NCT03630705

Safety and Immunogenicity of a 3-Dose Schedule of an Investigational Quadrivalent Meningococcal Conjugate Vaccine when Administered Concomitantly with Routine Pediatric Vaccines in Healthy Infants and Toddlers

Phase III, open-label, randomized, parallel-group, active-controlled, multi-center study to describe the immunogenicity and safety of a 3-dose immunization schedule of MenACYW conjugate vaccine or a 4-dose immunization schedule of a licensed quadrivalent meningococcal conjugate vaccine when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in Mexico and to describe the immunogenicity and safety of a 3-dose immunization schedule of MenACYW conjugate vaccine when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in the Russian Federation.

• Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	MET33
Development Phase:	Phase III
Sponsor:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, Pennsylvania (PA) 18370-0187, USA
Investigational Product(s):	MenACYW conjugate vaccine: Meningococcal Polysaccharide (Serogroups A, C, Y, and W) Tetanus Toxoid Conjugate Vaccine
Form / Route:	Liquid Solution / Intramuscular (IM)
Indication For This Study:	MenACYW conjugate vaccine administered to healthy infants and toddlers at 2 to 12 months of age
Version and Date of the SAP core body part:	Version 1.0, dated 09 November 2022

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List of Abbreviations

AE	adverse event
AESI	adverse event of special interest
AR	adverse reaction
BL	blood sample
CI	confidence interval
CRF	For trials using paper data collection: case report form
D	day
eCRF	For trials using EDC: electronic case report form
FAS	full analysis set
GM	geometric mean
LLOD	lower limit of detection
LLOQ	lower limit of quantification
MD	missing data
NA	Not applicable
PC	phone call
PP	per-protocol analysis set
PV	Pharmacovigilance
Q1; Q2; Q3	first quartile; second quartile (median); third quartile
SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SC	screening
SD	standard deviation
V	visit
Vac	vaccination

1 Introduction

The MenACYW conjugate vaccine is designed for the immunization of individuals of all ages (infants 6 weeks of age and older through and including older adults > 56 years of age) against IMD. The purpose of the vaccine is to provide broad coverage against circulating meningococcal strains from serogroups A, C, Y, and W. Compared to a previous Sanofi Pasteur meningococcal conjugate vaccine, Menactra®, the MenACYW conjugate vaccine is prepared using tetanus toxoid as the carrier protein. Conjugation of PS antigens to a protein carrier can induce T-cell-dependent immune responses, which are anticipated to give rise to higher antibody titers, longer duration of the immune response, and enhanced immunologic memory that allows for a booster response. The program targets licensure of the MenACYW conjugate vaccine in many countries in North America, Europe, Latin America, Africa, the Middle East, and Asia Pacific.

The MenACYW conjugate vaccine is designed to cover broader age groups than those covered by Menomune® -A/C/Y/W-135 and Menactra®. Menactra® has been very successful since its licensure in 2005; however, it is not licensed in Europe and is not indicated in persons 8 months of age or younger or 56 years of age and older. While Menomune® -A/C/Y/W-135 and Menactra® are currently licensed in different parts of the world, the MenACYW conjugate vaccine is being developed by Sanofi Pasteur to ultimately replace Menomune® -A/C/Y/W-135 and Menactra® in the global market as a quadrivalent meningococcal conjugate vaccine indicated in infants/toddlers, children, adolescents, adults, and older adults > 56 years of age. Meningococcal PS vaccines have 2 important limitations: a) the antibody response is age-dependent, with infants giving the poorest response; and b) PS alone are T-cell independent immunogens, and therefore no anamnestic response is seen. The immunogenicity of PS vaccines in infants and children has been shown to be improved by conjugating the PS to protein carriers. Among the key advantages expected of the tetanus carrier is improved immunogenicity in infants and older adults. Pre-clinical studies using a mouse model and investigating different carriers, showed significant levels of PS-specific total immunoglobulin (Ig) G and bactericidal responses in response to the formulations with tetanus toxoid as a carrier. Early Phase I/II trials including those with the final formulation (MET39 and MET44) showed the potential of the candidate vaccine as a very good immunogen in all age groups, including young infants and older adults. The MenACYW conjugate vaccine was found to be immunogenic and well tolerated; it did not raise any safety concerns in the above trials using the final formulation or in the earlier trials.

MenACYW conjugate vaccine is being developed for the infant/toddler population in the international region as a 3-dose series. This Phase III study (MET33) will generate data to primarily support the licensing of the MenACYW conjugate vaccine in the 2 international countries being targeted in the study and also in other countries outside of the US and EU with an infant/toddler indication from 6 weeks of age. The purpose of the MET33 study is to describe the safety profile and immunogenicity of the MenACYW conjugate vaccine and the comparator Menveo® when administered with routine pediatric vaccines given to healthy infants and toddlers.

2 Study Objectives

2.1 Primary Objective(s)

Immunogenicity

1) To describe the vaccine seroprotection (antibody titer ≥1:8) to the antigens (meningococcal serogroups A, C, Y, and W) present in MenACYW conjugate vaccine or Menveo® measured by serum bactericidal assay using human complement (hSBA), for Groups 1 and 2, when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in Mexico
2) To describe the vaccine seroprotection (antibody titer ≥1:8) to the antigens (meningococcal serogroups A, C, Y, and W) present in MenACYW conjugate vaccine measured by hSBA, for Group 3, when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in the Russian Federation

2.2 Secondary Objective(s)

Immunogenicity

1) To describe the hSBA vaccine seroresponse to the antigens (meningococcal serogroups A, C, Y, and W) for Groups 1 and 2, 30 days after the last vaccination of the infant series (2nd dose of MenACYW conjugate vaccine and 3rd dose of Menveo®), when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in Mexico
2) To describe the hSBA vaccine seroresponse to the antigens (meningococcal serogroups A, C, Y, and W) for Group 3, 30 days after the last vaccination of the infant series (2nd dose of MenACYW conjugate vaccine), when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in the Russian Federation
3) To describe the immunogenicity profile of routine pediatric vaccines when administered concomitantly with MenACYW conjugate vaccine (Groups 1 and 3), Menveo® (Group 2), or when administered alone (Group 4)
4) To describe the hSBA antibody responses against meningococcal serogroups A, C, Y, and W when MenACYW conjugate vaccine and Menveo® are administered concomitantly with routine pediatric vaccines in Mexico and the Russian Federation (Groups 1, 2, and 3)
5) To describe the antibody titers to the antigens (meningococcal serogroups A, C, Y, and W) present in MenACYW conjugate vaccine and Menveo® measured by serum bactericidal assay using baby rabbit complement (rSBA) before the first vaccination (Visit 1) and 30 days after the last vaccination of the infant series (2nd dose of MenACYW conjugate vaccine and 3rd dose of Menveo®), when administered concomitantly with routine pediatric vaccines in a subset of subjects (100 subjects per group in Groups 1 and 3, and 50 subjects in Group 2) in Mexico

6) To describe the antibody titers to the antigens (meningococcal serogroups A, C, Y, and W) present in MenACYW conjugate vaccine measured by rSBA before the first vaccination (Visit 1) and 30 days after the last vaccination of the infant series (2nd dose of MenACYW conjugate vaccine), when administered concomitantly with routine pediatric vaccines in a subset of subjects (100 subjects in Group 3) in the Russian Federation
7) To describe the antibody titers to the antigens (meningococcal serogroups A, C, Y, and W) present in MenACYW conjugate vaccine and Menveo® measured by rSBA before the first vaccination (Visit 1) and 30 days after the last vaccination in the second year of life, when administered concomitantly with routine pediatric vaccines in a subset of subjects (100 subjects in Group 1, and 50 subjects in Group 2) in Mexico
8) To describe the antibody titers to the antigens (meningococcal serogroups A, C, Y, and W) present in MenACYW conjugate vaccine measured by rSBA before the first vaccination (Visit 1) and 30 days after the last vaccination in the second year of life, when administered concomitantly with routine pediatric vaccines in a subset of subjects (100 subjects in Group 3) in the Russian Federation**Observational Objective(s)**

Safety

1) To describe the safety profile of MenACYW conjugate vaccine and Menveo® when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in Mexico (Group 1 vs Group 2)
2) To describe the safety profile of MenACYW conjugate vaccine when administered concomitantly with routine pediatric vaccines in healthy infants and toddlers in the Russian Federation (Group 3)
3) To describe the safety profile of routine pediatric vaccines in healthy infants and toddlers in Mexico (Groups 1 and 2) and the Russian Federation (Groups 3 and 4)

3 Description of the Overall Trial Design and Plan

3.1 Study Design

This is a Phase III, open-label, randomized, parallel-group, active-controlled, multi-center study to describe the immunogenicity and safety of a 3-dose immunization schedule of MenACYW conjugate vaccine or a 4-dose immunization schedule of a licensed quadrivalent meningococcal conjugate vaccine (Menveo® [Meningococcal {Groups A, C, Y and W-135} Oligosaccharide Diphtheria CRM_{197} Conjugate Vaccine]) when administered concomitantly with routine pediatric vaccines (Prevnar 13®, Hexacima®, RotaTeq®, and M-M-R®II) in healthy infants and toddlers aged 2 to 12 months in Mexico, and to describe the immunogenicity and safety of a 3-dose immunization schedule of MenACYW conjugate vaccine when administered concomitantly with routine pediatric vaccines (Prevnar 13®, Pentaxim®, ENGERIX-B®, and MMR) in healthy infants and toddlers aged 2 to 12 months in the Russian Federation.

A total of 525 subjects will be enrolled. Approximately 300 healthy, meningococcal-vaccine naïve infants aged 2 months will be randomized in a 2:1 ratio in Mexico, and 225 healthy,

meningococcal-vaccine naïve infants aged 2 months will be randomized in a 2:1 ratio in the Russian Federation into the following groups:

Mexico

Group 1: MenACYW conjugate vaccine at 2, 6, and 12 months of age + routine pediatric vaccines at 2, 4, 6, and 12 months of age

Group 2: Menveo® at 2, 4, 6, and 12 months of age + routine pediatric vaccines at 2, 4, 6, and 12 months of age

The Russian Federation

Group 3: MenACYW conjugate vaccine at 3, 6, and 12 months of age + routine pediatric vaccines at 2, 3, 4.5, 6, and 12 months of age

Group 4: Routine pediatric vaccines at 2, 3, 4.5, 6, and 12 months of age

All subjects in Groups 1 and 2 will receive either MenACYW conjugate vaccine or Menveo® concomitantly with the following routine pediatric vaccines in accordance with available official recommendations ***in Mexico***:

- Prevnar 13® (pneumococcal 13-valent conjugate vaccine [PCV13]) at 2, 4, 6, and 12 months of age*
- Hexacima® (DTaP-IPV-HB-Hib) at 2, 4, 6, and 12 months of age
- RotaTeq® (pentavalent rotavirus vaccine [RV5]) 2, 4, and 6 months of age
- MMR (measles, mumps, rubella [MMR] vaccine) at 12 months of age

All subjects in Group 3 will receive MenACYW conjugate vaccine concomitantly with the following routine pediatric vaccines; and all subjects in Group 4 will receive the following routine pediatric vaccines alone in accordance with the National Immunization Calendar (NIC) recommendations in ***the Russian Federation***:

- Prevnar 13® (pneumococcal 13-valent conjugate vaccine [PCV13]) at 2 and 4.5 months of age*
- Pentaxim® (DTaP-Hib-IPV) at 3, 4.5, and 6 months of age†
- ENGERIX-B® (hepatitis B vaccine) at 6 months of age‡
- MMR vaccine at 12 months of age§

*No immunogenicity endpoints will be measured for this vaccine in the Russian Federation. The PCV13 routine vaccine recommended at 15 months of age in the Russian Federation is considered as out of scope for this study and will not be provided by the Sponsor but procured by the sites as per their standard practices.

† The 4th dose of Pentaxim®, which is administered at 18 months of age, is considered out of the scope of the study, and it will not be provided by the Sponsor but procured by the sites as per their standard practices. Subjects will be instructed to receive it for completion of the Pentavalent series as per the NIC of the Russian Federation.

‡ In the event ENGERIX B® cannot be supplied in the Russian Federation, a locally licensed monovalent hepatitis B vaccine will be administered instead. Further details will be provided in the CSR.

§ In the event M-M-R®II combination vaccine cannot be supplied or is unavailable in the Russian Federation, locally licensed MMR or MM+R vaccines will be administered instead. Further details will be provided in the CSR.

Visit 0 = Screening visit for subjects in the Russian Federation only.
In the Russian Federation, Visit 0 and Visit 1 may take place on the same day, or Visit 1 may take place up to 5 days after Visit 0.

Blood sampling

Subjects will provide blood samples for immunogenicity assessments according to the following schedules:

Mexico

Groups 1 and 2:

- A blood sample at baseline (before the first study vaccination at Visit 1)
- A blood sample 30 days (+14 days) after the 2nd dose of MenACYW conjugate vaccine or 3rd dose of Menveo® (Visit 4)
- A blood sample 30 days (+14 days) after the 3rd dose of MenACYW conjugate vaccine or the 4th dose of Menveo® (Visit 6)

The Russian Federation

Group 3:

- A blood sample at baseline (before the first study vaccination, at Visit 0)
- A blood sample 30 days (+14 days) after the 2nd dose of MenACYW conjugate vaccine (Visit 5)
- A blood sample 30 days (+14 days) after the 3rd dose of MenACYW conjugate vaccine (Visit 7)

Group 4:

- A blood sample at baseline (before the first study vaccination, at Visit 0)
- A blood sample 30 days (+14 days) after the 1st dose of ENGERIX B® and 3rd dose of Pentaxim® vaccines (Visit 5)
- A blood sample 30 days (+14 days) after the 1st dose of MMR vaccine (Visit 7)

Collection of Safety Data

All subjects will be followed for safety from Visit 1 to the last study visit.

