8 though ***D***ay 30.

The severity of solicited local AEs ***(administration site event)***, including redness at injection site, swelling of skin at injection site, and hardness of skin at injection site will be summarized according to categories based on linear measurement: None (0mm); Mild (1mm to 25mm); Moderate (26mm to 50mm); Severe (>50mm).

Injection site tenderness and systemic reactions (except fever) occurring up to 7 days after each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Fever, derived from measured body temperature (≥38.0°C/100.4°F), will be summarized according to “Grade 0 (none)” (< 38.0°C), “mild “(≥ 38.0 - 38.9°C), “moderate” (≥ 39.0 - 39.9°C) and “severe” (≥ 40.0°C).

Each solicited local ***(administration site)*** and systemic AE will also be further summarized as “none” versus “any” (for fever the latter will be ≥38.0°C/100.4°F).

Use of antipyretics and analgesics will be summarized by type of use (prophylactic versus treatment) and percentage of subjects (with 95% CI) reporting use.

Body temperature will be summarized by 0.5°C increments from 36.0°C up to ≥40°C and will be broken down according by route of measurement, if applicable. Frequencies and percentages of subjects (with 95% CI) with temperatures ≥38.0°C and temperatures ≥40.0°C will also be presented.

9.6.1.2. Analysis of Unsolicited Adverse Events

This analysis applies to all AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in AE eCRF, with a start date on or after the date of first vaccination. All AE starting prior to the first vaccination will only be listed. The original verbatim terms used by investigators to identify AEs in the eCRFs will be mapped to preferred terms using the MedDRA dictionary. The AEs will then be grouped by MedDRA preferred terms into frequency tables (with 95% CI) according to system organ class.

All reported AEs, as well as AEs judged by the investigator as related to study vaccine, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccine group and by interval of study observation. When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Serious adverse events, withdrawal due to AEs and AESIs will be described in detail.

Separate summaries will be produced for the following categories:

- Serious adverse events (SAE)
- Adverse events related to vaccine
- Adverse events of special interest
- Adverse event leading to withdrawal
- Adverse events leading to a medically attended visit

These summaries will also be presented by the 0-6/6-12 months safety follow-up periods after the last study vaccination.

Data listings of all AEs will be provided by subject. In addition, AEs in the categories above will be provided as listed data.

Local and systemic AEs will be analyzed by point estimates with associated 95% CIs [Clopper, 1934].

9.6.2. Between groups assessment

Not applicable.

9.7. Interpretation of analyses

Except for analyses on objectives with a pre-defined success criterion and an appropriate Type I error control (see Section 2), analyses will be descriptive with the aim to characterize the reactogenicity and immunogenicity within each group.

9.8. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

9.8.1. Sequence of analyses

Any analyses (including a possible interim analysis) will be conducted on data as clean as possible. All data will be analyzed at the end of the study, after availability of all immunogenicity and safety data, and presented in a final clinical study report.

If needed, an interim analysis including data up to visit 6 may be performed. Selected immunogenicity objectives and solicited safety data from all timepoints up to Visit 6 will be analyzed. The interim analysis will be performed by an independent statistician, in order to maintain the observer-blindness of the study. Study unblinding will be performed only after the final database lock. The data or the results from this interim analysis will not be used to make any decision on the continuation of this trial. Therefore no impact on the conduct or subsequent full data analysis of the trial is expected. All remaining objectives and remaining safety data will be analyzed at the end of the study and an integrated clinical report will be generated, which contains all data from the trial.

9.8.2. Statistical considerations for interim analyses

If performed, interim analysis will be conducted on final immunogenicity data and the safety analyses will be descriptive. Therefore no statistical adjustment for the interim analysis is required.

10. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, public disclosure requirements and publications must be fulfilled.

10.1. Electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with an electronic format in read only mode of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

10.2. Subject Diary

Electronic Diaries (eDiaries), hereafter referred to as Subject Diaries will be the only source document allowed for solicited local ***(administration site)*** and systemic adverse events (including body temperature measurements), starting after the initial 30 minute post-vaccination period at the clinic. The following additional rules apply to documentation of safety information collected in the Subject Diary.

The Investigator or delegated staff should monitor the Subject's Diary status throughout the study for compliance and any solicited local ***(administration site)*** and systemic adverse events that were of concern to the subject's parent(s)/LAR(s).

- No corrections or additions of data recorded by the parent(s)/LAR(s) will be allowed once diary completion for that day has been performed.
- The Subject Diary will be designed in such a way as to prevent any blank, incomplete or biologically implausible entries. Parent(s)/LAR(s) will be instructed to fully complete the Subject Diary each day, as per the instructions provided.
- At or just in advance of each subject visit, site staff must review the Subject Diary data via the provider's web portal. It is necessary for site staff to acknowledge in the source documents that review of Subject Diary data for the preceding post-vaccination period has been performed. It is necessary for the investigator to acknowledge in the eCRF that the review of Subject Diary data has been performed for each subject. This is to be performed at Visit 6 (Day 331), when the subjects' parent/LAR returns the Subject Diary to the clinic..
- For vaccination visits, site staff must ensure that each subject's Diary is prepared for data capture in the ensuing post-vaccination period by confirming the visit within the eDiary/eDiary system.
- Any new safety information reported during the site visit (including a solicited reaction) cannot be entered into the Subject Diary. Such information must be described in the source notes as a verbally-reported event. Any adverse reaction reported in this fashion must be described as an unsolicited reaction and therefore entered on the adverse event page of the eCRF.

10.3. Study Monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst other items, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

10.4. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures, otherwise, the minimum retention period will default to 25 years after completion of the study report.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

10.5. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

10.6. Definition of medical device AE, adverse device effect (ADE), serious adverse device effect (SADE) and unanticipated SADE (USADE)

10.6.1. Definition of medical device AE and adverse device effect (ADE)

- 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 considered related to a medical device or not. 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 a medical device. This definition includes events related to the medical device or comparator and events related to the procedures involved.
- An adverse device effect (ADE) is an AE related to the use of a medical device. This definition includes any AE resulting from:
  - insufficient or inadequate instructions for use (i.e. user error), or
  - any malfunction of a medical device, or
  - intentional misuse of the medical device.

10.6.2. Definition of medical device SAE, SADE and USADE

A medical device SAE is any serious adverse event 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
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
Serious Adverse Device Effect (SADE) definition
• A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event. • Any device deficiency that might have led to an SAE if appropriate action had not been taken or circumstances had been less fortunate.
Unanticipated SADE (USADE) definition
• An USADE (also identified as UADE in US Regulations 21 CFR 813.3), is a serious adverse device effect that by its nature, incidence, severity or outcome has not been identified in the current version of the IB.

10.6.3. Recording and reporting of medical device AE, ADEs, SADEs and USADE

- Any device deficiency must be reported to GSK within 24 hours after the investigator determines that the event meets the definition of a device deficiency.
- E-mail/Facsimile transmission of the paper 'Medical device or combination product with device deficiency/incident report form' is the preferred method to transmit this information to the sponsor.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of 'Medical device or combination product with device deficiency/incident report form' sent by overnight mail or courier service.

- Contacts for reporting can be found in the Investigator's instruction within the 'Medical device or combination product with device deficiency/incident report form' and for guidance on reporting also refer to Section 7.9.
- GSK will review all device deficiencies, 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. Posting of information on publicly available clinical trial registers and publication policy

GSK assures that the key design elements of this protocol will be posted on the GSK website and in publicly accessible database(s) such as clinicaltrials.gov, in compliance with the current regulations.

GSK also assures that results of this study will be posted on the GSK website and in publicly accessible regulatory registry(ies) within the required time-frame, in compliance with the current regulations. The minimal requirement is to have primary endpoint summary results disclosed at latest 12 months post primary completion date (PCD) and to have secondary endpoint disclosed at latest 12 months after the last subject last visit (LSLV) as described in the protocol.

As per EU regulation, summaries of the results of GSK interventional studies (phase I-IV) in paediatric conducted in at least one EU member state will be posted on publicly available EMA registers within 6 months of EoS (as defined in the protocol) in the concerned EU member state. However, where, for scientific reasons detailed in the protocol, it is not possible to submit a summary of the results within 6 months in the concerned EU member state, the summary of results shall be submitted as soon as it is available. In this case, the protocol shall specify when the results are going to be submitted, together with a justification.

GSK also aims to publish the results of these studies in searchable, peer reviewed scientific literature and follows the guidance from the International Committee of Medical Journal Editors.

10.8. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

10.9. Data Sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non- GSK studies, to investigate further about the study product(s) and other product(s), and /or the disease/condition under investigation and related diseases and conditions.

11. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

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APPENDIX A LABORATORY ASSAYS

MenB serum bactericidal assays using human complement (hSBA):

Serum bactericidal activity against rMenB+OMV NZ will be determined by performing hSBA against a standard panel consisting of 4 meningococcal B test strains M14459, 96217, NZ98/254 and M13520. Each of these strains measures bactericidal activity primarily directed against one of the major bactericidal antigens included in the vaccine: strain M14459 measures hSBA against the 741 part of the 936-741 antigen, also known as fHbp variant 1.1; strain 96217 measures hSBA against antigen 961c, also known as NadA; and strain NZ98/254 measures hSBA against PorA P1.4, the immunodominant antigen in the OMV NZ vaccine component; strain M13520 measures hSBA against the 287 part of the 287-953 antigen, also known as NHBA.

***PCV13* Electrochemiluminescence (ECL) assay:**

Pneumococcal serotype-specific immunoglobin G (IgG) antibodies (antibodies to against serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) will each be measured by validated multiplex assays (ECL1 and ECL2 assays). The antibody concentration will be determined by logistic log comparison of the assay curves with a standard reference serum sp007 available from the US FDA for which concentration of IgG to each of the 13 serotypes is known in µg/mL [Goldblatt, 2011]. The cut-off of the ECL assay for each of the anti-pneumococcal serotype serologies is set at the serotype-specific LLOQ. The ECL threshold for non-inferiority assessment has been defined to be of 0.35µg/mL and is under assessment by FDA.

Diphtheria toxoid (anti-diphtheria) and tetanus toxoid (anti-tetanus) ELISAs, and pertussis component ELISAs:

Specific antibodies against diphtheria toxoid (anti-diphtheria IgG's) and tetanus toxoid (anti-tetanus IgG's) will be measured by ELISA. The clinical acceptable cut-off of ELISA was set at 0.1 International Units per mL (IU/mL), which provided a conservative estimate of the percentage of subjects deemed to be protected [Melville-Smith, 1983; Camargo, 1984].

Antibodies (IgG's) against the pertussis components PT, FHA and PRN will be measured by ELISA to evaluate the immunogenicity of acellular *B. pertussis* containing vaccines. The seropositivity for all three pertussis antibodies will be based on new respective ELISA cut-offs,, where subjects with antibody concentration below the cut-off being considered seronegative.

In 2014, GSK decided to redevelop and re-validate ELISA's used for quantitative determination of anti-DI, -TE, -PT, -FHA and -PRN IgG in human serum in accordance with most recent CBER guidelines and accepted practices (guidance for Industry "Bioanalytical Method Validation" from September 2013). Set-up, qualification and validation of the new assays was performed from 2014 till 2016, and both assay units and assay cut-offs were adapted.

As per CBER's recommendation, all values within the analytical range of the new ELISA's (*i.e.* within [Lower Limit of Quantification (LLOQ)-Upper Limit of Quantification (ULOQ)]) will be used in estimating GMCs. The new ELISA's cut-off will be the LLOQ.

The newly validated DTPa ELISA's have a lower assay cut-off as compared to the initial ones. Prior to their qualification, the new ELISA's for PT, FHA and PRN were calibrated against the WHO International Standard (NIBSC 06/140). This allowed the expression of concentrations measured with the new ELISA's in IU/mL instead of the formerly used ELISA units per milliliter (ELU/mL).

Hepatitis B Virus surface Ab CLIA:

Antibodies against the hepatitis B surface antigen (anti-HBs) will be measured using a ChemiLuminescence ImmunoAssay (CLIA). The cut-off of the test has been set at 6.2 mIU/mL. An antibody concentration ≥10 mIU/mL defines seroprotection [CDC, 1991].

Poliovirus Sabin Type 1, Type 2 & Type 3 Ab neutralization tests:

Antibodies against poliovirus types 1, 2 and 3 will be determined by a virus micro-neutralization test adapted from the WHO Guidelines for WHO/EPI Collaborative Studies on Poliomyelitis [WHO, 1993]. Titres will be expressed in terms of the reciprocal of the dilution resulting in 50% inhibition.

Hib capsular polysaccharide polyribosyl-ribitol phosphate (PRP) ELISA:

Concentrations of IgG antibodies against the Hib polysaccharide PRP will be measured by an ELISA.

MMRV Luminex based immuno assay

Antibodies against measles, mumps and rubella viruses will be determined by a quantitative Luminex assay. Inactivated native viruses from measles, mumps, rubella and varicella are coupled to their assigned Luminex MicroPlex microsphere type. Each microsphere type has its own distinct fluorescent dye that can be recognized by excitation with a red laser. The red laser classifies the microsphere type and determines the corresponding assigned virus that is being detected. The microsphere are incubated with human serum. Following this, the microspheres are washed to remove excess sera and incubated with R-Phycoerythrin (PE) conjugated goat anti-human IgG antibody. The fluorescent signal from the PE-labeled conjugate, recognized by excitation with a green laser, is proportional to the anti-virus IgG concentration in the serum sample tested.

IgG concentrations are derived from the MMRV Secondary Standard curve, which is made of pooled sera with positive reactivity to all 4 viruses and is aligned to the WHO International reference standards for measles (NIBSC reference# 97/648), rubella (NIBSC reference# RUBI-1-94) and VZV (NIBSC reference# W1044) and to NIBSC QC Reagent for mumps (NIBSC reference# 15/B664). The MMRV Secondary Standard is 2-fold serially diluted in duplicate to create a 10-point (STD1 to STD10) standard curve for each virus. Standard curve for each virus is calculated using a 3 Parameter Logistic (3PL) curve fit. Results are expressed in milli International Unit/mL (mIU/mL) for measles and varicella, Arbitrary Unit/mL (AU/mL) for mumps, and International Unit/mL (IU/mL) for rubella.

This 4-plex (MMRV) immunoassay will only be used to quantify the IgG antibodies specific to measles, mumps, and rubella, while antibodies specific to varicella will be quantified with the anti-gE ELISA.

Varicella anti-gE ELISA

Anti-gE ELISA is a two-step ELISA based on the antibody and antigen interaction, which allows the detection and the quantification of specific IgG antibodies directed against gE in tested serum samples. Briefly, diluted serum samples are added onto a 96 polystyrene-well microplate pre-coated with gE. After a washing step, goat antibodies directed against human IgG antibodies and conjugated to HRP (a-IgG-HRP) are added and will bind to anti-gE IgG if present. After a washing step, the addition of a chromogen-substrate solution specific for HRP will provide means of detecting the anti- gE specific for the pre-coated antigen. The HRP catalyzes an enzymatic reaction which is stopped by the addition of sulfuric acid, resulting in a color change from blue to yellow. The optical density recorded is proportional to the concentration of the anti-gE antibodies present in the serum sample. Antibody titer is expressed in mIU/mL.

APPENDIX B CLINICAL LABORATORIES

Table 23 GSK Biologicals' Laboratories

Laboratory	Address
GSK Biological's *Vaccines Clinical Laboratory and Assay Portfolio (Vx CL&AP)*, Rixensart	**Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart – Belgium**
GSK Biological's *Vaccines Clinical Laboratory and Assay Portfolio (Vx CL&AP)*, Wavre-Nord Noire Epine	**Avenue Fleming, 20 - B-1300 Wavre - Belgium**
Nexelis Marburg *a Q2 Solutions Company*	**Emil-von-Behring-Str. 76 35041 Marburg Germany**

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals SA Vaccines R &D **Protocol Amendment 2**	
eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Amendment number:	Amendment 2
Amendment date:	11-Dec-2017
Co-ordinating author:	PPD

Rationale/background for changes:

- As the sponsorship of the study has changed from Novartis Vaccines to GSK Biologicals, the protocol has been transcribed to the GSK protocol template (DS-BIO-CLIN-1006 v1) and all applicable mandatory sections have been included.
- Based on feedback received from the Center for Biologics Evaluation and Research (CBER), the following changes in study design were implemented:
  - Only two study groups (Group A: rMenB+OMV NZ+PCV13 and Group B: Placebo+PCV13) are to be assessed, with both receiving certain ACIP recommended infant vaccinations. Study Group C has been removed. The study is to enrol 2700 subjects, in a 2:1 ratio.
  - Additional ACIP recommended routine infant vaccinations will be included in the assessment of immunogenicity, safety and tolerability as part of the secondary objectives.
  - The study will be observer-blind.
  - All subjects in both groups will have 3 blood draws at the same timepoints, ie, at 1 month post-third, pre- and post-fourth vaccinations. Group A will not comprise of sub-cohorts A1 and A2, and there will be no baseline blood draw.
  - The concomitant vaccines have been modified from the DTaP5/IPV/Hib (diphtheria, tetanus toxoids, five component acellular pertussis adsorbed, inactivated poliovirus and *Haemophilus influenzae* type b conjugate (Hib)) and human rotavirus (HRV) vaccines to DTPa-HBV-IPV (diphtheria, tetanus toxoids and acellular pertussis adsorbed, hepatitis B and inactivated poliovirus, *Pediarix*), HRV (*Rotarix*) and Hib (*Hiberix*) vaccines. In addition, measles, mumps and rubella vaccine (MMR, *MMR II*) and varicella (VV,

Varivax) vaccine have been included. All concomitant vaccines are now considered as investigational medicinal products (IMPs), ie study vaccines.

- Additionally, a single dose of Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine (DTPa, *Infanrix*) and the fourth dose of Hib vaccine will be administered at 13 months but not considered as IMPs (ie, not included in the safety & immunogenicity assessments).
- The success criteria for the primary and key secondary objectives have been updated.
- The immunogenicity responses for all concomitant IMP study vaccines (DTPa-HBV-IPV, Hib, HRV, MMR and V) will now be analyzed as secondary objectives. Corresponding endpoints have been included.
- An interim analysis *may* be performed at 1 month post-4th vaccination (ie, up to Visit 6). This analysis will evaluate all primary and secondary immunogenicity objectives and solicited safety data in all subjects, rather than only safety data from first 2000 enrolled subjects up to Visit 2 (1 month post-first vaccination). If this analysis was to be performed, the final analysis will then include all safety data up to Visit 7 (12 months after the 4th vaccination).
- Solicited local and systemic adverse events (AEs) will be collected for all subjects and not in only 50% of subjects.
- Three additional safety follow-up calls will be conducted. Safety follow-up calls will now take place every two months instead of every three months, starting at 1 month post-last blood draw at Visit 6 (ie, at Day 361, Day 421, Day 481, Day 541 and Day 601). The safety call between Visit 4 and Visit 5 at Day 211 has now been replaced by safety calls at Day 181 and Day 241.

- Details regarding the collection of a recruitment/randomization agreement have been removed as this process is not applicable in the country(ies) selected for the study. The first study visit will be performed at the clinic/study site.
- For testing of the 13 valent pneumococcal conjugate vaccine (PCV13) antigens, the enzyme linked immunosorbant assay (ELISA) has been replaced by GSK Biologicals'multiplex, immunochemistry assay based on mesoscale discovery technology (electro-chemiluminescence assay, ECL). The assay cut-offs are specific for each of the 13 serotypes.
- A pre-defined script to be used during clinic visits for providing instructions for safety monitoring to parent is now included.
- Arthritis has been included as an adverse event of special interest (AESI).

The major changes made to this amendment were with regard to transferring the protocol to the hybrid GSK protocol template (DS-BIO-CLIN-1006 v1) and the incorporation of protocol template-specified mandatory text. These changes have not been listed here. The changes with regard to CBER feedback in the main sections of the protocol are described here.

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

Objectives:

The study objectives (synopsis and Section 2.1) have been updated based on the feedback from CBER.

Primary safety objectives:

- To assess the safety and tolerability ***of*** ~~and~~ rMenB+OMV NZ ,~~and~~ PCV13 ***and other RIV*** when administered concomitantly~~/non-concomitantl~~y to healthy infants at 2, 4, 6 and 12 months of age, throughout the study duration.

Co-Primary immunogenicity objectives:

- ***To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4 and 6 months of age, at one month after the 3rd vaccination.***

  Criteria: The sufficiency of the immune response to rMenB+OMV NZ after the 3rd vaccination will be demonstrated if the adjusted lower confidence limit for the percentage of subjects achieving serum bactericidal assay using human complement (hSBA) titers ≥ Lower Limit of Quantitation (LLOQ) is ≥ 60% for the N. meningitidis serogroup B test strains M14459, 96217, NZ98/254 and ≥12% for strain M07-0241084 (individually); and is ≥10% for all strains combined (composite endpoint).

- To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered non-concomitantly with PCV13 ***and other RIV*** to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 4th vaccination

  ~~– Demonstration of the sufficiency of the immune response to rMenB+OMV NZ at one month after the 4th vaccination will be measured by the percentage of subjects with serum bactericidal assay using human complement (hSBA) titers ≥ Lower Limit of Quantitation (LLOQ) for each and for all the N. meningitidis serogroup B test strains M14459 (fHbp), 96217 (NadA), NZ98/254 (PorA) and M07-0241084 (NHBA)~~

  Criteria: The sufficiency of the immune response to rMenB+OMV NZ will be demonstrated if the adjusted lower confidence limit for the percentage of subjects achieving a 4-fold rise in hSBA titers (from pre-4th) is ≥70% for N. meningitidis serogroup B test strains M14459, 96217, NZ98/254 and ≥50% for strain M07-0241084 (individually); and is ≥40% for all strains combined (composite endpoint).

- ~~To demonstrate the immunological non-inferiority of rMenB+OMV NZ when administered concomitantly with PCV13 to healthy infants 2, 4, 6 and 12 months of age, compared to rMenB+OMV NZ without PCV13, at one month after the 4th vaccination.~~

  ~~Demonstration of the immunological non-inferiority of rMenB+OMV NZ after the 3rd and the 4th vaccination will be measured by~~

  - ~~the hSBA geometric mean titers (GMTs) against the M14459, 96217, NZ98/254 and M07-0241084 test strains~~
  - ~~*the percentage of subjects with hSBA titers ≥ LLOQ for each and for all the M14459, 96217, NZ98/254 and M07-0241084 test strains*~~

- To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ ***and other RIV*** to healthy infants 2, 4, 6 and 12 months of age, compared to PCV13 without rMenB+OMV NZ, at one month after the 3rd vaccination

- ~~To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ to healthy infants 2, 4, 6 and 12 months of age, compared to PCV13 without rMenB+OMV NZ, at one month after the 4th vaccination~~

  ~~*Demonstration of the immunological non-inferiority of PCV13 after the 3rd and the 4th vaccination will be measured by the enzyme linked immunosorbent assay (ELISA) geometric mean concentrations (GMCs) for each of the thirteen (4, 9V, 14, 18C, 19F, 23F, 6B, 1, 3, 5, 6A, 7F, 19A) PCV13 antigens.*~~

  Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the adjusted lower confidence limit for the between-group ratio of electrochemiluminescence (ECL) assay Geometric Mean Concentrations (GMCs) is >0.5 for each of the 13 PCV13 antigens.

Secondary objectives

- ***To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants 2, 4, 6 and 12 months of age, compared to PCV13 alone, at one month after the 4th vaccination.***

  Criteria: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of 2-sided 95% CI for the between-group-ratio of ECL assay GMCs is >0.5 for each of the 13 PCV13 antigens.

- ***To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants at 2, 4, 6 and 12 months of age compared to PCV13 alone, at both one month after the 3rd and the 4th vaccinations.***

Criteria: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of the 2-sided 95% CI for the group differences in percentage of subjects with IgG ≥ 0.35 µg/mL is > -10% for each of the 13 PCV13 antigens at one month after both 3rd and 4th vaccination.

- ***To demonstrate the immunological non-inferiority 2of DTaP-HBV-IPV and Hib vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy infants at 2, 4, and 6 months compared to DTaP-HBV-IPV and Hib vaccines concomitantly administered with PCV13 without rMenB+OMV NZ, at one month after the 3rd vaccination.***

  Criteria: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group differences is greater than the margin for each antigen at one month after 3rd vaccination.

- ***To demonstrate the immunological non-inferiority of MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy subjects at 12 months compared to MMR and VV vaccines concomitantly administered with PCV13, without rMenB+OMV NZ, at one month after vaccination.***

  Criteria: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group differences is greater than the margin for each antigen at one month after the MMR and VV vaccinations.

- To evaluate the immune response to rMenB+OMV NZ when administered concomitantly~~/non-concomitant~~ly with PCV13 ***and other RIV*** to healthy infants at 2, 4, 6 and 12 months of age***, at one month after the 3rd vaccination, at 6 months after the 3rd vaccination (immediately before the 4th vaccination), and at one month after the 4th vaccination against the M14459, 96217, NZ98/254 and M07-0241084 test strains.***

  - ~~at pre-vaccination (baseline) and six months after the 3rd vaccination (immediately before the 4th vaccination), as measured by the GMTs and the percentage of subjects with hSBA titers ≥ LLOQ against the M14459, 96217, NZ98/254 and M07-0241084 test strains~~

- ***To evaluate immune responses to routine infant vaccines DTaP-HBV-IPV, Hib, MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13.***

- ~~To evaluate the immune response to PCV13 when administered concomitantly/non-concomitantly with rMenB+OMV NZ to healthy infants at 2, 4, 6 and 12 months of age~~

  - ~~at one month after the 3rd and the 4th vaccination, as measured by the percentage of subjects with serum pneumococcal anticapsular polysaccharide IgG ≥ 0.35 µg/mL for each of the thirteen PCV13 antigens~~

Concomitant vaccines:

The concomitant non-study vaccines have been modified from the DTaP5/IPV/Hib (diphtheria, tetanus toxoids, five component acellular pertussis adsorbed, inactivated poliovirus and *Haemophilus influenzae* type b conjugate (Hib)) and rotavirus (HRV) vaccines to concomitant study vaccines DTPa-HBV-IPV (diphtheria, tetanus toxoids and acellular pertussis adsorbed, hepatitis B and inactivated poliovirus), HRV, Hib, MMR and VV vaccines. A fourth dose or Hib and a single dose of DTPa are now added as non-study vaccines.

Section 6.1.1:

~~The term 'study vaccine' refers to those vaccines~~ ***The study vaccines' (see glossary of terms) will be*** provided by the Sponsor.~~, which will be evaluated as part of the study objectives.~~ ***All study participants are expected to receive these vaccines as specified by the study schedule.*** The study vaccines specific to this study are described below.

- ~~**Vaccine A**: GSK Meningococcal group-B Vaccine, (rMenB+OMV NZ) (Bexsero®);~~
- ~~**Vaccine B**: Pneumococcal polysaccharide conjugate vaccine, (13-valent Pneumococcal Vaccine) (PCV13) (Prevnar13®);~~
- ***GSK Biologicals' Meningococcal group-B vaccine, (rMenB+OMV NZ, Bexsero);***
- ***Pneumococcal polysaccharide conjugate vaccine, (13-valent Pneumococcal Vaccine) (PCV13, Prevnar13);***
- ***Saline placebo vaccine.***

Standardized routine infant immunizations included in this study are described below.

- ***GSK Biologicals' Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine (DTPa-HBV-IPV, Pediarix): a 3 dose vaccine given by IM injection at 2, 4, and 6 months of age. A single-dose prefilled syringes contains a 0.5-mL suspension for injection.***
- ***GSK Biologicals' Rotavirus Vaccine, live, oral (HRV, Rotarix): a vaccine containing attenuated human strain P1AG1. HRV is a 2-dose vaccine typically given at 2 months and 4 months of age; it is available in single-dose vials of lyophilized vaccine, accompanied by a prefilled oral applicator of liquid diluent.***
- ***GSK Biologicals' Haemophilus influenzae type b vaccine (Hib, Hiberix): a vaccine indicated for active immunization for the prevention of invasive disease caused by Haemophilus influenzae type b. Hib is given as a 4 dose series by IM injection; the primary series consists of one dose each at 2, 4, and 6 months of age, followed by a booster dose at 12 through 15 months of age.***
- ***Merck's measles, mumps and rubella virus vaccine live (MMR, M-M-R II): a vaccine indicated for active immunization for simultaneous vaccination against measles, mumps, and rubella in individuals 12 months of age or older. Each lyophilized dose is approximately 0.5 mL after reconstitution and is administered by subcutaneous injection.***

- ***Merck's varicella zoster virus vaccine live (VV, Varivax): a vaccine indicated for active immunization for the prevention of varicella in individuals 12 months of age or older. Each dose is approximately 0.5 mL after reconstitution and is administered by subcutaneous injection.***

Section 6.1.2:.