- All subjects will be observed for 30 minutes after vaccination under the supervision of a responsible healthcare professional at each study site and any unsolicited

systemic AEs occurring during that time will be recorded as immediate unsolicited systemic AEs in the electronic case report form (eCRF).

- The subject's parent / guardian will record information in a diary card about solicited injection-site reactions and solicited systemic reactions from D0 to D7 after each vaccination and unsolicited AEs will be recorded from D0 after each vaccination until the subject returns for the next study visit.
- Serious AEs (including AEs of special interest [AESIs]) will be recorded in a diary card throughout the study. The subject's parent / guardian will be asked to notify the site immediately about any potential SAEs at any time during the trial.
- A member of the study staff will contact subject's parent / guardian by telephone 8 days (+2 days) after each vaccination visit to identify the occurrence of any SAE (including AESIs) not yet reported and to remind them to complete the diary card after each vaccination visit, and to bring it back to the next study visit.
- The completed diary cards will be collected and reviewed with the subject's parent / guardian at subsequent visits.
- A member of the study staff will contact the subject's parent / guardian by telephone 14 days (+2 days) before the first study visit of the subject's second year of life to identify the occurrence of any SAE not yet reported and to remind them to complete the diary card and to bring it back to the next study visit so it can be reviewed at the study site.

3.2 Study Plan

Vaccination

Subjects in Mexico will receive either MenACYW conjugate vaccine or Menveo®, with routine pediatric vaccines according to the vaccination schedule shown in Table 3.1.

Subjects in the Russian Federation will receive either MenACYW conjugate vaccine or routine pediatric vaccines alone, according to the vaccination schedule shown in Table 3.2.

Blood sampling

Subjects will provide blood samples for immunogenicity assessments at the following time points:

Mexico

Subjects in Groups 1 and 2: Visit 1 (pre-vaccination), Visit 4, and Visit 6 (see Table 3.1).

Russian Federation

Groups 3 and 4: Visit 0 (pre-vaccination), Visit 5, and Visit 7 (see Table 3.2)

For sites in the Russian Federation only:

Subjects enrolled at sites in the Russian Federation will also provide additional blood sample, (depending on local laboratory needs) for CBC and blood chemistry testing at Visit 0 (Screening

visit) and at Visit 7 in accordance with local regulations. Total blood volume collected will be approximately 6 mL per blood draw at Visit 0, Visit 5, and Visit 7. See Table 3.2.

The results of CBC and biochemistry laboratory tests, performed for the subject in terms of routine practice, can be used, if the tests were done within 7 days before Visit 0 and Visit 7. In this case, the blood sample volume collected at Visit 0 and Visit 7 will be 4 mL.

Laboratory tests done as part to the CBC and blood chemistry are presented in Section 9.3.3.1, and Section 9.3.3.2, respectively of the protocol.

Urine Sampling (for Sites in the Russian Federation Only)

Subjects enrolled at sites in the Russian Federation will also provide an approximately 8 mL urine sample (depending on local laboratory needs) for urinalysis before vaccination on Visit 0 and at Visit 7 per Health Authority request and in accordance with local regulations.

The results of urine laboratory tests, performed for the subject in terms of routine practice, can be used, if the tests were done within 7 days before Visit 0. In this case, no urine sample will be collected at Visit 0.

Laboratory tests done as part of the urinalysis are presented in Section 9.3.3.3 of the protocol

Table 3.1: Vaccination and BL schedule: Mexico (Groups 1 and 2)

Visit (V)	V01	V02	V03	V04	V05	V06*
Subject Age	**2 months**	**4 months**	**6 months**	**7 months**	**12 months***	**13 months**
Group 1	**MenACYW†** Prevnar 13® Hexacima® RotaTeq® **BL0001** (Pre-Vaccination)	Prevnar 13® Hexacima® RotaTeq®	**MenACYW†** Prevnar 13® Hexacima® RotaTeq®	**BL0002**	**MenACYW†** Prevnar 13® Hexacima® M-M-R®II	**BL0003**
Group 2	**Menveo®** Prevnar 13® Hexacima® RotaTeq® **BL0001** (Pre-Vaccination)	**Menveo®** Prevnar 13® Hexacima® RotaTeq®	**Menveo®** Prevnar 13® Hexacima® RotaTeq®	**BL0002**	**Menveo®** Prevnar 13® Hexacima® M-M-R®II	**BL0003**

*Per the current National Immunization Program (NIP) and Health Authority recommendations in Mexico, the varicella vaccine is administered at or after 12 months of age; it is not administered within the scope of the study. However, VARIVAX® vaccine will be provided by the Sponsor as a benefit vaccine as per standard practices and the current recommendations of the NIP in Mexico. The study personnel / Investigator will be responsible for administering this vaccine at V6 after the last blood sample (BL0003) of the study. No endpoints will be measured for this vaccine, even if it is administered at V6 of the study.

†MenACYW conjugate vaccine

Table 3.2: Vaccination, BL, and urinalysis schedule: the Russian Federation (Groups 3 and 4)

Visit (V)	V0	V01	V02	V03	V04	V05	V06	V07
Subject Age	**2 months**	**2 months**	**3 months**	**4.5 months**	**6 months**	**7 months**	**12 months**	**13 months**
Group 3	**BL0001** (6 mL) **UA** (8 mL)	Prevnar 13®†	**MenACYW*** Pentaxim®‡	Prevnar 13®† Pentaxim®‡	**MenACYW*** Pentaxim®‡ ENGERIX-B®§	**BL0002** (6 mL)	**MenACYW*** MMR**	**BL0003** (6 mL) **UA** (8 mL)
Group 4	**BL0001** (6 mL) **UA** (8 mL)	Prevnar 13®†	Pentaxim®‡	Prevnar 13®† Pentaxim®‡	Pentaxim®‡ ENGERIX-B®§	**BL0002** (6 mL)	MMR**	**BL0003** (6 mL) **UA** (8 mL)

V: Visit, UA: Urinalysis

*MenACYW conjugate vaccine

† No immunogenicity endpoints will be measured for this vaccine in the Russian Federation. The PCV13 routine vaccine recommended at 15 months of age in the Russian Federation is considered as out of scope for this study and will not be provided by the Sponsor but procured by the sites as per their standard practices.

‡The 4th dose of Pentaxim®, which is administered at 18 months of age, is considered out of the scope of the study, and it will not be provided by the Sponsor but procured by the sites as per their standard practices. Subjects will be instructed to receive it for completion of the Pentavalent series as per the NIC of the Russian Federation recommendation.

§In the event ENGERIX B® cannot be supplied in the Russian Federation, a locally licensed monovalent hepatitis B vaccine will be administered instead. Further details will be provided in the CSR.

** In the event M-M-R®II combination vaccine cannot be supplied or is unavailable in the Russian Federation, locally licensed MMR or MM+R vaccines will be administered instead. Further details will be provided in the CSR.

Table 3.3: Study procedure for the Group 1

Phase III Trial, Group 1 (Mexico): 6 Visits, 4 Vaccination Visits, 4 Telephone Calls, 3 Blood Samples, 11 Months Duration Per Subject for Subjects Randomized to Receive MenACYW Conjugate Vaccine

Visit / Telephone Call	**Visit 1**	**TC1***	**Visit 2**	**Visit 3**	**TC2***	**Visit 4**	**TC3†**	**Visit 5**	**TC4***	**Visit 6**
Approximate Subject Age	**2 months‡**	**-**	**4 months**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
Trial timelines (days, months)	D0	V01 +8 days	V01 + 60 days	V02 + 60 days	V03 + 8 days	V03 + 30 days	V05 14 days	V04 5 months	V05 +8 days	V05 + 30 days
Time windows (days)	NA	+2 days	+14 days	+14 days	+2 days	+14 days	+2 days	+14 days	+2 days	+14 days
Informed consent form signed and dated	**X**									
Inclusion/exclusion criteria	**X**									
Collection of demographic data	**X**									
Medical history§ (including history of maternal immunization against tetanus)	**X**									
Physical examination and temperature	**X****		**X††**	**X††**				**X††**		
Review of temporary contraindications for blood sampling (BL) ‡‡§§	**X**					**X**				**X**
BL (6 mL)	**BL0001**					**BL0002**				**BL0003**
Review of warnings and precautions and / or contraindications for vaccinations	**X**		**X**	**X**				**X**		
Review conditions for withdrawal***	**X**		**X**	**X**				**X**		
Contact IRT system for randomization / allocation of subject number / vaccine assignment	**X**									
Contact IRT system for vaccine dose number for all vaccines to be given			**X**	**X**				**X**		
Vaccination with MenACYW conjugate vaccine	**X**			**X**				**X**		
Prevnar 13® vaccination†††	**X**		**X**	**X**				**X**		

Visit / Telephone Call	**Visit 1**	**TC1***	**Visit 2**	**Visit 3**	**TC2***	**Visit 4**	**TC3†**	**Visit 5**	**TC4***	**Visit 6**
Approximate Subject Age	**2 months‡**	**-**	**4 months**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
RotaTeq® vaccination†††	**X**		**X**	**X**						
Hexacima® vaccination†††	**X**		**X**	**X**				**X**		
M-M-R®II vaccinations†††								**X**		
Immediate surveillance (30 min)	**X**		**X**	**X**				**X**		
Diary Card (DC):										
Provided	**DC1**		**DC2**	**DC3**		**DC4**		**DC5**		
Reviewed			**DC1**	**DC2**		**DC3**		**DC4**		**DC5**
Collected			**DC1**	**DC2**		**DC3**		**DC4**		**DC5**
Telephone call		**X**			**X**		**X**		**X**	
Recording of solicited injection site and systemic reactions	**X**		**X**	**X**				**X**		
Recording of unsolicited AEs		**Reported from D0 to D30 after each vaccination visit**								
Reporting of SAEs / AESIs‡‡‡	**To be reported throughout the study period**									
Collection of reportable concomitant medications	**To be reported throughout the study period**									
Trial termination record										**X**

Abbreviations: V: Visit; TC: telephone call; D: Day; IRT: interactive response technology; AE: adverse event; SAE: serious adverse event; AESI: adverse event of special interest

*This call is made 8 days after the respective vaccinations. If Day 8 falls on a weekend or holiday, the telephone call may be made on the following business day. During this telephone call, the staff will find out whether the subject experienced any SAEs (including AESIs) not yet reported and will remind the subject's parent/guardian to continue using the diary card, to bring the diary card to the study center at the next visit, and confirm the date and time of the next visit.

†This call is made 14 days before V05 to remind the subject's parent / guardian of the forthcoming visit. If the subject's participation is discontinued due to withdrawal of / guardian consent or for other reasons, the staff will check over the phone to see if the subject received any medications or treatments or experienced any parental SAEs (including AESIs) that have not yet been reported to the study personnel and will make arrangements to retrieve the diary card.

‡"2 months" means from the 2nd month after birth to the day before the 3rd month after birth (2 months to 2 months 29 days).

§Medical history to include history of maternal immunization against tetanus.

**Physical examination to be performed as per routine standard of care. Temperature needs to be measured before each vaccination and recorded in the source documents. As a reminder, a temperature ≥38.0°C is a temporary contraindication. The vaccine should not be administered until the condition has resolved or the febrile event has subsided.

††Physical examination should be performed on the basis of relevant medical history at the time of the visit, according to the investigator's clinical judgment. Temperature needs to be measured before each vaccination and recorded in the source documents. As a reminder, a temperature ≥38.0°C is a temporary contraindication. The vaccine should not be administered until the condition has resolved or the febrile event has subsided

‡‡Should a subject receive oral or injectable antibiotic therapy within 3 days prior to the second blood draw, the investigator will postpone that blood draw until it has been 3 days since the subject last received oral or injectable antibiotic therapy. Postponement must still be within the timeframe for blood draw. If postponement would result in the sample collection falling outside of this timeframe, the blood sample should be collected without postponement, and it should be documented appropriately that the sample was taken less than 3 days after stopping antibiotic treatment

§§If vaccines are to be administered at the same visit, blood sample will be drawn prior to vaccinations.

***Conditions for withdrawal are listed in Section 5.2.8 of the protocol.

†††Routine vaccines should be administered in accordance with available official recommendations in Mexico.

‡‡‡AESIs will be collected throughout the trial, as SAEs, to ensure that the events are communicated to the Sponsor in an expedited manner and followed up until the end of the follow-up period or resolution, as per the assigned causality.

Table 3.4: Study procedure for the Group 2

Phase III Trial, Group 2 (Mexico): 6 Visits, 4 Vaccination Visits, 5 Telephone Calls, 3 Blood Samples, 11 Months Duration Per Subject for Subjects Randomized to Receive Menveo Vaccine

Visit / Telephone Call (TC)	**Visit 1**	**TC1***	**Visit 2**	**TC2***	**Visit 3**	**TC3***	**Visit 4**	**TC4†**	**Visit 5**	**TC5***	**Visit 6**
Approximate Subject Age	**2 months‡**	**-**	**4 months**	**-**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
Trial timelines (days, months)	D0	V01 +8 days	V01+60 days	V02 +8 days	V02 +60 days	V03 +8 days	V03 +30 days	V05 -14 days	V04 -5 months	V05 +8 days	V05 +30 days
Time windows (days)	NA	+2 days	+14 days	+2 days	+14 days	+2 days	+14 days	+2 days	+14 days	+2 days	+14 days
Informed consent form signed and dated	**X**										
Inclusion/exclusion criteria	**X**										
Collection of demographic data	**X**										
Medical history§	**X**										
Physical examination and temperature	**X****		**X††**		**X††**				**X††**		
Review of temporary contraindications for blood sampling (BL) ‡‡ §§	**X**						**X**				**X**
BL (6 mL)	**BL0001**						**BL0002**				**BL0003**

Visit / Telephone Call (TC)	**Visit 1**	**TC1***	**Visit 2**	**TC2***	**Visit 3**	**TC3***	**Visit 4**	**TC4†**	**Visit 5**	**TC5***	**Visit 6**
Approximate Subject Age	**2 months‡**	**-**	**4 months**	**-**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
Review of warnings and precautions and / or contraindications for vaccinations	**X**		**X**		**X**				**X**		
Review conditions for withdrawal***	**X**		**X**		**X**				**X**		
Contact IRT system for randomization / allocation of subject number / vaccine assignment	**X**										
Contact IRT system for vaccine dose number for all vaccines to be given			**X**		**X**				**X**		
Vaccination with Menveo®	**X**		**X**		**X**				**X**		
Prevnar 13® vaccination†††	**X**		**X**		**X**				**X**		
RotaTeq® vaccination†††	**X**		**X**		**X**						
Hexacima® vaccination†††	**X**		**X**		**X**				**X**		
M-M-R®II vaccinations†††									**X**		
Immediate surveillance (30 min)	**X**		**X**		**X**				**X**		
Diary Card (DC):											
Provided	**DC1**		**DC2**		**DC3**		**DC4**		**DC5**		
Reviewed			**DC1**		**DC2**		**DC3**		**DC4**		**DC5**
Collected			**DC1**		**DC2**		**DC3**		**DC4**		**DC5**
Telephone call		**X**		**X**		**X**		**X**		**X**	
Recording of solicited injection site and systemic reactions	**X**		**X**		**X**				**X**		
Recording of unsolicited AEs	**Collected from D0 to D30 after each vaccination visit**										
Reporting of SAEs / AESIs§§§	**To be reported throughout the study period**										
Collection of reportable concomitant medications	**To be reported throughout the study period**										
Trial termination record											**X**

Abbreviations: V: Visit; TC: telephone call; D: Day; IRT: interactive response technology; AE: adverse event; SAE: serious adverse event; AESI: adverse event of special interest

*This call is made 8 days after the respective vaccinations. If Day 8 falls on a weekend or holiday, the telephone call may be made on the following business day. During this telephone call, the staff will find out whether the subject experienced any SAEs (including AESIs) not yet reported and will remind the subject's parent/guardian to continue using the diary card, to bring the diary card to the study center at the next visit, and confirm the date and time of the next visit

†This call is made 14 days before V05 to remind the subject's parent / guardian of the forthcoming visit. If the subject's participation is discontinued due to withdrawal of / guardian consent or for other reasons, the staff will check over the phone to see if the subject received any medications or treatments or experienced any parental SAEs (including AESIs) that have not yet been reported to the study personnel and will make arrangements to retrieve the diary card.

‡"2 months" means from the 2nd month after birth to the day before the 3rd month after birth (2 months to 2 months 29 days).

§Medical history to include history of maternal immunization against tetanus.

**Physical examination to be performed as per routine standard of care. Temperature needs to be measured before each vaccination and recorded in the source documents. As a reminder, a temperature ≥38.0ºC is a temporary contraindication. The vaccine should not be administered until the condition has resolved or the febrile event has subsided.

††Physical examination should be performed on the basis of relevant medical history at the time of the visit, according to the investigator's clinical judgment. Temperature needs to be measured before each vaccination and recorded in the source documents. As a reminder, a temperature ≥38.0°C is a temporary contraindication. The vaccine should not be administered until the condition has resolved or the febrile event has subsided.

‡‡Should a subject receive oral or injectable antibiotic therapy within 3 days prior to the second blood draw, the investigator will postpone that blood draw until it has been 3 days since the subject last received oral or injectable antibiotic therapy. Postponement must still be within the timeframe for blood draw. If postponement would result in the sample collection falling outside of this timeframe, the blood sample should be collected without postponement, and it should be documented appropriately that the sample was taken less than 3 days after stopping antibiotic treatment.

§§If vaccines are to be administered at the same visit, blood sample will be drawn prior to vaccinations.

***Conditions for withdrawal are listed in Section 5.2.8 of the protocol.

†††Routine vaccines should be administered in accordance with available official recommendations in Mexico.

§§§AESIs will be collected throughout the trial, as SAEs, to ensure that the events are communicated to the Sponsor in an expedited manner and followed up until the end of the follow-up period or resolution, as per the assigned causality.

Table 3.5: Study procedure for the Group 3

Phase III Study, Group 3 (Russian Federation): 7 or 8 Visits, 5 Vaccination Visits, 5 Telephone Calls, 3 Blood Samples, 11 Months Duration per Subject for Subjects Randomized to Receive MenACYW Conjugate Vaccine

Visit / Telephone Call	**V0**	**V01**	**TC1***	**V02**	**TC2***	**V03**	**V04**	**TC3***	**V05**	**TC4†**	**V06**	**TC5***	**V07**
Approximate Subject Age	**2 months‡**	**2 months**	**-**	**3 months**	**-**	**4.5 months**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
Trial timeline (days, months)		Day 0 + 1 day	V01 +8 days	V01 +30 days	V02 +8 days	V02 +45 days	V03 +45 days	V04 +8 days	V04 +30 days	V06 -14 days	V05 +5 months	V06 +8 days	V06 +30 days

Visit / Telephone Call	**V0**	**V01**	**TC1***	**V02**	**TC2***	**V03**	**V04**	**TC3***	**V05**	**TC4†**	**V06**	**TC5***	**V07**
Approximate Subject Age	**2 months‡**	**2 months**	**-**	**3 months**	**-**	**4.5 months**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
Time windows (days)	NA	+4 days	+2 days	+14 days	+2 days	+14 days	+14 days	+2 days	+14 days	+2 days	+14 days	+ 2 days	+14 days
Informed consent form signed and dated	X	X											
Inclusion / exclusion criteria	X	X											
Collection of demographic data	X	X											
Medical history§	X	X											
Urine Sample, (8 mL)**	X												X
Physical examination & temperature	X	X††		X‡‡		X‡‡	X‡‡				X‡‡		
Review of temporary contraindications for Blood Sampling (BL)§§***	X								X				X
BL	BL0001 6 mL†††								BL0002 6 mL‡‡‡				BL0003 6 mL†††
Review of warnings and precautions and / or contraindications to vaccinations		X		X		X	X				X		
Review conditions for withdrawal§§§		X		X		X	X				X		

Visit / Telephone Call	**V0**	**V01**	**TC1***	**V02**	**TC2***	**V03**	**V04**	**TC3***	**V05**	**TC4†**	**V06**	**TC5***	**V07**
Approximate Subject Age	**2 months‡**	**2 months**	**-**	**3 months**	**-**	**4.5 months**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
Contact IRT system for randomization / allocation of subject number / vaccine assignment		X											
Contact IRT system for vaccine dose number for all vaccines to be given				X		X	X				X		
Vaccination with MenACYW conjugate vaccine				**X**			**X**				**X**		
ENGERIX B®**†††† vaccination**							**X**						
Prevnar 13® vaccination††† †		**X**				**X**							
Pentaxim® vaccination††† †				**X**		**X**	**X**						
MMR vaccination††† †											**X**		
Immediate surveillance (30 minutes)		X		X		X	X				X		
Telephone Call			X		X			X		X		X	
Diary Card (DC):													
Provided		DC1		DC2		DC3	DC4		DC5		DC6		

Visit / Telephone Call	**V0**	**V01**	**TC1***	**V02**	**TC2***	**V03**	**V04**	**TC3***	**V05**	**TC4†**	**V06**	**TC5***	**V07**
Approximate Subject Age	**2 months‡**	**2 months**	**-**	**3 months**	**-**	**4.5 months**	**6 months**	**-**	**7 months**	**-**	**12 months**	**-**	**13 months**
Reviewed				DC1		DC2	DC3		DC4		DC5		DC6
Collected				DC1		DC2	DC3		DC4		DC5		DC6
Collection of reportable medications	To be reported throughout the study period												
Recording of solicited injection-site and systemic reactions		X		X		X	X				X		
Recording of unsolicited AEs	Collected from D0 to D30 after each vaccination visit												
Reporting of SAEs / AESIs‡‡‡‡	To be reported throughout the study period												
Trial Termination													X

Abbreviations: V: Visit; TC: telephone call; D: Day; IRT: interactive response technology; AE: adverse event; SAE: serious adverse event; AESI: adverse event of special interest

*This call is made 8 days after the respective vaccinations. If Day 8 falls on a weekend or holiday, the telephone call may be made on the following business day. During this telephone call, the staff will find out whether the subject experienced any SAEs (including AESIs) not yet reported and will remind the subject's parent/guardian to continue using the diary card, to bring the diary card to the study center at the next visit, and confirm the date and time of the next visit

†This call is made 14 days before V06 to remind the subject's parent(s) of the forthcoming visit. If the subject's participation is discontinued due to withdrawal of parental / guardian consent or for other reasons, the staff will check over the phone to see if the subject received any medications or treatments or experienced any SAEs (including AESIs) that have not yet been reported to the study personnel and will make arrangements to retrieve the diary card.

‡"2 months" means from the 2nd month after birth to the day before the 3rd month after birth (2 months to 2 months 29 days).

§ Medical history to include history of maternal immunization against tetanus.

** The results of urine laboratory tests, performed for the subject in terms of routine practice, can be used, if the tests were done within 7 days before Visit 0. In this case, no urine sample will be collected at Visit 0.

††Physical examination to be performed as per routine standard of care. Temperature needs to be measured before each vaccination and recorded in the source documents.

‡‡ Physical examination should be performed on the basis of relevant medical history at the time of the visit, according to the investigator's clinical judgment. Temperature needs to be measured before each vaccination and recorded in the source documents. As a reminder, a temperature ≥37.0°C in the Russian Federation is a temporary contraindication. The vaccine should not be administered until the condition has resolved or the febrile event has subsided.

§§Should a subject receive oral or injectable antibiotic therapy within 3 days prior to the second blood draw, the investigator will postpone that blood draw until it has been 3 days since the subject last received oral or injectable antibiotic therapy. Postponement must still be within the timeframe for blood draw. If postponement would result

in the sample collection falling outside of this timeframe, the blood sample should be collected without postponement, and it should be documented appropriately that the sample was taken less than 3 days after stopping antibiotic treatment.

*** If vaccines are to be administered at the same visit, blood sample will be drawn prior to vaccinations.

††† The results of CBC and biochemistry laboratory tests, performed for the subject in terms of routine practice, can be used, if the tests were done within 7 days before Visit 0 and Visit 7. In this case, the blood sample volume collected at Visit 0 and Visit 7 will be 4 mL.

‡‡‡The minimal blood volume required for study immunogenicity objectives is 4 mL at Visit 0 and Visit 7 and 6 mL at Visit 5.

§§§Conditions for withdrawal are listed in Section 5.2.8 of the protocol.

****In the event ENGERIX B® cannot be supplied in the Russian Federation, a locally licensed monovalent hepatitis B vaccine will be administered instead. Further details will be provided in the CSR.

††††Routine vaccines should be administered in accordance with official recommendations in the Russian Federation. In the event M-M-R®II combination vaccine cannot be supplied or is unavailable in the Russian Federation, locally licensed MMR or MM+R vaccines will be administered instead. Further details will be provided in the CSR.

‡‡‡‡AESIs will be collected throughout the trial, as SAEs, to ensure that the events are communicated to the Sponsor in an expedited manner and followed up until the end of the follow-up period or resolution, as per the assigned causality.

Table 3.6: Study procedure for the Group 4

Phase III Study, Group 4 (Russian Federation): 7 or 8 Visits, 5 Vaccination Visits, 5 Telephone Calls, 3 Blood Samples, 11 Months Duration per Subject for Subjects Randomized to Receive Routine Pediatric Vaccines

Telephone Call	**V0**	**V01**	**TC1***	**V02**	**TC2***	**V03**	**V04**	**TC3***	**V05**	**TC4***	**V06**	**TC5†**	**V07**
Approximate Subject Age	**2 months‡**	**2 months**	**-**	**3 months**	**-**	**4.5 months**	**6 months**		**7 months**	**-**	**12 months**	**-**	**13 months**
Trial timeline (days, months)		Day 0 + 1 day	V01 +8 days	V01 +30 days	V02 +8 days	V02 +45 days	V03 +45 days	V04 +8 days	V04 +30 days	V06 -14 days	V05 +5 months	V06 +8 days	V06 +30 days
Time windows (days)	NA	+4 days	+2 days	+14 days	+2 days	+14 days	+14 days	+2 days	+14 days	+2 days	+14 days	+2 days	+14 days
Informed consent form signed and dated	X	X											
Inclusion / exclusion criteria	X	X											
Collection of demographic data	X	X											
Medical history§	X	X											
Urine Sample (8mL)**	X												X
Physical examination & temperature	X	X††		X		X‡‡	X‡‡				X‡‡		

Telephone Call	**V0**	**V01**	**TC1***	**V02**	**TC2***	**V03**	**V04**	**TC3***	**V05**	**TC4***	**V06**	**TC5†**	**V07**
Approximate Subject Age	**2 months‡**	**2 months**	**-**	**3 months**	**-**	**4.5 months**	**6 months**		**7 months**	**-**	**12 months**	**-**	**13 months**
Review of temporary contraindications for Blood Sampling (BL)§§***	X							X					X
BL	BL00016 mL†††								BL00026 mL‡‡‡				BL00036 mL†††
Review of warnings and precautions and / or contraindications to vaccinations		X		X		X	X				X		
Review conditions for withdrawal§§§		X		X		X	X				X		
Contact IRT system for randomization / allocation of subject number / vaccine assignment		X											
Contact IRT system for vaccine dose number for all vaccines to be given				X		X	X				X		
ENGERIX B® vaccination**†††							**X**						
Prevnar 13® vaccination†††		**X**				**X**							
Pentaxim® vaccination†††				**X**		**X**	**X**						
MMR vaccination†††											**X**		
Immediate surveillance (30 minutes)		X		X		X	X				X		
Telephone Call			X		X			X		X		X	
Diary Card (DC):													
Provided		DC1		DC2		DC3	DC4		DC5		DC6		
Reviewed				DC1		DC2	DC3		DC4		DC5		DC6

Telephone Call	V0	V01	TC1*	V02	TC2*	V03	V04	TC3*	V05	TC4*	V06	TC5†	V07
Approximate Subject Age	**2 months‡**	**2 months**	**-**	**3 months**	**-**	**4.5 months**	**6 months**		**7 months**	**-**	**12 months**	**-**	**13 months**
Collected				DC1		DC2	DC3		DC4		DC5		DC6
Collection of reportable concomitant medications	To be reported throughout the study period.												
Recording of solicited injection and systemic reactions		X		X		X	X				X		
Recording of Unsolicited AEs	Collected from D0 to D30 after each vaccination visit												
Reporting of SAEs / AESIs‡‡‡‡	To be reported throughout the study period												
Trial Termination													X

Abbreviations: V: Visit; TC: telephone call; D: Day; NA: not applicable; IRT: interactive response technology; AE: adverse event; SAE: serious adverse event; AESI: adverse event of special interest

*This call is made 8 days after the respective vaccinations. If Day 8 falls on a weekend or holiday, the telephone call may be made on the following business day. During this telephone call, the staff will find out whether the subject experienced any SAEs (including AESIs) not yet reported and will remind the subject's parent/guardian to continue using the diary card, to bring the diary card to the study center at the next visit, and confirm the date and time of the next visit.