- ~~Diphtheria and Tetanus Toxoids and [five component] acellular Pertussis Adsorbed, Inactivated Poliovirus and *Haemophilus influenzae* type b (Tetanus Toxoid Conjugate) Vaccine (DTaP5/IPV/Hib). Two types of DTaP5/IPV/Hib containing the same antigen components will be used in this study. In one, available in the United States, the Hib component is lyophilized and the vaccine needs reconstitution; the other, not available in the United States but available in several European countries and elsewhere, is fully liquid and does not need reconstitution. The non-antigenic ingredients of the two formulations (e.g. the excipients) are not identical. It is possible that the immune response to the two vaccines may not be identical; the potential clinical significance of this difference is not well understood. Where available the US licensed product will be used preferentially, but the antigenically identical alternative will be used where the US licensed product is not available.~~

- ~~Rotavirus Vaccine, Live, Oral (RV): a vaccine containing attenuated human strain P1AG1 (RV1). RV1 is a two dose vaccine typically given at 2 months and 4 months of age; it is available in single-dose vials of lyophilized vaccine, accompanied by a prefilled oral applicator of liquid diluent.~~

The following non-study vaccine will be administered to all subjects in both groups at 13 months of age, at Visit 6 (Day 331).

- ***GSK Biologicals' GSK Biologicals' Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed Vaccine (DTPa, Infanrix): DTPa is a 5 dose vaccine given by IM injection at 2, 4, and 6 months of age, with a booster dose at 15 to 20 months of age and another booster dose at 4 to 6 years of age. A single-dose prefilled syringes contains a 0.5-mL suspension for injection.***

In order to complete the dosage scheme for DTPa, a single dose of DTPa (Infanrix) will be administered IM as a booster dose at Visit 6 (as subjects would have already received 3 doses of Pediarix as an IMP study-vaccine).

In addition, all subjects will receive the fourth dose of Hib (Hiberix) as a non-study vaccine during Visit 6. Details on the vaccine are provided in Section 6.1.1.

Endpoints (Synopsis and section 9.1 and 9.2)

Safety endpoints:

Only AESIs predefined as serious will be recorded as SAEs in the eCRF, and not all AESIs. Arthritis has been included as an AESI. The safety endpoint of summarizing the percentage of subjects with AESIs has been modified.

- The percentage of subjects with solicited local AEs and systemic AEs during the 7 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, ~~4, 7 and 11~~***2, 3 and 5***).

- ***The percentage of subjects with solicited systemic AEs of parotid/salivary gland swelling, fever and rash during the 30 days (including the day of vaccination) after the 4th vaccination (Visit 5).***
- The percentage of subjects with ***all*** ~~any other (~~unsolicited~~)~~ AEs ***(including*** SAEs, AEs leading to withdrawal, ***AESIs*** and medically attended AEs) during 30 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, ***2,3,and 5*** ~~4, 7 and 11~~).
- The percentage of subjects with SAEs, AEs leading to withdrawal***, AESIs*** and medically attended AEs from study Day 1 (Visit 1) until study end (12 months after last study vaccination, Visit ~~17~~***7***).
- ~~The percentage of subjects with AESI and seizures from study Day 1 (Visit 1) until study end (12 months after last study vaccination, Visit 17).~~

Primary immunogenicity endpoints:

Primary immunogenicity endpoints have been updated based on changes made to the study objectives. Endpoints for the different routine infant vaccines (now considered study vaccines) have been included.

- For rMenB+OMV NZ, the following endpoints will be calculated:
- at one month after the 3rd ~~and the 4th~~ vaccinations.
  - the percentage of subjects with hSBA titers ≥ LLOQ for each of the M14459, 96217, NZ98/254 and M07-0241084 test strains
  - the percentage of subjects with hSBA titers ≥ LLOQ for all strains combined (composite endpoint)
  - ~~the hSBA GMTs~~
- ***At one month after the 4th vaccination:***
  - ***the percentage of subjects with 4-fold rise (from pre-4th vaccination) in hSBA titers for each of the M14459, 96217, NZ98/254 and M07-0241084 test strains.***
  - ***the percentage of subjects with 4-fold rise (from pre-4th vaccination) in hSBA titers for all strains combined (composite endpoint).***

  A 4-fold rise in hSBA titers is defined as:
  - ***if pre-vaccination titer <Limit of Detection (LOD), then a post-vaccination titer ≥LLOQ;***
  - ***if pre-vaccination titer is ≥LOD but ≤LLOQ, then a post-vaccination titer ≥4 times the LLOQ;***

  if pre-vaccination titer is ≥ LLOQ, then a post-vaccination titer ≥4 times the pre-vaccination titer.
- For PCV13 the following endpoint will be calculated at one month after the 3rd ~~and the 4th~~ vaccination:
  - the ELISA GMCs for each of the 13 PCV13 antigens.

Secondary immunogenicity endpoints:

For rMenB+OMV NZ, the following secondary endpoints will be calculated:

- ~~at pre-vaccination (baseline) and~~ ***At 1*** six months after the 3rd vaccination (immediately before the 4th vaccination):
  - the percentage of subjects with hSBA titers ≥***5 and*** ≥***8*** ~~LLOQ~~ for each of the M14459, 96217, NZ98/254 and M07-0241084 test strains
  - the hSBA GMTs
- ***At 6 months after the 3rd vaccination (immediately before the 4th vaccination):***
  - ***the percentage of subjects with hSBA titers ≥ LLOQ for each of the M14459, 96217, NZ98/254 and M07-0241084 test strains.***
  - ***the percentage of subjects with hSBA titers ≥ 5 and ≥8 for each of the M14459, 96217, NZ98/254 and M07-0241084 test strains.***
  - ***the hSBA GMTs against each strain.***
- ***At 1 month after the 4th vaccination:***
  - ***the percentage of subjects with hSBA titers ≥LLOQ; for each of the M14459, 96217, NZ98/254 and M07-0241084 test strains.***
  - ***the percentage of subjects with hSBA titers ≥5 and ≥8 for each of the M14459, 96217, NZ98/254 and M07-0241084 test strains.***
  - ***hSBA GMTs and geometric mean ratios (GMRs) over pre-4th vaccination.***
  - ~~Additionally, the GMR will be calculated at one month after the 4th vaccination to pre-fourth vaccination.~~

For PCV13 the following endpoint will be calculated:

- ***At one month after the 4th vaccination the ECL GMCs for each of the 13 PCV13 antigens.***
- At one month after the 3rd and the 4th vaccination, the percentage of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 µg/mL

For DTPa-HBV-IPV, the following endpoints will be calculated at one month after the 3rd vaccination

- ***Pertussis:***
  - ***GMCs against the 4 pertussis antigens (pertussis toxin [PT], pertactin [PRN], filamentous hemagglutinin [FHA]).***
- ***Hepatitis B:***
  - ***Percentage of subjects with concentrations ≥10 mIU/mL and ≥100 mIU/mL.***
  - ***Anti-HBsAg GMTs.***

- ***Polio:***
  - ***Percentage of subjects with anti-polio type 1, 2 and 3 neutralization antibody titers ≥ 8.***
- ***Diphtheria and Tetanus:***
  - ***Percentage of subjects with anti-diphtheria and anti-tetanus concentrations ≥ 0.1 IU/mL and ≥ 1 IU/mL.***
  - ***Anti-diphtheria and anti-tetanus antibody GMTs.***
- ***Hib:***
  - ***The percentage of subjects with anti-PRP concentration ≥0.15 µg/mL and ≥1 µg/mL.***

For VV, the following endpoint will be calculated at 1 month after the 4th vaccination:

- ***Seroresponse, defined as post-vaccination anti-VZV antibody concentration ≥75 mIU/mL among children who were seronegative (antibody concentration <25 mIU/mL) before vaccination.***

For MMR, the following endpoints will be calculated at 1 month after the 4th vaccination:

- ***Seroresponse as defined by the post-vaccination anti-measles virus antibody concentration ≥ 200 mIU/mL (ELISA, Enzygnost) in subjects seronegative (antibody concentration <150 mIU/mL) before vaccination.***
- ***Seroresponse as defined as post-vaccination anti-mumps virus antibody concentration ≥ a threshold in subjects seronegative (antibody concentration < assay cut-off) before vaccination.***

  Note: A suitable ELISA assay for analysis of anti-mumps virus antibody concentrations is yet to be selected and/or developed.
- ***Seroresponse, defined as post-vaccination anti-rubella virus antibody concentration ≥10 IU/mL (ELISA, Enzygnost) in subjects seronegative (antibody concentration <4 IU/mL) before vaccination.***

Interim analysis:

Details regarding the interim analysis have been modified to include immunogenicity analysis and to change the time point of the analysis.

Section 9.10:

An interim analysis may be performed to provide Health Authorities with ~~preliminary~~ information on the ***primary and secondary objectives and solicited*** **safety** ***data. The analyses if performed will occur at 1 month post-4th vaccination (ie, up to Visit 6 at Day 331).*** ~~and tolerability of rMenB+OMV NZ and PCV13 administered either concomitantly or alone, one month after the third dose.~~ The interim analysis will include ~~approximately the first 2000~~ ***all*** enrolled subjects.~~, and the analyses will be performed on safety data from Visit 1 to Visit 4 (i.e. two months post first vaccination).~~This analysis

~~will be descriptive. For 2nd and 3rd vaccination the data will be provided as available at that point in time for these subjects.~~ ***The interim analysis will include all enrolled subjects. All immunogenicity objectives will be analyzed. The safety analysis will be performed on solicited safety data from all timepoints up to Visit 6. The interim analysis will be performed by independent statistician, in order to maintain the observer-blindness of the study. Study unblinding will be performed only after database lock.*** The data or the results from this interim analysis will not be used to make any decision on the continuation of this open label trial. Therefore no impact on the conduct or subsequent full data analysis of the trial is expected.

~~A final analysis related to the tolerability, safety and immunogenicity may be also performed after all subjects complete Visit 13 (i.e. one month post 4th vaccination).The locked data or the results from this final analysis will include the relevant endpoints, analysis sets and methods aligning with the primary and secondary immunogenicity objectives of this study and with the safety objectives of this study up to the above mentioned timepoint. If this final analysis is performed, the~~ ***The*** remaining safety analyses up to ***study termination at Visit 7*** will be provided in an ~~addendum to the Clinical Study Report~~ ***additional study report***

Change in assay for PCV13:

ELISA has been replaced by the ECL assay for testing the PCV13 antigens, and the assay cut-off has been updated.

Criterion for objectives:

Demonstration of the immunological non-inferiority of PCV13 after the 3rd and the 4th vaccination will be measured by the ~~enzyme linked immunosorbent~~ ***pneumococcal polysaccharide (PnPs) multiplex electrochemiluminescence (ECL)*** assay ~~(ELISA)~~ geometric mean concentrations (GMCs) for each of the thirteen (4, 9V, 14, 18C, 19F, 23F, 6B, 1, 3, 5, 6A, 7F, 19A) PCV13 antigens

Laboratory assays (Section 5.7.3 and Appendix 1):

~~The antibody responses to PCV13 will be determined by ELISA. The ELISA measures 13 serotype specific anti *S. pneumoniae* IgG capsular polysaccharide antibodies present in human sera. The respective antibody concentration is measured in µg/mL.~~

~~Beside the classical Sandwich ELISA there are alternative anti-*S. pneumoniae* IgG detection methods developed (e.g. Multiplex fluorescent bead assay).~~

Pneumococcal serotype specific total IgG antibodies (antibodies to 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) will each be measured by a multiplex immunochemistry assay based on mesoscale discovery technology (electro-chemiluminescence assay, ECL)

The antibody concentration will be determined by logistic log comparison of the ECL curves with a standard reference serum SP007 available from the US Food and Drug Administration (FDA). The cut-off of the assay was defined at serotype-specific LLOQ for each serology (see). The ECL assay has been bridged to the WHO reference 22F-ELISA and the derived threshold for IPD licensure for the ECL assay, corresponding to the 0.35µg/mL, was found to be 0.32µg/mL.

Other indicators of reactogenicity

Solicited indicators of reactogenicity updated to include medically attended fever and use of analgesics/antipyretics. Fever is now categorized as a solicited systemic AE.

Other solicited adverse events (Section 7.1.3.3):

~~Fever, defined as body temperature ≥38°C; temperature should be taken preferably via the rectal route, ideally at same time each day. Other routes of temperature measurement include axillary, oral and tympanic.~~

The following will also be measured and recorded as other solicited events in the Subject Diary (eDiary).

- ***Body temperature *,***
- ***Medically-attended fever, and***
- ***Use of analgesics/antipyretics for either prophylactic or treatment purposes.***

GlaxoSmithKline Biologicals SA

Vaccines R &D

Protocol Amendment 3

eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Amendment number:	Amendment 3
Amendment date:	25 APR 2018
Co-ordinating author:	PPD

Rationale/background for changes:

- The definition of a qualified healthcare professional has been clarified to include any licensed or certified health care professional, rather than only state-licensed health care professionals.
- The text regarding allocation of treatment numbers to subjects has been corrected. Individual treatment numbers will now be allocated to subjects for each vaccine, instead of a single treatment number throughout the study.
- The definition of 4-fold rise in hSBA titers has been corrected.
- The storage temperature for the *Varivax* vaccine is updated to range from -15°C to -25°C. The range for acceptable temperature excursions is changed accordingly.
- The formulation for the HRV vaccine (*Rotarix*) in Table 10 is updated to describe details related to the lyophilized formulation ('HRV lyo').
- Minor changes to text on location of administration of the placebo vaccine, route of administration for all vaccines and recording of injection site in the eDiary, to align with the dosage and administration table (Table 11).
- The time period for recording of concomitant vaccinations modified to begin from birth, rather than 14 days (28 for live attenuated vaccines) before the first study vaccine dose.
- Text in various sections updated to clarify that solicited AEs ongoing after the 7-day reporting period will continue to be collected in the eDiaries, until resolution.
- Time intervals for analyses of unsolicited AEs clarified to specify that only certain AEs (SAEs, AEs leading to withdrawal, medically attended AEs etc.) will be analyzed after 30 days post-vaccination.
- Other minor changes to correct typos, and improve clarity and alignment within the document.

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

Definition of Qualified Healthcare Provider (Glossary and Section 5.6.6)

~~***A state licensed***~~ ***Any licensed or certified*** healthcare professional who is permitted by institutional policy to perform protocol required procedures, and who is identified within the Study Staff Signature Log.

Allocation of treatment numbers

Section 5.2.2.2 Treatment allocation to the subject:

The treatment numbers will be allocated by dose ***for Bexsero and placebo vaccines***. ***Treatment allocation will be by components for all other products.*** ~~Throughout the study, a single treatment number will identify the vaccine doses to be administered to the same subject.~~

Section 6.4 Replacement of unusable vaccine doses:

The investigator will use SBIR to obtain the replacement vaccine number. The replacement numbers will be allocated by dose ***for Bexsero and placebo vaccines. Replacement numbers will be allocated by components for all other products***. The system will ensure, in a blinded manner, that the replacement ***treatment*** ~~vial~~ matches the formulation the subject was assigned to by randomization.

Clarification of definition of 4-fold rise (Synopsis and Section 9.1.2 Primary immunogenicity endpoints)

The second criterion of the definition has been updated as below:

A 4-fold rise in hSBA titers is defined as

- if pre-vaccination titer <Limit of Detection (LOD), then a post-vaccination titer ≥LLOQ;
- if pre-vaccination titer is ≥LOD but ~~≤~~<LLOQ, then a post-vaccination titer ≥4 times the LLOQ;
- if pre-vaccination titer is ≥LLOQ, then a post-vaccination titer ≥4 times the pre-vaccination titer.

Section 6.2 Storage and handling of study and non-study vaccines

Varivax storage temperature updated as follows:

For the *Varivax* vaccine, the antigen is to be stored ~~at - 20C/-4 F~~ ***between - 25C/-13 F to - 15C/5 F*** and the diluent at +4°C/+39.2 F.

Range for temperature excursions updated accordingly and clarification that the process for reporting of temperature exclusions applies to NIMPs as well:

Any temperature excursion outside the range of 2.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) or ***below - 25C/-13 or*** above -15.0°C/***5 F*** (for -20°C/-4°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form (Advanced Temperature Excursion Analysis and Management [ATEAM]). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor. ***This applies for the NIMPs as well.***

Formulation of *Rotarix* vaccine (Table 10 Study Vaccines)

The vaccine name and formulation was updated to the HRV lyophilized version.

Rotarix	~~HRV~~ ***HRV lyo***	~~HRV RIX4144=$10^{6.5}$ $CCID_{50}$~~ ***HRV RIX4144 live attenuated >=$10^{6.0}$$CCID_{50}$***	Lyophilized vaccine in a monodose glass vial.
	HRV Diluent	$CaCO_3$=60mg	Diluent for lyophilized vaccine (calcium carbonate liquid antacid) supplied separately in a prefilled oral applicator

Overview of Study Design (Figure 1)

The first safety follow-up visit was corrected to 'D16', as it previously read 'D15'.

Update of ICH version (Section 5.1 Regulatory and ethical considerations, including the informed consent process and Section 12 References)

The ICH GCP version was updated to reflect the most recent version.

Properly constituted IRB/EC is defined in ICH Guideline for GCP E6 (~~R1~~ ***R2***), Section 3 [ICH, ~~1997~~ ***2018***].

The corresponding reference was updated as follows:

ICH (***2018*** ~~1997~~) ICH Harmonized Tripartite ICH Guideline for Good Clinical Practices E6(~~R1~~ ***R2): Integrated Addendum to E6(R1);.*** Federal Register, ~~62 (90): 25691-25709~~ ***83 (41): 8882-8883***.

List of Study Procedures (Table 5)

The row for inclusion/exclusion criteria check was updated to remove the check at Visit 4 (Day 151). The row for training of eDiary was changed at Visit 5 (Day 301) from an open bullet indicating it is not required to be documented in the eCRF to a closed bullet (ie, required to be documented in the eCRF).

Location of administration of placebo, update of route of vaccine administration and recording of injection site (Section 6.3 Dosage and administration of study vaccines)

The location for administration of the placebo vaccines was included in the text.

The rMenB+OMV NZ***/Placebo*** vaccine should be administered at least 2.5 cm above the injection site of Hib, when concomitantly administered on the same thigh.

The route of administration was updated to refer to the Dosage and Administration table instead:

Standard immunization practices are to be observed and care should be taken to administer the injection ~~intramuscularly~~ ***by the route described in Table 11 below***.

Text added to clarify that site of injection should be recorded in the eDiary as well:

The exact anatomic location of each injection must be carefully recorded in the Medical Chart and in the eCRF, ***as well as the Subject eDiary***.

Clarification of timepoint of assessment of exclusion criteria (Section 6.5 Contraindications to subsequent vaccination)

The text on assessment of exclusion criteria was clarified to describe that it should be performed before each dose.

- Any occurrence of an event listed in the exclusion criteria which must be always re-assessed by the investigator before administration of the ~~second~~ ***next*** dose of study vaccine. Refer to Section 4.2.

Time period of recording of concomitant vaccinations (Section 6.7.1 Recording of concomitant medications/products and concomitant vaccinations)

Criteria updated to record concomitant vaccinations from birth, rather than 14/28 days prior to first study vaccine dose.

- Any concomitant vaccination administered in the period starting ***from birth*** ~~14 days (or 28 days for live attenuated vaccines) before the first dose of study vaccine(s)~~ and ending at the last study visit (Day -14 to Day 661).

Recording of solicited AEs only via eDiary (Section 7.1.1 Definition of an adverse event)

Solicited AEs are derived from organized data collection systems, such as Subject Diaries~~, or interview~~.

Recording of solicited AEs in the eCRF (Section 7.1.3.3 Other solicited adverse events)

Solicited local and systemic AEs that continued beyond day 7 after vaccination will not need to be entered as an AE in the AE eCRF or the subject's source document. They will be collected via the eDiary.

Note: Any solicited adverse event that meets any of the following criteria must be entered into subjects' source document (see Section 10.2) and also as an adverse event on the Adverse Event eCRF:

- ~~Solicited local or systemic adverse event that continues beyond day 7 after vaccination.~~

- Solicited local or systemic adverse event that leads to a visit to a healthcare provider (medically attended adverse event, see Section 7.2.3.4).
- Solicited local or systemic adverse event leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator (adverse event leading to withdrawal).

Solicited AEs ongoing after 7 days post-vaccination

Solicited AEs that are ongoing after the 7-day reporting period will continue to be recorded in the eDiary until resolution or up to 30 days post-vaccination (ie, recording period for unsolicited AEs).

Table 5 List of Study Procedures: Footnote added as ***[11] Solicited AEs that are ongoing after the 7-day reporting period will continue to be recorded in the eDiary until resolution.***

Section 5.6.15.1.1: The subject's parent(s)/LAR(s) will receive daily reminders via the Subject Diary device's in-built audio-visual alarms to alert the user to complete the diary during the post vaccination period, which is from Day 1 to Day 7 after Visits 1, 2 and 3 ***(or until the solicited AE resolves***) and from Day 1 to Day 30 after Visit 5.

Section 7.1.3: The term "reactogenicity" refers to solicited signs and symptoms ("solicited adverse events") occurring in the hours and days following a vaccination, to be collected by the parent(s)/LAR(s) for 7 consecutive days (***with ongoing solicited AEs collected until resolution or day 30, whichever occurs first***), using a pre-defined Subject Diary.

Table 13 Footnote 2: Solicited AEs to be collected for 7 days following each vaccination, ***with ongoing solicited AEs collected until resolution or day 30, whichever occurs first***. Specific solicited systemic AEs (parotid gland swelling, fever, rash) will be collected for 30 days following MMR/V vaccination a Visit 5 (Day 301).

Pre-vaccination temperature recording (Section 5.6.9 Assess pre-vaccination body temperature)

Included a cross-reference to section on contraindications to subsequent vaccinations:

If the subject has fever [fever is defined as a body temperature ≥38.0°C/100.4°F regardless the location of measurement on the day of vaccination], the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 6 ***and Section 6.5***).

Alignment of post-vaccination procedures with Table 5 (Section 5.6.13 Post-vaccination procedures)

The following post-vaccination procedures for each subject will be performed on Day 1 (Visit 1), Day 61 (Visit 2), Day 121 (Visit 3), and Day 301 (Visit 5). ***After vaccination, the subject will be observed for at least 30 minutes including observation for solicited and unsolicited AEs.*** Record all safety data collected during this time in the subject's source document and eCRF.

Safety information to be reported by parent(s)/LAR(s) (Section 7.2.3.2.1 Assessment of intensity and Section 10.2 Subject Diary)

Text updated in various sections to make clear that it is the parent/LAR who will be providing the safety information, and not the subject.

Section 5.6.15: The ~~subjects~~/subjects' parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.

Section 5.6.15.1: Subject Diary assignment and use (at Visit 1 and Visit 5):

- Each subject's parent~~(s)~~/LAR~~(s)~~ will be assigned a Subject Diary and shown how to use the device – this will include how to access the diary, performing test data entry on sample questions, and how to charge and store the device.
- The subject's parent~~(s)~~/LAR~~(s)~~ will self-select a numeric access code secret to themselves. The same individual should preferably make the assessments and complete the Subject Diary throughout the relevant reporting period.

Section 5.6.15.2: Safety follow-up calls are calls made to the subject'***s parent(s)/LAR(s)*** by a qualified healthcare professional designated on the site log.

Section 5.7: Any sample testing will be done in line with the consent of the individual ~~subject~~/subject's parent(s)/LAR(s).

Section 7.2.3.2.1: Every effort should be made by the investigator to evaluate safety information reported by a subject***'s parent(s)/LAR(s)*** for an underlying diagnosis and to capture this diagnosis as the event in the AE page.

Section 10.2: The Investigator or delegated staff should monitor the Subject's Diary status throughout the study for compliance and any solicited local and systemic adverse events that were of concern to the subject***'s parent(s)/LAR(s)***.

Time period for collecting SAEs, medically attended AEs, AEs leading to withdrawal (Section 7.2.1 Time period for detecting and recording adverse events and serious adverse events)

Text updated to clarify that recording of these AEs will start from Day 1, which would also be at the first receipt of study vaccines.

The time period for collecting and recording SAEs will begin at ***Day 1 (ie,*** the first receipt of study vaccines***)*** and will end 12 months following administration of the last dose of study vaccine for each subject ***(ie, study end)***. See Section 7.3 for instructions on reporting of SAEs.

All medically attended AEs will be collected and recorded from ***Day 1 (ie,*** the time of the first receipt of study vaccines) until ***12 months following administration of the last dose of study vaccine for each subject (ie,*** study end).

All AEs/SAEs leading to withdrawal from the study will be collected and recorded from ***Day 1 (ie***, the time of the first receipt of study vaccines) until ***12 months following administration of the last dose of study vaccine for each subject (ie,*** study end***)***.

The time period for collecting and recording of AESIs will begin at ***Day 1 (ie,*** the first receipt of study vaccines***)*** and will end 12 months following administration of the last dose of study vaccines ***(ie, study end)***.

Alignment of terminology for visits and follow-up calls (Section 7.2.3.1 Active questioning to detect adverse events and serious adverse events)

Pre-defined scripts will be used during the ***clinic*** ~~safety~~ visits and ***safety*** follow-up calls for instructing parents in recording safety data.

Analyses of unsolicited AEs clarified (Section 9.5 Derived and Transformed data)

The time intervals for the analysis of unsolicited AEs have been updated to clarify that only certain AEs will be analyzed post-30 days after vaccination.

- *Unsolicited AEs*: ~~For unsolicited AEs, the entire study period will be divided into the following disjoint intervals: Day 1 through Day 30, Day 31 until next vaccination or study termination~~. ***Unsolicited AEs are collected for 30 days post vaccination, and are analyzed as such without division of the time interval***
- ***AEs/SAEs leading to withdrawal from the study, SAEs (including SAEs related to study vaccine(s)), SAEs related to study participation or concurrent GSK medication/vaccine, AESIs, and Medically attended AEs: The entire study period will be divided into the following disjoint intervals: Day 1 through Day 30 post vaccination, Day 31 post vaccination until either the next vaccination or study termination.***

Footnote for Table 19 (GMTs at One Month Post 3rd Dose and GMRs at One Month Post 4th rMenB+OMV NZ Vaccination)

The footnote for Table 19 has been removed as it is no longer applicable.

~~Post 3rd vaccination results not available for M07-0241084. It is calculated assuming that post-3rd vaccination GMT is -0.3 to the post-4th vaccination GMT, because a difference of -0.2 to -0.4 were observed from the data of the other three strains.~~

Time period for investigator acknowledgment (Section 10.2 Subject Diary)

Added time period to specify when investigator needs to provide acknowledgment of subject diary review.

It is necessary for the investigator to acknowledge in the eCRF that the review of Subject Diary data has been performed for each subject. ***This is to be performed at Visit 6 (Day 331), when the subjects' parent/LAR returns the Subject Diary to the clinic.***

GlaxoSmithKline Biologicals SA

Vaccines R &D

Protocol Amendment 4

eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Amendment number:	Amendment 4
Amendment date:	02 NOV 2018
Co-ordinating author:	PPD

Rationale/background for changes:

- Request received from the Center for Biologics Evaluation and Research (CBER) on 10 JUL 2018 to amend the study protocol to revise the endpoints and success criteria based on using the strain M10713, for evaluating immune responses to the NHBA antigen. Sections describing endpoints, success criteria for sufficiency of immune response and all corresponding statistical methods have been updated accordingly. The testing strategy for the primary objective has been reduced from 5 hypotheses families to 3.
- CBER asked to maintain a sample size of 2700 and hence the power has been recalculated, based on certain assumptions and sufficiency criteria for the M10713 strain and composite endpoint.
- Exclusion criteria modified to allow administration of one dose of Hepatitis B vaccine (HBV) up to 4 weeks prior to informed consent, in line with local practice.
- Confirmation of hSBA cut-off values following completion of the assay validation, including both lower limits of detection (LODs) and lower limits of quantitation (LLOQs) for the 3 MenB indicator strains M14459 (fHbp), 96217(NadA) and NZ98/254 (PorA).
- In alignment with eDiaries, clarified that site staff to contact the subjects parents/LAR(s) when subjects experienced either solicited or unsolicited AE(s) that required hospitalization or visit to the emergency room or medically attended events that were of concern to the parents/LAR(s).
- Temperature categories for grading of fever in Fahrenheit updated.
- Other minor changes to correct typos, and improve clarity and alignment within the document.