†This call is made 14 days before V06 to remind the subject's parent(s) of the forthcoming visit. If the subject's participation is discontinued due to withdrawal of parental / guardian consent or for other reasons, the staff will check over the phone to see if the subject received any medications or treatments or experienced any SAEs (including AESIs) that have not yet been reported to the study personnel and will make arrangements to retrieve the diary card.

‡"2 months" means from the 2nd month after birth to the day before the 3rd month after birth (2 months to 2 months 29 days).

§Medical history to include history of maternal immunization against tetanus.

** The results of urine laboratory tests, performed for the subject in terms of routine practice, can be used, if the tests were done within 7 days before Visit 0. In this case, no urine sample will be collected at Visit 0.

††Physical examination to be performed as per routine standard of care. Temperature needs to be measured before each vaccination and recorded in the source documents.

‡‡Physical examination should be performed on the basis of relevant medical history at the time of the visit, according to the investigator's clinical judgment. Temperature needs to be measured before each vaccination and recorded in the source documents. As a reminder, a temperature ≥37.0°C in the Russian Federation is a temporary contraindication. The vaccine should not be administered until the condition has resolved or the febrile event has subsided.

§§Should a subject receive oral or injectable antibiotic therapy within 3 days prior to the second blood draw, the investigator will postpone that blood draw until it has been 3 days since the subject last received oral or injectable antibiotic therapy. Postponement must still be within the timeframe for blood draw. If postponement would result in the sample collection falling outside of this timeframe, the blood sample should be collected without postponement, and it should be documented appropriately that the sample was taken less than 3 days after stopping antibiotic treatment.

***If vaccines are to be administered at the same visit, blood sample will be drawn prior to vaccinations.

†††The results of CBC and biochemistry laboratory tests, performed for the subject in terms of routine practice, can be used, if the tests were done within 7 days before Visit 0 and Visit 7. In this case, the blood sample volume collected at Visit 0 and Visit 7 will be 4 mL.

‡‡‡The minimal blood volume required for study immunogenicity objectives is 4 mL at Visit 0 and Visit 7 and 6 mL at Visit 5.
§§§Conditions for withdrawal are listed in Section 5.2.8 of the protocol.
****In the event ENGERIX B® cannot be supplied in the Russian Federation, a locally licensed monovalent hepatitis B vaccine will be administered instead. Further details will be provided in the CSR.
††††Routine vaccines should be administered in accordance with available official recommendations in the Russian Federation. In the event M-M-R®II combination vaccine cannot be supplied or is unavailable in the Russian Federation, locally licensed MMR or MM+R vaccines will be administered instead. Further details will be provided in the CSR.
‡‡‡‡AESIs will be collected throughout the trial, as SAEs, to ensure that the events are communicated to the Sponsor in an expedited manner and followed up until the end of the follow-up period or resolution, as per the assigned causality.

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

4.1.1 Immunogenicity

4.1.1.1 Immunogenicity Endpoints

The primary endpoints for the evaluation of immunogenicity are:

- Meningococcal serogroups A, C, Y, and W antibody titers ≥ 1:8 measured by hSBA, assessed at 30 days after the last vaccination in the second year of life with MenACYW conjugate vaccine or Menveo® in Mexico (Group 1 and Group 2)
- Meningococcal serogroups A, C, Y, and W antibody titers ≥ 1:8 measured by hSBA assessed at 30 days after the last vaccination in the second year of life with MenACYW conjugate vaccine in the Russian Federation (Group 3)

4.1.1.2 Immunogenicity Assessment Methods

All assays will be performed at GCI, Swiftwater, Pennsylvania (PA) or at a qualified contract laboratory for GCI. The assay method to be used is summarized below. Laboratory technicians conducting the immunogenicity assays will be blinded to the group to which each subject was assigned.

Antibodies to meningococcal antigens (hSBA Method)

Functional meningococcal antibody activity against serogroups A, C, Y, and W will be measured in hSBA. Two-fold dilutions of test sera are prepared in sterile 96-well microtiter plates. Serogroup-specific meningococcal bacteria along with human complement are added to the serum dilutions and allowed to incubate. After this incubation period, an agar overlay medium is added to the serum/complement/bacteria mixture, allowed to harden, and then incubated overnight at 37°C with 5% carbon dioxide (CO_2). Bacterial colonies present in the wells are then counted. The endpoint titer is determined by the reciprocal serum dilution yielding ≥ 50% killing as compared to the mean of the complement control wells. The lower limit of quantitation (LLOQ) of the hSBA assay is a titer of 1:4.

This method will be performed on all samples collected from Groups 1, 2, and 3.

4.1.2 Safety

There are no primary objectives for safety.

4.2 Secondary Endpoints and Assessment Methods

4.2.1 Immunogenicity

4.2.1.1 Immunogenicity Endpoints

The secondary endpoints for the evaluation of immunogenicity are:

1) Meningococcal serogroups A, C, Y, and W antibody titers measured by hSBA, before the first vaccination (Visit 1) and 30 days after the last vaccination of the infant series with MenACYW conjugate vaccine or Menveo® (2nd dose of MenACYW conjugate vaccine and 3rd dose of Menveo® in Mexico (Group 1 and Group 2) (vaccine seroresponse[a])
2) Meningococcal serogroups A, C, Y, and W antibody titers measured by hSBA, before the first vaccination (Visit 1) and 30 days after the last vaccination of the infant series with MenACYW conjugate vaccine (2nd dose of MenACYW conjugate vaccine) in the Russian Federation (Group 3) (vaccine seroresponse)
3) The following serological endpoints will be described for Mexico (Groups 1 and 2):

- Day 0 (before the first vaccinations with Hexacima® and RotaTeq®):
  - Anti-pertussis antibody concentrations (PT and FHA)
  - Anti-rotavirus serum immunoglobulin (Ig) A antibody concentrations
- 30 days after the 6-months vaccinations with Prevnar 13® and RotaTeq®:
  - Anti-pneumococcal antibody concentrations for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
  - Anti-pneumococcal antibody concentrations (PCV13) ≥ 0.35 µg/mL and 1.0 µg/mL for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
  - Anti-rotavirus serum IgA antibody concentrations
  - Anti-rotavirus serum IgA antibody concentrations with ≥3-fold and ≥4-fold rise over baseline
- 30 days after the 12-months vaccinations with M-M-R®II, Prevnar 13®, and Hexacima®:
  - Antibody concentrations/titers for all antigens
  - Anti-pneumococcal antibody concentrations (PCV13) ≥ 0.35 µg / mL and 1.0 µg / mL for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
  - Anti-measles antibody concentrations (serostatus cutoff 255 mIU / mL)

[a] hSBA vaccine seroresponse for serogroups A, C, W, and Y is defined as:

- For a subject with a pre-vaccination titer < 1:8, the post-vaccination titer must be ≥ 1:16.
- For a subject with a pre-vaccination titer ≥ 1:8, the post-vaccination titer must be ≥ 4-fold greater than the pre-vaccination titer.

- Anti-mumps antibody concentrations (serostatus cutoff: 10 Mumps Ab units / mL)
- Anti-rubella antibody concentrations (serostatus cutoff: 10 IU / mL)
- Anti-tetanus antibody concentrations ≥ 0.1 IU / mL and 1.0 IU / mL
- Anti-diphtheria antibody concentrations ≥ 0.1 IU / mL and 1.0 IU / mL
- Anti-pertussis (PT and FHA) vaccine response[a]
- Anti-poliovirus types 1, 2, and 3 antibody titers ≥ 1:8
- Anti-PRP antibody concentrations ≥ 0.15 µg / mL and 1.0 µg / mL
- Anti-HBs concentrations ≥ 10 mIU / mL and 100 mIU / mL

The following serological endpoints will be described for the Russian Federation (Groups 3 and 4):

- Day 0 (before the first vaccination with Pentaxim®):
  - Anti-pertussis antibody concentrations (PT and FHA)
- 30 days after the 6-months vaccinations with Pentaxim® and ENGERIX-B®:
  - Antibody concentrations/titers for all antigens
  - Anti-tetanus antibody concentrations ≥ 0.1 IU / mL and 1.0 IU / mL
  - Anti-diphtheria antibody concentrations ≥ 0.1 IU / mL and 1.0 IU / mL
  - Anti-pertussis (PT and FHA) vaccine response
  - Anti-poliovirus types 1, 2, and 3 antibody titers ≥ 1:8
  - Anti-PRP antibody concentrations ≥ 0.15 µg / mL and 1.0 µg / mL
  - Anti-HBs concentrations ≥ 10 mIU / mL and 100 mIU / mL
- 30 days after the 12-months vaccination with MMR:
  - Antibody concentrations for measles, mumps and rubella
  - Anti-measles antibody concentrations (serostatus cutoff 255 mIU / mL)
  - Anti-mumps antibody concentrations (serostatus cutoff: 10 Mumps Ab units / mL)
  - Anti-rubella antibody concentrations (serostatus cutoff: 10 IU / mL)

4) The following serological endpoints will be assessed for Groups 1, 2, and 3:

- D0 (before first vaccination) for Group 1, Group 2, and Group 3:

[a] Pertussis vaccine response definition:

- If the pre-vaccination concentration is >= 4 x LLOQ, then the post-vaccination concentration is >= pre-vaccination concentration
- If the pre-vaccination concentration is < 4 x LLOQ, then the post-booster vaccination concentration is >= 4 x LLOQ

- hSBA meningococcal serogroups A, C, Y, and W antibody titers

- 30 days after the 6-month vaccination (after the 2nd dose) with MenACYW conjugate vaccine for Group 1 and Group 3:
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers
  - Titer distribution and reverse cumulative distribution curves (RCDCs)
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 1:4 and ≥ 1:8
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 4-fold rise from pre-vaccination (D0) to post-vaccination
- 30 days after the 6-month vaccination (after the 3rd dose) with Menveo vaccine for Group 2:
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers
  - Titer distribution and RCDCs
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 1:4 and ≥ 1:8
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 4-fold rise from pre-vaccination (D0) to post-vaccination
- 30 days after the 12-month vaccination (after the 3rd dose) with MenACYW conjugate vaccine for Group 1 and Group 3:
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers
  - Titer distribution and RCDCs
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 1:4 and ≥ 1:8
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 4-fold rise from pre-vaccination (D0) to post-vaccination
  - hSBA meningococcal serogroups A, C, Y, and W vaccine seroresponse
- 30 days after the 12-month vaccination (after the 4th dose) with Menveo vaccine for Group 2:
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers
  - Titer distribution and RCDCs
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 1:4 and ≥ 1:8
  - hSBA meningococcal serogroups A, C, Y, and W antibody titers ≥ 4-fold rise from pre-vaccination (D0) to post-vaccination
  - hSBA meningococcal serogroups A, C, Y, and W vaccine seroresponse

5) Meningococcal serogroups A, C, Y, and W antibody titers measured by rSBA, before the first vaccination (Visit 1) and 30 days after the last vaccination of the infant series with MenACYW conjugate vaccine or Menveo® (2nd dose of MenACYW conjugate vaccine and 3rd dose of Menveo®) in Mexico (Group 1 and Group 2)
6) Meningococcal serogroups A, C, Y, and W antibody titers measured by rSBA, before the first vaccination (Visit 1) and 30 days after the last vaccination of the infant series with

MenACYW conjugate vaccine (2nd dose of MenACYW conjugate vaccine) in the Russian Federation (Group 3)

7) Meningococcal serogroups A, C, Y, and W antibody titers measured by rSBA, before the first vaccination (Visit 1) and 30 days after the last vaccination in the second year of life with MenACYW conjugate vaccine or Menveo® in Mexico (Group 1 and Group 2)
8) Meningococcal serogroups A, C, Y, and W antibody titers measured by rSBA before the first vaccination (Visit 1) and 30 days after the last vaccination in the second year of life with MenACYW conjugate vaccine in the Russian Federation (Group 3)

4.2.1.2 Immunogenicity Assessment Methods

All assays will be performed at GCI, Swiftwater, Pennsylvania (PA) or at a qualified contract laboratory for GCI. The assay method to be used is summarized below. Laboratory technicians conducting the immunogenicity assays will be blinded to the group to which each subject was assigned.