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

Objectives:

The study objectives (synopsis and Section 2.1) and endpoints (synopsis and Sections 9.1 and 9.2) have been updated based on the feedback from CBER to change the NHBA strain.

Co-Primary immunogenicity objectives:

- To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4 and 6 months of age, at one month after the 3rd vaccination.

  *Criteria: The sufficiency of the immune response to rMenB+OMV NZ after the 3rd vaccination will be demonstrated if the adjusted lower confidence limit for the percentage of subjects achieving serum bactericidal assay using human complement (hSBA) titers ≥ Lower Limit of Quantitation (LLOQ) is ≥ 60% for the N. meningitidis serogroup B test strains M14459, 96217, NZ98/254 and ≥~~12~~ **55**% for strain ~~M07-0241084~~ **M10713** (individually); and is ≥~~10~~ **43**% for all strains combined (composite endpoint).*

- To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered non-concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 4th vaccination

  *Criteria: The sufficiency of the immune response to rMenB+OMV NZ will be demonstrated if the adjusted lower confidence limit for the percentage of subjects achieving a 4-fold rise in hSBA titers (from pre-4th) is ≥70% for N. meningitidis serogroup B test strains M14459, 96217, NZ98/254 and ≥~~50~~ **66**% for strain ~~M07-0241084M10713~~ **M10713** (individually); and is ≥~~40~~**48**% for all strains combined (composite endpoint).*

Secondary objective:

- To evaluate the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 3rd vaccination, at 6 months after the 3rd vaccination (immediately before the 4th vaccination), and at one month after the 4th vaccination against the M14459, 96217, NZ98/254 and ~~M07-0241084~~ ***M10713*** test strains.

Immunogenicity endpoints:

Primary:

For rMenB+OMV NZ, the following endpoints will be calculated:

- At one month after the 3rd vaccination
  - the percentage of subjects with hSBA titers ≥LLOQ; for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~ ***M10713*** test strains.
  - the percentage of subjects with hSBA titers ≥LLOQ for all strains combined (composite endpoint).

- At one month after the 4th vaccination
  - the percentage of subjects with 4-fold rise (from pre-4th vaccination) in hSBA titers for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~***M10713*** test strains.

Secondary:

For rMenB+OMV NZ, the following secondary endpoints will be calculated:

- At 1 month after the 3rd vaccination
  - the percentage of subjects with hSBA titers ≥5 and ≥8 for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~ ***M10713*** test strains.
- At 6 months after the 3rd vaccination (immediately before the 4th vaccination)
  - the percentage of subjects with hSBA titers ≥LLOQ for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~ ***M10713*** test strains.
  - the percentage of subjects with hSBA titers ≥5 and ≥8 for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~ ***M10713*** test strains.
- At 1 month after the 4th vaccination
  - the percentage of subjects with hSBA titers ≥LLOQ; for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~ ***M10713*** test strains.
  - the percentage of subjects with hSBA titers ≥5 and ≥8for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~ ***M10713*** test strains.

Strain name and cut-off in Table 7:

Table 7 Humoral Immunity (Antibody determination)

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
Serum	N Men B ***M10713*** ~~M07-0231084~~ (NHBA) Ab	MSBA	In house	1/DIL	***16*** 4	GSK Biologicals *** or laboratory designated by GSK Biologicals	1.1
	N Men B NZ98/254 (PorA) Ab				6		1.2
	N Men B M14459 (fHbp) Ab				5		1.3
	N Men B 96217 (NadA) Ab				15		1.4

Rationale for the study and study design (Synopsis and Section 1.2.1)

Furthermore, the immune response of the rMenB+OMV NZ vaccine against test strains M14459 (fHbp), 96217 (NadA), NZ98/254 (PorA P1.4) and ~~M07_024108~~ ***M10713*** (NHBA) has not been evaluated yet in a ***US*** pediatric population. These strains are considered by the Center for Biologics Evaluation and Research (CBER) to better evaluate the protective response of the vaccine in the US population.

Statistical methods

Statistical methods in Section 9 were updated to reflect the change in strain and reduction in number of hypotheses families.

Section 9.3 Determination of sample size:

With 250 per group for PCV13 antigen tests and 1170 evaluable subjects from Group MenB+PCV for rMenB+OMV NZ strain tests, there is approximately ~~90%~~ ***94%*** probability to reject all 23 null hypotheses, and to claim that the primary objectives of the study will be met according to the predefined success criteria, assuming that the correlation between the rMenB+OMV NZ strains is 25%.

Table 17 Power to Show Non-Inferiority PCV13 Co-Primary Objective and Sufficiency rMenB+OMV NZ Co-Primary Objectives

	With 20% Correlation[1]	With 25% Correlation[1]	With 50% Correlation[1]
	N=1170 (N=250 for PCV13)	N=1170 (N=250 for PCV13)	N=1170 (N=250 for PCV13)
Success: - Non-inferiority for GMCs for 13 antigens after the 3rd vaccination Sufficiency for % of subjects with titers ≥ LLOQ per each strain and all 4 strains combined (composite response) after the 3rd vaccination Sufficiency for % of subjects with 4-fold increase (from pre-4th to post-4th) per each strain and all 4 strains combined (composite response) after the 4th vaccination			
Probability of rejection of all 23 null hypotheses	~~75~~ ***80%***	~~90~~ ***94%***	~~99~~ ***98%***

[1] With correlation assumption, it is assuming that there is a certain level of dependence between the results among the strains/antigens. The correlation value can range from 0 to 1. The higher the value is, the more dependent the results are among the strains. From V72P13 and V72P13E1 T***1***~~0~~ strains results it showed that the between-strain correlation ***is*** ~~are~~ mostly within the range of ~~0.25~~ 0.17 and 0.4***57***. ***For robustness of results power calculations were repeated using correlation matrices between strains with pairwise correlations taken from V72P13 and V72P13E1 where needed imputed 0.25. The resulting power is 95% for individual pairwise correlations, which is consistent with the result 94% when all pairwise correlations are chosen constant equal to 0.25.***

The power was estimated using simulations: 10.000 simulations per case. SAS ~~9.2~~ **9.4** was used for the calculations.

e. For testing Family 2 ***and 3*** ~~***to 5***~~, 1170 normal distributed data were drawn for one single group and for the four strains, according to the GMTs (for post 3rd dose) and GMRs (for post 4th dose), as shown in Table 19, and the SDs as described below in Table 18. Correlation structure was made for between-antigen correlation of 20%, 25% or 50%, respectively.

f. Testing of Family 1: Shifted one-sided t-test was used to test the hypotheses and output the p-values (for the power calculation no center effect was assumed).

g. ~~P-values were adjusted for multiplicity using the "Bonferroni-Holm" method (SAS procedure PROC MULTTEST, option "holm").~~

h. ***P-values*** ~~Adjusted p-values~~ were compared with the Family Wise Error Rate (FWER) to conclude on whether the null hypotheses were rejected or not. FWER for Family 1 is initially set to be 1/3 of the full alpha, which is rounded to 0.017. The FWER for Family 2 is 0.033 and the initial FWER is 0 for Family 3~~, 4 and 5~~. No multiplicity adjustment was performed within a family. Within family 1 all thirteen antigens were tested with the full FWER for family 1. If one or more test fail to reject the hypothesis for the antigen, family 1 fails. Consequently the alpha of family 1 will not be propagated (see bullet h. below).

i. Preparing for testing Family 2 ***and 3*** ~~**to 5**~~, percentages were obtained by dichotomizing the normal random sets that were generated at step b, by applying the cut-off of LLOQ value for post 3rd or a cut-off of 4 at post 4th. By applying a cut-off of 4 to the GMRs at post-4th this will generate a percentage of subjects who had a 4-fold increase for their GMT value at post-4th compared to pre-4th, which is an approximation of the percentage of 4-fold increase using LOD and LLOQ values. ~~A check step has been performed by comparing the percentages generated from this step with the true percentages observed from clinical trials. The results were comparable~~. Per time point, composite endpoint was derived by looking into each subject if the titer is above the cut-off for all 4 individual strains and if it is then this subject is counted as "1". Otherwise the subject is counted as "0".

j. For each of the ten percentages for rMenB+OMV NZ (4 individual strains and 1 composite endpoint at post-3rd and post-4th) the lower confidence limit was generated by fitting the binomial distribution with the percentage obtained from the previous step and alpha that has been assigned or propagated to the family. If the lower confidence limit is greater or equal to the sufficiency margin, then the null hypothesis is rejected for the single test. For Family 2 and 3, it is required to reject the null hypothesis for each and all of strain M14459, 96217,~~ and~~ NZ98/254, ***M10713, and the composite endpoint*** to be able to claim the success of the family. ~~For family 4 and 5, it is required to reject the null hypothesis for both M07-0241084 and the composite endpoint to be able to claim the family success.~~

k. Testing of Family 2: with the given FWER, if the LCL is greater or equal to 0.7 for each of strain M14459, 96217 and NZ98/254, ***greater or equal to 0.66 for strain M10713, and greater or equal to 0.48 for the composite endpoint***, then Family 2 is claimed for success. The FWER for Family 2 is set up as 2/3 of the full alpha, which is rounded to ~~0.33~~ ***0.033.***

l. Testing of Family 3: with the given FWER, if the LCL is greater or equal to 0.6 for each of strain M14459, 96217 and NZ98/254, ***greater or equal to 0.55 for strain M10713, and greater or equal to 0.43 for the composite endpoint***, then Family 3 is claimed for success. ~~Propagate the FWER from Family 3 to Family 4 and continue to test Family 4. Otherwise, if Family 3 fails, then stop testing globally.~~

m. ~~Testing of Family 4: with the given FWER, if the LCL is greater or equal to 0.5 for strain M07-0241084 and the LCL is greater or equal to 0.4 for the composite endpoint, then Family 4 is claimed for success. Propagate FWER from Family 4 to Family 5 and continue to test Family 5. Otherwise, if Family 4 fails, then stop testing globally.~~

n. ~~Testing of Family 5: with the given FWER, if the LCL is greater or equal to 0.12 for strain M07-0241084 and the LCL is greater or equal to 0.1 for the composite endpoint, then Family 5 is claimed for success. Otherwise, Family 5 fails.~~

Table 18 Two-sided 80% Upper Confidence Limit of Standard Deviations

	80% UCL of SDs	
rMenB+OMV NZ strains	**Post 4th vaccination (SDs of GMR)**	**Post 3rd vaccination (SDs of GMT)**
M14459	0.54372	0.32221
96217[2]	0.77760	0.36900
NZ98/254	0.51794	0.51967
~~M07-0241084~~***M10713***	0.66918	~~0.39959~~ ***0.522***
PCV13 antigen[1]	**Post 3rd vaccination (SDs of GMC)**	
1	0.36629	
3	0.41572	
4	0.34261	
5	0.43567	
6A	0.39552	
6B	0.44974	
7F	0.34352	
9V	0.32548	
14	0.32445	
18C	0.33550	
19A	0.24439	
19F	0.45638	
23F	0.37567	

[1.] Estimated SDs for PCV13 antigens are the upper confidence limit of 2-sided 80% CI of back-calculated SDs, based on information (degree of freedom, 95% CI) provided on table 17 of *Prevnar13* Package Insert (dated on July 2016) and assuming the underlying SDs fulfill a t-distribution.

[2] The SD for 96217 at post-4th vaccination GMR is not available and hence assumed the SD of GMR from Strain GB394.

~~[3] For strain M07-0241084 as no pre-4th vaccination data available, GMR was calculated by post-4th/post-3rd vaccination.~~

Table 20 Limit of Detection and Lower Limit of Quantitation for rMenB+OMV NZ strains

rMenB+OMV NZ strains	**LOD**	**LLOQ**
~~M07-024180~~ ***M10713***	4***3***	4 ***16***
NZ98/254	4	6
M14459	3	5
96217	6	15

LOD: lower limit of detection, LLOQ: lower limit of quantitation.

Note: ***Other than for strain M10713, t***~~T~~hese LODs and LLOQs were cited from the latest lab assay ~~pre-~~validation report released in **2018**~~Q2 2017~~. ***For strain M10713, the LOD and LLOQ are to be determined***. ~~They are subject to change upon CBER's potential comments on the validation plan.~~

b. The percentage of subjects with titers ≥ LLOQ is more than or equal to 60% for strain M14459, 96217 and NZ98/254, and more than or equal to ~~12 %~~ ***55%*** for strain ~~M07-0241084~~ ***M10713***, and more than or equal to ~~10%~~ ***43%*** for all 4 rMenB+OMV NZ strains at one month post 3rd vaccination.

c. Percentage of subjects with 4-fold increase is more than or equal to 70% for strain M14459, 96217 and NZ98/254, and more than or equal to ~~50%~~***66%*** for strain ~~M07-0241084~~ ***M10713***, and more than or equal to ~~40%~~***48%*** for all four rMenB+OMV NZ strains , at one month post 4th vaccination.

Section 9.7.1 Within groups assessment:

For each study group, at each timepoint that a blood sample result is available, the following endpoints will be assessed related to MenB strains and PCV13 strains:

- the percentage of subjects with hSBA titers ≥ LLOQ for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~***M10713*** test strains
- the percentage of subjects with hSBA titers ≥ LLOQ for all strains combined (composite endpoint)
- the percentage of subjects with hSBA titers ≥5 and ≥8 for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~***M10713*** test strains
- the percentage of subjects with 4-fold increase at post-4th (from ~~post-3rd~~ ***pre-4th***) for each of the M14459, 96217, NZ98/254 and ~~M07-0241084~~***M10713*** test strains.

9.7.3.1 Statistical Hypotheses:

In total 23 hypotheses will be tested for the primary objectives. A step-wise statistical approach will be used for the 23 statistical tests. The hypotheses will be grouped into ~~five~~ ***three*** families, based on the different immunogenicity objectives:

Family 1:13 statistical hypotheses related to non-inferiority of PCV13 with rMenB+OMV NZ as measured by the GMCs of the 13 PCV13 antigens, after the 3rd vaccination.

Family 2: ~~3~~ ***5*** statistical hypotheses related to sufficiency objective on percentage of subjects with 4-fold increase at post 4th dose (from pre-4th dose) for each of the ~~three~~ ***four*** strains M14459, 96217, NZ98/254***, and M10713, and the composite endpoint***

Family 3: ~~3~~ ***5*** statistical hypotheses related to sufficiency objective on percentage of subjects with hSBA titers ≥ LLOQ at post 3rd dose for each of the ~~three~~ ***four*** strains M14459, 96217, NZ98/254***, and M10713, and the composite endpoint.***

~~Family 4: 2 statistical hypotheses related to sufficiency objective on percentage of subjects with 4-fold increase at post 4th dose (from pre-4th dose) for strain M07-021084 and for all strains combined~~

~~Family 5: 2 statistical hypotheses related to sufficiency objective on percentage of subjects with hSBA titers ≥ LLOQ at post 3rd dose for strain M07-021084 and for all strains combined~~

Families 2 and ~~5~~***3 a***re related to the *"sufficiency of the immune response to rMenB+OMV NZ"* objective at one month post~~3rd~~***-3rd*** ~~4th~~ dose and one month post-***4th*** dose***, respectively***.

*Family 2**:** Percentage of subjects with 4-fold increase for each of the ~~three~~ **four** strains (M14459, 96217, NZ98/254, **M10713) and the composite endpoint**, post-4th*

Sufficiency will be claimed for each of the ***4*** strains ***and the composite endpoint***, separately, if the following null hypothesis will be rejected:

H_{02j}: $P_{Aj} < A_j$ vs. H_{12j} : $P_{Aj} \geq A_j$,

where P_{Aj} denotes the percentage of subjects with 4-fold increase at one month after the 4th vaccination (from pre-4th dose) for strain j, (j=1, 2, 3, ***4, and composite***), which is assumed to be at least 90% for each of the three strains M14459, 96217, and NZ98/254, ***72% for strain M10713, and 54% for the composite endpoint (estimate based on assumption of 25% correlation)***. Aj represents the level of sufficient immune response for each of the ~~three~~ ***four*** strains ***and composite endpoint***. The immune response at one month following the 4th vaccination, will be sufficient for rMenB+OMV against each individual strain if the lower limit of the adjusted CI for the percentage of subjects with 4-fold increase is ≥70% for strains M14459, 96217, NZ98/254***, ≥66% for strain M10713, and ≥48% for the composite endpoint.***

***Family 3:** Percentage of subjects with hSBA≥LLOQ for each of the **four** strains (M14459, 96217, NZ98/254, **M10713) and the composite endpoint**, post-3rd*

Sufficiency will be claimed for each of the 4 strains, separately, if the following null hypothesis will be rejected:

H_{03j} : $P_{Aj} < A_j$ vs. H_{13j} : $P_{Aj} \geq A_j$,

where P_{Aj} denotes the percentage of subjects hSBA≥LLOQ at one month after the 3rd vaccination for strain j, j=1, 2, 3,***4, and composite)***), which is assumed to be at least 90% for strains M14459 and 96217, 80% for NZ98/254, ***61% for strain M10713, and 49% for the composite endpoint***. Aj represents the level of sufficient immune response for each of the ***four*** strains ***and composite endpoint (estimate based on assumption of 25% correlation)***. The immune response at one month following the 3rd vaccination, will be sufficient for rMenB+OMV NZ against each individual strain if the lower limit of the adjusted CI for the percentage of subjects with hSBA titer ≥LLOQ is ≥60% for strains M14459, 96217, NZ98/254***, ≥55% for strain M10713, and ≥43% for the composite endpoint.***

***Family 3:** Percentage of subjects with hSBA≥LLOQ for each of the ~~three~~ **four** strains (M14459, 96217, NZ98/254, **M10713) and the composite endpoint**, post-3rd*

Sufficiency will be claimed for each of the ~~3~~ 4 strains, separately, if the following null hypothesis will be rejected:

H_{03j} : $P_{Aj} < A_j$ vs. H_{13j} : $P_{Aj} \geq A_j$,

where P_{Aj} denotes the percentage of subjects hSBA≥LLOQ at one month after the 3rd vaccination for strain j, j=1, 2, 3,***4, and composite)***), which is assumed to be at least 90% for strains M14459 and 96217, ~~and~~ 80% for NZ98/254, ***61% for strain M10713, and 49% for the composite endpoint***. Aj represents the level of sufficient immune response for each of the ~~three~~ ***four*** strains ***and composite endpoint***. The immune response at one

month following the 3rd vaccination, will be sufficient for rMenB+OMV NZ against each individual strain if the lower limit of the adjusted CI for the percentage of subjects with hSBA titer ≥LLOQ is ≥60% for strains M14459, 96217, NZ98/254***, ≥55% for strain M10713, and ≥43% for the composite endpoint.***

~~***Family 4:*** *Percentage of subjects with 4-fold increase for strain M07-0241084 and for the four strains combined, post-4th*~~

~~Sufficiency will be claimed, if the following null hypothesis will be rejected:~~

~~$H_{04j}: P_{Aj} < A_j$ vs. $H_{14j}: P_{Aj} \geq A_j$,~~

~~where P_{Aj} denotes the percentage of subjects with 4-fold increase at one month after the 4th vaccination for strain M07-0241084 (j=1) and for the composite endpoint with all 4 strains combined (j=2), which are assumed to be 60% and 45% respectively. Aj represents the sufficient level for this composite endpoint, and is set to 50% for A_1 and 40% for A_2. The immune response at one month following the 4th vaccination, will be sufficient for M07-0241084, if the lower limit of the adjusted CI for the percentage of subjects with 4-fold increase for M07-0241084 is ≥50%.The immune response at one month following the 4th vaccination, will be sufficient for rMenB+OMV NZ for all strains combined, if the lower limit of the adjusted CI for the percentage of subjects with 4-fold increase for all strains combined is ≥40%.~~

~~***Family 5:*** *Percentage of subjects with hSBA≥LLOQ for strain* M07-0241084 *and for the four strains combined, post-3rd*~~

~~Sufficiency will be claimed, if the following null hypothesis will be rejected:~~

~~$H_{05j}: P_{Aj} < A_j$ vs. $H_{12j}: P_{Aj} \geq A_j$,~~

~~where P_{Aj} denotes the percentage of subjects with hSBA≥LLOQ at one month after the 3rd vaccination for strain M07-0241084 (j=1) and for the composite endpoint with all 4 strains combined (j=2), which are assumed to be 18% and 15% respectively. Aj represents the sufficient level for this composite endpoint, and is set to 12% for A_1 and 10% for A_2 The immune response at one month following the 3rd vaccination, will be sufficient for M07-0241084, if the lower limit of the adjusted CI for the percentage of subjects with hSBA titer ≥LLOQ for M07-0241084 is ≥12%.The immune response at one month following the 3rd vaccination, will be sufficient for rMenB+OMV NZ for all strain combined, if the lower limit of the adjusted CI for the percentage of subjects with hSBA titer ≥ LLOQ for all strains combined is ≥10%.~~

The initial α is 0.0167 (2-sided) for Family 1 and 0.033 (2-sided) for Family 2, while it is zero for ~~all the other~~ families ***family 3***.

Step 1: Family 1 and Family 2

i. Start test Family 1 and Family 2 as the first step. ~~Within Family 1, the Bonferroni-Holm method will be applied to correct for multiplicity.~~

- For testing Family 1 the hypotheses per antigen the p-values will be obtained from an ANOVA model, with vaccination group and center as independent variables and log transformed titers/concentrations as response variable. ~~P-values will be adjusted by the Bonferroni-Holm method, new (Bonferroni stepdown) p-values will be generated and outputted.~~
- The null hypotheses will be rejected if the ~~new, for multiplicity adjusted~~ p-values < FWER ***for all thirteen antigens***.
- For testing Family 2, if the lower limits of the 2-sided ***96.96***~~98.33~~% CIs are greater or equal to the sufficiency margins for each of the ~~three~~ ***four*** strains ***and composite endpoint***, then Family 2 is successful. Otherwise, Family 2 fails.

ii. If Family 1 had succeeded and Family 2 not, then propagate ½ FWER from Family 1 to Family 3 and the other ½ FWER to Family 2, and re-test Family 2 with the new FWER which is the sum of the original FWER from Family 2 plus the ½ of FWER from Family 1. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 2 and propagate full FWER to Family 3; otherwise Family 2 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 1.

iii. If Family 2 had succeeded and Family 1 not, then propagate ½ FWER from Family 2 to Family 3 and the other ½ FWER to Family 1, and re-test Family 1 with the new FWER which is the sum of the original FWER from Family 1 plus the ½ of FWER from Family 2. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 1 and propagate full FWER to Family 3; otherwise Family 1 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 2.

iv. If both Family 1 and Family 2 had succeeded, then the FWER from both families were propagated to Family 3, and proceed to test Family 3;

v. If both Family 1 and Family 2 had failed, then no FWER will be propagated to Family 3, and we stop the testing globally and success can be claimed on none of the ~~five~~ ***three*** families.

~~Step 2: Family 3:~~

~~If all null hypotheses are rejected, the propagate full FWER to Family 4; otherwise stop testing globally, and success can be claimed for Family 1 and/or Family 2 whichever was successful in Step 1.~~

~~Step 3: Family 4:~~

~~If all null hypotheses are rejected, the propagate full FWER to Family 5; otherwise stop testing globally, and success can be claimed for Family 3, as well as for Family 1 and/or Family 2 whichever was successful in Step 1.~~

Step 4 *2*: Family ~~5~~ *3*:

If all null hypotheses are rejected, then propagate 1/2 FWER to Family 1 and Family 2 respectively, and success can be claimed for Family 3~~, Family 4 and Family 5~~ as well as for Family 1 and/or Family 2 whichever was successful in Step 1; otherwise stop testing globally, and success can be claimed for ~~Family 3 and Family 4, as well as for~~ Family 1 and/or Family 2 whichever was successful in Step 1.

Step ~~5~~3: Family 1 or Family 2:

In case one of Family 1 and Family 2 failed in step 1, with the alpha passed down by Family ~~5~~*3*, it is possible to re-test the family that failed once and again test it with ½ FWER from Family ~~5~~*3*. If all null hypotheses are rejected, then success can be claimed for this family as well.

Appendix A Laboratory Assays

MenB serum bactericidal assays using human complement (hSBA):

Serum bactericidal activity against rMenB+OMV NZ will be determined by performing hSBA against a standard panel consisting of 4 meningococcal B test strains M14459, 96217, NZ98/254 and ~~M07-0241084~~***M10713***. Each of these strains measures bactericidal activity primarily directed against one of the major bactericidal antigens included in the vaccine: strain M14459 measures hSBA against the 741 part of the 936-741 antigen, also known as fHbp variant 1.1; strain 96217 measures hSBA against antigen 961c, also known as NadA; and strain NZ98/254 measures hSBA against PorA P1.4, the immunodominant antigen in the OMV NZ vaccine component; strain M10713 measures hSBA against ***the 287 part of the 287-953*** antigen ~~287-953~~, also known as NHBA.

Exclusion criteria for enrolment

Exclusion criteria in Section 4.3 modified to allow for administration of one dose of HBV up to 4 weeks prior to study enrollment**.**

- Received a dose of DTPa-HBV-IPV ~~(receipt of one dose of HBV at birth is allowed),~~ HRV, MMR, VV and/or Hib at any time prior to informed consent. ***Receipt of one dose of HBV up to 4 weeks prior to informed consent is allowed.***

Collection of body temperature measurements post-vaccination

Clarified that collection of 30-minutes post-vaccination solicited data includes body temperature measurement.

Section 5.6.1 Data collected from subject:

- 30 minutes post-vaccination immediate reactions: signs or symptoms of anaphylaxis, allergic phenomena (such as rashes, itching, or other allergic manifestations). Solicited local and systemic data (including e.g., use of medication to treat or prevent fever and/or pain, ***body temperature measurement).***

Section 5.6.13 Post-vaccination procedures

After vaccination, the subject will be observed for at least 30 minutes including observation for solicited and unsolicited AEs, ***including measurement of body temperature***.

Table 5 List of study procedures:

30 Minutes post-injection assessment (including body temperature measurement).

Typo in Section 7.1.1 (missing text)

All other AEs will be recorded ***as UNSOLICITED A***Es.

Typo in strain number of *Rotarix* vaccine (Table 10 Study Vaccines)

The strain number in Table 10 the for the *Rotarix* vaccine was corrected.

Rotarix	HRV lyo	HRV RIX4144***4414*** live attenuated >=$10^{6.0}$$CCID_{50}$	Lyophilized vaccine in a monodose glass vial.

Section 5.4 Method of blinding – alignment within section:

To work in an observer-blind manner, vaccine preparation and administration will be done by authorized medical personnel who will not participate in any of the study clinical evaluation assays ***or procedures***.

See the glossary of terms for definition of unblinded study staff. Two teams of study personnel will hence be set up:

- A team of unblinded personnel (responsible for the preparation and the administration of the vaccines).
- A team of blinded personnel (responsible for the clinical evaluation of the subjects ***including blood sampling***).

Access to SBIR:

Both blinded and unblinded study staff will have access to the SBIR system, in alignment with blinding strategy for the study.

Section 5.2.2.2.1 Study group and treatment number allocation:

After obtaining the signed and dated ICF from the subject's parent/ LAR and having checked the eligibility of the subject, the site staff ~~in charge of the vaccine administration~~ will access SBIR.

Section 5.2.2.2.2 Treatment number allocation for subsequent doses:

For each dose subsequent to the first dose, the study staff ~~in charge of the vaccine administration~~ will access SBIR, provide the subject identification number, and the system will provide a treatment number consistent with the allocated study group.

Treatment allocation to the subject (correction from amendment 3):

Text in section 5.2.2.2 updated based on change made in amendment 3.

The treatment numbers will be allocated by dose for Bexsero and placebo vaccines. Treatment allocation will be by components for all other products. ~~Throughout the study, a single treatment number will identify the vaccine doses to be administered to the same subject.~~

Definition of unsolicited AEs:

Text in section 7.1.4 Unsolicited Adverse Events updated to clarify that solicited AEs ongoing after the reporting period will be recorded in the eDiary, and not considered as unsolicited AEs

An unsolicited adverse event is an adverse event that was not solicited using a Subject Diary and that was spontaneously communicated by a parent(s)/LAR(s) who has signed the informed consent ~~or a solicited local or systemic AE that continues beyond the solicited period after vaccination~~.

Subject Diary Alerts:

Text in section 5.6.15.1.1 updated to clarify that site staff to contact subjects parents/LAR(s) when subjects experienced both solicited or unsolicited AE(s) that required hospitalization or visit to the emergency room or medically attended events that were of concern to the parents/LAR(s).

- Subject experienced ~~an unsolicited~~ ***any*** AE that required hospitalization or visit to the emergency room or medically attended events that were of concern to the parents/LAR(s).

Temperature grading for fever

The temperature grading in Table 15 for fever was updated to avoid measurements up to 2 decimal places.

Table 15 Grading of Solicited Systemic Adverse Events for All Subjects

Adverse Events	Grading of Severity			
	Grade 0	Mild	Moderate	Severe
Fever[2]	<38.0°C (<100.4°F)	≥38.0 - 38.9°C (≥100.4 – ~~102.02~~ ***102.1***°F)	≥39.0 - 39.9°C (≥102.2 – ~~103.82~~ ***103.9***°F)	≥40.0°C (≥ 104.0°F)

Correction on correlate of protection:

Text in section 5.7.3 Laboratory assays was corrected to clarify that serum bactericidal activity is a surrogate of protection. Text on lab blinding was removed as already stated previously.

The immunogenicity of rMenB+OMV NZ is assessed in this study by measuring the serum bactericidal activity, which is a functional measure of the ability of antibodies, in conjunction with human complement, to kill meningococci, and is widely accepted and generally recognized as the serological ~~correlate~~ ***surrogate*** of protection. ~~Testing will be conducted in a blinded manner towards the study group and the visit~~.

Addition of text on possible testing of other meningococcal strains:

Text in section 5.7 Biological sample handling and analysis updated to include possible testing of additional meningococcal strains if needed.

Although not planned, additional serologic testing may be performed in the future to further characterize the antibody response to the antigens included in the ***study vaccines or*** concomitantly administered vaccines (e.g. Rotavirus [HRV], ***hSBA against other meningococcal strains***).

Correction on typos in storage temperatures:

The typos in representing the temperature in section 6.2 Storage and handling of study and non-study vaccines is corrected.

For the *Varivax* vaccine, the antigen is to be stored between -25 C/-13 F to -15 C/5 F and the diluent at +4°C/+39.2 F.

Any temperature excursion outside the range of 2.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) or below -25 C/-13 or above -15.0°C/5 F (for -20°C/-4°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form (Advanced Temperature Excursion Analysis and Management [ATEAM]).

Correction in cut-off for pneumococcal anti-capsular polysaccharide IgG in Section 9.7.4.1:

For the binary variables, percentage of subjects with hSBA titer ≥ LLOQ, percentage of subjects with serum pneumococcal anti-capsular polysaccharide ***IgG ≥0.35***~~0.32~~ µg/mL, the number and percentage of subjects and associated 2-sided 95% Clopper-Pearson CIs will be computed for each antigen/strain by vaccine group and by time-point.

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Vaccines R &D

Protocol Amendment 5

eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Amendment number:	Amendment 5
Amendment date:	10 April 2020
Co-ordinating author:	PPD

Rationale/background for changes:

This protocol amendment 5 outlines measures that may be applicable during special circumstances (e.g., COVID-19 pandemic). The purpose of the amendment is to protect subject's welfare, and as far as possible ensure the potential benefit to the subject and promote data integrity.

The measures include the following,

- Allow increased flexibility in schedule and procedures to optimize site staff and patient safety.
- Assist the ability to complete study specific visits and procedure in order to preserve study integrity.
- Allow specific reporting of COVID-19 events for surveillance and increased specificity for safety data analysis at study conclusion.
- The definition of 'medically attended AEs' has been revised to include tele visit.

If possible all study specified visits and procedures should be completed according to the protocol, taking into account clinical judgment and local public health guidance to protect the safety of staff and subjects.

Additional revisions:

- A minor typographical error in grading of severity solicited local adverse events for all subjects in Table 14 has been corrected.
- The range for temperature excursions for non-investigational medicinal products (NIMPs) has been corrected in Section 6.2.
- Risk assessment of *Bexsero* has been aligned as per the EU-RMP.
- A minor typographical error in the footnote of Table 3 has been corrected.
- The definition of 'enrolled set' (Section 9.4.1) has been revised to align with the study.

- Minor revisions have been made in the sections 5.2.2.2.1, 5.6.4 and 9.4.4.1 to align with the revised definition of 'enrolled set'.

- Footnotes 1 and 2 from Table 5 (List of study procedures) have been converted into notes to address the minor finding from the site audit conducted in 2019.
- Addition of the 'Subject ID assignment' to pre-vaccination procedures (Section 5.6.2).
- The location for the administration of MMR and VV vaccines has been corrected to deltoid region.
- A minor typographical error in recording of concomitant vaccination has been corrected.

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

List of Abbreviations

COVID-19 ***Coronavirus Disease 2019***

Glossary of Terms

Enrolled	***All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments and are randomized and have received a Subject ID, regardless of the subject's treatment status in the study.***
Medically attended adverse events:	Symptoms or illnesses requiring hospitalization, or emergency room visit, or ***tele visit or*** visit to/by a health care provider.

Section 1.3.1. Risk Assessment

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
IMP: *Bexsero*		
~~Important potential risk: **Decrease of immunogenicity secondary to prophylactic use of acetaminophen (paracetamol)**~~	~~Prophylactic use of acetaminophen (paracetamol) reduces the incidence and severity of fever without affecting the immunogenicity of either Bexsero or routine vaccines [Prymula, 2014]. The effect of antipyretics other than acetaminophen (paracetamol) on the immune response has not been studied.~~	~~Prophylactic use of analgesic/antipyretic medications to reduce febrile reactions during the first 7 days after vaccination is allowed on a voluntary basis, but must be recorded in the source document and the eCRF (see Section 6.7.1).~~

Section 3. Study design overview

Addition of the following footnote to Figure 1 (Overview of study design)

Refer to Section 7.2.4 for study procedures to be considered during special circumstances

Addition of the following statement to the section,

Refer to Section 7.2.4 for study procedures to be considered during special circumstances.

Table 3 Overview of the Study Design: Blood Draws and Study vaccines

Clinic Visits (Study Day)		**Visit 1 (Day 1)**	**Visit 2 (Day 61)**	**Visit 3 (Day 121)**	**Visit 4 (Day 151)**	**Visit 5 (Day 301)**	**Visit 6 (Day 331)**
Months of Age (MoA)		**~2 MoA[1]**	**4 MoA**	**6 MoA**	**7 MoA**	**12 MoA**	**13 MoA[3]**
Study Vaccines	Group MenB+PCV N=1800	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	*Blood Draw[23]*	*Blood Draw[23]* rMenB+ OMV NZ PCV13	*Blood Draw[23, 34]*
	Group Placebo+PCV N=900	Placebo PCV13	Placebo PCV13	Placebo PCV13		*Blood Draw[23]* Placebo PCV13	
Routine Study Vaccines	**Both groups** N=2700	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV Hib		MMR VV	

MOA, months of age.

.[1]. Age at enrollment can be between 6 weeks to 12 weeks of age.

~~[2]. Study visits do not include safety follow-up calls. Although not conducted face-to-face, safety calls are intended for safety data collection. For details, refer to Table 5.~~

[23] Blood draw to be performed prior to any vaccination (study or non-study).

[43]. Subjects in both groups will receive a fourth dose of Hib vaccine (*Hiberix*) and a single dose of DTPa vaccine (*Infanrix*) at 13 months of age, i.e. Visit 6 at Day 331. No immunogenicity or safety assessments will be performed following these Visit 6 vaccinations.

Note: Study visits do not include safety follow-up calls. Although not conducted face-to-face, safety calls are intended for safety data collection. For details, refer to Table 5.

- **Sampling schedule**: The sampling schedule for both study groups is the same. Three blood samples of approximately 5 mL each are to be taken at:
  - Visit 4 (Day 151), i.e., 30 (***-9*** ~~-7~~ to +***30***~~14~~) days after the 3rd vaccination.
  - Visit 5 (Day 301), i.e., 180 (-7 to ***91***~~+21~~) days after the 3rd vaccination (pre-4th vaccination).
  - Visit 6 (Day 331), i.e., 30 (~~-7~~ ***-9*** to +~~14~~***30***) days after the 4th vaccination.

The same above changes in sampling schedule are applicable for Section 5.6.11.1 (Blood sampling for immune response assessments)

Section 5.2.2.2.1. Study group and treatment number allocation

~~Enrolled subjects~~ ***Subjects*** will be randomized in the SBIR system to one of the two groups in a 2:1 ratio to receive:

Section 5.5. Outline of study procedures

Table 5 List of Study Procedures

Age (approximate)	**~2 moa**			**4 moa**			**6 moa**		**7 moa**			**12 moa**		**13 moa**						**24 moa**
Epoch	**Epoch 001**											**Epoch 002**			**Epoch 003**					
Type of contact [1, 2]	**Visit 1**	**SFC 1**	**SFC 2**	**Visit 2**	**SFC 3**	**SFC 4**	**Visit 3**	**SFC 5**	**Visit 4**	**SFC 6**	**SFC 7**	**Visit 5**	**SFC 8**	**Visit 6**	**SFC 9**	**SFC 10**	**SFC 11**	**SFC 12**	**SFC 13**	**Visit 7**[10]
Visit window (days) [2 4]	**-5**	**-3/+3**	**3/+3**	**-*317*/+21**	**-3/+3**	**-3/+3**	**-*317*/+21**	**-3/+3**	**-*97*/+*30* ~~14~~**	**-3/+3**	**-3/+3**	**-7/+*91* ~~21~~**	**-3/+3**	**-*97*/+*30* ~~14~~**	**-7/+21**	**7/+21**	**-7/+21**	**-7/+21**	**-7/+21**	**--7/+21**
Recording of all unsolicited AEs within 30 days post-vaccination*11*	●	●	●	●	●	●	●	●	●			●	●	●						
Recording of SAEs, medically attended AEs, AEs leading to withdrawal and AESIs [7, *11*]	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Note: Procedures to be performed prior to any vaccination.
Although not conducted face-to-face, safety follow-up calls are intended for safety data collection and considered equivalent to clinic study visits.
The double line after Visit 6 at Day 331 indicates the potential interim analysis time period.
~~[1. Although not conducted face-to-face, safety follow-up calls are intended for safety data collection and considered equivalent to clinic study visits.]~~
~~[2. Procedure to be performed prior to any vaccination.]~~
[10]. Visit 7 may be a telephone contact during special circumstances. Refer to Section 5.6.15 for study procedures to be considered during special circumstances.
[11]. COVID-19 infection related AEs and SAEs should be recorded in a separate eCRF.

Table 6 Intervals Between Study Visits

Interval	Optimal length of interval	Allowed interval[1, 2]
Visit 1 → Visit 2	60 days	~~53~~ ***29*** days - 81 days
Visit 2 → Visit 3	60 days	~~53~~ ***29*** days - 81 days
Visit 3 → Visit 4	30 days	~~23~~***21*** days - ~~44~~ ***60*** days
Visit 3 → Visit 5	180 days	173 days - ~~201~~ ***271*** days
Visit 5 → Visit 6	30 days	~~23~~ ***21*** days - ~~44~~ ***60*** days
Visit 5 → Visit 7 (Study termination	360 days	353 days - 381 days

[1]The investigator should arrange study visits within this interval as subjects may not be eligible for inclusion in the PPS cohorts if they make the study visit outside this interval. All visits and windows should be planned using the day of last vaccination as the reference point

[2]The allowed interval have been modified in keeping with ACIP guidelines to facilitate the safe scheduling of study visits during the COVID-19 pandemic situation.

Section 5.6. Detailed description of study procedures.

During special circumstances, exemplified by the COVID-19 pandemic, certain study procedures may be adapted to protect the subject and promote data integrity. Refer to Section 5.6.15 for further details.

Section 5.6.2. Pre-vaccination procedures (Visit 1)

A pre-vaccination clinic visit may occur within 5 days prior and up to the time of the vaccination Visit 1 on Day 1 (inclusive). The following procedures will be performed for each potential subject prior to the first vaccination at Visit 1:

- Obtaining informed consent from subject's parent(s)/LAR(s).
- ***Subject ID assignment***

Section 5.6.4 Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 4.2 and 4.3 ~~before~~ ***after obtaining informed consent and before randomization*** ~~enrolment~~.

Section 5.6.15. Study procedures during special circumstances

During special circumstances (e.g., COVID-19 pandemic), the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied. For the duration of such special circumstances, the following measures may be implemented for enrolled participants:

- ***Visit 7 (Day 661): the study conclusion visit has been revised to allow for either a site visit or concluding telephone call. The telephone call is intended for safety data collection and will be considered equivalent to study conclusion visit.***
- ***The existing protocol-specified windows have been modified in keeping with ACIP guidelines to facilitate the safe scheduling of study visits (See allowed interval in Table 6).***

- ***If despite best efforts it is not possible to collect blood samples within the allowed interval between visits (see Table 6), then the interval may be extended as per the clinical judgement of the investigator.***
- ***If despite best efforts it is not possible to give the vaccine dose within the allowed interval between visits (see Table 6), then the interval may be extended as per the clinical judgement of the investigator and in keeping with ACIP guidelines.***

Impact on the per protocol set for immunogenicity will be determined on a case by case basis.

Section 5.6.16. Recording of AEs, SAEs and AESIs

- Refer to Section 7.2 for procedures for the investigator to record AEs, SAEs, and AESIs. Refer to Section 7.3 for guidelines and how to report SAE and AESI reports to GSK Biologicals. ***Refer to Section 7.2.4 for recording of COVID-19 infection related AEs.***

Section 5.6.17. Study conclusion

- The study termination visit will occur on Day 661 (Visit 7). The termination visit will be a clinic visit ***or a telephone call.***
- At this clinic visit ***or telephone call***, subject's parent(s)/LAR(s) will be interviewed for any SAEs, medically attended AEs, AESIs (pIMDs, seizures, and/or arthritis) experienced by their child. Any concomitant medications associated with those events will also be collected and recorded in the subject's records and on the eCRF.
- At this visit ***or telephone call***, subject's parent(s)/LAR(s) will be asked if they can be contacted to participate in any future related study. If the subject's parent(s)/LAR(s) are not interested in participating in future studies the reason for refusal will be documented in the subject's eCRF.

Early termination:

If the Early Termination is at a clinic visit, the following procedures will be performed: review of Subject Diary (if applicable), review of systems, interview of subject's parent(s)/LAR(s) to collect: unsolicited AEs, AESI, SAEs, medically attended AEs, AEs leading to vaccine/study withdrawal, interview of subject's parent(s)/legal guardian(s) to collect concomitant medications/ vaccinations, symptom-directed physical assessment including vital signs (at a minimum rectal (subjects <12 months of age) or axillary (≥12 months of age) body temperature preferably) and a check of general appearance, and blood sampling for immunogenicity ***(if applicable at the visit).***

Section 5.7. Biological sample handling and analysis

Refer to Section 5.6.15 for changes in biological samples collection that may be implemented during special circumstances.

Section 6.2. Storage and handling of study and non-study vaccines

Any temperature excursion outside the range of 2.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) or below -25 C/-13 or above -15.0°C/5 F (for -20°C/-4°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form (Advanced Temperature Excursion Analysis and Management [ATEAM]). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor~~. This applies for the NIMPs as well~~. Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines.

Section 6.3. Dosage and administration of study vaccines

Refer to Section 5.6.15 in case of a change in the allowed interval to receive the vaccine dose between visits during special circumstances.

The MMR and VV vaccines should be given subcutaneously in the upper arm (***deltoid*** ~~triceps~~ region) by inserting the needle in a pinched-up fold of skin and subcutaneous tissue to prevent injection into muscle.

Section 6.7.1. Recording of concomitant medications/products and concomitant vaccinations

- Any concomitant vaccination administered in the period starting from birth and ending at the last study visit (Day ~~-14~~ ***of birth*** to Day 661).

Section 7.2.1. Time period for detecting and recording adverse events and serious adverse events

Table 13 Reporting Periods for Collecting Safety Information

Event	Pre-V1[1]	V1 D 1	D16	D31	V2 D61 (2 M post-vacc 1)	D76	D91	V3 D121 (2 M post-vacc 1)	D136	V4 D151 (1 M post-vacc 3)	D181	D241	V5 D301 (6 M post-vacc 3)	D316	V6 D331 (1 M post-vacc 4)	D361, 421, 481, 541, 601	V7 D661 (12 M post-vacc 4)
COVID-19 infection related AEs																	

M: month, V: Visit; D: Day, AE: adverse event, SAE: serious adverse event, AESI: adverse event of special interest, ***COVID-19***: ***Coronavirus Disease 2019***.

Section 7.2.3.2.1. Assessment of intensity

Table 14 Grading of Solicited Local Adverse Events for All Subjects

Adverse Events	Grading of Severity			
	Grade 0	Mild	Moderate	Severe
Tenderness or discomfort to touch at injection site	None	Minor ~~light~~ reaction to touch	Cried or protested to touch	Cried when injected limb was moved
Hardness of skin at the injection site Swelling of skin at the injection site Redness at injection site	0 mm	1 - ~~24~~ ***25*** mm	~~25~~ ***26*** - <50 mm	~~≥~~>50 mm

Section 7.2.4. Recording of AEs related to COVID-19

For COVID-19 infection related AEs, sites should follow routing AE/SAE processes as outlined in the protocol using the following terms per WHO defined case definitions:

- ***Suspected COVID-19 infection***
- ***Probable COVID-19 infection***
- ***Confirmed COVID-19 infection.***

Section 7.2.4.1. WHO Case Definition

- ***Suspected case***

  A. A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), AND a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset;

  OR

  B. A patient with any acute respiratory illness AND having been in contact (see definition of "contact" below) with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset;

  OR

  C. A patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; AND requiring hospitalization) AND in the absence of an alternative diagnosis that fully explains the clinical presentation.

- ***Probable case***

  A. A suspect case for whom testing for the COVID-19 virus is inconclusive (Inconclusive being the result of the test reported by the laboratory).

  OR

  B. A suspect case for whom testing could not be performed for any reason.

  Confirmed case

  A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms.

- ***A contact is a person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case:***
  - ***Face-to-face contact with a probable or confirmed case within 1 meter and for more than 15 minutes;***
  - ***Direct physical contact with a probable or confirmed case;***
  - ***Direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment; 2OR***
  - ***Other situations as indicated by local risk assessments.***

  Note: for confirmed asymptomatic cases, the period of contact is measured as the 2 days before through the 14 days after the date on which the sample was taken which led to confirmation.

Section 9.4.1. All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments ***and are randomized and have received a Subject ID***, regardless of the subject's ~~randomization and~~ treatment status in the study ~~and received a Subject ID~~.

Section 9.4.4.1. Full Analysis Set Immunogenicity

All subjects in the All Enrolled Set ~~who are randomized~~, receive at least one study vaccination and provide immunogenicity data at ***either*** one month after their 4th vaccination ***or~~/~~*** one month after their 3rd vaccination for at least 1 antigen/strain.

Section 9.4.5. Per Protocol (PP) Set for Immunogenicity Set

Examples for subjects excluded due to other reasons than protocol deviations are:

- who did not comply with the post Dose 3 (for primary 3 doses) or post Dose 4 (for the 4th dose) blood sample schedule ***(Table 6),*** ~~i.e. 23 to 44 days post the vaccination~~.

GlaxoSmithKline Biologicals SA

Vaccines R &D

Protocol Amendment 6

eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Amendment number:	Amendment 6
Amendment date:	9 December 2021
Co-ordinating author:	PPD (post-CBER feedback)

Rationale/background for changes:

- This study was initiated in July 2018 but faced difficulties in meeting the expected recruitment levels in spite of various efforts put in place i.e. protocol changes, recruitment material developed with a third party and additional investigators meetings. Despite all actions taken, the recruitment rate remained well below expectations. In addition, the COVID-19 (Coronavirus Disease 2019) pandemic has impacted enrolment as well as the timely completion of the scheduled vaccinations as well as collection of blood samples for already enrolled subjects.
- As a result of both the continual slow enrolment and the impact of COVID-19 pandemic, it was estimated by the study team that reaching the target of 2700 subjects would only be possible with a significant extension of study timelines that would negatively impact the study feasibility.
- The study team investigated alternative study endpoints in order to deliver a meaningful analysis with adequate power in a reasonable time. The change of the endpoints for assessment of rMenB+OMV NZ sufficiency after the 4th dose allowed to decrease the number of enrolled subjects to about 1200, permitting to end the study in a reasonable time and also fulfill the commitment in the initial Pediatric Study Plan (iPSP).
- The revised study design will still allow a powered primary assessment of the sufficiency of rMenB+OMV NZ response after 3rd dose and 4th dose. The study will also assess as a powered primary objective, the non-inferiority of the immune response to PCV-13 after the 3rd dose, when co-administered with rMenB+OMV NZ and other routine vaccines compared with routine vaccines alone.

Overall, the following changes are being implemented in the study:

- The criteria for the primary and key secondary objectives have been updated,

  - The sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants after the 3rd vaccination has been revised.
  - The sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV after the 4th vaccination has been revised.
- The total number of enrolled subjects was revised (N)=1200,
  - Group MenB+PCV: N=800
  - Group Placebo+PCV: N=400
- Assuming a 40% drop-out rate and with 1200 subjects to be enrolled in a 2:1 ratio, 480 evaluable subjects from Group MenB+PCV and 240 evaluable subjects from Group Placebo+PCV would be evaluable for the primary analyses.

Additional revisions:

- NHBA strain was revised based on Center for Biologics Evaluation and Research (CBER) recommendation to use the strain M13520, for evaluating immune responses to the NHBA antigen.
- Based on CBER recommendation, geometric mean concentrations (GMCs) will be included as an endpoint for evaluating NI for each of the individual antigens in the MMR and VV vaccines. GMCs, being a continuous variable and more sensitive to detect small differences between groups, is considered more appropriate for this assessment. The seroresponse rates of antigens tested for polio, MMR and VV vaccines will be assessed in a descriptive manner.
- The end of Study (EoS) has been changed from Last Subject Last Visit (LSLV) to the date of the last testing/reading released of the Human Biological Samples (HBS), related to primary and secondary endpoints. EoS must be achieved no later than 8 months after LSLV.
- The revision in the number of subjects assigned to different immunogenicity subsets belonging to either Group MenB+PCV (800 subjects will be randomly assigned into 3 subsets) or Group Placebo+PCV (400 subjects will be randomly assigned into 2 subsets).
- In method of blinding, the laboratory in charge of the testing will be blinded to the treatment as well as to the subject number. In addition, a different subject code will be used for each timepoint tested.
- Change in placebo volume from 0.5 mL to 0.65 mL, also adding a footnote – between 0.6 mL and 0.8 mL, full volume to be administered; to be aligned with the Certificate of Analysis and product lifecycle management (PLM) dictionary.
- Emergency unblinding contact information has been revised.
- The serological assays for the determination of antibodies against measles, mumps, rubella and varicella viruses are yet to be selected and/or developed and therefore the endpoints have been revised to reflect this change.

- For sequence of analyses, if needed, an interim analysis including data up to Visit 6 may be performed on final immunogenicity data. Selected immunogenicity objectives and solicited safety data from all timepoints up to Visit 6 will be analyzed.
- Additional information on medical device deficiencies and medical device AE have been provided in Section 7.9 and Section 10.6, respectively.
- The objectives and endpoints have been presented in a tabular format under Section 2 and the endpoints description have been removed from Section 9.

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

Co-ordinating authors: PPD Scientific Writing.

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List of abbreviations

HBS	***Human Biological Samples***
iPSP	***initial Pediatric Study Plan***
NadA:	Neisseria~~l~~ adhesin A
NHBA:	Neisseria***l*** Heparin Binding Antigen

Section 1.1: Background

GSK recombinant Meningococcal B vaccine with the OMV derived from New Zealand strain) is based on three proteins: i) factor H binding protein (fHbp), ii) Neisseria~~l~~ adhesin A (NadA) and iii) Neisseria***l*** Heparin Binding Antigen (NHBA) or 287

Synopsis and Section 1.2.1: Rationale for the study

Previous clinical studies conducted in Europe~~an~~ and other countries ***worldwide*** have shown that rMenB+OMV NZ demonstrated a robust immune response in all age groups against indicator test strains, H44/76 (fHbp), 5/99 (NadA), NZ98/254 (PorA P1.4) and M10713 (NHBA) and that the concomitant administration of rMenB+OMV NZ does not clinically interfere with response to the vaccine antigens present in routinely administered infant vaccines: diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type b, inactivated poliomyelitis, hepatitis B, measles, mumps, rubella, varicella and 7-valent pneumococcal conjugate vaccine. However, no data on the concomitant use of rMenB+OMV NZ with PCV13 and routine infant vaccines in North American infants is currently available.

~~Furthermore, the immune response of the rMenB+OMV NZ vaccine against~~ ***~~additional~~*** ~~test strains M14459 (fHbp), 96217 (NadA), NZ98/254 (PorA P1.4) and M10713 (NHBA) has not been evaluated yet in a US pediatric population.~~ ***~~These~~ 3 strains M14459 (fHbp), 96217 (NadA), NZ98/254 (PorA P1.4) in addition to M13520 strain*** are considered by the Center for Biologics Evaluation and Research (CBER) to better evaluate the protective response of the vaccine ~~in the US population~~.

Synopsis and Section 2: Objectives and endpoints

Objectives	Endpoints
Primary Safety	
• To assess the safety and tolerability of rMenB+OMV NZ, PCV13 and other RIV when administered concomitantly to healthy infants at 2, 4, 6 and 12 months of age, throughout the study duration.	• The percentages of subjects with solicited local Adverse events (AEs) and systemic AEs during the 7 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with solicited systemic AEs of parotid/salivary gland swelling, fever and rash during the 30 days (including the day of vaccination) after the 4th vaccination (Visit 5). • The percentages of subjects with all unsolicited AEs (including SAEs, AEs leading to withdrawal, AESIs, and medically attended AEs) during 30 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with SAEs, AEs leading to withdrawal, AESIs and medically attended AEs from study Day 1 (Visit 1) until study end (12 months after last study vaccination, Visit 7).
Co-Primary Immunogenicity	
• To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4 and 6 months of age, at one month after the 3rd vaccination. *Criteria: The sufficiency of the immune response to rMenB+OMV NZ* ***at one month*** *after the 3rd vaccination will be demonstrated if the adjusted lower confidence limit for the percentage of subjects achieving serum bactericidal assay using human complement (hSBA) titers ≥ Lower Limit of Quantitation (LLOQ) is ≥ 60% for the N. meningitidis serogroup B test strains* ***M14459, 96217, NZ98/254, M13520 (individually); and is ≥ 50% for all strains combined (composite endpoint).*** ~~*M14459, 96217, NZ98/254 and ≥55% for strain M10713 (individually); and is ≥43% for all strains combined (composite endpoint).*~~	• At 1 month after the 3rd vaccination ***(Day 151):*** – the percentages of subjects with hSBA titers ≥LLOQ; for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains. – the percentages of subjects with hSBA titers ≥LLOQ for all strains combined (composite endpoint).

Objectives	Endpoints
• To demonstrate the sufficiency of the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 4th vaccination. *Criteria: The sufficiency of the immune response to rMenB+OMV NZ will be demonstrated if the adjusted lower confidence limit for the percentage of subjects* ***with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) is ≥75% for the individual N. meningitidis serogroup B test strains and is ≥65% for all strains combined (composite endpoint)*** *~~achieving a 4-fold rise in hSBA titers (from pre-4th vaccination) is ≥70% for N. meningitidis serogroup B test strains M14459, 96217, NZ98/254 and ≥66% for strain M10713 (individually); and is ≥48% for all strains combined (composite endpoint).~~*	• At 1 month after the 4th vaccination ***(Day 331):*** – the percentages of subjects with ~~4-fold rise (from pre-4th vaccination) in~~ hSBA ***titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) for each of the test strains*** ~~for each of the M14459, 96217, NZ98/254 and M10713 test strains.~~ – the percentages of subjects with ~~4-fold rise (from pre-4th vaccination) in~~ hSBA ***titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217)*** for all strains combined (composite endpoint).
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants 2, 4 and 6 months of age, compared to PCV13 without rMenB+OMV NZ, at one month after the 3rd vaccination. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the adjusted lower confidence limit for the between-group ratio of electrochemiluminescence (ECL) assay GMCs is >0.5 for each of the 13 PCV13 antigens.*	• At 1 month after the 3rd vaccination ***(Day 151):*** – the ECL GMCs for each of the 13 PCV13 antigens.
Secondary Immunogenicity	
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants 2, 4, 6 and 12 months of age, compared to PCV13 and other RIV alone, at one month after the 4th vaccination. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of 2-sided 95% CI for the between-group-ratio of ECL assay GMCs is >0.5 for each of the 13 PCV13 antigens.*	• At one month after the 4th vaccination ***(Day 331)***: – The ECL GMCs for each of the 13 PCV13 antigens.
• To demonstrate the immunological non-inferiority of PCV13 when administered concomitantly with rMenB+OMV NZ and other RIV to healthy infants at 2, 4, 6 and 12 months of age compared to PCV13 and other RIV alone, at both one month after the 3rd and the 4th vaccinations. *Criterion: The immunological non-inferiority of PCV13 will be demonstrated if the lower limit of the 2-sided 95% CI for the group differences in percentage of subjects with IgG ≥ 0.35 µg/mL is >-10% for each of the 13 PCV13 antigens at one month after both 3rd and 4th vaccination.*	• At 1 month after the 3rd ***(Day 151)*** and the 4th vaccination ***(Day 331):*** – Percentages of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 µg/mL for each of the 13 PCV13 antigens.