Antibodies to meningococcal antigens (hSBA Method)

Functional meningococcal antibody activity against serogroups A, C, Y, and W will be measured in hSBA. Two-fold dilutions of test sera are prepared in sterile 96-well microtiter plates. Serogroup-specific meningococcal bacteria along with human complement are added to the serum dilutions and allowed to incubate. After this incubation period, an agar overlay medium is added to the serum/complement/bacteria mixture, allowed to harden, and then incubated overnight at 37°C with 5% carbon dioxide (CO_2). Bacterial colonies present in the wells are then counted. The endpoint titer is determined by the reciprocal serum dilution yielding ≥ 50% killing as compared to the mean of the complement control wells. The lower limit of quantitation (LLOQ) of the hSBA assay is a titer of 1:4.

This method will be performed on all samples collected from Groups 1, 2, and 3.

Antibodies to Meningococcal Antigens (rSBA Method)

Functional meningococcal antibody activity against serogroups A, C, Y, and W will be measured in an SBA utilizing baby rabbit complement. Two-fold dilutions of test sera are prepared in sterile 96-well microtiter plates. Serogroup-specific meningococcal bacteria along with baby rabbit complement are added to the serum dilutions and allowed to incubate. After this incubation period, 10 microliters (µL) of the serum/complement/bacteria mixture is removed and added to a blood agar plate using the tilt method, and then incubated overnight at 37°C with 5% CO_2. Bacterial colonies present on the blood agar plate are then counted. The bactericidal titer of each sample is expressed as the final reciprocal dilution yielding ≥ 50% killing as compared to the T60[a] (average number of bacteria in each control well after incubation) colony-forming unit.

The LLOQ of the rSBA assay is a titer of 1:4.

This method will be performed on all samples collected from a subset of subjects (100 subjects per group in Groups 1 and 3, and 50 subjects in Group 2).

[a] T60: Time of incubation duration of 60 minutes

Anti-Measles Antibodies

The purpose of the Bulk Measles IgG EIA (Enzyme Immunoassay) is to detect total IgG antibody to measles virus before and after vaccination with a measles-containing vaccine. Plates are coated in house using inactivated measles antigen that is bound to solid phase microtiter plates. The antigen is derived from Measles Edmonston strain-infected Vero cells. Serum or plasma is added to the coated plates and samples positive for measles antibodies will bind to the measles antigen-coated plates, forming antibody-antigen complexes. The bound antibody-antigen complexes can then be detected using an Alkaline Phosphatase labeled anti-human IgG. Color development occurs as a result of the addition of an enzyme-specific substrate Phenolphthalein Monophosphate. The color intensity is then measured spectrophotometrically with the highest intensity of color correlating to a high level of measles antibody and lowest color intensity correlating to low levels of measles antibody. Quantitation of the human IgG antibody to measles virus or titer is determined by comparison of the resulting optical density (OD) to a standard curve. The reference standard is a pool of human sera that has been calibrated against the WHO anti-measles reference standard, lot NIBSC 66/202. The concentration of anti-measles antibody in a sample is reported in milli-International Units per milliliter of serum (mIU/mL). The clinical endpoint for the measles assay is 255 mIU/mL and the LLOQ is 60 mIU/mL a sample is reported in milli-International Units per milliliter of serum (mIU/mL). The clinical endpoint for the measles assay is 255 mIU/mL and the LLOQ is 60 mIU/mL.

This method will be performed on BL0003 collected from all subjects in Mexico and in the Russian Federation.

Anti-Mumps Antibodies

The purpose of the mumps enzyme-linked immunosorbent assay (ELISA) is to detect IgG antibody to mumps virus before and after vaccination with a mumps virus-containing vaccine.The assay uses an earlier passage of the Jeryl Lynn® mumps virus (Jeryl Lynn® 135 [JL135],<12 passages) which is considered to be a wild-type (WT)-like strain. The reactivity of the sera to the mumps antigens prepared from uninfected Vero cells (denoted as tissue culture control [TCC] wells) is subtracted from that of JL135-infected Vero cells. JL135 mumps virus antigen or TCC is bound to solid phase microtiter plates and serum containing mumps antibody is added. The mumps antibody bound to the WT mumps antigen-coated plates forms an antibody-antigen complex. The bound antibody-antigen complex is then detected using an enzyme-labeled antihuman IgG. Color development occurs with the addition of a substrate and color intensity is measured spectrophotometrically. Results are obtained as a difference of the average duplicate of each optical density (OD) of JL135 mumps antigen wells and the average duplicate OD of TCC wells for each serum sample (noted as delta optical density [DOD]). Quantitation of the human IgG antibody to mumps virus, or antibody concentration, is determined by comparison of the resulting test DOD to a standard curve. The reference standard is an individual human serum. Results for the assay are reported as the concentration of antibody in Mumps antibody units/mL. The clinical endpoint and the LLOQ for the mumps assay is 10 Mumps Ab units/mL.

This method will be performed on BL0003 collected from all subjects in Mexico and in the Russian Federation.

Anti-Rubella Antibodies

The purpose of the Bulk Rubella IgG EIA (Enzyme Immunoassay) is to detect total IgG antibody to rubella virus before and after vaccination with a rubella-containing vaccine. Plates are coated in house using inactivated rubella antigen that is bound to solid phase microtiter plates. The antigen is derived from Rubella HPV-77 infected Vero cells. Serum is added to the coated plates and samples positive for rubella antibodies will bind to the rubella antigen-coated plates, forming antibody-antigen complexes. The bound antibody-antigen complexes can then be detected using an Alkaline Phosphatase labeled anti-human IgG. Color development occurs as a result of the addition of an enzyme-specific substrate, Phenolphthalein Monophosphate. The color intensity is then measured spectrophotometrically with the highest intensity of color correlating to a high level of rubella antibody and lowest color intensity correlating to low levels of rubella antibody.

Quantitation of the human IgG antibody to rubella virus or titer is determined by comparison of the resulting analysis OD to a standard curve. The reference standard is an individual human serum that has been calibrated against the WHO anti-rubella reference standard. The concentration of anti-rubella antibody in a sample is reported in International Units per milliliter of serum (IU/mL). The clinical endpoint for the rubella assay is 10 IU/mL and the LLOQ is 5 IU/mL

This method will be performed on BL0003 collected from all subjects in Mexico and in the Russian Federation.

Anti-Pneumococcal Antibodies

The pneumococcal capsular PS (PnPS) IgG Electrochemiluminscent (ECL) assay is used to quantitate the amount of anti-Streptococcus pneumoniae PS (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F) antibodies in human serum. In this method, purified antigen of 8 PnPS are coated into defined spots within the wells of a 96-well microtiter plate by MesoScale Discovery (MSD) using 3 types of plates to cover all 21 PnPS. Diluted serum samples (test samples, reference standard, and quality controls), pre-treated with pneumococcal cell wall absorbents (to reduce the interference of non-specific Antibodies in the assay), are incubated in the wells. Specific antibodies in the serum samples bind to the immobilized antigen. Unbound antibodies are washed from the wells, and SULFO-TAG-conjugated anti-human immunoglobulin is added. The antibody conjugate binds to the antigen-antibody complex. Excess conjugate is washed away, and read buffer is added. The plate is read using electrochemiluminescence on an MSD imager. The intensity of the generated light is proportional to the amount of specific antibody bound to the antigen-coated spots. An international reference standard assayed on each plate is used to calculate the amount of anti-pneumococcal IgG antibody (µg / mL) in human serum. The LLOQ for all PnPS serotypes is 0.15 µg/mL.

This method will be performed on BL0002 samples from all groups.

Anti-Diphtheria, Tetanus and Pertussis Antibodies

The DTP (Diphtheria, Tetanus and Pertussis) ECL is a multiplexed serological assay which allows for the simultaneous quantification of human antibodies to 6 specific antigens including Diphtheria Toxoid (DT), Tetanus Toxoid (TT), and 4 Pertussis antigens: PT, FHA, FIM and PRN. In this assay, each well of a 96-well microtiter plate is pre-coated in precise positions with the 6

different antigens in a multi-spot fashion. Following incubation with serum samples, antigen-specific antibodies bind to the respective antigens. The captured antibodies are then detected using a sulfotag conjugated anti-human IgG conjugate. Electrical stimulation of the conjugate in the presence of a chemiluminescent substrate results in the generation of a light signal from each specific spot that is captured by a camera in relative light units. The signal generated is directly proportional to the amount of antibodies present in the sample, which is quantified using software and based on an established reference standard sample curve. The LLOQ for Diphtheria is 0.005 IU/mL, the LLOQ for Tetanus is 0.01 IU/mL and the LLOQ for Pertussis antigens is 2.00 EU/mL.

This method will be performed on BL0003 samples collected from all subjects in Mexico. This method will be performed on BL0002 samples collected from all subjects in the Russian Federation. Only PT and FHA Pertussis antigens will be analyzed in this testing.

Anti-Polio (types 1, 2, and 3) Antibodies

Anti-poliovirus types 1, 2, and 3 will be measured by neutralization assay. Serial dilutions of sera are mixed with challenge poliovirus and incubated with cultured Vero cells that are sensitive to poliovirus. Specific neutralizing antibody contained in the sera bind to and neutralize the challenge poliovirus. The neutralized poliovirus does not affect cellular viability and these cells continue to metabolize and release CO_2, reducing the pH of the culture medium. Cell survival correlates with the change in the pH indicator (phenol red to yellow at pH < 7.0) contained in the medium. In the absence of neutralizing antibody, the challenge poliovirus reduces cellular metabolism and CO_2 production. Therefore, the pH does not decrease and a color change is not detected. The poliovirus mouse inoculation test (MIT) measures the functional serum antibody response to poliovirus by utilizing Vero cells (African green monkey kidney cells) and wild type poliovirus strains 1, 2, and 3 (Mahoney, MEF-1, and Saukett, respectively) as the challenge virus. The Karber method is used to determine the serum dilution that neutralized 50% of the challenge virus. Results are expressed as titers (1 / dilution). The LLOQ of the anti-poliovirus types 1, 2, and 3 assays is 4 (1 / dil).

This method will be performed on BL0003 samples collected from all subjects in Mexico. This method will be performed on BL0002 samples collected from all subjects in the Russian Federation.

Anti-*Haemophilus influenzae* type b *(Anti-PRP) Antibodies*

Anti-PRP concentrations will be measured using a Farr-type radioimmunoassay (RIA). Serum levels of anti-Hib Capsular PRP antibody are determined by RIA, in which serum samples are incubated with radiolabeled PRP (^{3}H-PRP) in the presence of ^{36}Cl (volume marker). Specific antibody binds to tritiated capsular PS to form antigen-antibody complexes. These complexes are precipitated with ammonium sulfate and collected by centrifugation. The radioactivity is measured in the precipitated pellet, in counts per minute and is proportional to the amount of anti-Hib capsular PS antibody present in the serum sample. The concentration of anti-PRP antibody in the serum sample is determined from the concentration response curve generated by the titration results of dilutions of the reference standard analyzed in the assay. Results are reported in µg/mL by comparison to the Center for Biologics Evaluation and Research, Lot No. 1983 reference standard. The LLOQ of the anti-PRP RIA is 0.06 µg / mL.

This method will be performed on BL0003 samples collected from all subjects in Mexico. This method will be performed on BL0002 samples collected from all subjects in the Russian Federation.

Anti-hepatitis B Antibodies

Anti-hepatitis B antibody will be measured by the commercially available VITROS ECi/ECiQ Immunodiagnostic System using chemiluminescence detection technology. The VITROS ECi Immunodiagnostic system uses an antibody mediated antigen sandwich formation to detect the presence of anti-HBs antigen total immunoglobulin in human serum. This involves the reaction of anti-HBs antigen in the sample with plasma-derived HBs antigen (ad and ay subtypes) coated onto the wells. A horseradish peroxidase (HRP)-labeled HBs antigen conjugate (ad and ay subtypes) then complexes with the bound anti-hepatitis Bs (HepBs), forming an antigen sandwich. Substrate is then added which catalyzes HRP, producing light. The light signals are read by the VITROS ECi/ECiQ. Immunodiagnostic System and the amount of HRP conjugate bound is directly proportional to the concentration of anti-HepBs Antibodies present in the sample. Results are reported in milli-international units (mIU) / mL by comparison to a calibrator provided by the manufacturer that has been calibrated according to the World Health Organization (WHO) First International Reference Preparation for antibody to HBs antigen (1977). The LLOQ is 5 mIU / mL.

This method will be performed on BL0003 samples collected from all subjects in Mexico. This method will be performed on BL0002 samples collected from all subjects in the Russian Federation.

Anti-Rotavirus IgA Antibodies

Anti-rotavirus IgA Antibodies in human serum will be measured by ELISA. Microtiter plates are coated with rabbit anti-rotavirus antibody and then viral lysate (positive wells) or control cell lysate (negative wells) is added. Diluted serum samples (test samples, reference standard, and quality controls) are incubated in the wells. Unbound antibodies are washed from the wells, and enzyme-conjugated anti-human IgA is added. The enzyme conjugate binds to the antigen-antibody complex. Excess conjugate is washed away and a specific colorimetric substrate is added. Bound enzyme catalyzes a hydrolytic reaction, which causes color development. A reference standard assayed on each plate is used to calculate the amount of specific anti-rotavirus IgA antibody in the units assigned by the reference standard (units [U] / mL of serum).

This method will be performed on BL0002 samples collected from all subjects in Mexico.

The priority of titration for subjects receiving MenACYW conjugate vaccine (Group 1) or Menveo® vaccine (Group 2), and DTaP-IPV-HB-Hib, Rotavirus, PCV13 vaccines is as follows: hSBA, anti-PT, anti-FHA, anti-Hep B, anti-PRP, anti-polio 1, 2, and 3, anti-tetanus, and anti-diphtheria, anti-pneumococcal serotypes 1, 3, 5, 6A, 7F, 19A, 4, 6B, 9V, 14, 18C, 19F, and 23F and anti-Rotavirus (IgA).