Objectives	Endpoints
• To demonstrate the immunological non-inferiority of DTaP-HBV-IPV and Hib vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy infants at 2, 4, and 6 months compared to DTaP-HBV-IPV and Hib vaccines concomitantly administered with PCV13 without rMenB+OMV NZ, ***in terms of D, T, PT, FHA, PRN, Hep B and Hib***, at one month after the 3rd vaccination. *Criterion: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group differences is greater than the* ***pre-specified margin†*** *for each antigen at one month after 3rd vaccination.*	• At 1 month after the 3rd vaccination ***(Day 151)***: – GMCs against the 3 pertussis antigens (Pertussis toxin [PT], pertactin [PRN], filamentous hemagglutinin [FHA]). – Percentages of subjects with ***anti-HBs antibody*** concentrations ≥10 mIU/mL. – Percentages of subjects with anti-diphtheria and anti-tetanus concentrations ≥0.1 IU/mL. – Percentages of subjects with anti-PRP concentration ≥0.15 µg/mL and ≥1 µg/mL.
• To demonstrate the immunological non-inferiority of MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 to healthy subjects at 12 months compared to MMR and VV vaccines concomitantly administered with PCV13, without rMenB+OMV NZ, at one month after vaccination. *Criterion: The immunological non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI for the between-group ratio of GMCs is >0.67 at one month after the MMR and VV vaccinations.*	• ***At 1 month after the 4th vaccination (Day 331):*** – ***GMCs against measles, mumps, rubella and VZV antigens.***
• To evaluate the immune response to rMenB+OMV NZ when administered concomitantly with PCV13 and other RIV to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 3rd vaccination, at 6 months after the 3rd vaccination (immediately before the 4th vaccination), and at one month after the 4th vaccination against the M14459, 96217, NZ98/254 and ~~M10713~~ ***M13520*** test strains.	• At 1 month after the 3rd vaccination ***(Day 151)*** – the percentages of subjects with hSBA titers ≥5, ≥8 ***and ≥16*** for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains. – ***the*** hSBA geometric mean titers (GMTs) ***against each strain***. • At 6 months after the 3rd vaccination (***Day 301,*** immediately before the 4th vaccination): – the percentages of subjects with hSBA titers ≥LLOQ for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains. – the percentages of subjects with hSBA titers ≥5 and ≥8 for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains. – the hSBA GMTs against each strain. • At 1 month after the 4th vaccination ***(Day 331):*** – the percentages of subjects with hSBA titers ≥LLOQ for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains. – the percentages of subjects with hSBA titers ≥5 for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains. – the hSBA GMTs and geometric mean ratios (GMRs) over pre-4th vaccination against each strain.

Objectives	Endpoints
	– ***the percentages of subjects with 4-fold rise* in hSBA titers (from pre-4th vaccination) for each of the M14459, 96217, NZ98/254 and M13520 test strains.***
• To evaluate immune responses to routine infant vaccines DTaP-HBV-IPV, Hib, MMR and VV vaccines when administered concomitantly with rMenB+OMV NZ and PCV13 ***to healthy infants at 2, 4, 6 and 12 months of age, at one month after the 3rd vaccination, at 1 month after the 4th vaccination***.	• At 1 month after the 3rd vaccination ***(Day 151)***: – Percentages of subjects with ***anti-HBs antibody*** concentrations ≥100 mIU/mL. – Anti-HBsAg ***GMCs***. – Percentages of subjects with anti-diphtheria and anti-tetanus concentrations ≥1 IU/mL. – Anti-diphtheria and anti-tetanus antibody GMCs. – ***Percentage of subjects with anti-polio type 1, 2 and 3 neutralization antibody titers ≥8.*** • At 1 month after the 4th vaccination ***(Day 331)***: – Seroresponse, defined as post-vaccination anti-VZV virus, anti-measles virus, anti-mumps virus and anti-rubella virus antibody concentration^ ***≥ a protective threshold among subjects who were seronegative (antibody concentration <assay cut-off)*** before vaccination. ^ A suitable ELISA assay for analysis ***of anti-VZV, anti-measles virus,*** anti-mumps virus ***and anti-rubella virus*** antibody concentrations is yet to be selected and/or developed. ~~Seroresponse, defined as post-vaccination anti-VZV antibody concentration ≥75 mIU/mL (ELISA, Enzygnost) among children who were seronegative (antibody concentration <25 mIU/mL) before vaccination.~~ ~~Seroresponse as defined by the post-vaccination anti-measles virus antibody concentration ≥ 200 mIU/mL (ELISA, Enzygnost) in subjects seronegative (antibody concentration <150 mIU/mL) before vaccination~~ ~~Seroresponse, defined as post-vaccination anti-rubella virus antibody concentration ≥10 IU/mL (ELISA, Enzygnost) in subjects seronegative (antibody concentration <4 IU/mL) before vaccination~~

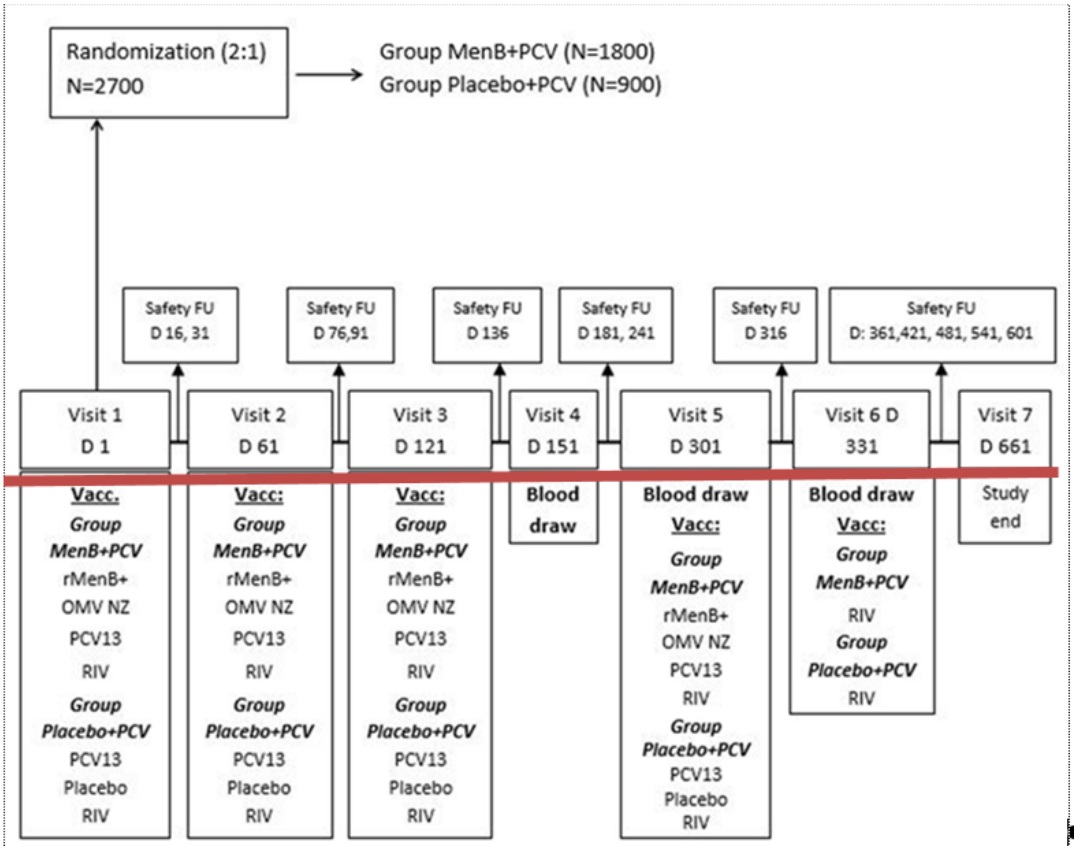
* A 4-fold rise in hSBA titers is defined as
- if pre-vaccination titer <Limit of Detection (LOD), then a post-vaccination titer ≥ 4 times the LOD or ≥ LLOQ, whichever is greater;
- if pre-vaccination titer is ≥LOD but <LLOQ, then a post-vaccination titer ≥4 times the LLOQ;
- if pre-vaccination titer is ≥LLOQ, then a post-vaccination titer ≥4 times the pre-vaccination titer,

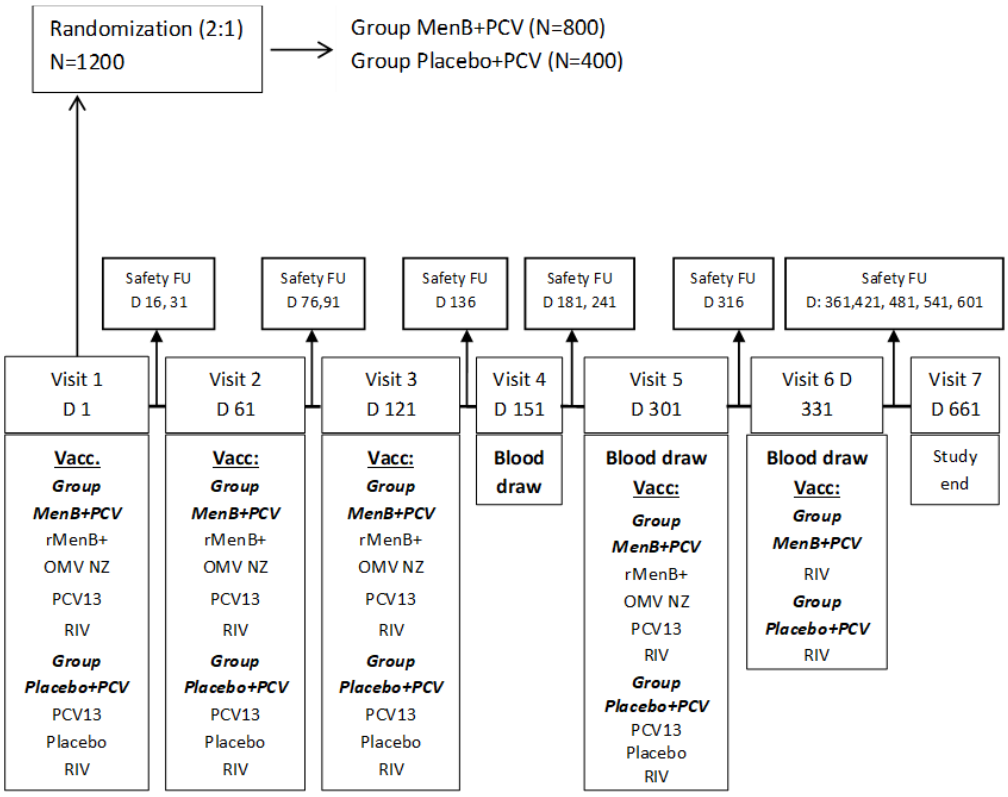
where pre-vaccination titer=pre-4th dose titers (Day 301)

† The endpoints and thresholds used for evaluation of the non-inferiority criteria for DTaP-HBV-IPV and Hib vaccines can be found in Table 21 and the thresholds and endpoint for evaluating the non-inferiority criteria for MMR and VV vaccines can be found in Table 22.

Note 1: In the context of this study, the following are considered as RIVs at different visits:
Visit 1 and Visit 2: DTPa-HBV-IPV, HRV, Hib;
Visit 3: DTPa-HBV-IPV, Hib;
Visit 5: MMR and VV.

Synopsis and Section 3: Study design

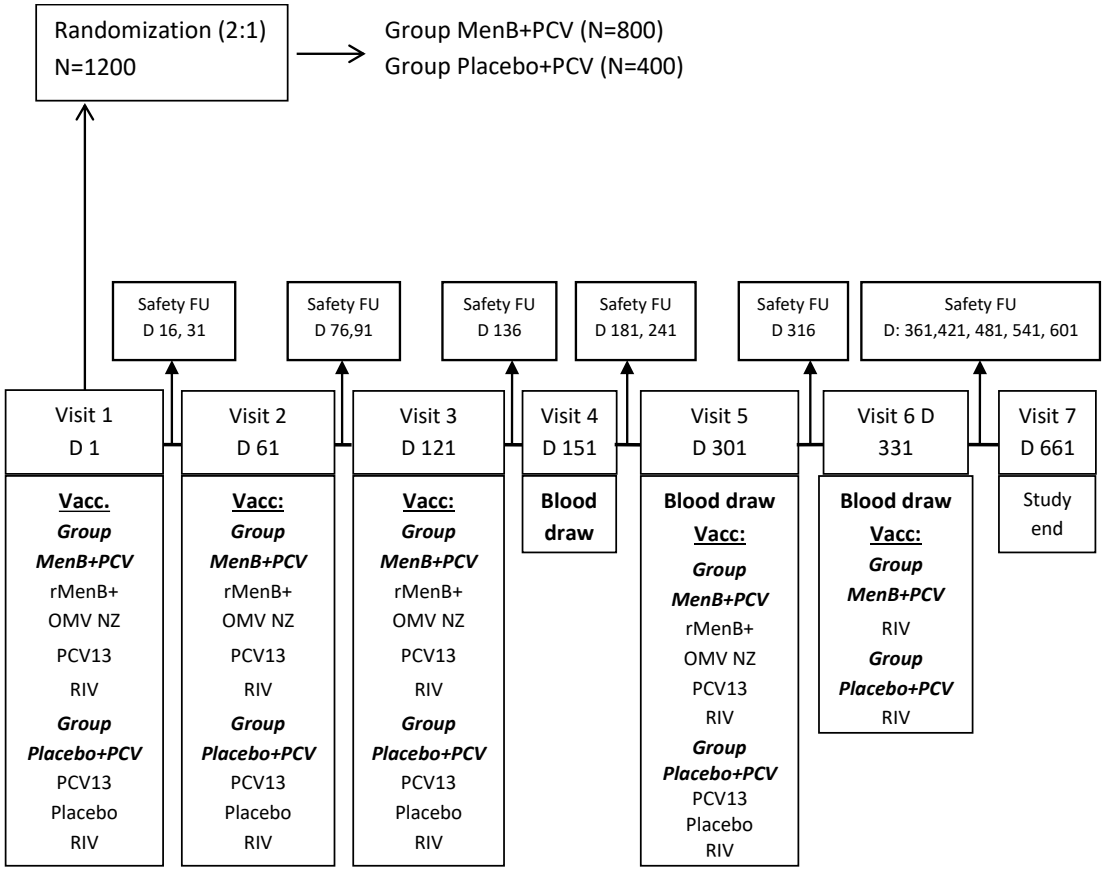




- End of Study (EoS): ***Date of the last testing/reading released of the Human Biological Samples (HBS) related to primary and secondary endpoints. Study completion must be achieved no later than 8 months after LSLV***.~~Last subject last visit (Visit 7).~~

Synopsis Table 1 and Section 3 Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min – Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
MenB+PCV	~~1800~~***800***	6 weeks – 12 weeks	x	x	x
Placebo+PCV	~~900~~***400***	6 weeks – 12 weeks	x	x	x



Number of subjects: Target enrolment is approximately ~~2700~~***1200*** subjects who will be randomly assigned to the 2 study groups in a 2:1 ratio.

Table 3 Overview of the Study Design: Blood Draws and Study vaccines

Clinic Visits (Study Day)		**Visit 1 (Day 1)**	**Visit 2 (Day 61)**	**Visit 3 (Day 121)**	**Visit 4 (Day 151)**	**Visit 5 (Day 301)**	**Visit 6 (Day 331)**
Months of Age (MoA)		**~2 MoA[1]**	**4 MoA**	**6 MoA**	**7 MoA**	**12 MoA**	**13 MoA[3]**
Study Vaccines	Group MenB+PCV N=~~1800~~ ***800***	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	*Blood Draw[2]*	*Blood Draw[2]* rMenB+ OMV NZ PCV13	*Blood Draw[2, 3]*
	Group Placebo+PCV N=~~900~~ ***400***	Placebo PCV13	Placebo PCV13	Placebo PCV13		*Blood Draw[2]* Placebo PCV13	
Routine Study Vaccines	**Both groups** N=~~2700~~ ***1200***	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV Hib		MMR VV	

Section 4.1: Number of subjects/centers

Approximately ~~2700~~ ***1200*** healthy subjects, 42 through 84 days of age (i.e. 6 through 12 weeks) will be enrolled in a 2:1 ratio.

Section 5.2.2.2.1: Study group and treatment number allocation

The target will be to enroll approximately ~~2700~~ ***1200*** eligible subjects who will be randomly assigned to 2 study groups in a 2:1 ratio.

Section 5.2.3: Allocation of subjects to assay subsets

Group MenB+PCV: All ~~1800~~ ***800*** subjects will be randomly assigned into one of the 4 ***3*** immunogenicity subsets:

- Subset A1: ~~385~~ ***400*** subjects
- Subset A2: ~~515~~ ***200*** subjects
- Subset A3: ~~385~~ ***200*** subjects
- ~~Subset A4: 515 subjects~~

Group Placebo+PCV: A total of ~~900~~ ***400*** subjects will be randomly assigned into 2 immunogenicity subsets:

- Subset B1: ~~100~~ ***200*** subjects
- Subset B2: ~~385~~ ***200*** subjects

In addition, subjects from subset B1 (~~100~~***200*** subjects) will also be tested against the 4 serogroup B strains at all 3 timepoints in order to maintain the observer-blindness of the study during the blood sample testing.

Section 5.3: Method of blinding

~~The laboratory in charge of the laboratory testing will be blinded to the treatment, subject and visit number, and codes will be used to link the subject, visit and study (without any link to the treatment attributed to the subject) to each sample.~~

The laboratory in charge of the laboratory testing will be blinded to the treatment as well as to the subject number. In addition, a different subject code will be used for each timepoint tested. This subject coding will prevent the laboratory from linking the consecutive visits to a specific subject.

The serological data, which would lead to the unblinding of the study groups, will not be available during the course of the study to any investigator or any person involved in the clinical conduct of the study (including data cleaning).

Section 5.7.3: Laboratory assays

Table 7 Humoral Immunity (Antibody determination)

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
Serum	N Men B ~~M10713~~***M13520*** (NHBA) Ab*,∞	***hSBA***	***In house***	***1/dil***	***4***~~TBD~~	GSK Biologicals *** or laboratory designated by GSK Biologicals	***1*** ~~1.1~~
	Corynebacterium diphtheriae.Diphtheria Toxoid Ab.IgG	ELI	In-house	IU/mL	~~0.057~~ ***0.030***	GSK Biologicals *** or laboratory designated by GSK Biologicals	10
	Clostridium tetani.Tetanus Toxoid Ab.IgG	ELI	In-house	IU/mL	~~0.043~~ ***0.037***		11
Serum	Measles Virus Ab.IgG	ELI*	~~Siemens Enzygnost Anti-measles virus IgG~~ ***TBD***	***TBD***	***TBD***	GSK Biologicals*** or laboratory designated by GSK Biologicals	8
	Rubella Virus Ab.IgG	ELI*	~~Siemens Enzygnost Anti-rubella virus IgG~~ ***TBD***	***TBD***	***TBD***	GSK Biologicals*** or laboratory designated by GSK Biologicals	9
	Mumps Virus Ab IgG	ELI*	TBD	TBD	TBD	GSK Biologicals*** or laboratory designated by GSK Biologicals	6

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
	Varicella Zoster Virus Ab.IgG	ELI*	***TBD***	***TBD***	***TBD***	GSK Biologicals*** or laboratory designated by GSK Biologicals	7

~~∞ The NHBA indicator strain is provisional and assay cut-offs may be adjusted during further characterization of the assay, should another indicator strain be used to characterize NHBA bactericidal responses.~~

Section 5.7.4: Immunological read-outs

In case of insufficient blood sample volume to perform assays for all antibodies, the samples will be analyzed according to priority ranking provided in Table 8. Testing against RIV antigens will be performed in immunogenicity subsets as defined in Section 5.2.3 and in Table 9.

Table 8 Immunological Read-outs

Blood sampling timepoint		Group Name	Max No. subjects[a]	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Visit 4 (Day 151)	Post-Vacc 3	Group MenB+PCV, Placebo+PCV[b]	~~1900~~***1000***	hSBA-~~M10713~~***M13520*** hSBA-NZ98/254 hSBA-M14459 hSBA-96217	***1*** ~~1.1~~ ~~1.2~~ ~~1.3~~ ~~1.4~~
		Groups MenB+PCV, Placebo+PCV	~~770~~***800***	Anti-PCV13 (1,3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)	2
		Groups MenB+PCV, Placebo+ PCV	~~2700~~***1200***	DT,TT, PT, FHA, PRN, HBs, Polio, Hib[a]	See Table 9 [a]
Visit 5 (Day 301)	Pre-Vacc 4	Groups MenB+PCV Placebo+PCV[b]	~~1900~~***1000***	hSBA-~~M10713~~***M13520*** hSBA-NZ98/254 hSBA-96217 hSBA-M14459	***1*** ~~1.1~~ ~~1.2~~ ~~1.3~~ ~~1.4~~
		Groups MenB+PCV and Placebo+PCV	~~2185~~***1200***	MMR, V	See Table 9[a]
Visit 6 (Day 331)	Post-Vacc 4	Groups MenB+PCV Placebo+PCV[b]	~~1900~~***1000***	hSBA-~~M10713~~***M13520*** hSBA-NZ98/254 hSBA-96217 hSBA-M14459	***1*** ~~1.1~~ ~~1.2~~ ~~1.3~~ ~~1.4~~
		Groups MenB+PCV Placebo+PCV	~~770~~***800***	Anti-PCV13 (1,3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)	2
		Groups MenB+PCV and Placebo+PCV	~~2185~~***1200***	M,M,R, V[a]	See Table 9 [a]

[b] A subset (B1) of ~~100~~ ***200*** subjects (~~65~~ ***120*** evaluable) from Group Placebo+PCV will be tested against the 4 serogroup B strains in order to maintain the observer-blindness of the study during blood sample testing.

Table 9 Immunogenicity Subsets

Blood sampling timepoint		Group Name	Subset	Max No. subjects	Component	Components priority rank
Type of contact and timepoint	**Sampling timepoint**					
Visit 4 (Day 151)	Post-Vacc 3	Group MenB+PCV	A1	~~385~~***400***	MenB PCV13 Hib Pertussis	1 2 3 4
			A2	~~515~~***200***	MenB Hib Pertussis ***HepB***	1 2 3 ***4***
			A3	~~385~~***200***	MenB ***Pertussis*** ***Polio*** ~~HepB~~ ~~Diphtheria~~ ~~Tetanus~~	 1 2 3 4
			~~A4~~	~~515~~	~~MenB~~ ~~Polio~~	~~1~~ ~~2~~
		Group Placebo+PCV	B1	~~100~~ ***200***	MenB ***PCV13*** Hib Pertussis ~~Polio~~	1 2 3 4
			B2	~~385~~***200***	PCV13 Hib Pertussis HepB Diphtheria Tetanus ***Polio***	1 2 3 4 5 6 ***7***
			~~B3~~	415***50***	~~***PCV13***~~ ~~Hib~~ ~~Pertussis~~ ~~Polio~~	~~1~~ ~~2~~ ~~3~~ ~~***4***~~
Visit 5 (Day 301)	Pre-Vacc 4	Group MenB+PCV	A1	~~385~~ ***400***	MenB ***Measles*** ***Rubella*** ***Mumps*** Varicella	1 ***2*** ***2*** ***2*** 3
			A2	~~515~~ ***200***	MenB Varicella Measles ***Rubella*** ***Mumps***	1 2 3 ***3*** ***3***
			A3	~~385~~ ***200***	MenB ***Varicella*** ***Measles*** Mumps Rubella	1 ***2*** ***3*** 3 3

Blood sampling timepoint		Group Name	Subset	Max No. subjects	Component	Components priority rank
Type of contact and timepoint	**Sampling timepoint**					
		Group Placebo+PCV	B1	~~100~~ ***200***	MenB ***Measles*** ***Rubella*** ***Mumps*** ***Varicella*** ~~Varicella~~ ~~Measles~~	1 ***2*** ***2*** ***2*** ***3*** ~~2~~ ~~3~~
			B2	~~385~~ ***200***	~~Mumps~~ Varicella Measles Rubella ***Mumps***	~~1~~ 1 2 3 ***~~4~~***
			~~B3~~	415 ***~~50~~***	***~~Measles~~*** ***~~Mumps~~*** ***~~Rubella~~*** ***~~Varicella~~*** ~~Varicella~~ ~~Measles~~	***~~1~~*** ***~~1~~*** ***~~1~~*** ***~~2~~*** ~~1~~ ~~2~~
Visit 6 (Day 331)	Post-Vacc 4	Group MenB+PCV	A1	~~385~~***400***	MenB PCV13 ***Measles*** ***Rubella*** ***Mumps*** ***Varicella*** ~~Varicella~~ ~~Measles~~	1 2 ***3*** ***3*** ***3*** ***4*** ~~3~~
			A2	~~515~~***200***	MenB Varicella Measles ***Rubella*** ***Mumps***	1 2 3 ***3*** ***3***
			A3	~~385~~ ***200***	MenB ***Varicella*** ***Measles*** Mumps Rubella	1 2 3 ***3*** ***3***
		Group Placebo+PCV	B1	~~100~~ ***200***	MenB ***PCV13*** ***Measles*** ***Rubella*** ***Mumps*** ***Varicella*** ~~Varicella~~ ~~Measles~~	1 ***2*** ***3*** ***3*** ***3*** ***4***
			B2	~~385~~***200***	PCV13 ~~Mumps~~ Varicella Measles Rubella ***Mumps***	1 2 3 3 3

Blood sampling timepoint		Group Name	Subset	Max No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint					
			~~B3~~	~~415~~***~~50~~***	~~Varicella~~ ~~Measles~~ ***~~PCV13~~*** ***~~Measles~~*** ***~~Mumps~~*** ***~~Rubella~~*** ***~~Varicella~~***	~~1~~ ~~2~~ ***~~2~~*** ***~~2~~*** ***~~3~~***

Note: The individual components and the order of testing is given below:
MenB: hSBA-~~M10713~~***M13520***, hSBA-NZ98/254, hSBA-M14459, hSBA-96217.

Section 5.7.5: Immunological correlates of protection

- ~~Anti-varicella concentrations ≥75 U/mL (ELISA), ~~***≥threshold to be defined based on the ELISA assay to be selected (ELISA),***
- ~~Anti-measles ≥200 mIU/mL (ELISA),~~ ***≥threshold to be defined based on the ELISA assay to be selected (ELISA),***
- ~~Anti-rubella ≥10 IU/mL (ELISA) ~~***≥threshold to be defined based on the ELISA assay to be selected (ELISA)***

Section 6.1.1: Study vaccines

Table 10 Study Vaccines

Treatment name	Vaccine	Formulation	Presentation	Volume to be administered*	Number of doses
Placebo	Placebo (NaCl)	NaCl=150mM	Prefilled syringe (liquid)	***0.65 mL****~~0.5 mL~~	4

Note: Refer to the SPM for the volume after reconstitution. The fourth dose of Hib vaccine will be administered as an non-study vaccine at 13 months of age.
$CCID_{50}$=median Cell Culture Infective Dose (quantity of virus causing infection in 50% of exposed cells)
$CaCO_3$: Calcium carbonate, HRV: Human Rotavirus, Hib: Haemophilus influenzae type b vaccine, DT: diphtheria toxoid, TT: tetanus Toxoid, PT: pertactin, FHA: filamentous hemagglutinin, PRN: Pertactin, HbsAg: Hepatitis B surface antigen, PRP: Polyribosyl-ribitol phosphate, MMR: measles, mumps, rubella.
*** ****The volume of the saline syringe may be between 0.6mL and 0.8 mL. The full volume is to be injected.***

Section 7.7: Emergency unblinding

GSK Biologicals' Contact information for Emergency Unblinding

24/24 hour and 7/7 day availability

GSK Biologicals' Central Safety Physician:

For US only:
+ ~~1 844 344 4389~~ ***1 844 446 3133*** (GSK Biologicals Central Safety Physician on-call)

GSK Biologicals' Central Safety Physician Back-up:

For US only:
~~+ 1 844 344 4390~~ ***877.870.0019***

Section 7.9: ***Medical device deficiencies***

The study intervention is a combination product constituted of a device and biologic product (e.g. pre-filled syringes). Refer to the Glossary of Terms for the definition of combination product and medical device deficiency.

Section 7.9.1: ***Detection, follow-up and prompt reporting of medical device deficiency***

The investigator is responsible for the detection, documentation and prompt reporting of any medical device deficiency occurring during the study to GSK. This applies to any medical device provided for the conduct of the study.

Device deficiencies will be reported to GSK within 24 hours after the investigator determines that the event meets the protocol definition of a device deficiency. Refer to Section 10.6 for definitions and details on recording and reporting of these events.

The sponsor will be the contact for the receipt of device deficiency reports.

The investigator will ensure that follow-up includes any additional investigations to elucidate the nature and/or causality of the deficiency. Follow-up applies to all participants, including those who discontinue study intervention or the study.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator and reported to GSK within 24 hours.

Section 7.9.2: ***Regulatory reporting of medical device deficiency when used as combination product***

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 fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies. Refer to section 10.6.3. for details of reporting.

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.

Section 9.1: Determination of sample size

The study sample size was determined by ***the revised study design while maintaining an adequate study power to achieve all co-primary objectives previously discussed with CBER. The multiplicity issue was dealt with by controlling the global α at the level of 0.05 for all associated statistical hypotheses. This reduced number will also allow for enrolment of the minimum number of subjects exposed to rMenB+OMV NZ to fulfill initial Pediatric Study Plan (iPSP) commitment. The sample sizes were also calculated for the secondary objectives to evaluate the non-inferiority of RIV antigens. The evaluation will be performed by calculating the between-group differences and their 2-sided 95% CI. Therefore, no multiplicity was adjusted for the secondary objectives.***

~~This reduced number will also allow for enrolment of the minimum number of subjects exposed to rMenB+OMV NZ to fulfill initial Pediatric Study Plan (iPSP) commitment.For the co-primary objectives, multiplicity issue was dealt with by controlling the global α at the level of 0.05 for all associated statistical hypotheses.~~

Study Sample Size Determination by Co-Primary Objectives

For the immune response to PCV13, sample size calculations were based on data from the PCV13 package insert and for the immune response to rMenB+OMV NZ sample size calculations were based on data from the previous GSK (legacy Novartis) V72P13 and V72P13E1, ***and from V72_56*** study.

Using the procedure described in this section, the power for the study is presented in Table 17. In case of ~~35~~***40***% drop-out rate and ~~2700~~***1200*** subjects to be enrolled in a 2:1 ratio, ~~1170~~***it's assumed that 480*** evaluable subjects from Group MenB+PCV and ~~585~~***240*** evaluable subjects from Group Placebo+PCV could participate with data to the analyses. For PCV13 antigens a sample size of ~~250 there~~***240*** will ~~be~~***have*** approximately 99% power to show non-inferiority for all antigens. For MenB strains ~~1170~~***it's assumed that 480*** evaluable samples from Group MenB+PCV will be evaluated in the analyses. With ~~250~~ ***240*** per group for PCV13 antigen tests and ~~1170~~***480*** evaluable subjects from Group MenB+PCV for rMenB+OMV NZ strain tests, there is approximately ~~94~~***99***% probability to reject all 23 null hypotheses, and to claim that the primary objectives of the study will be met according to the predefined success criteria. ~~assuming that the correlation between the rMenB+OMV NZ strains is 25%.~~

Table 17 Power to Show Non-Inferiority PCV13 Co-Primary Objective and Sufficiency rMenB+OMV NZ Co-Primary Objectives

Co-Primary Objective	**Power**
Non-inferiority for GMCs for 13 antigens after the 3rd vaccination	***99.0***%
Sufficiency for % of subjects with titers ≥ LLOQ per each strain and all 4 strains combined (composite response) after the 3rd vaccination	***99.8%***
Sufficiency for % of subjects with ~~with 4-fold increase (from pre-4th to post-4th) per each strain~~ ***hSBA titers≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217) individually*** and all 4 strains combined (composite response) after the 4th vaccination	***>99.999%***
Probability of rejection of all 23 null hypotheses.	~~98~~***98.8%***

The power was estimated using simulations: 10.000 simulations per case. SAS 9.4 was used for the calculations.

A short summary on the steps of simulation is presented below, in the example of ***40***% drop-out. The definition for families is given in Section 9.5.3.1.

In total 10.000 simulations were performed, each simulation consisted of:

a. For testing Family 1, ~~250~~ ***240*** normal distributed data were drawn for two groups and hence two times thirteen antigens, according to the ratio of GMC and the SD, as described below Table 18. For PCV13, a GMC ratio of 0.8 was assumed. Correlation structure was made for between-antigen correlation of ~~20%, 25% or 50%, respectively.~~***0%***.

b. For testing Family 2 and 3, ~~1170~~ ***480*** normal distributed data were drawn for one single group and for the four strains, according to the GMTs (for post 3rd dose) and ~~GMRs~~***GMTs*** (for post 4th dose), as shown in Table 19, and the SDs as described below Table 18. Correlation structure was made for between-antigen correlation of ~~20%, 25% or 50%, respectively~~ 0%.

c. Testing of Family 1: Shifted one-sided t-test was used to test the hypotheses and output the p-values (for the power calculation no center effect was assumed).

d. P-values were compared with the Family Wise Error Rate (FWER) to conclude on whether the null hypotheses were rejected or not. FWER for Family 1 is initially set to be 1/3 of the full alpha, which is rounded to 0.017. The FWER for Family 2 is 0.033 and the initial FWER is 0 for Family 3. No multiplicity adjustment was performed within a family. Within family 1 all thirteen antigens were tested with the full FWER for family 1. If one or more test fail to reject the hypothesis for the antigen, family 1 fails. Consequently the alpha of family 1 will not be propagated (see bullet h. below).

e. Preparing for testing Family 2 and 3, percentages were obtained by dichotomizing the normal random sets that were generated at step b, by applying the cut-off of LLOQ value for post 3rd or a cut-off of ~~4 at post 4th. By applying a cut-off of 4 to the GMRs at post-4th this will generate a percentage of subjects who had a 4-fold increase for their GMT value at post-4th compared to pre-4th, which is an approximation of the percentage of 4-fold increase using LOD and LLOQ values~~ ***8 (for strains M14459, NZ98/254, M13520) or 16 (for strain 96217)*** at post 4th. Per time point, composite endpoint was derived by looking into each subject if the titer is above the cut-off for all 4 individual strains and if it is then this subject is counted as "1". Otherwise the subject is counted as "0".

f. For each of the ten percentages for rMenB+OMV NZ (4 individual strains and 1 composite endpoint at post-3rd and post-4th) the lower confidence limit was generated by fitting the binomial distribution with the percentage obtained from the previous step and alpha that has been assigned or propagated to the family. If the lower confidence limit is greater or equal to the sufficiency margin, then the null hypothesis is rejected for the single test. For Family 2 and 3, it is required to reject the null hypothesis for each and all of strain M14459, 96217, NZ98/254, ~~M10713~~ ***M13520***, and the composite endpoint to be able to claim the success of the family.

g. Testing of Family 2: with the given FWER, if the LCL is greater or equal to 0.~~7~~***75*** for each of strain M14459, 96217 and NZ98/254, ~~M10713~~ ***M13520***, and greater or equal to ***0.65*** for the composite endpoint, then Family 2 is claimed for success. The FWER for Family 2 is set up as 2/3 of the full alpha, which is rounded to 0.033.

h. Alpha propagation between Family 1, 2 and 3 was calculated for 4 scenarios:

   5) If Family 1 had succeeded and Family 2 not, then propagate ½ FWER from Family 1 to Family 3 and the other ½ FWER to Family 2, and re-test Family 2 with the new FWER which is the sum of the original FWER from Family 2 plus the ½ of FWER from Family 1. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 2 and propagate full FWER to Family 3; otherwise Family 2 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 1.

   6) If Family 2 had succeeded and Family 1 not, then propagate ½ FWER from Family 2 to Family 3 and the other ½ FWER to Family 1, and re-test Family 1 with the new FWER which is the sum of the original FWER from Family 1 plus the ½ of FWER from Family 2. If the null hypothesis were rejected during the 2nd time test, then claim the success of Family 1 and propagate full FWER to Family 3; otherwise Family 1 fails and proceed with Family 3 testing with the ½ of FWER propagated from Family 2.

   7) If both Family 1 and Family 2 had succeeded, then the FWER from both families were propagated to Family 3, and proceed to test Family 3;

   8) If both Family 1 and Family 2 had failed, then no FWER will be propagated to Family 3, and we stop the testing globally.

i. Testing of Family 3: with the given FWER, if the LCL is greater or equal to 0.6 for each of strain M14459, 96217 and NZ98/254, ~~M10713~~ ***M13520***, and greater or equal to ***0.5*** for the composite endpoint, then Family 3 is claimed for success.

j. Add a loop on top of the aforementioned steps for 10.000 times simulation, summarize the probability of have all hypotheses rejected for each and all families, and the probability to meet all the primary objectives.

k. ~~For each of the scenarios (correlation=0.2, 0.25 or 0.5), 10,000 times simulation was performed.~~

Based on the immune response observed in infant data from V72P13E1 and the PCV13 package insert, the following assumptions were made for the calculations:

1. As estimate for the SD of the different antigens and strains was taken the upper 80% CI limits of the common standard deviations for the $\log_{10}$ titers observed in GSK (legacy Novartis) study V72P13E1 and $\log_{10}$ ELISA concentrations observed from PCV13 studies. SDs that were used in the sample size calculations are presented in the table below.

Table 18 Two-sided 80% Upper Confidence Limit of Standard Deviations

	80% UCL of SDs	
rMenB+OMV NZ strains	**Post 4th vaccination (SDs of ~~GMR~~*GMT*)**	**Post 3rd vaccination (SDs of GMT)**
M14459	***0.***~~5437~~***45097***	0.~~32221~~***32193***
~~96217[2]~~96217	***0.***~~7776~~***063454***	0.36900
NZ98/254	***0.***~~5179~~***453796***	0.~~519675~~1***670***
~~M10713~~***M13520***[2]	~~0.66918~~***36477***	***0.***~~522~~***45023***
PCV13 antigen[1]	**Post 3rd vaccination (SDs of GMC)**	
1	0.36629	
3	0.41572	
4	0.34261	
5	0.43567	
6A	0.39552	
6B	0.44974	
7F	0.34352	
9V	0.32548	
14	0.32445	
18C	0.33550	
19A	0.24439	
19F	0.45638	
23F	0.37567	

[1] Estimated SDs for PCV13 antigens are the upper confidence limit of 2-sided 80% CI of back-calculated SDs, based on information (degree of freedom, 95% CI) provided on table 17 of *Prevnar13* Package Insert (dated on July 2016) and assuming the underlying SDs fulfill a t-distribution.

[2] The assumptions for M13520 are cited from study V72_56 (205240) ad-hoc analysis on additional NHBA strains.

2. Underlying GMC ratios for each PCV13 antigens is assumed as 0.8.
3. For the sufficiency objectives, the following mean ($\log_{10}$(GMT)) were used in simulation based on observed data from previous studies (studies V72P13 and V72P13E1 ***and V72_56***):

Table 19 GMTs at One Month Post 3rd Dose and GMRs at One Month Post 4th rMenB+OMV NZ Vaccination

rMenB+OMV NZ strains	**~~GMRs* Post 4th vaccination~~**	**~~GMTs* Post 3rd vaccination~~**	**GMTs* Post 4th vaccination**	**GMTs* Post 3rd vaccination**
~~M10713~~***M13520***	~~0.99~~	~~-1.35362~~	***1.41225***	***1.10501***
NZ98/254	~~1.27~~	~~1.15836~~	***1.57978***	1.15836
M14459	~~1.25~~	~~1.26400~~	***1.53148***	1.26400
96217	~~-1.36~~	~~2.66753~~	***3.06183***	2.66753

*log-scale

4. The LODs and LLOQs associated with the 4-fold definition are assumed to be:

Table 20 Limits of Detection and Lower Limits of Quantitation for rMenB+OMV NZ strains

rMenB+OMV NZ strains	LOD	LLOQ
M13520 ~~M10713~~	~~***34***~~	~~***164***~~
NZ98/254	4	6
M14459	3	5
96217	6	15

LOD: lower limit of detection, LLOQ: lower limit of quantitation.
Note: **Other than for strain *M13520***, these LODs and LLOQs were cited from the latest lab assay validation report released in 2018. ***For strain M13520, the LOD and LLOQ are validated and cited from the latest laboratory assay validation report released in 2020.***

5. That each comparison is statistically independent.

In conclusion, assuming a ~~35~~ ***40***% drop-out rate in this age group, a total of ~~2700~~ ***1200*** subjects to be enrolled in a 2:1 ratio, which will lead to ~~1170~~ ***480*** subjects evaluable in Group MenB+PCV and ~~250~~ ***240*** subjects evaluable in Group Placebo+PCV, there will be 99% power to conclude that:

a. 13 PCV13 antigens will be shown non-inferiority of PCV13 vaccine when co-administrated with rMenB+OMV NZ vaccine, compared to PCV13 vaccine that is administered alone, at one month post 3rd vaccination

b. The percentage of subjects with titers ≥ LLOQ is more than or equal to 60% for strain M14459, 96217, NZ98/254 and ***M13520***, and more than or equal to ~~55% for strain M10713, and more than or equal to 43~~ ***50***% for all 4 rMenB+OMV NZ strains at one month post 3rd vaccination.

c. Percentage of subjects with 4-fold increase is more than or equal to ~~70~~ 7***5***% for strain M14459, 96217, NZ98/254 and ***M13520***, and more than or equal to ~~66% for strain M10713, and more than or equal to 48~~ ***65***% for all four rMenB+OMV NZ strains, at one month post 4th vaccination***.***

Determination of the Immunogenicity Subsets Sizes for the Secondary Objectives:

As described in detail in Table 9, due to limited serum volume available subjects will be randomly assigned to test different sets of RIV antigens. Subjects will either be assigned to subset A1, A2, A3 in Group MenB+PCV or assigned to subset B1, B2 and B3 in Group Placebo+PCV (see Section 5.2.3). The below tables (Table 21 and Table 22) show the likelihood to achieve non-inferiority for each of the RIV antigens (excluding polio) with the proposed sample sizes for immunogenicity subsets.

Table 21 Non-inferiority to Each Individual Antigen (excluding polio antigens) in Pediarix and Hiberix, at One Month After 3rd Dose of rMenB+OMV NZ - Preliminary Results^

Component	Endpoint	Non-inferiority Margin	Reference		# Evaluable Subjects per Immunogenicity Subset (MenB group/Placebo group)*	Power
			Difference in %/ratio (Co-ad vs routine)	% in Routine group or SD		
Diphtheria	%≥0.1IU/mL	10%	Diff=0%	99.4%	120/120	>99.9%
Tetanus	%≥0.1IU/mL	5%	Diff=0%	100%	120/120	>99.9%
PT	GMC	1.5	Ratio=0.8	SD: 0.31535	480/240	88.7%
FHA	GMC	1.5	Ratio=0.84	SD: 0.3111	480/240	98.3%
PRN	GMC	1.5	Ratio=0.83 [b]	SD: 0.43427	480/240	79.1%
HepB	%≥10.0mIU/mL	10%	diff=-2%	97.7%	120/120	93.5%
Hib	%≥0.15µg/mL	5%	diff=0% [a]	96.6%	360/240	91.2%
	%≥1µg/mL	10%	diff=0%	81.2%	360/240	86.7%

^ Data Not Quality-Assured

Abbreviations: SD, standard deviation; GMC, geometric mean concentrations; PT, pertussis toxin; FHA, Filamentous hemagglutinin; PRN, pertactin; HepB, Hepatitis B; Hib, Haemophilus influenzae type B; diff, difference

Note: The sources are V72P13 clinical study report and the package insert of vaccines, unless otherwise indicated. SDs for Pertussis were taken from GSK study Hib-97.

[a] Source: V72P12 CSR.

[b] Source: V72P16 Table 14.2.1.14 Group B+OMV versus MenC.

* Considering an estimated drop-out rate of 40%.

Table 22 Non-inferiority to Each Individual Antigen in MMR II and Varivax, at One Month After 4th Dose of rMenB+OMV NZ - Preliminary Results^

Component	Endpoint	Non-inferiority Margin	Reference		# Evaluable Subjects per Immunogenicity Subset (MenB group/Placebo group)*	Power
			Ratio (co-ad vs routine)	SD		
Measles	GMC	1.5	ratio=0.9	SD: 0.451	480/240	95.5%
Mumps	GMC	1.5	ratio=0.9	SD: 0.381	480/240	99.1%
Rubella	GMC	1.5	ratio=0.9	SD: 0.341	480/240	99.8%
Varicella	GMC	1.5	ratio=0.9	SD: 0.452	480/240	95.5%

^ Data Not Quality-Assured

[1] Reference for sample size calculation: GSK Study MMR-161.

[2] Reference for sample size calculation: GSK Study MMR-160.

* Considering an estimated drop-out rate of 40%.

Section 9.4.7: Subgroups

- ~~GMTs,~~ GMCs and ratios of GMCs for PVC (Group MenB+PCV vs Group Placebo+PCV) at one month after the 3rd and 4th vaccination.
- ***Percentages of subjects with hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217); for each of the 4 rMenB+OMV NZ strains and for the combined strains at one month after the 4th vaccination in Group MenB+PCV and Group Placebo+PCV.***

Analyses will be performed on the ~~PPS~~***FAS***.

~~Safety endpoints may also be analyzed for each category of the subgroup factors gender, race and ethnic origin. The subgroup analyses will include summaries by vaccination of i) subjects with solicited AE (per solicited AE), ii) subjects with any unsolicited AE, iii) subjects with any possibly or probably related unsolicited AE and iv) subjects with serious unsolicited AEs.~~

Additional subgroup analyses may be performed to assess the impact of COVID-19. More details will be provided in the Statistical Analysis Plan.

Section 9.7: Analysis of immunogenicity

The primary population for the primary immunogenicity analyses will be the PPS. ***All primary and selected secondary immunogenicity analyses (NI assessments to other RIVs) will be repeated on the FAS.***~~; however, selected analyses will be repeated on the FAS.~~

Section 9.7.1: Within groups assessment

For each study group, at each timepoint that a blood sample result is available, the following endpoints will be assessed related to MenB strains and PCV13 strains:

- the percentages of subjects with hSBA titers ≥ LLOQ for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains
- the percentages of subjects with hSBA titers ≥ LLOQ for <u>all</u> strains combined (composite endpoint) ***at one month after the 3rd vaccination***
- the percentages of subjects with hSBA titers ≥5 for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains
- ***the percentages of subjects with hSBA titers ≥8 for each of the test strains (M14459, NZ98/254 and M13520), ≥16 for test strain 96217, and for all strains combined (composite endpoint) at one month after the 4th vaccination***
- the percentages of subjects with 4-fold increase at post-4th (from pre-4th) for each of the M14459, 96217, NZ98/254 and ~~M10713~~***M13520*** test strains
- ~~the percentages of subjects with 4-fold increase at post-4th vaccination (from pre-4th vaccination) for <u>all</u> strains combined (composite endpoint)~~
- GMCs/GMTs and within group GMRs (for post-4th versus pre-4th titers) will be tabulated for antibodies for each antigen
- the ECL GMCs and within group GMRs for each of the 13 PCV13 antigens ***at one month post 4th vaccination***
- Percentage of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 μg/mL at one month after the 3rd and 4th vaccination in Group MenB+PCV and Group Placebo+PCV**.**

The CIs will be calculated for 2-sided 95% for exploratory objectives, and CIs will be calculated for given alpha at the testing step for the primary objectives that are associated with statistical hypothesis testing.

Endpoints associated with the other RIV will be assessed within-group ~~for their endpoints mentioned in the endpoints section~~ ***as specified in Section 9.2. The 2-sided 95% CI will be calculated***.

Section 9.7.2: Between groups assessment

For PCV antigens, at each blood sampling timepoint and for each antibody for which results are available, ***for Diphtheria, Tetanus, Pertussis and Hepatitis B antigens, at blood sampling timepoint one month after the 3rd vaccination and for each antibody for which results are available:***

- The CI of the between-group GMC ratios (i.e. study group minus control group) will be computed using an analysis of variance (ANOVA) model on the logarithm10 transformation of the concentrations.

For Diphtheria, Tetanus, Hib and Hepatitis B antigens, at blood sampling timepoint one month after the 3rd vaccination, for VV and MMR, at blood sampling timepoint one month after the 4th vaccination and for each antibody for which results are available:

- ***The CI of the between-group percentage differences (i.e. study group minus control group) will be computed.***

Section 9.7.3: Analysis of Primary Immunogenicity

Section 9.7.3.1: Statistical Hypotheses

In total 23 hypotheses will be tested for the primary objectives. A step-wise statistical approach will be used for the 23 statistical tests. The hypotheses will be grouped into ***three*** families, based on the different immunogenicity objectives:

Family 2: ***5*** statistical hypotheses related to sufficiency objective on percentages of subjects with ***hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217)*** **4-** ~~fold increase~~ at ***one month*** post 4th dose ~~(from pre-4th dose) for each of the four strains M14459, 96217, NZ98/254, and M10713~~, and the composite endpoint

Family 3: ***5*** statistical hypotheses related to sufficiency objective on percentages of subjects with hSBA titers ≥ LLOQ at ***one month*** post 3rd dose for each of the four strains M14459, 96217, NZ98/254, and ~~M10713~~***M13520***, and the composite endpoint**.**

The statistical hypotheses for each and all families are formulated as below:

Family1*: Non-inferiority of rMenB+OMV NZ + PCV13 to PCV13 with respect to GMCs* ***at one month post 3rd vaccination***

Family 2: *Percentage of subjects with* ***hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217)*** ~~*4-fold increase for each of the four strains (M14459, 96217, NZ98/254, M10713)*~~ *and the composite endpoint,* ***at one month*** *post-4th* ***vaccination***

Sufficiency will be claimed for each of the 4 strains and the composite endpoint, separately, if the following null hypothesis will be rejected:

H_{02j}: $P_{Aj} < A_j$ vs. H_{12j} : $P_{Aj} \geq A_j$,

where P_{Aj} denotes the percentage of subjects with ***hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217)*** ~~4-fold increase~~ at one month after the 4th vaccination ~~(from pre-4th dose)~~ for strain j, (j=1, 2, 3, 4, and composite), which is assumed to be at least 90% for each of the ~~three~~ ***individual*** strains M14459, 96217, ~~and~~ NZ98/254, ***and M13520*** ~~72% for strain M10713~~, and ~~54~~***at least 70%*** for the composite endpoint (estimate based on assumption of ~~25~~***0***% correlation). Aj represents the level of sufficient immune response for each of the four strains and composite endpoint. The immune response at one month following the 4th vaccination, will be sufficient for rMenB+OMV against each individual strain if the lower limit of the adjusted CI for the percentage of subjects with ***hSBA titers ≥ 8 (for strains M14459, NZ98/254, M13520) and ≥16 (for strain 96217)***~~4-fold increase~~ is ≥~~70~~***75***% for ***the individual N. meningitidis serogroup B test*** strains ~~M14459, 96217, NZ98/254, ≥66% for strain M10713~~, and ***is*** ≥~~48~~***65***% for the composite endpoint.

Family 3: *Percentage of subjects with hSBA≥LLOQ for each of the four strains (M14459, 96217, NZ98/254, ~~M10713~~**M13520**) and the composite endpoint,* ***one month post*** *post-3rd.* ***vaccination***

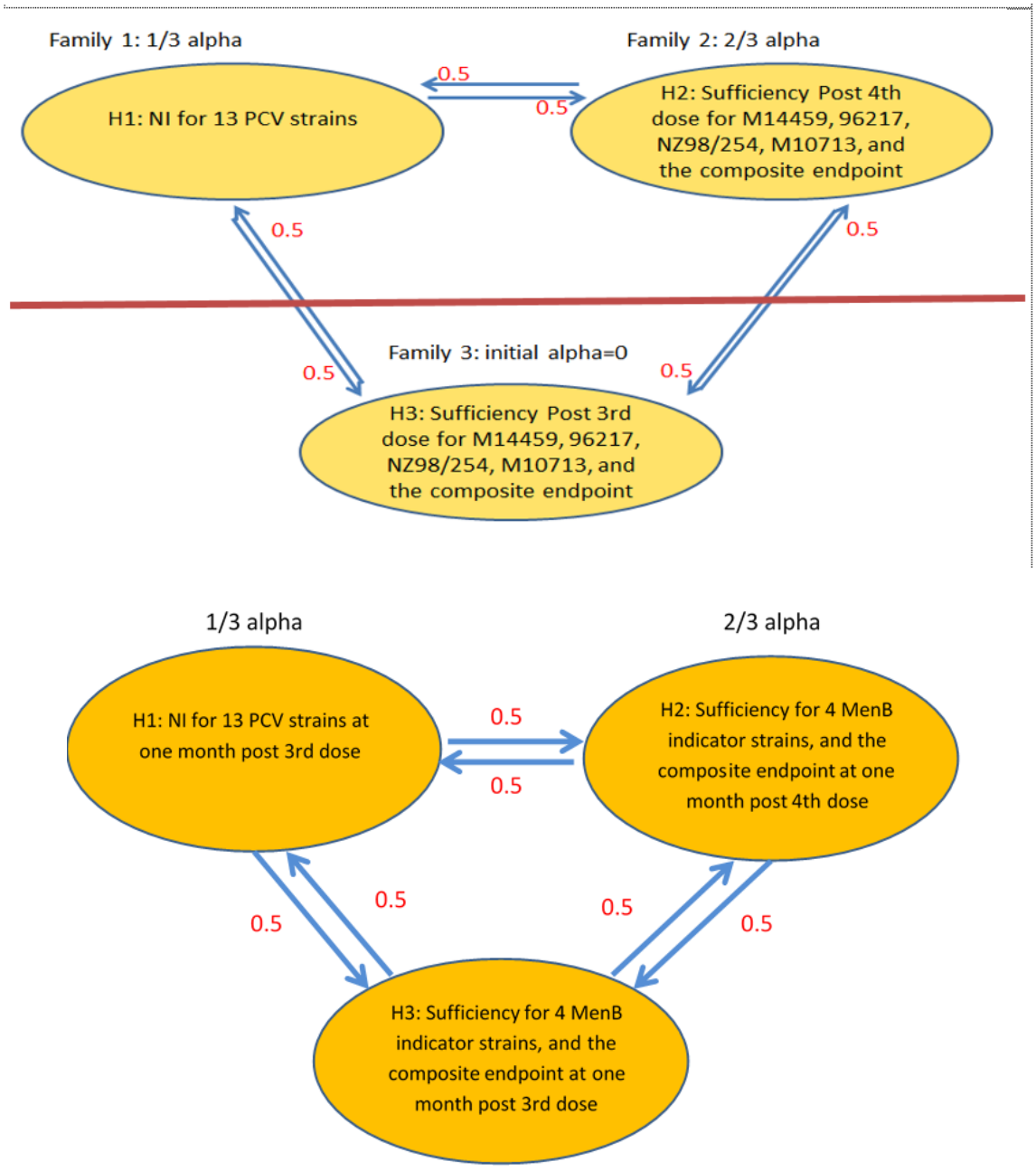
Sufficiency will be claimed for each of the 4 strains, separately, if the following null hypothesis will be rejected:

H_{03j} : $P_{Aj} < A_j$ vs. H_{13j} : $P_{Aj} \geq A_j$,

where P_{Aj} denotes the percentage of subjects hSBA≥LLOQ at one month after the 3rd vaccination for strain j, j=1, 2, 3,***4,*** and composite)), which is assumed to be at least 90% for strains M14459, and 96217, 80% for ***M13520, and 70% for*** NZ98/254~~, 61% for strain M10713~~***M13520***, and ~~49~~***at least 60***% for the composite endpoint (estimate based on assumption of ~~25~~***0***% correlation). Aj represents the level of sufficient immune response for each of the four strains and composite endpoint. The immune response at one month following the 3rd vaccination, will be sufficient for rMenB+OMV NZ against each individual strain if the lower limit of the adjusted CI for the percentage of subjects with hSBA titer ≥LLOQ is ≥60% for ***the N. meningitidis serogroup B test*** strains ~~M14459, 96217, NZ98/254, ≥55% for strain M10713,~~ and ≥~~43~~***50***% for the composite endpoint**.**

Section 9.7.3.2: Statistical Methods

Figure 2 Testing Strategy for *Three* Families with Corresponding α Propagation



The initial α is 0.0167 (2-sided) for Family 1 and 0.033 (2-sided) for Family 2, while it is zero for ***family 3***.