The priority of titration for subjects receiving MenACYW conjugate vaccine (Group 1) or Menveo® vaccine (Group 2), and DTaP-IPV-HB-Hib Measles, Mumps, Rubella, PCV13 vaccines is as follows: hSBA, anti-PT, anti-FHA, anti-Hep B, anti-PRP, anti-polio (types 1, 2, and 3) anti-tetanus, anti-diphtheria, anti-measles, anti-mumps, anti-rubella, anti-pneumococcal serotypes 1, 3, 5, 6A, 7F, 19A, 4, 6B, 9V, 14, 18C, 19F, and 23F.

For subjects receiving MenACYW conjugate vaccine and DTaP-IPV//Hib vaccine and hepatitis B vaccine (Group 3) the priority of titrations is as follows: hSBA, anti-PT, anti-FHA, anti-PRP, anti-polio 1, 2, and 3, anti-tetanus, anti-diphtheria and anti-HepB.

For subjects receiving DTaP-IPV//Hib and hepatitis B vaccines only (Group 4), the priority of titrations is as follows: anti-PT, anti-FHA, anti-PRP, anti-polio 1, 2, and 3, anti-tetanus, and anti-diphtheria and anti-Hep B.

For subjects receiving PCV-13 vaccine only (Group 4), the priority of titrations is as follows: anti-pneumococcal serotypes 1, 3, 5, 6A, 7F, 19A, 4, 6B, 9V, 14, 18C, 19F, and 23F.

For subjects receiving MenACYW conjugate vaccine, and MMR vaccine (Group 3), the priority of titrations is as follows: hSBA, anti-measles, anti-mumps, anti-rubella.

For subjects receiving MMR vaccine only (Group 4), the priority of titrations is as follows: anti-measles, anti-mumps, anti-rubella.

4.2.2 Efficacy

No clinical efficacy data will be obtained in the study.

4.2.3 Safety

There are no secondary objectives for safety.

4.3 Observational Endpoints and Assessment Methods

4.3.1 Immunogenicity

There are no observational objectives for immunogenicity in this study.

4.3.2 Safety

4.3.2.1 Safety Definitions

The following definitions are taken from the International Conference on Harmonization ICH E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

Adverse Event (AE):

An AE is any untoward medical occurrence in a patient or in a clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Therefore, an AE may be:

- A new illness
- The worsening of a pre-existing condition
- An effect of the vaccination, including the comparator
- A combination of the above
- All AEs include serious and non-serious AEs.
- Surgical procedures are not AEs; they are the actions taken to treat a medical condition. It is the condition leading to the action taken that is the AE (if it occurs during the study period).

Pre-existing medical conditions are not to be reported as AEs. However, if a pre-existing medical condition worsens following study interventions in frequency or intensity, or if according to the Investigator there is a change in its clinical significance, this change should be reported as an AE (exacerbation). This applies equally to recurring episodes of pre-existing conditions (e.g., asthma) if the frequency or intensity increases post-vaccination.

Serious Adverse Event (SAE):

Serious and *severe* are not synonymous. The term *severe* is often used to describe the intensity of a specific event as corresponding to Grade 3. This is not the same as *serious* which is based on subject / event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations.

An SAE is any untoward medical occurrence that at any dose

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability / incapacity
- Is a congenital anomaly / birth defect
- Is an important medical event (IME)

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as IMEs that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the health of the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These IMEs should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse, new-onset diabetes, or autoimmune disease.

Adverse Reaction:

All noxious and unintended responses to a medicinal product related to any dose should be considered ARs.

(The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility).

The following additional definitions are used by Sanofi Pasteur:

Immediate Event/Reaction:

Immediate events are recorded to capture medically relevant unsolicited systemic AEs (including those related to the product administered) that occur within the first 30 minutes after vaccination.

Solicited Reaction:

1) A solicited reaction is an "expected" AR (sign or symptom) observed and reported under the conditions (nature and onset) prelisted in the protocol and CRB.
2) By definition, solicited reactions are to be considered as being related to the product administered.

For injectable vaccines, solicited reactions can either be solicited injection site reactions or solicited systemic reactions.

The assessment of these reactions by the investigator is mandatory.

Unsolicited AE / AR:

An unsolicited AE is an observed AE that does not fulfill the conditions prelisted in the CRB in terms of diagnosis and/or onset window post-vaccination. For example, if vomiting between D0 and D7 is a solicited reaction (ie, prelisted in the protocol and CRB), then a vomiting starting on D7 is a solicited reaction, whereas vomiting starting on D8 post-vaccination is an unsolicited AE. Unsolicited AEs includes both serious (SAEs) and non-serious unsolicited AEs.

Injection Site Reaction:

An injection site reaction is an AR at and around the injection site. Injection site reactions are commonly inflammatory reactions. They are considered to be related to the product administered.

Systemic AE:

Systemic AEs are all AEs that are not injection or administration site reactions. They therefore include systemic manifestations such as vomiting, fever, as well as localized or topical

manifestations that are not associated with the vaccination or administration site (e.g., erythema that is localized but that is not occurring at the injection site).

Adverse Event of Special Interest (AESI):

An AESI is an event for which ongoing monitoring and rapid communication by the Investigator to the Sponsor must be done.

Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the study Sponsor to other parties (e.g., regulators) might also be warranted.

4.3.2.2 Safety Endpoints

The observational endpoints for the evaluation of safety are:

- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, relationship to vaccination, and whether the event led to early termination from the study, of any unsolicited systemic AEs reported in the 30 minutes after each vaccination
- Occurrence, time of onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited (prelisted in the subject's diary card and CRF) injection site reactions occurring up to D07 after each vaccination
- Occurrence, time of onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited (prelisted in the subject's diary card and CRF) systemic reactions occurring up to D07 after each vaccination
- Occurrence, nature (MedDRA preferred term), time of onset, duration, intensity, action taken, relationship to vaccination, and whether the event led to early termination from the study, of unsolicited AEs up to D30 after each vaccination
- Occurrence, nature (MedDRA preferred term), time of onset, duration, seriousness criteria, relationship to vaccination, outcome, and whether the event led to early termination from the study, of SAEs (including AESIs) throughout the trial from D0 to the last study visit**Safety Assessment Methods**

At each vaccination visit, the Investigator or a delegate will perform a physical examination on the basis of relevant medical history according to the Investigator's clinical judgment and will ask the parent / guardian about any solicited reactions and unsolicited AEs recorded in the diary card, as well as about any other AEs that may have occurred since the previous visit. All relevant data will be transcribed into the CRB according to the instructions provided by the Sponsor.

4.3.2.3.1 Immediate Post-vaccination Observation Period

Subjects will be kept under observation for 30 minutes after each vaccination to ensure their safety. The post-vaccination observation should be documented in the source document. Any AE that occurs during this period will be noted on the source document and recorded in the CRB, as follows:

- Unsolicited systemic AEs will be recorded as immediate AEs in the CRB (presence marked as "yes" and details collected).
- Solicited and unsolicited injection site reactions and solicited systemic reactions will be recorded in the CRB in the same way as any reactions starting on the day of vaccination.
- SAEs will be recorded in the CRB and reported to the Sponsor in the same way as any other SAEs.

4.3.2.3.2 Reactogenicity (Solicited Reactions from Day 0 to Day 7 After Each Meningococcal Vaccination)

After the first vaccination, subject's parents / guardians will be provided with a diary card, a digital thermometer, and a flexible ruler, and will be instructed how to use them. The following items will be recorded by the subjects in the diary card on the day of vaccination and for the next 7 days (i.e., D0 to D7) until resolution:

- Daily temperature, with the route by which it was taken
- Daily measurement or intensity grade of all other solicited injection site and systemic reactions
- Action taken for each event (e.g., medication)

  The action(s) taken by the parent or guardian to treat and/or manage any **solicited reactions** will be classified in the CRB using the following list (all applicable items should be checked):

  - None
  - Medication
  - Health care provider contact
  - Hospitalized
  - Discontinuation of study vaccination
- Whether the AE was related to the investigational product (for injection site events linked to routine pediatric vaccines)

  The Investigator will assess the causal relationship between the AE and the investigational product as either "Not related" or "Related".

Subject's parents / guardians will be contacted by telephone 8 days after vaccination visit, to remind them to record all safety information in the diary card.

If the timing of the telephone call should fall on a weekend or a holiday, the call should be made on the next business day. If contact is not made on the designated day, study staff will continue calling until contact is made. Every telephone attempt and its outcome will be documented in the source document.

Table 4.1 and Table 4.2, respectively, present the injection site reactions and systemic reactions that are prelisted in the diary cards and CRB, together with the intensity scales.

Table 4.3: Solicited injection site reactions: terminology, definitions, and intensity scales

CRB term (MedDRA lowest level term [LLT])	Injection site tenderness	Injection site erythema	Injection site swelling
(MedDRA preferred term)	Injection site pain	Injection site erythema	Injection site swelling
Diary card term	Tenderness	Redness	Swelling
Definition	Pain when the injection site is touched or injected limb mobilized	Presence of a redness including the approximate point of needle entry	Swelling at or near the injection site Swelling or edema is caused by a fluid infiltration in tissue or cavity and, depending on the space available for the fluid to disperse, swelling may be either soft (typically) or firm (less typical) to touch and thus can be best described by looking at the size of the swelling
Intensity scale*	Grade 1: Minor reaction when injection site is touched Grade 2: Cries or protests when injection site is touched Grade 3: Cries when injected limb is mobilized, or the movement of the injected limb is reduced	Grade 1: > 0 to < 25 mm Grade 2: ≥ 25 to < 50 mm Grade 3: ≥ 50 mm	Grade 1: > 0 to < 25 mm Grade 2: ≥ 25 to < 50 mm Grade 3: ≥ 50 mm

* For the subjective reaction of tenderness, parents /guardians will record the intensity level (Grade 1, 2, or 3) in the diary card. For the measurable reactions of redness and swelling, they will record just the size of the reaction, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis.

Table 4.4: Solicited systemic reactions: terminology, definitions, and intensity scales

CRB term (MedDRA LLT)	Fever	Vomiting	Crying abnormal	Drowsiness	Appetite lost	Irritability
Data analysis term (MedDRA preferred term)	Pyrexia	Vomiting	Crying	Somnolence	Decreased appetite	Irritability
Diary card term	Temperature	Vomiting	Abnormal crying	Drowsiness	Loss of appetite	Irritability

Definition	Elevation of temperature to ≥°38.0°C* (≥ 100.4°F)	Vomiting does not include spitting up	Inconsolable crying without a determined reason	Reduced interest in surroundings, or increased sleeping	See intensity scale	An excessive response to stimuli: increased fussiness, whining, and fretfulness despite attempts to comfort the infant and despite caregiver responses that would normally be soothing
Intensity scale*	Grade 1: ≥ 38.0°C to < 38.5°C **or** ≥ 100.4°F to < 101.3°F	Grade 1: 1 episode per 24 hours	Grade 1: < 1 hour	Grade 1: Sleepier than usual or less interested in surroundings	Grade 1: Eating less than normal	Grade 1: Easily consolable
	Grade 2: ≥ 38.5°C to < 39.5°C **or** ≥ 101.3°F to < 103.1°F	Grade 2: 2–5 episodes per 24 hours	Grade 2: 1–3 hours	Grade 2: Not interested in surroundings or did not wake up for a feed / meal	Grade 2: Missed 1 or 2 feeds / meals completely	Grade 2: Requiring increased attention
	Grade 3: ≥ 39.5°C **or** ≥ 103.1°F	Grade 3: ≥ 6 episodes per 24 hours or requiring parenteral hydration	Grade 3: > 3 hours	Grade 3: Sleeping most of the time or difficult to wake up	Grade 3: Refuses ≥ 3 feeds / meals or refuses most feeds / meals	Grade 3: Inconsolable

* For the Russian Federation, febrile illness is defined as temperature ≥ 37°C). A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided.

† For all reactions but fever, parents / guardians will record the intensity level (Grade 1, 2, or 3) in the diary card. For fever, they will record the body temperature, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis based on the unit used to measure the temperature and the intensity scale.

Important notes for the accurate assessment of temperature:

Parents / guardians are to measure body temperature once per day, preferably always at the same time. The optimal time for measurement is the evening, when body temperature is the highest. Temperature is also to be measured at the time of any apparent fever. The observed daily temperature and the route of measurement are to be recorded in the diary card and the highest temperature will be recorded by the site in the CRB. The preferred route for this study is axillary. Pre-vaccination temperature is also systematically collected by the investigator on the source document. Tympanic thermometers must not be used.

4.3.2.3.3 Unsolicited Adverse Events

In addition to recording solicited reactions, parents / guardians will be instructed to record any other medical events that may occur during the 30-day period following vaccination. Local reactions will be collected after all vaccinations. Space will be provided in the diary card for this purpose.

Information on SAEs will be collected and assessed throughout the study, from the time of vaccination to 30 days after the last vaccination or study visit. Any SAE occurring at any time during the study will be reported by the Investigator in the CRB according to the completion instructions provided by the Sponsor; this includes checking the "Serious" box on the AE CRF and completing the appropriate Safety Complementary Information CRFs. All information concerning the SAE is to be reported either as part of the initial reporting or during follow-up reporting if relevant information became available later (e.g., outcome, medical history, results of investigations, copy of hospitalization reports in case a subject experiences febrile convulsion (neurological event associating fever and seizure), the assessment will be performed according to the "Guideline for definition and collection of cases of febrile convulsion," and this event will be considered an SAE.

For each unsolicited AE (whether serious or non-serious), the following information is to be recorded:

- Start and stop date[a]

[a] The stop date of all related AEs will be actively solicited. For other events, the investigator will provide the stop date when it becomes available. AEs for which no stop date was obtained during the course of the study will be considered as ongoing at the end of the study.