Section 9.8.1.1: Analysis of Solicited Adverse events

~~Due to ediary software issue, a subject may be able to enter solicited adverse events in the ediary more than once per day, giving rise to duplicated records in the database. Discrepant records will not be included in the solicited safety analysis, but will be reported in the listing.~~

Section 9.8.1.2: Analysis of Unsolicited Adverse Events

Local and systemic AEs will be analyzed by point estimates with associated 95% CIs [Clopper, 1934].

Section 9.8.1.2: Between groups assessment

Not applicable.

Section 9.10.1: Sequence of analyses

Any analyses (including a possible interim analysis) will be conducted on data as clean as possible. All data will be analyzed at the end of the study, after availability of all immunogenicity and safety data, and presented in a final clinical study report.

If needed, an interim analysis including data up to visit 6 may be performed. Selected immunogenicity objectives and solicited safety data from all timepoints up to Visit 6 will be analyzed. The interim analysis will be performed by an independent statistician, in order to maintain the observer-blindness of the study. Study unblinding will be performed only after the final database lock. The data or the results from this interim analysis will not be used to make any decision on the continuation of this trial. Therefore no impact on the conduct or subsequent full data analysis of the trial is expected. All remaining objectives and remaining safety data will be analyzed at the end of the study and an integrated clinical report will be generated, which contains all data from the trial.

~~Any analyses (including a possible interim analysis) will be conducted on data as clean as possible. If performed, the analyses will be done stepwise:~~

- ~~An interim analysis may be performed to provide Health Authorities with information on the primary and secondary objectives and safety data. The analyses if performed will occur at 1 month post-4th vaccination (ie, up to Visit 6).~~

  ~~The interim analysis will include all enrolled subjects.~~

  ~~All immunogenicity objectives will be analyzed. The safety analysis will be performed on solicited safety data from all timepoints up to Visit 6.~~

  ~~The interim analysis will be performed by independent statistician, in order to maintain the observer-blindness of the study. Study unblinding will be performed only after the final database lock. The data or the results from this interim analysis will not be used to make any decision on the continuation of this trial. Therefore no impact on the conduct or subsequent full data analysis of the trial is expected.~~

- ~~The remaining safety analyses up to study termination at Visit 7 will be provided in an additional study report.~~

~~An integrated clinical study report containing all data will be written and made available to the investigators.~~

Section 9.10.2: Statistical considerations for interim analyses

~~Interim analysis will be conducted on final immunogenicity data and the safety analyses will be descriptive. Therefore no statistical adjustment for the interim analysis is required.~~

If performed, interim analysis will be conducted on final immunogenicity data and the safety analyses will be descriptive. Therefore no statistical adjustment for the interim analysis is required.

Section 10.6: ***Definition of medical device AE, adverse device effect (ADE), serious adverse device effect (SADE) and unanticipated SADE (USADE)***

Section 10.6.1: ***Definition of medical device AE and adverse device effect (ADE)***

- ***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 considered related to a medical device or not. 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 a medical device. This definition includes events related to the medical device or comparator and events related to the procedures involved.***
- ***An adverse device effect (ADE) is an AE related to the use of a medical device. This definition includes any AE resulting from:***
  - ***insufficient or inadequate instructions for use (i.e. user error), or***
  - ***any malfunction of a medical device, or***
  - ***intentional misuse of the medical device.***

Section 10.6.2: ***Definition of medical device SAE, SADE and USADE***

A medical device SAE is any serious adverse event 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***

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] (include after consultation with study safety physician)***
Serious Adverse Device Effect (SADE) definition
• ***A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.*** • ***Any device deficiency that might have led to an SAE if appropriate action had not been taken or circumstances had been less fortunate.***
Unanticipated SADE (USADE) definition
• ***An USADE (also identified as UADE in US Regulations 21 CFR 813.3), is a serious adverse device effect that by its nature, incidence, severity or outcome has not been identified in the current version of the IB.***

Section 10.6.3: ***Recording and reporting of medical device AE, ADEs, SADEs and USADE***

- ***Any device deficiency must be reported to GSK within 24 hours after the investigator determines that the event meets the definition of a device deficiency.***
- ***E-mail/Facsimile transmission of the paper 'Medical device or combination product with device deficiency/incident report form' is the preferred method to transmit this information to the sponsor.***
- ***In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of 'Medical device or combination product with device deficiency/incident report form' sent by overnight mail or courier service.***
- ***Contacts for reporting can be found in Section 7.9.***
- ***GSK will review all device deficiencies, 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.***

APPENDIX A: LABORATORY ASSAYS

MenB serum bactericidal assays using human complement (hSBA):

Serum bactericidal activity against rMenB+OMV NZ will be determined by performing hSBA against a standard panel consisting of 4 meningococcal B test strains M14459, 96217, NZ98/254 and ~~M10713~~***M13520***. Each of these strains measures bactericidal activity primarily directed against one of the major bactericidal antigens included in the vaccine: strain M14459 measures hSBA against the 741 part of the 936-741 antigen, also known as fHbp variant 1.1; strain 96217 measures hSBA against antigen 961c, also known as NadA; and strain NZ98/254 measures hSBA against PorA P1.4, the immunodominant antigen in the OMV NZ vaccine component; strain ~~M10713~~***M13520*** measures hSBA against the 287 part of the 287-953 antigen, also known as NHBA.

Mumps, measles, rubella and varicella tests:

Serological assays for the determination of antibodies against measles, mumps, rubella and varicella viruses ***are yet to be developed and/or selected. They*** will be performed on human serum by ELISA GSK Biologicals, or in another validated laboratory designated by GSK Biologicals using standardized and validated procedures.

~~Anti-measles antibody concentrations will be measured using a commercial ELISA kit (Enzygnost/Dade Behring), which is based on a single point quantification antibody method (sera tested at a 1:231 dilution). This corresponds to a measles antibody concentration of 150 mIU/mL (as determined by the manufacturer using a standard serum); all sera which have titres equal to or above this value (i.e. are positive at this dilution) are considered as positive.~~

~~Anti-rubella antibody concentrations will be measured using also commercial ELISA kits (Enzygnost/Dade Behring), which are also based on a single point quantification Ab method.~~

~~Anti-VZV IgG Ab concentrations will be measured by using the commercial CE labeled ELISA kit Enzygnost from Siemens (former Dade Behring). Solid phase of the assay (96 wells microplate) consist of wells coated with native VZV antigens derived from cells infected with VZV Virus (Ellen strain, ATCC VR-586).~~

~~The anti-mumps IgG antibody (Ab) concentrations will be measured by using an ELISA assay. Because of the unavailability of the ELISA assay that was used by GSK in other clinical development programs (MMR-161 GSK study), a new ELISA has either to be developed and validated by GSK or selected from commercially-available sources of validated ELISA against anti-mumps IgG antibodies. This selection is not yet made.~~

~~Therefore the type of ELISA, the assay characteristics (e.g. validated assay cut-offs and units) will be determined during the course of the study. Any of these changes will be documented in a protocol amendment or in the clinical study report.~~

APPENDIX B CLINICAL LABORATORIES

~~GSK Vaccines GmbH~~ **~~Clinical Laboratory Sciences~~,*~~Nexelis~~*** ***~~Marburg GmbH~~*** **Marburg, Germany**	**Emil-von-Behring-Str. 76** **35041 Marburg** **Germany**

GlaxoSmithKline Biologicals SA

Vaccines R &D

Protocol Amendment 7

eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Amendment number:	Amendment 7
Amendment date:	24 October 2022
Co-ordinating author:	PPD

Rationale/background for changes:

The purpose of the amendment 7 is to shorten the safety follow-up period to 6 months in subjects who have not reached the 6-month safety follow-up after the last dose, at the time this amendment takes effect. Shortening the safety follow-up period is not expected to jeopardize the safety of subjects, as rMenB+OMV NZ has been extensively studied in long term clinical studies in infants, and has showed a favorable benefit-risk profile. In addition, more than 700 out of 1200 subjects enrolled in the study will be followed up for 12 months after the last vaccination.

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

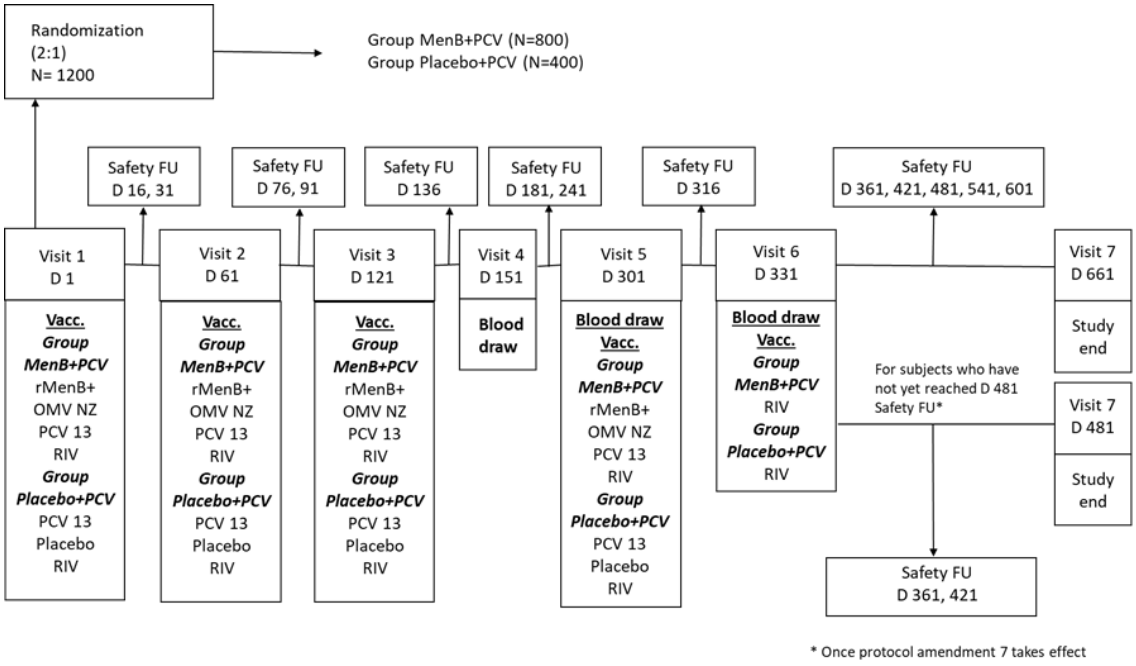
Synopsis and Section 2: Objectives and endpoints

Objectives	Endpoints
Primary Safety	
• To assess the safety and tolerability of rMenB+OMV NZ, PCV13 and other RIV when administered concomitantly to healthy infants at 2, 4, 6 and 12 months of age, throughout the study duration.	• The percentages of subjects with solicited local Adverse events (AEs) and systemic AEs during the 7 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with solicited systemic AEs of parotid/salivary gland swelling, fever and rash during the 30 days (including the day of vaccination) after the 4th vaccination (Visit 5). • The percentages of subjects with all unsolicited AEs (including SAEs, AEs leading to withdrawal, AESIs, and medically attended AEs) during 30 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5).

Objectives	Endpoints
	• The percentages of subjects with SAEs, AEs leading to withdrawal, AESIs and medically attended AEs from study Day 1 (Visit 1) until study end (***6 months or*** 12 months after last study vaccination, Visit 7¥).

¥ Visit 7 will occur either on Day 481 (for subjects who have not yet reached the 6-month follow-up after the last dose at the time protocol amendment 7 takes effect) or on Day 661 (for all other subjects).

Figure 1: Overview of Study Design - V71_57



Synopsis and Section 3: Study design

- Duration of the study:
  - Epoch 001 Primary starting at Visit 1 (Day 1) and ending at Visit 5 (Day 301).
  - Epoch 002: Secondary starting at Visit 5 (Day 301) and ending at Visit 6 (Day 331).
  - Epoch 003: Safety follow-up period starting at Visit 6 (Day 331) and ending at Visit 7 (***Day 481 or*** Day 661). ***For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will take place on Day 481.***
- Primary completion date (PCD): Visit 7 (***Day 481 or*** Day 661).

Table 5: List of Study Procedures

Age (approximate)	~2 moa			4 moa			6 moa		7 moa			12 moa		13 moa						24 moa
Epoch	Epoch 001											Epoch 002			Epoch 003					
Type of contact	Visit 1	SFC 1	SFC 2	Visit 2	SFC 3	SFC 4	Visit 3	SFC 5	Visit 4	SFC 6	SFC 7	Visit 5	SFC 8	Visit 6	SFC 9	SFC 10	SFC 11	*Visit 7*[10]	SFC 12	SFC 13	Visit 7[11]
Timepoints	Day 1	Day 16	Day 31	Day 61	Day 76	Day 91	Day 121	Day 136	Day 151	Day 181	Day 241	Day 301	Day 316	Day 331	Day 361	Day 421	Day 481		Day 541	Day 601	Day 661
Days post-vaccination [1]	0	15 days post-vacc 1	30 days post-vacc 1	60 days post-vac 1	15 days post vacc 2	30 days post vacc 2	60 days post-vacc 2	15 days post-vacc 3	30 days post-vacc 3	60 days post-vacc 3	120 days post-vacc 3	180 days post-vacc 3	15 days post-vacc 4	30 days post-vacc 4	60 days post-vacc 4	120 days post-vacc 4	180 days post-vacc 4		240 days post-vacc 4	300 days post-vacc 4	360 days post-vacc 4
Visit window (days) [2]	-5	-3/+3	-3/+3	-31/+21	-3/+3	-3/+3	-31/+21	-3/+3	-9/+30	-3/+3	-3/+3	-7/+91	-3/+3	-9/+30	-7/+21	-7/+21	-7/ +21		-7/ +21	-7/ +21	-7/ +21

[10] For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481.

Table 6: Intervals Between Study Visits

Interval	Optimal length of interval	Allowed interval[1, 2]
Visit 1 →Visit 2	60 days	*29* days – 81 days
Visit 2 → Visit 3	60 days	*29* days – 81 days
Visit 3 → Visit 4	30 days	*21* days – *60* days
Visit 3 → Visit 5	180 days	173 days – 271 days
Visit 5 → Visit 6	30 days	*21* days – *60* days
Visit 5 → Visit 7 (Study termination)[3]	***180 days***	***173 days – 271 days***
Visit 5 → Visit 7 (Study termination)	360 days	353 days – 381 days

[1]The investigator should arrange study visits within this interval as subjects may not be eligible for inclusion in the PPS cohorts if they make the study visit outside this interval. All visits and windows should be planned using the day of last vaccination as the reference point

[2]The allowed interval have been modified in keeping with ACIP guidelines to facilitate the safe scheduling of study visits during the COVID-19 pandemic situation.

[3] For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481.

Section 5.6.15: Study procedures during special circumstances

- Visit 7 (***Day 481 or*** Day 661): the study conclusion visit has been revised to allow for either a site visit or concluding telephone call. The telephone call is intended for safety data collection and will be considered equivalent to study conclusion visit.

Section 5.6.16.2: Safety follow-up calls

During the course of the trial, a total of ***10 or*** 13 safety follow-up calls will be performed. Safety follow-up calls will be performed on the following time points:

Treatment period:

- Day 16 and Day 31 (15 and 30 days post 1st vaccination, respectively),
- Day 76 and Day 91 (15 and 30 days post 2nd vaccination, respectively),
- Day 136, Day 181 and Day 241 (15, 60 and 120 days post 3rd vaccination, respectively),
- Day 316 (15 days post 4th vaccination).

Follow-up period:

- Day 361, Day 421, Day 481, Day 541 and Day 601 (60, 120, 180, 240, and 300 days post 4th vaccination, respectively). ***For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481 instead of the follow-up call, and follow-up calls at Day 541 and Day 601 will not be performed.***

Section 5.6.17: Study conclusion

- The study termination visit will occur on ***Day 481 or*** Day 661 (Visit 7). The termination visit will be a clinic visit.
- If Visit 7 is performed by telephone for subjects who are impacted by protocol amendment 7, reconsenting should be performed as per site and IRB guidelines.

Section 6.7.1: Recording of concomitant medications/products and concomitant vaccinations

- Any concomitant vaccination administered in the period starting from birth and ending at the last study visit (Day of birth to ***Day 481 or*** Day 661).

Section 7.2.1 Time period for detecting and recording adverse events and serious adverse events

The time period for collecting and recording SAEs will begin at Day 1 (ie, the first receipt of study vaccines) and will end ***6 months or*** 12 months following administration of the last dose of study vaccine for each subject (ie, study end). See Section 7.3 for instructions on reporting of SAEs.

All medically attended AEs will be collected and recorded from Day 1 (ie, the time of the first receipt of study vaccines) until ***6 months or*** 12 months following administration of the last dose of study vaccine for each subject (ie, study end).

All AEs/SAEs leading to withdrawal from the study will be collected and recorded from Day 1 (ie, the time of the first receipt of study vaccines) until ***6 months or*** 12 months following administration of the last dose of study vaccine for each subject (ie, study end).

The time period for collecting and recording of AESIs will begin at Day 1 (ie, the first receipt of study vaccines) and will end ***6 months or*** 12 months following administration of the last dose of study vaccines (ie, study end). See section 7.3 for instructions on reporting of AESIs. Details regarding follow-up of AEs, SAEs and AESIs are given in Section 7.4

Table 13: Reporting Periods for Collecting Safety Information

Event	Pre-V1[1]	V1 D 1	D16	D31	V2 D61 (2 M post-vacc 1)	D76	D91	V3 D121 (2 M post-vacc 2)	D136	V4 D151 (1 M post-vacc 3)	D181	D241	V5 D301 (6 M post-vacc 3)	D316	V6 D331 (1 M post-vacc 4)	D361, 421, 481, 541, 601[2]	V7 *D481 or* D661 (*6 M or* 12 M post-vacc 4)[3]

[2]For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, follow-up calls on Day 481, Day 541 and Day 601 will not be performed.
[3]For subjects who have not yet reached the 6-month safety follow-up after the last dose at the time protocol amendment 7 takes effect, Visit 7 will occur on Day 481.

Section 7.7 Emergency unblinding

~~Any emergency unblinding must be fully documented by using the Emergency Unblinding Documentation Form, which must be appropriately completed by the investigator and sent within 24 hours to GSK Biologicals.~~

Section 9.2.1 ~~All~~ Enrolled Set

Section 9.2.2 ~~All~~ Exposed Set

All subjects in the ***E***nrolled ***S***et who receive a study vaccination.

Section 9.6 Analysis of Safety

Distribution of subjects by vaccinations will be summarized by vaccine group for the ***E***nrolled ***S***et. The primary analysis will be performed on the ~~all~~ ***E***xposed ***S***et

Section 9.3 Derived and transformed data

Immunogenicity:

- The GMTs/***GMCs*** calculations are performed by taking the anti-log of the mean of the log titer transformations. Values to be used for the antibody concentrations/titres below the assay cut-off will be described in the Statistical Analysis Plan (SAP).

Section 9.5.1 Between group assessments

- the ECL GMCs ~~and within group GMRs~~ for each of the 13 PCV13 antigens at one month post 4th vaccination

The CIs will be calculated ~~for~~ ***as*** 2-sided 95% ~~for exploratory objectives~~, and ~~CIs~~ will be calculated ~~for~~ ***at*** given alpha at the testing step for the primary objectives that are associated with statistical hypothesis testing.

Section 9.6.1.2 Analysis of Unsolicited Adverse Events

Separate summaries will be produced for the following categories:

- Serious adverse events (SAE)
- Adverse events related to vaccine
- Adverse events of special interest
- Adverse event leading to withdrawal
- Adverse events leading to a medically attended visit

These summaries will also be presented by the 0-6/6-12 months safety follow-up periods after the last study vaccination.

GlaxoSmithKline Biologicals SA Vaccines R &D **Protocol Amendment 8**	
eTrack study number and Abbreviated Title	205239 (MENB REC 2ND GEN [V72_57])
IND number	IND-011561
EudraCT number	2016-003268-37
Amendment number:	Amendment 8
Amendment date:	19 Dec 2023
Co-ordinating author:	PPD
Rationale/background for changes: The purpose of the amendment 8 is to align the protocol with the recent update of CDC's ACIP for the US NIP (National Immunization Program). According to this ACIP update, the 20-valent pneumococcal conjugate vaccine (PCV20) is listed as one of the recommended vaccines for the immunization of pneumococcal disease in children in U.S while PCV13 is no longer recommended for full series of pneumococcal vaccination. Children who received 3 PCV13 doses before 12 months but have not received their fourth booster dose, have now the option to receive PCV20 or PCV13. Therefore, to incorporate the current ACIP recommendations, subjects who have not reached their visit 5 at the time when this protocol amendment becomes effective have the option to receive either PCV13 or PCV20 based on the investigator judgment and/or parent's preference.	

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

Synopsis, Section 3: STUDY DESIGN OVERVIEW, Section 5.2.2.2.1. Study group and treatment number allocation

- **Study groups**:
  - Group MenB+PCV: rMenB+OMV NZ given concomitantly with PCV13 at 2, 4, 6, and 12 months of age. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment 8 becomes effective.*** Subjects ***will*** also ~~received~~***receive*** routine infant vaccines (DTPa-HBV-IPV, HRV, Hib, MMR, VV) at applicable timepoints.

– Group Placebo+PCV: Placebo and PCV13 at 2, 4, 6, and 12 months of age. ***Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment 8 becomes effective.*** Subjects ***will*** also ~~received~~***receive*** routine infant vaccines (DTPa-HBV-IPV, HRV, Hib, MMR, VV) at applicable timepoints.

Table 2: Study Groups and Treatment Foreseen in the Study

Treatment name	Vaccine name	Study Groups	
		MenB+PCV	Placebo+PCV
Bexsero	rMenB+OMV NZ	X	-
Prevnar13	PCV13*	X	X
Prevnar 20	***PCV20****	***X***	***X***
Placebo	NaCl	-	X
Pediarix	DTPa-HBV-IPV	X	X
Rotarix	HRV	X	X
Hiberix	Hib	X	X
M-M-R II	MMR	X	X
Varivax	VV	X	X

Note: Subjects in both groups will receive a fourth dose of Hib vaccine (*Hiberix*) and a single dose of DTPa vaccine (*Infanrix*) as non-study vaccines at Visit 6.

**** Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.***

Synopsis and Section 2: OBJECTIVES AND ENDPOINTS:

Objectives	Endpoints
Primary Safety	
• To assess the safety and tolerability of rMenB+OMV NZ, PCV13 and other RIV when administered concomitantly to healthy infants at 2, 4, 6 and 12 months of age, throughout the study duration.	• The percentages of subjects with solicited local Adverse events (AEs***) (administration site event***) and systemic AEs during the 7 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with solicited systemic AEs of parotid/salivary gland swelling, fever and rash during the 30 days (including the day of vaccination) after the 4th vaccination (Visit 5). • The percentages of subjects with all unsolicited AEs (including serious adverse events [SAEs], AEs leading to withdrawal, adverse event of special interest [AESIs], and medically attended AEs) during 30 days (including the day of vaccination) after the 1st, the 2nd, the 3rd and the 4th vaccination (Visits 1, 2, 3 and 5). • The percentages of subjects with SAEs, AEs leading to withdrawal, AESIs and medically attended AEs from study Day 1 (Visit 1) until study end (6 months or 12 months after last study vaccination, Visit 7¥).

Note 1: To control the risk of erroneously concluding, a hierarchical procedure will be used for the multiple confirmatory study objectives, with the possibility to conclude on the study success based on the results of the co-primary objectives alone (refer to Section 9.5.3 for details).

Section: GLOSSARY OF TERMS

Co-administered (concomitant) products	***A product given to clinical trial participants as required in the protocol as part of their standard care for a condition which is not the indication for which the IMP is being tested and is therefore not part of the objective of the study.***
Comparator	***Any product used as a reference (including placebo, marketed product, GSK or non-GSK) for an investigational product being tested in a clinical trial. This is any product that is being used to assess the safety, efficacy, or other measurable value against the test product (IMP).***
Investigational vaccine: **(Synonym of Investigational Medicinal Product)**	***A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form.*** ~~A pharmaceutical form of an active ingredient being tested in a clinical trial, including a product with a marketing authorization when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.~~
Placebo	***An inactive substance or treatment that looks the same as, and is given in the same way as, an active drug or intervention/treatment being studied.***

Section: TRADEMARKS

Trademarks not owned by the GSK group of companies	Generic description
M-M-R II (Merck & Co., Inc.)	Measles, Mumps and Rubella Virus Vaccine Live
Prevnar 13 (Wyeth Pharmaceuticals Inc./Pfizer Inc.)	Pneumococcal polysaccharide conjugate vaccine (13-valent, adsorbed)
Prevnar 20 (Wyeth Pharmaceuticals Inc./Pfizer Inc.)	***Pneumococcal polysaccharide conjugate vaccine (20-valent, adsorbed)***
Varivax (Merck & Co., Inc.)	Varicella Virus Vaccine Live

Section 1.3.1: Risk Assessmentz

Important Potential/ Identified Risk	Data/Rationale for Risk	Mitigation Strategy
IMP: *Prevnar13 and *Prevnar20****		
Hypersensitivity and anaphylaxis	Hypersensitivity including anaphylaxis to one or several components of the vaccine can rarely occur. Hypersensitivity reaction including face oedema, dyspnea, bronchospasm is listed in the clinical trial section of *Prevnar13* US PI as well as anaphylactic /anaphylactoid reaction including shock is a listed adverse reaction from the post-marketing experience.	Anaphylaxis following vaccine administration constitutes an absolute contraindication to subsequent vaccination (see Section 6.5). The subjects will be observed closely for at least 30 minutes following the administration of the vaccines, with appropriate medical treatment readily available in case of anaphylaxis (see Section 5.6.12).
Seizures (including febrile seizures)	The febrile convulsion is a risk for the infant and toddler groups. Seizures (including febrile seizures) are listed adverse reactions in the clinical trial section of *Prevnar13* US PI.	Close monitoring of all seizures, as reported in Section 7.1.6.1. Seizure will be monitored through the AESI collection.
Fever	Fever may occur following vaccination. Fever is listed in the *Prevnar13* **and *Prevnar20*** US PI.	Any febrile illness constitutes a contraindication to administration of the vaccine at the time scheduled for vaccination e study (see Section 6.5). Prophylactic use of analgesic/antipyretic medications during the first 7 days after vaccination is allowed on a voluntary basis, but must be recorded in the source document and in the eCRF (see Section 6.7.1).
Erythema multiforme	Erythema multiforme is listed in the post-marketing section of the *Prevnar13* US PI.	No specific mitigation in this study: SAE collection is part of the study protocol.

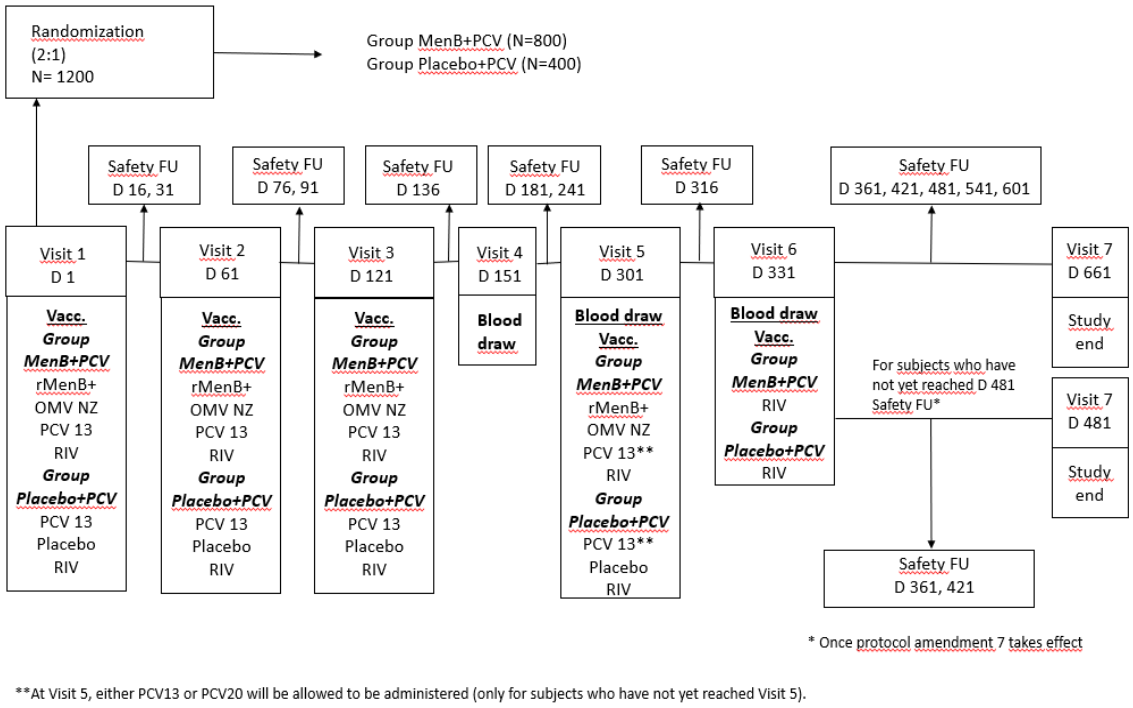
* information derived from the publicly available data.

Note 1: According to the label, events from clinical trials and post marketing experience of Prevnar and Prevnar 13 are applicable also to Prevnar 20

Section 1.3.2: Benefit Assessment

- Receiving *Prevnar 13* during the study may prevent occurrence of pneumococcal pneumonia and invasive disease caused by 13 *Streptococcus pneumoniae* strains (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F). ***For subjects who received PCV20 at visit 5, PCV20 may prevent occurrence of pneumococcal pneumonia and invasive disease caused by 20 Streptococcus pneumoniae strains (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F).***

Figure 1 Overview of Study Design – V72_57



**At Visit 5, either PCV13 or PCV20 will be allowed to be administered (only for subjects who have not yet reached Visit 5).

Section 1.3.3: Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to subjects participating in this study, the potential or identified risks in association with the rMenB+OMV NZ, PCV13 ***(or PCV20),*** DTPa-HBV-IPV, HRV, Hib, MMR and VV vaccines are justified by the potential benefits (prevention) that may be afforded to subject(s) receiving these vaccines.

Table 24 Overview of the Study Design: Blood Draws and Study vaccines

Clinic Visits (Study Day)		**Visit 1 (Day 1)**	**Visit 2 (Day 61)**	**Visit 3 (Day 121)**	**Visit 4 (Day 151)**	**Visit 5 (Day 301)**	**Visit 6 (Day 331)**
Months of Age (MoA)		**~2 MoA[1]**	**4 MoA**	**6 MoA**	**7 MoA**	**12 MoA**	**13 MoA[3]**
Study Vaccines	Group MenB+PCV N=800	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	rMenB+ OMV NZ PCV13	*Blood Draw[2]*	*Blood Draw[2]* rMenB+ OMV NZ ~~PCV13~~***PCV13[4]***	*Blood Draw[2, 3]*
	Group Placebo+PCV N=400	Placebo PCV13	Placebo PCV13	Placebo PCV13		*Blood Draw[2]* Placebo ~~PCV13~~***PCV13[4]***	
Routine Study Vaccines	**Both groups** N=1200	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV HRV Hib	DTPa-HBV-IPV Hib		MMR VV	

[4] Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.

Section 5.1. Regulatory and ethical considerations, including the informed consent process

Potential subjects' parent(s)/LAR(s) or the designee must be informed that their participation is voluntary. They will be required to physically or digitally sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, HIPAA requirements, privacy and data protection requirements, where applicable, and the IRB/IEC or study center.

Table 5: List of Study Procedures

Age (approximate)	~2 moa			4 moa			6 moa		7 moa			12 moa		13 moa						24 moa
Epoch	Epoch 001											Epoch 002			Epoch 003					
Type of contact	Visit 1	SFC 1	SFC 2	Visit 2	SFC 3	SFC 4	Visit 3	SFC 5	Visit 4	SFC 6	SFC 7	Visit 5	SFC 8	Visit 6	SFC 9	SFC 10	SFC 11	*Visit 7*[10]	SFC 12	SFC 13	Visit 7[11]
Timepoints	Day 1	Day 16	Day 31	Day 61	Day 76	Day 91	Day 121	Day 136	Day 151	Day 181	Day 241	Day 301	Day 316	Day 331	Day 361	Day 421	Day 481		Day 541	Day 601	Day 661
Days post-vaccination [1]	0	15 days post-vacc 1	30 days post-vacc 1	60 days post-vac 1	15 days post vacc 2	30 days post vacc 2	60 days post-vacc 2	15 days post-vacc 3	30 days post-vacc 3	60 days post-vacc 3	120 days post-vacc 3	180 days post-vacc 3	15 days post-vacc 4	30 days post-vacc 4	60 days post-vacc 4	120 days post-vacc 4	180 days post-vacc 4		240 days post-vacc 4	300 days post-vacc 4	360 days post-vacc 4
Visit window (days) [2]	-5	-3/+3	-3/+3	-31/+21	-3/+3	-3/+3	-31/+21	-3/+3	-9/+30	-3/+3	-3/+3	-7/+91	-3/+3	-9/+30	-7/+21	-7/+21	-7/+21		-7/+21	-7/+21	-7/+21
Sampling timepoints									Post-vacc 3			Pre-vacc 4		Post-vacc 4				-			-
Informed consent [3]	●																				
Check inclusion/exclusion criteria	●			O			O					O									
Medical history	●																				
History directed physical examination	O																				
Symptom directed physical examination				O			O		O			O		O				O			O
Check contraindications and warnings and precautions to vaccination	O			O			O					O									
Pre-vaccination body temperature	●			●			●					●									
Study group and/or treatment number allocation	●																				

Age (approximate)	**~2 moa**			**4 moa**			**6 moa**		**7 moa**			**12 moa**		**13 moa**						**24 moa**
Epoch	**Epoch 001**											**Epoch 002**			**Epoch 003**					
Type of contact	**Visit 1**	**SFC 1**	**SFC 2**	**Visit 2**	**SFC 3**	**SFC 4**	**Visit 3**	**SFC 5**	**Visit 4**	**SFC 6**	**SFC 7**	**Visit 5**	**SFC 8**	**Visit 6**	**SFC 9**	**SFC 10**	**SFC 11**	***Visit 7*[10]**	**SFC 12**	**SFC 13**	**Visit 7[11]**
Timepoints	**Day 1**	**Day 16**	**Day 31**	**Day 61**	**Day 76**	**Day 91**	**Day 121**	**Day 136**	**Day 151**	**Day 181**	**Day 241**	**Day 301**	**Day 316**	**Day 331**	**Day 361**	**Day 421**	**Day 481**		**Day 541**	**Day 601**	**Day 661**
Days post-vaccination [1]	**0**	**15 days post-vacc 1**	**30 days post-vacc 1**	**60 days post-vac 1**	**15 days post vacc 2**	**30 days post vacc 2**	**60 days post-vacc 2**	**15 days post-vacc 3**	**30 days post-vacc 3**	**60 days post-vacc 3**	**120 days post-vacc 3**	**180 days post-vacc 3**	**15 days post-vacc 4**	**30 days post-vacc 4**	**60 days post-vacc 4**	**120 days post-vacc 4**	**180 days post-vacc 4**		**240 days post-vacc 4**	**300 days post-vacc 4**	**360 days post-vacc 4**
Visit window (days) [2]	**-5**	**-3/+3**	**-3/+3**	**-31/+21**	**-3/+3**	**-3/+3**	**-31/+21**	**-3/+3**	**-9/+30**	**-3/+3**	**-3/+3**	**-7/+91**	**-3/+3**	**-9/+30**	**-7/+21**	**-7/+21**	**-7/ +21**		**-7/ +21**	**-7/ +21**	**-7/ +21**
Sampling timepoints									**Post-vacc 3**			**Pre-vacc 4**		**Post-vacc 4**				**-**			**-**
Treatment number allocation for subsequent doses				●			●					●									
Vaccines administration [4]	●			●			●					●[13]		● [5]							
Recording of solicited local ***(administration site)*** and systemic AEs (Days 1–7 post vaccination) in eDiary [9]	O			O			O					O									

[13] Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time the protocol amendment 8 becomes effective.

Intervals Between Study Visits

Interval	Optimal length of interval	Allowed interval[1, 2]
Visit 1 →Visit 2	60 days	*29* days – 81 days
Visit 2 → Visit 3	60 days	*29* days – 81 days
Visit 3 → Visit 4	30 days	*21* days – *60* days
Visit 3 → Visit 5	180 days	173 days – 271 days
Visit 5 → Visit 6	30 days	*21* days – *60* days
Visit 5→ Visit 7 (Study termination)[3]	180 days	173 days – ~~271~~***201*** days
Visit 5→ Visit 7 (Study termination)	360 days	353 days – 381 days

Section 5.6.5. Collect demographic data

At Visit 1, record demographic data such as date of birth, gender, race and ethnic origin, weight and length in the subject's eCRF.

Collection of sex, race and ethnicity data is necessary to assess and monitor the diversity of the trial participants, and to determine if the trial participants are truly representative of the impacted population.

Section 5.6.14.1. Follow-up safety clinic visit

~~A blood sample will also be collected for immunological evaluation at both visits.~~

At both visits on Day 151 (Visit 4) and Day 331 (Visit 6), the eDiary should be returned to the study site ~~and~~ ***; the eDiary dispensed at Day 1 (Visit1) will be*** collected ***at Day 151 (Visit 4) and the eDiary dispensed at Day 301 (Visit 5) will be collected at Day 331 (Visit 6).*** For details on the eDiary see Section 5.6.16.1. ***A blood sample will also be collected for immunological evaluation at both visits.***

Section 5.7.3. Laboratory assays

Serological testing for immunogenicity to rMenB+OMV NZ, PCV13 ***(same pneumococcal serotypes panel (PCV13)will be used for subjects who received PCV20)*** and the other routine vaccines will be performed at a GSK Biologicals' laboratory(ies) or in a laboratory(ies) designated by GSK Biologicals using standardized and validated procedures (refer to Table 7).

Table7: Humoral Immunity (Antibody determination)

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
Serum	N Men B M13520 (NHBA) Ab*,∞	hSBA	In house	1/dil	4	~~GSK Biologicals *** or laboratory~~***Laboratory*** designated by GSK Biologicals	1
	N Men B NZ98/254 (PorA) Ab				4		
	N Men B M14459 (fHbp) Ab				3		

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
	N Men B 96217 (NadA) Ab				6		
Serum	Streptococcus pneumoniae.Polysaccharide 01 Ab.IgG	ECL	In house	µg/mL	0.08	GSK Biologicals ***	2
	Streptococcus pneumoniae.Polysaccharide 03 Ab.IgG				0.075		
	Streptococcus pneumoniae.Polysaccharide 04 Ab.IgG				0.061		
	Streptococcus pneumoniae.Polysaccharide 05 Ab.IgG				0.198		
	Streptococcus pneumoniae.Polysaccharide 06A Ab.IgG				0.111		
	Streptococcus pneumoniae.Polysaccharide 06B Ab.IgG				0.102		
	Streptococcus pneumoniae.Polysaccharide 07F Ab.IgG				0.063		
	Streptococcus pneumoniae.Polysaccharide 09V Ab.IgG				0.066		
	Streptococcus pneumoniae.Polysaccharide 14 Ab.IgG				0.16		
	Streptococcus pneumoniae.Polysaccharide 18C Ab.IgG				0.111		
	Streptococcus pneumoniae.Polysaccharide 19A Ab.IgG				0.199		
	Streptococcus pneumoniae.Polysaccharide 19F Ab.IgG				0.163		
	Streptococcus pneumoniae.Polysaccharide 23F Ab.IgG ~~0~~				0.073		
Serum	Corynebacterium diphtheriae.Diphtheria Toxoid Ab.IgG	ELI	In-house	IU/mL	0.030	GSK Biologicals ~~*** or laboratory designated by GSK Biologicals~~***	10
	Clostridium tetani.Tetanus Toxoid Ab.IgG	ELI	In-house	IU/mL	0.037		11
	Bordetella pertussis.Filamentous Hemaglutinin Ab.IgG	ELI	In-house	IU/mL	2.046		4
	Bordetella pertussis.Pertussis Toxin Ab.IgG	ELI	In-house	IU/mL	2.693		4

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**	Overall testing prioritization
	Bordetella pertussis.Pertactin Ab.IgG	ELI	In-house	IU/mL	2.187		4
	Poliovirus Sabin Type 1 Ab	NEU	In-house	ED50	8		12
	Poliovirus Sabin Type 2 Ab	NEU	In-house	ED50	8		14
	Poliovirus Sabin Type 3 Ab	NEU	In-house	ED50	8		13
	Hepatitis B Virus.Surface Ab	CLIA	CCI	mIU/mL	6.2		5
Serum	Haemophilus influenzae type b.Polyribosyl Ribitol Phosphate Ab	ELI*	In-house	µg/mL	0.066	GSK Biologicals ~~*** or laboratory designated by GSK Biologicals~~***	3
Serum	Measles Virus Ab.IgG	~~ELI*~~***Luminex***	~~TBD~~***In-house***	~~TBD~~***mIU/mL***	TBD	~~GSK Biologicals*** or laboratory~~***Laboratory*** designated by GSK Biologicals	8
Serum	Rubella Virus Ab.IgG	~~ELI*~~***Luminex***	~~TBD~~***In-house***	~~TBD~~***IU/mL***	TBD	~~GSK Biologicals*** or laboratory~~***Laboratory*** designated by GSK Biologicals	9
Serum	Mumps Virus Ab IgG	~~ELI*~~***Luminex***	~~TBD~~***In-house***	~~TBD~~***AU/mL***	TBD	~~GSK Biologicals*** or laboratory~~***Laboratory*** designated by GSK Biologicals	6
Serum	Varicella Zoster Virus Ab.IgG	ELI*	~~TBD~~***In-house***	~~TBD~~***mIU/mL***	~~TBD~~***7.0***	GSK Biologicals*** ~~or laboratory designated by GSK Biologicals~~	7

Ab: Antibody, CLIA: Chemiluminescence immunoassay, IgG: Immunoglobin G, µg: Microgram, mL: Milliliter, 1/dilution: reciprocal of the dilution; ECL: Electrochemiluminescent Assay, ELI: enzyme-linked immunosorbent assay (ELISA), MSBA: manual human serum bactericidal assay (hSBA), NEU: neutralization assay, TBD: to be determined.
*Type of assays, the validated assay cut-offs and units might be subject to change during the course of the study (e.g. in case of requalification, revalidation or standardization, availability of assays). In case testing is done at another GSK designated laboratory, the validated assay cut-off may also need to be adapted accordingly. Any of these changes will be documented in a protocol amendment or clinical study report. The LLOQ for the meningococcal B strains are provided in Table 20 ~~(to be determined for the NHBA strain)~~.
**Refer to Appendix B for the laboratory addresses.

GSK Biologicals laboratory refers to the ***Vaccines Clinical Laboratory ~~Sciences (CLS~~***and Assay Portfolio (Vx CL&AP***) in Rixensart, Belgium; Wavre, Belgium~~; Marburg, Germany~~.
Additional exploratory testing on the vaccine and/or on the disease under study may be performed if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

Section 5.7.5: Immunological correlates of protection

For the following antigens in the DTPa-HBV-IPV, Hib, MMR and VV vaccines, an immunological correlate of protection has been established:

- Anti-D and anti-T concentrations ≥0.1 IU/mL (ELISA),
- Anti-HBS concentration ≥10 mIU/mL (ELISA),
- Polio ≥8 ED50 for types 1, 2 and 3 (neutralizing antibody assay),
- Anti-PRP concentrations ≥0.15 µg/mL (ELISA),
- ~~Anti-varicella concentrations ≥threshold to be defined based on the ELISA assay to be selected (ELISA),~~
- Anti-measles ≥threshold to be defined ~~based on the ELISA assay to be selected (ELISA),~~
- Anti-mumps ≥threshold to be defined ~~based on the ELISA assay to be selected (ELISA),~~
- Anti-rubella ≥threshold to be defined ~~based on the ELISA assay to be selected (ELISA)~~
- The immunological assay results will be communicated to the investigator as soon as they become available. For antigens for which an immunological correlate of protection has been established, the following applies.

Section 6.1.1. Study vaccines

The study vaccines' (see glossary of terms) will be provided by the Sponsor. All study participants are expected to receive these vaccines as specified by the study schedule. The study vaccines specific to this study are described below. ***In exceptional circumstances, in order to avoid any inconvenience for the participant, a commercially-acquired vaccine with identical formulation and brand of that reported in the study protocol can be administered to the participant. In this case this subject will still be eligible for the Per Protocol Set.***

- GSK Biologicals' Meningococcal group-B vaccine, (rMenB+OMV NZ, *Bexsero*);
- Pneumococcal polysaccharide conjugate vaccine, (13-valent Pneumococcal Vaccine) (PCV13, *Prevnar13*) ***(In order to comply with the change in the US NIP, 20-valent Pneumococcal Vaccine (PCV20, Prevnar20) will also be allowed as specified in the protocol);***
- Saline placebo vaccine.

Table 10 Study Vaccines

Treatment name	Vaccine	Formulation	Presentation	Volume to be administered*	Number of doses
Bexsero	rMenB+OMV NZ	rp936-741=50µg; rp287-953=50µg; rp961c=50µg; OMV NZ98/254=25µg; $Al(OH)_3$=1.5mg; Histidine=776µg; NaCl=3.125mg; Sucrose=10mg; water=0.5ml	Prefilled syringe (liquid)	0.5 mL	4
Prevnar13	PCV13	PS1=2.2µg CRM197; PS3=2.2µg CRM197; PS4=2.2µg CRM197; PS5=2.2µg CRM197; PS6A=2.2µg CRM197; PS6B=4.4µg CRM197; PS7F=2.2µg CRM197; PS9V=2.2µg CRM197; PS14=2.2µg CRM197; PS18C=2.2µg CRM197; PS19A=2.2µg CRM197; PS19F=2.2µg CRM197; PS23F=2.2µg CRM197; $AlPO_4$=125µg Al3+	Homogenous white suspension after shaking (in pre-filled syringe)	0.5 mL	4**
Prevnar20	***PCV20***	***PS1(2.2 µg)[1,2]; PS3(2.2 µg)[1,2]; PS4(2.2 µg)[1,2]; PS5(2.2 µg)[1,2]; PS6A(2.2 µg)[1,2]; PS6B(4.4 µg)[1,2]; PS7F(2.2 µg)[1,2]; PS8(2.2 µg)[1,2]; PS9V(2.2 µg)[1,2]; PS10A(2.2 µg)[1,2]; PS11A(2.2 µg)[1,2]; PS12F(2.2 µg)[1,2]; PS14(2.2 µg)[1,2]; PS15B(2.2 µg)[1,2]; PS18C(2.2 µg)[1,2]; PS19A(2.2 µg)[1,2]; PS19F(2.2 µg)[1,2]; PS22F(2.2 µg)[1,2]; PS23F(2.2 µg)[1,2]; PS33F(2.2 µg)[1,2]***	***Suspension for injection (in pre-filled-syringe)***	***0.5 mL***	***1***
Placebo	Placebo (NaCl)	NaCl=150mM	Prefilled syringe (liquid)	0.65 mL*	4
Pediarix	DTPa-HBV-IPV	DT>=30IU; TT>=40IU; PT=25µg; FHA=25µg; PRN=8µg; HbsAg=10µg; Inactivated Poliovirus type 1 (Mahoney strain)=40DU; Inactivated Poliovirus type 2 (MEF-1	Prefilled syringe (liquid)		3

Treatment name	Vaccine	Formulation	Presentation	Volume to be administered*	Number of doses
		strain)=8DU; Inactivated Poliovirus type 3 (Saukett strain)=32DU; Aluminium=700µg Al3+			
Hiberix	Hib	PRP=10µg; TT~=25µg; Aluminium=120µg Al3+	Lyophilized cake to be reconstituted with saline diluent	0.5 mL	3
	Hib diluent (NaCl)	NaCl=150mM	Vial (liquid)		
Rotarix	HRV Lyo	HRV RIX4414 live attenuated >=$10^{6.0}CCID_{50}$	Lyophilized vaccine in a monodose glass vial.	1 mL	2
	HRV Diluent	$CaCO_3$=60mg	Diluent for lyophilized vaccine (calcium carbonate liquid antacid) supplied separately in a prefilled oral applicator		
M-M-R II	MMR-II	Measles>=1.000TCID50; Mumps=12.500TCID50; Rubella=1.000TCID50	Lyophilized pellet in a vial for reconstitution with water for injections	0.5 mL	1
	MMR-II diluent (Water for injection)	Water=0.5ml	vial		
Varivax	Varivax	Oka=1350pfu	Monodose vial of lyophilized vaccine	0.5 mL	1
	Varivax Diluent (Water for injection)	Water=0.5ml	vial		

***** For subjects who receive PCV20 at Visit 5, only 3 doses of PCV13 will be administered.***
[1]conjugated to CRM_{197} (51 µg);
[2]adsorbed on $AlPO_4$ (0.125 mg Al^{3+}); Water for injections

Section 6.3. Dosage and administration of study vaccines

Study vaccines should be administered using the preferred site locations shown in Table 11 below. The rMenB+OMV NZ/Placebo vaccine should be administered at least 2.5 cm above the injection site of Hib, when concomitantly administered on the same thigh. Similarly, PCV13 ***(or PCV20)*** should be administered at least 2.5 cm above the injection site of DTPa-HBV-IPV, when concomitantly administered on the same thigh. The HRV vaccine will be administered orally. The MMR and VV vaccines should be given subcutaneously in the upper arm (deltoid region) by inserting the needle in a pinched-up fold of skin and subcutaneous tissue to prevent injection into muscle.

Table 11 Dosage and Administration

Study group	Type of contact and timepoint	Treatment name	Vol. administered	Route[1]	Site[3]		
					Location	Directionality[2]	Laterality
MenB+PCV	Visit 1 (Day 1)	*Bexsero*	0.5 mL	IM	Thigh	Upper	Right
		Prevnar13	0.5 mL	IM	Thigh	Upper	Left
	Visit 2 (Day 61)	*Pediarix*	0.5 mL	IM	Thigh	Lower	Left
		Hiberix	0.5 mL	IM	Thigh	Lower	Right
	Visit 3 Day 121)	*Rotarix*[4]	1 mL	O	-	-	-
	Visit 5 (Day 301)	*Bexsero*	0.5 mL	IM	Thigh	-	Right
		Prevnar13[5]	0.5 mL	IM	Thigh	-	Left
		M-M-R II	0.5 mL	SC	Arm	Upper	Right
		Varivax	0.5 mL	SC	Arm	Upper	Left
Placebo+PCV	Visit 1 (Day 1)	*Placebo*	0.5 mL	IM	Thigh	Upper	Right
		Prevnar13	0.5 mL	IM	Thigh	Upper	Left
	Visit 2 (Day 61)	*Pediarix*	0.5 mL	IM	Thigh	Lower	Left
		Hiberix	0.5 mL	IM	Thigh	Lower	Right
	Visit 3 (Day 121)	*Rotarix*[4]	1 mL	O	-	-	-
	Visit 5 (Day 301)	*Placebo*	0.5 mL	IM	Thigh	-	Right
		Prevnar13[5]	0.5 mL	IM	Thigh	-	Left
		M-M-R II	0.5 mL	SC	Arm	Upper	Right
		Varivax	0.5 mL	SC	Arm	Upper	Left

[5] Either PCV13 or PCV20 is allowed to be given at 12 months of age (Visit 5) for subjects who have not reached Visit 5 at the time this protocol amendment becomes effective.

Section 6.5. Contraindications to subsequent vaccination

The following events constitute absolute contraindications to further administration of ~~rMenB+OMV NZ, PCV 13 and other RIVs.~~***vaccines specified in the protocol.*** If any of these events occur during the study, the subject must not receive additional doses of vaccines but may continue other study procedures at the discretion of the investigator (see Section 7.4).

Section 7.3.2. Contact information for reporting serious adverse events and AESIs

Study Contact for Reporting SAEs and AESIs
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs and AESIs
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance**

US sites only: Fax: 1-610-787-7053 ***Email: RIX.CT-Safety-Vac@gsk.com***

Section 7.7: Emergency unblinding

GSK Biologicals' Contact information for Emergency Unblinding **24/24 hour and 7/7 day availability**
GSK Biologicals' Central Safety Physician: For US only: +1 844 446 3133 (GSK Biologicals Central Safety Physician on-call) **GSK Biologicals' Central Safety Physician Back-up:** For US only: ~~877.870.0019~~***GSK Helpdesk: 1-877-528-7677 or 1 877-221-2913*** ***Email: GSKClinicalSupportHD@gsk.com***

~~**Section 9.2.3.3: Overall Safety Set**~~

~~All subjects who are in the Solicited Safety Set and/or Unsolicited Safety Set.~~

~~Subjects will be analyzed as "treated" (i.e., according to the vaccine a subject received, rather than the vaccine to which the subject may have been randomized).~~

Section 9.2.4. Full Analysis Set (FAS) *for* Immunogenicity ~~Set~~

Section 9.2.4.1. Full Analysis Set Immunogenicity

All subjects in the ~~Enrolled Set, who receive at least one study vaccination~~***Exposed Set*** and provide immunogenicity data at either one month after their 4th vaccination or one month after their 3rd vaccination for at least 1 antigen/strain.

Section 9.2.7. Subgroups

The following descriptive analyses will be performed by gender, race, ~~and~~ ethnic origin ***and age group (6-8 weeks or ≥8 weeks)*** for the following parameters:

Section 9.4. Analysis of demographics

Distributions of subjects by sex, race, ethnic origin~~ and~~, geographic region ***and age group (6-8 weeks or ≥8 weeks)*** will be summarized overall and by vaccine group.

Section 9.5.1. Within groups assessment

For each study group, at each timepoint that a blood sample result is available, the following endpoints will be assessed related to MenB strains and PCV13 strains:

- GMCs/GMTs and within group GMRs (for post-4th versus pre-4th titers) will be tabulated for antibodies for each antigen
- ~~the ECL GMCs for each of the 13 PCV13 antigens at one month post 4th vaccination~~

- Percentage of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 μg/mL at one month after the 3rd and 4th vaccination in Group MenB+PCV and Group Placebo+PCV.

Section 9.5.4.1. Statistical Methods

Concentration and titer data will be summarized by vaccine group, ~~meningococcal B~~ strain and time point. Additionally, within-subject GMRs will be computed for GMTs at one month after 4th vaccination versus pre-4th vaccination. As for GMTs, the GMRs and 95% CIs will be computed by exponentiating (base 10) the corresponding means and 95% CIs from an ANOVA model.

9.6. Analysis of safety

Distribution of subjects by vaccinations will be summarized by vaccine group for the ~~Enrolled~~***Exposed*** Set. The primary analysis will be performed on the ~~***E***xposed ***S***et.~~ ***Solicited/Unsolicited Safety Set.***

Section 9.6.1.1: Analysis of Solicited Adverse events

Post-vaccination solicited AE reported from Day 1 to Day 7 will be summarized for the interval Day 1-7 (and Day 1-3, Day 4-7 if needed) by maximal severity and by vaccine group, excluding the 30-minute measurement, which will be summarized separately. For MMR and VV-specific solicited systemic AEs (parotid/salivary gland swelling, fever, rash) collected after Visit 5 (30 minutes through Day 30 post-vaccination), the study period will be divided into the following intervals: 6 hours through **D**ay 7, **D**ay 8 though **D**ay 30.

Use of antipyretics and analgesics will be summarized by ~~frequency by~~ type of use (prophylactic versus treatment) and percentage of subjects (with 95% CI) reporting use.

Section 12: REFERENCES

Adams DA, Thomas KR, Jajosky R, Foster L, Sharp P, Onweh DH, Schley AW, Anderson WJ. Summary of Notifiable Infectious Diseases and Conditions — United States, 2014. *Morb Mortal Wkly Rep (MMWR)* 2016; 63:1-152

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Section 5.6.1: Data collected from subjects, Section 5.6.13: Post-vaccination procedures, Section 7.1.3.3: Other solicited adverse events, Section 9.6.1.1: Analysis of Solicited Adverse events, and Section 10.2: Subject Diary

Solicited Local ***(administration site)***

5.6.16.1. Subject Diary, 7.1.3.1. Solicited local adverse events (administration site event), Grading of Solicited Local Adverse Events (Administration Site Event) for All Subjects, 9.6.1.1. Analysis of Solicited Adverse events

solicited local AEs ***(administration site event),***

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