- Intensity of the event:
  - For measurable unsolicited AEs that are part of the list of solicited reactions, the size of the AE as well as the temperature for fever will be collected and analyzed based on the corresponding scale used for solicited reactions.
  - All other unsolicited AEs will be classified according to the following intensity scale:
    - Grade 1: A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
    - Grade 2: A type of adverse event that is usually alleviated with additional therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
    - Grade 3: A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.
- Whether the AE was related to the investigational product (for unsolicited systemic AEs)

  The Investigator will assess the causal relationship between the AE and the investigational product as either “Not related” or “Related”.
- Action taken for each AE (e.g., medication)

  The action(s) taken by the parent or guardian to treat and/or manage any unsolicited AEs will be classified in the CRB using the following list (all applicable items should be checked):
  - None
  - Medication
  - Health care provider contact
  - Hospitalized
  - Discontinuation of study vaccination
- Whether the AE was serious

  For each SAE, the investigator will complete all seriousness criteria that apply (outcome, elapsed time, and relationship to study procedures)

- Whether the AE caused study discontinuation

4.3.2.3.4 Adverse Events of Special Interest

An AESI is defined as event for which ongoing monitoring and rapid communication by the Investigator to the Sponsor must be done. The following AEs will be captured as AESIs throughout the study:

- Generalized seizures (febrile and non-febrile)
- Kawasaki disease
- Guillain-Barré syndrome
- Idiopathic thrombocytopenic purpura (ITP)

These events have been listed as AESIs based on the feedback received from the European Union regulators.

No safety concerns relating to these AESIs have been identified with the use of MenACYW conjugate vaccine in the completed clinical trials. Because of their medical importance and to ensure expedited communication to the Sponsor, these AESIs are to be considered and collected as SAEs and reported to the Sponsor according to the procedure described in Section 10 of the study protocol.

4.3.2.3.5 Assessment of Causality

The Investigator will assess the ***causal relationship*** between each unsolicited systemic AE and the product administered as either ***not related*** or ***related***, based on the following definitions:

Not related – The AE is clearly / most probably caused by other etiologies such as an underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the AE is incompatible with a causal relationship; or the AE started before the first vaccination (screening phase, if applicable)

Related – There is a "reasonable possibility" that the AE was caused by the product administered, meaning that there is evidence or arguments to suggest a causal relationship

Note: By convention, all AEs reported at the injection site (whether solicited or unsolicited) and all solicited systemic AEs are considered to be related to the administered product and therefore are referred to as reactions and do not require the Investigator's opinion on relatedness.

Adverse events likely to be related to the product, whether serious or not, that persist at the end of the study will be followed up by the Investigator until their complete disappearance or the stabilization of the subject's condition. The Investigator will inform the Sponsor of the date of final disappearance of the event or the date of "chronicity" establishment.

4.4 Derived Endpoints: Calculation Methods

4.4.1 Safety

4.4.1.1 Solicited Reactions

4.4.1.1.1 Daily Intensity

All daily records for solicited reactions will be derived into daily intensity according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing (Unknown).

For the derivation of daily intensities, the following sequential steps will be applied:

1) Solicited reactions (except Fever/Pyrexia) with CRF presence recorded as "No" and with all daily records missing (Unknown) then all daily intensities will be derived as None.
2) For non-measurable solicited reactions, daily intensities will correspond to daily records reported in the clinical database. For measurable solicited reactions the daily measurements reported in the clinical database will be converted based upon the intensity scales defined in the protocol; this assumes a reaction that is too large to measure (non-measurable, "NM") is Grade 3. Note the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator

Note: The maximum intensity on the ongoing period is derived from the record of the maximum intensity/measurement after the end of the solicited period following the rule described above.

4.4.1.1.2 Maximum Intensity

Maximum overall intensity is derived from the daily intensities computed as described in Section 4.4.1.1.1 of the protocol and is calculated as the maximum of the daily intensities over the period considered.

4.4.1.1.3 Presence

Presence is derived from the maximum overall intensity over the time period considered:

- None: No presence
- Grade 1, Grade 2, or Grade 3: Presence
- Missing or Unknown: Missing presence

Participants with at least one non-missing presence for a specific endpoint will be included in the analysis. Conversely, those without a non-missing presence will not be included in the analysis of the endpoint.

The time period is displayed as D0-D3, D4-D7, D8 and later.

4.4.1.1.4 Time of Onset

Time of onset is derived from the daily intensities computed as described in Section 4.4.1.1.1 It corresponds to the first day with intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (i.e., reaction occurs over two separate periods of time intervened by at least one daily intensity Missing or None) then the time of onset is the first day of the first occurrence.

4.4.1.1.5 Number of Days of Occurence During the Solicited Period

Number of days of occurrence over the period considered is derived from the daily intensities computed as described in Section 4.4.1.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. Number of days of occurrence on the solicited period with a specified intensity may also be derived.

4.4.1.1.6 Overall Number of Days of Occurrence

If a reaction is ongoing at the end of the solicited period, then the overall number of days of occurrence is derived from the daily intensities and the end date of the reaction after the end of the solicited period.

The overall number of days of presence is:

> (End date – last vaccination date) + (number of days of occurrence within the solicited period) – length of the solicited period + 1

If the end date is missing or incomplete (contains missing data), the overall number of days of occurrence will be considered as Missing.

4.4.1.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed as described in Section 4.4.1.1.1 and the maximum intensity on the ongoing period. The investigator's ongoing flag is not used because the measurement would determine the ongoing status of the reaction.

- Ongoing: if the last daily intensity of the solicited period is at least Grade 1 and the maximum intensity on the ongoing period is at least Grade 1
- Not ongoing: if the last daily intensity of the solicited period is None or the maximum intensity on the ongoing period is None.
- Missing: all other conditions (in this case, it is not included in the denominator of the ongoing analysis in the safety tables)

4.4.1.2 Unsolicited AEs

Unsolicited AEs includes both serious (SAEs) and non-serious unsolicited AEs.

4.4.1.2.1 Presence

An observation will be considered an event if it has at least a verbatim term and is not a Grade 0 intensity event.

Grade 0 events are not included in safety analysis but are included in separate listings.

4.4.1.2.2 Intensity

Intensity will be defined according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

If the unsolicited AE is measurable and its preferred term is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule of the intensity scales defined in the **Error! Reference source not found.** and **Error! Reference source not found.** for that measurable injection site or systemic reaction. Note the intensity could be considered as "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement >0 mm but < 25 mm in adults).

Intensity for the other unsolicited AEs will correspond to the value reported in the CRF.

The maximum intensity corresponds to the highest intensity for a unique term.

4.4.1.2.3 Last Vaccination

Last vaccination before an unsolicited AE is derived from the start date of the unsolicited AE provided in the CRF and is calculated as follows:

- If an unsolicited AE has a complete start date and different to any of the vaccination dates, the start date is used to determine the last vaccination before the unsolicited AE
- If the start date is missing or partially missing, or equal to any vaccination date, then the visit number in the "Appeared after Visit" or similar field, is used to determine the last vaccination before the unsolicited AE.

4.4.1.2.4 Time of Onset

Time of onset is derived from the start date of the unsolicited AE and the date of last vaccination as described in Section **Error! Reference source not found.**:

Time of Onset = start date of the unsolicited AE – date of last vaccination before the unsolicited AE

The time of onset is considered as missing only if one or both of the dates are missing or partially missing.

The unsolicited AEs will be analyzed "Within 30 days" after each vaccination, which corresponds to AEs with a time of onset between 0 and 30 days after vaccination or missing. An AE with missing time of onset will be considered to have occurred just after the last vaccination (computed according to the section **Error! Reference source not found.**), so will be included in these tables.

Time of onset period is displayed as D0-D3, D4-D7, D8-D14, D15 or later, and Missing.

Note: Unsolicited AE that occurred before vaccination (negative time of onset) or with a time of onset higher than defined above will not be included in analysis but will be listed separately.

4.4.1.2.5 Duration

Duration is derived from the start and end dates of the unsolicited AE:

Duration = End date of unsolicited AE - start date of unsolicited AE + 1.

The duration is considered as missing only if one or both of the start and end dates of the unsolicited AE is missing or partially missing.

4.4.1.3 Serious Adverse Events

An event will be considered as a serious event if "Yes" is checked for "Serious" in the CRF.

SAEs will be analyzed throughout the study using the following periods:

- Within 7 days after vaccination
- Within 30 days after vaccination
- During the study (i.e., all SAEs occurred during the study)

4.4.1.4 Adverse Events of Special Interest

An event will be considered as an AESI if "Yes" is checked for "Is the event an AESI?" in the CRF.

AESIs will be analyzed throughout the study using the following periods:

- Within 7 days after vaccination
- Within 30 days after vaccination

During the study (i.e., all SAEs occurred during the study)

4.4.1.5 Other Safety Endpoints

4.4.1.5.1 Action Taken

This information will be summarized as collected, including missing observations. No derivation or imputation will be done.

4.4.1.5.2 Seriousness

This information will be summarized as collected for SAEs. No derivation or imputation will be done.

4.4.1.5.3 Outcome

This information will be summarized as collected for Unsolicited non-serious AEs and SAEs. No derivation or imputation will be done.

4.4.1.5.4 Causal relationship

By convention, all events reported at the injection site (either solicited or unsolicited) will be considered as related to the administered product and then referred to as reactions. In a same way, all solicited systemic events pre-listed in

the CRF are also considered as related to vaccination and will be considered as reactions.

- For unsolicited systemic AE, missing relationship to IMP will be considered as related to study vaccines at the time of analysis.
- The missing relationship to study procedures for SAEs will not be imputed.

4.4.1.5.5 Adverse Events Leading to Study Discontinuation

This information will be summarized as collected. A flag is available in the clinical database for all AEs in order to identify AEs leading to discontinuation before the end of active phase.

In general, the items that are counted are:

- Disposition table: A participant who, on the "Completion at End of Study" form question "What was the participant's status?" has "Adverse Event" checked.
- Safety overview table: A participant who has either on the "Completion at End of Study" form, question" What was the participant's status?" has "Adverse Event" checked or lists a solicited AE that has "Caused Study Termination" checked that is at least Grade 1 or an unsolicited AE that has "Caused Study Discontinuation" checked that is at least Grade 1 or missing and is within the time period indicated.
- System Organ Class (SOC)/Preferred Term (PT) table: A solicited AE that has "Caused Study Termination" checked that is at least Grade 1 or an unsolicited AE that has "Caused Study Discontinuation" checked that is at least Grade 1 or missing and is within the time period indicated.

4.4.2 Immunogenicity

4.4.2.1 Computed Values at a specific Blood Sample

In order to appropriately manage extreme values (undetectable responses < the lower limit of quantitation [LLOQ] and ≥ the upper limit of quantitation [ULOQ]) for analysis purposes, the following computational rule is applied to the values provided in the clinical database for each blood sample drawn:

- If a value is < LLOQ, then use the computed value LLOQ/2
- If a value is between ≥ LLOQ and < ULOQ, then use the value
- If a value is ⩾ ULOQ, then use the computed value ULOQ

4.4.2.2 Seroprotection

hSBA vaccine seroprotection is defined as: hSBA titers ≥ 1:8.

4.4.2.3 Fold-rise

The derived endpoint fold-rise is driven by both baseline and post-baseline computed values and is computed as follows. Generally, for extreme values, this algorithm minimizes the numerator and maximizes the denominator.

- If the baseline computed value is < LLOQ and the post-baseline computed value is < LLOQ, then the fold-rise is 1
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is ≥ LLOQ, then the fold-rise is post-baseline computed value / baseline computed value
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is < LLOQ, then the fold-rise is (LLOQ/2) / baseline computed value
- If the baseline computed value is < LLOQ and the post-baseline computed value is ≥ LLOQ, then the fold-rise is post-baseline computed value /LLOQ

Note: If baseline or post-baseline is missing, then fold-rise is missing.

4.4.2.4 A/C/Y/W Vaccine Seroresponse

hSBA vaccine seroresponse for serogroups A, C, Y, and W is defined as either:

- Post-vaccination hSBA titers ≥ 1:16, if pre-vaccination hSBA titers < 1:8 or
- At least a 4-fold increase in hSBA titers from pre- to post-vaccination, if pre-vaccination hSBA titers ≥ 1:8.

4.4.3 Derived Other Variables

4.4.3.1 Age for Demographics

The age of a subject in the study was the calendar age in day at the time of inclusion.

4.4.3.2 Subject Duration

The duration of a subject in the study is computed as follows: Maximum (date of last visit, date of term form) – (date of Visit 1) +1.

The duration of a subject in the study including follow-up is computed as follows: Maximum (date of last visit, date of term form, last date of follow-up contact) – (date of Visit 1) +1.

4.4.3.3 Duration of the Study

The duration of the study (until last visit) is computed as follows: Maximum of all subjects (date of last visit, date of termination form) – minimum for all subjects (date of Visit 1) +1.

The duration of the study (including follow-up) is computed as follows: Maximum of all subjects (date of last visit, date of termination form, date of last follow-up contact) – minimum for all subjects (date of visit V01) +1

5 Statistical Methods and Determination of Sample Size

The statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics platform using SAS® Version 9.4 software or later.

The results of the statistical analysis will be available in the final clinical study report (CSR).

The CI for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (1), i.e., using the inverse of the beta integral with SAS®.

For immunogenicity and efficacy results, assuming that Log10 transformation of the titers / data follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log10 (titers / data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide geometric means (GMs) and their 95% CI.

For descriptive purposes, the following statistics will be presented:

Table 5.1: Descriptive statistics produced

Baseline characteristics and follow-up description	Categorical data	Number of subjects. Percentage of subjects.
	Continuous data	Mean, standard deviation, quartiles, minimum, and maximum.
Clinical safety results	Categorical data	Solicited: Number and percentage (95% CIs) of subjects. Unsolicited: Number and percentage (95% CIs) of subjects, and number of events.
Immunogenicity results	Categorical data (seroprotection, seroconversion, cutoff)	Number and percentage (95% CIs) of subjects.
	Continuous data (titer / data†)	Log10: Mean and standard deviation. Anti-Log10 (work on Log10 distribution, and anti-Log10 applied): Geometric mean, 95% CI of the geometric mean, quartiles, minimum, and maximum. Graphical representation by Reverse Cumulative Distribution Curve (RCDC).

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Primary Objective

5.1.1.1 Hypotheses

No hypotheses will be tested. Descriptive statistics will be presented.

5.1.1.2 Statistical Methods

Descriptive analyses on meningococcal serogroups A, C, Y, and W measured by hSBA for Groups 1, 2, and 3, 30 days after the last vaccination in the second year

of life with MenACYW conjugate vaccine or Menveo® will be computed on the following parameter:

- Percentage of subjects with titer ≥ 1:8 and 95% CI

The 95% CI will be computed using the exact binomial distribution (Clopper-Pearson method).

5.1.2 Hypotheses and Statistical Methods for Secondary Objective

5.1.2.1 Hypotheses

No hypotheses will be tested. Descriptive statistics will be presented.

5.1.2.1.1 Statistical Methods for Secondary Objectives 1, 2 and 4

Descriptive analyses on meningococcal serogroups A, C, Y, and W measured by hSBA, for Groups 1, 2, and 3, before the first vaccination, 30 days after the last vaccination of the infant series with MenACYW conjugate vaccine or Menveo® (2nd dose of MenACYW conjugate vaccine and 3rd dose of Menveo®) and 30 days after the last vaccination in the second year of life will include but not limited to the following:

- GMT and 95% CI
- Titer distribution and RCDCs
- Percentage of subjects with titer ≥ 1:4 and ≥ 1:8 and 95% CI
- Percentage of subjects with titer ≥ 4-fold rise from pre-vaccination to post-infant vaccination, and 95% CI
- Percentage of subjects with titer ≥ 4-fold rise from pre-vaccination to post-12-month vaccination, and 95% CI
- Percentage of subjects with hSBA vaccine seroresponse[a] and 95% CI

In general, categorical variables will be summarized and presented by frequency counts, percentages, and CIs. The 95% CIs of point estimates will be calculated using the normal approximation for quantitative data and the exact binomial distribution (Clopper-Pearson method) for proportions. For GMTs and geometric mean concentrations (GMCs), 95% CIs of point estimates will be calculated using a normal approximation assuming they are log-normally distributed.

5.1.2.1.2 Statistical Methods for Secondary Objectives 5, 6, 7, 8

Descriptive analysis on meningococcal serogroups A, C, Y, and W measured by rSBA before the first vaccination, 30 days after the last vaccination of the infant

series (2nd dose of the MenACYW conjugate vaccine and 3rd dose of Menveo®), and 30 days after the last vaccination of the second year of life with MenACYW conjugate vaccine or Menveo® in a subset of subjects (100 subjects per group in Groups 1 and 3, and 50 subjects in Group 2) will include but not limited to the following parameters:

- GMT and 95% CI
- Titer distribution and RCDCs
- Percentage of subjects with titer ≥ 1:8 and ≥ 1: 128 and 95% CI
- Percentage of subjects with titer ≥ 4-fold rise from pre-vaccination to post-vaccination, and 95% CI
- Percentage of subjects with rSBA vaccine seroresponse and 95% CI

5.1.2.1.3 Statistical Methods for Secondary Objective 3

The analyses on the concomitant vaccines will include GMT and titer distribution or GMC, and RCDC, as well as percentage of subjects with:

The Russian Federation[a]:

- 30 days after vaccination with MMR vaccine at 12 months of age
    - Anti-measles antibody concentrations (serostatus cutoff: 255: mlIU/mL).
    - Anti-mumps antibody concentrations (serostatus cutoff: 10 Mumps ab units/mL).
    - Anti-rubella antibody concentrations (serostatus cutoff: 10 IU/mL).
- Before the first vaccination with Pentaxim®
    - Anti-pertussis (PT and FHA) antibody concentrations
- 30 days after the last vaccination with Pentaxim® at 6 months of age:
    - Anti-tetanus antibody concentrations ≥ 0.1 IU / mL and 1.0 IU / mL
    - Anti-diphtheria antibody concentrations ≥ 0.1 IU / mL and 1.0 IU / mL

[a] Anti-pneumococcal antibody concentrations will not be determined in this study for the Russian Federation.

- Anti-pertussis (PT and FHA) antibody concentrations (vaccine response)
- Anti-poliovirus types 1, 2, and 3 antibody titers ≥ 1:8
- Anti-PRP antibody concentrations and ≥ 0.15 µg / mL and 1.0 µg / mL

- 30 days after vaccination with ENGERIX-B®[a] at 6 months of age
  - Anti-HBs antigen concentrations ⩾ 10 mIU / mL and ⩾ 100 mIU / mL

Mexico:

- 30 days after vaccination with M-M-R®II at 12 months of age
  - Anti-measles antibody concentrations (serostatus cutoff: 255 mIU/mL)
  - Anti-mumps antibody concentrations (serostatus cutoff: 10 Mumps Ab units/mL)
  - Anti-rubella antibody concentrations (serostatus cutoff: 10 IU/mL)
- 30 days after vaccination with Prevnar 13® at 6 months of age and the last vaccination with Prevnar 13® at 12 months of age
  - Anti-pneumococcal antibody concentrations ≥ 0.35 µg/mL and 1.0 µg/mL for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
- Before the first vaccination with Hexacima®
  - Anti-pertussis[b] (PT and FHA) antibody concentrations
- 30 days after the last vaccination with Hexacima® vaccine at 12 months of age

[a] In the event ENGERIX B® cannot be supplied in the Russian Federation, a locally licensed monovalent hepatitis B vaccine will be administered instead. Further details will be provided in the CSR.

[b] Pertussis vaccine response is defined as:

- If the pre-vaccination concentration is < 4 x LLOQ, then the post-vaccination concentration is ≥ 4 x LLOQ;
- If the pre-vaccination concentration is ≥ 4 x LLOQ, then the post vaccination concentration is ≥ the pre-vaccination concentration

- Anti-tetanus antibody concentrations ≥ 0.1 IU/mL and 1.0 IU/mL
- Anti-diphtheria antibody concentrations ≥ 0.1 IU / mL and 1.0 IU / mL
- Anti-pertussis (PT and FHA) antibody concentrations and pertussis vaccine response
- Anti-poliovirus types 1, 2, and 3 antibody titers ≥ 1:8
- Anti-PRP antibody concentrations and ≥ 0.15 µg / mL and 1.0 µg / mL
- Anti-HBs antigen concentrations ≥ 10 mIU / mL and ≥ 100 mIU / mL

- Before the first vaccination and 30 days after the last vaccination with RotaTeq® at 6 months of age
  - Anti-RV IgA ≥ 3-fold and ≥ 4-fold antibody titers from baseline to 30 days after last dose

5.1.3 Statistical Methods for Observational Objectives

No hypotheses will be tested. Descriptive statistics will be presented. Safety results will be described for subjects in all study groups. The main parameters for the safety endpoints will be described by 95% CI using the exact binomial method (Clopper-Person method) (1).

5.1.4 Complementary analysis

5.1.4.1 Sensitivity Analysis due to COVID-19 Pandemic

The impact of COVID-19 pandemic situation on study conduction will be summarized through impact on visit procedures, study completion and major/critical protocol deviations due to COVID-19.

The subjects impacted by COVID-19 pandemic situation will be defined as the subjects with at least one major/critical protocol deviation due to COVID-19 or who did not complete the study due to COVID-19. If more than 10% of subjects are impacted as per this definition, baseline and demographics characteristics, and the main immunogenicity and safety endpoints will also be summarized in the subsets of subjects impacted/ non-impacted subjects to assess the potential impact of COVID-19 situation on study outcome.

5.1.4.2 Subgroup analysis

Additional subgroup analyses by gender and race based on PPAS will be provided for primary and main secondary immunogenicity endpoints.

The gender subgroup analyses will have two categories (Female and Male), and the race subgroup analyses will have four categories (White, Black, Asian, and Other).

The following parameters will be assessed 30 days after the last vaccination in the second year and 30 days after the last vaccination in infant series:

- hSBA GMTs with 95% CI
- Percentage of subjects with hSBA titer ≥ 1:8 and 95% CI
- Percentage of subjects with hSBA vaccine seroresponse and 95% CI

The safety overview will be also described by race and gender

5.2 Analysis Sets

5.2.1 Per-Protocol Analysis Set

The per-protocol analysis set (PPAS) is a subset of the FAS. Immunogenicity analyses will primarily be performed on the per-protocol analysis sets. There will be 2 PPAS corresponding to the 2 FAS:

- PPAS for infant vaccination (PPAS1)
- PPAS for 2nd year of life vaccination (PPAS2)

5.2.1.1 Per-Protocol Analysis Set 1 (PPAS1)

Serology obtained from the last blood sample of infancy vaccination stage for all antigens will be used for immunogenicity analyses of infant stage of the study.

The subjects presenting with at least one of the following relevant protocol deviations during infancy will be excluded from the PPAS1:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not complete the vaccination schedule
- Subject received a vaccine other than the one that he / she was randomized to receive

- Preparation and / or administration of vaccine was not done as per-protocol
- Subject did not receive vaccine in the proper time window, according to the Tables of Study Procedures. All infant stage vaccines (including concomitant vaccines) need to be received as scheduled in the Table of Study Procedures.
- Subject did not provide post-dose serology sample during infancy in the proper time window or a post-dose serology sample was not drawn
- Subject received a protocol-prohibited therapy, medication or vaccine

In addition to the reasons listed above, subjects will also be excluded from the PPAS1 if their infancy post vaccination serology sample did not produce a valid test result (ie, results for all antigens are missing).

In the event of a local or NIC with a pandemic influenza or coronavirus vaccine or any other vaccine as needed, subjects who receive 1 or more doses of the pandemic influenza or coronavirus vaccine at any time during the study will not be withdrawn from the study.

5.2.1.2 Per-Protocol Analysis Set 2 (PPAS2)

Serology obtained from the last blood sample of 2nd year of life for all antigens will be used for immunogenicity analyses of 2nd year of the study.

The subjects presenting with at least one of the following relevant protocol deviations during 2nd year of life will be excluded from the PPAS2:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not complete the vaccination schedule including the infant and the 2nd year of the study
- Subject received a vaccine other than the one that he / she was randomized to receive during both the infant and 2nd year of the study
- Preparation and / or administration of vaccine was not done as per-protocol during both the infant and 2nd year of the study
- Subject did not receive vaccine in the proper time window. All 2nd year of life vaccines (including concomitant vaccines) need to be received as scheduled in the Table of Study Procedures.
- Subject did not provide post-dose serology sample during infancy in the proper time window or a post-dose serology sample was not drawn during 2nd year of life

- Subject received a protocol-prohibited therapy, medication or vaccine

In addition to the reasons listed above, subjects will also be excluded from the PPAS2 if their 2nd year of life post vaccination serology sample did not produce a valid test result (ie, results for all antigens are missing).

In the event of a local or NIC recommendation or requirement with a pandemic influenza or coronavirus vaccine or any other vaccine as needed, subjects who receive 1 or more doses of the pandemic influenza or coronavirus vaccine at any time during the study will not be withdrawn from the study.

5.2.2 Full Analysis Set

There will be 2 full analysis sets (FAS) for this study:

- FAS for infant vaccination (FAS1). The full analysis set 1 (FAS1) is defined as the subset of all randomized subjects who received at least 1 dose of the study vaccine in infancy (<12 months of age) and have a valid post-vaccination serology result in infancy. All subjects will be analyzed according to the treatment group to which they were randomized.
- FAS for 2nd year of life vaccination (FAS2). The full analysis set 2 (FAS2) is defined as the subset of all randomized subjects who received at least 1 dose of the study vaccine in the 2nd year of life (≥12 months of age) and have a valid post-vaccination serology result in the 2nd year of life. All subjects will be analyzed according to the treatment group to which they were randomized.

Immunogenicity analyses will be performed on the FAS for exploratory purposes.

5.2.3 Safety Analysis Set

The safety analysis set (SafAS) is defined as those subjects who have received at least 1 dose of the study vaccine and have any safety data available.

All subjects will have their safety analyzed after each dose according to the vaccine they actually received at that dose. For ‘any dose’ safety analyses, all subjects will have their safety analyzed according to the vaccine received at the first dose.

Safety data recorded for a vaccine received out of protocol design will be excluded from the analysis (and listed separately).

5.2.3.1 Overall Safety Analysis Set for Any Dose

The overall safety analysis set (SafAS) is defined as those subjects who have received at least 1 dose of the study vaccines and have any safety data available.

All subjects will have their safety analyzed after any dose according to the vaccine received at the first dose.

Safety data recorded for a vaccine received out of the protocol design will be excluded from the analysis (and listed separately).

5.2.3.2 Safety Analysis Set for Vaccination at 2 Months of Age

The safety analysis set 1 for vaccination at around 2 months of age (SafAS1) is defined as those subjects who have received the study vaccine at Visit 1 around 2 months of age and have any safety data available. All subjects will have their safety analyzed after the Visit 1 dose according to the vaccines they actually received at Visit 1.

Safety data recorded for a vaccine received out of the protocol design at Visit 1 will be excluded from the analysis (and listed separately).

5.2.3.3 Safety Analysis Set for Vaccination at 3 Months of Age for Russia Only

The safety analysis set 2 (SafAS2) is defined as those subjects who have received the study vaccine at Visit 2 at around 3 months of age for Group 3 and Group 4 in Russia and have any safety data available. Subjects will have their safety analyzed after this dose according to the vaccines they actually received at Visit 2.

Safety data recorded for a vaccine received out of the protocol design at Visit 2 will be excluded from the analysis (and listed separately).

5.2.3.4 Safety Analysis Set for Vaccination at 4 Months of Age for Mexico only

The safety analysis set 3 (SafAS3) is defined as those subjects who have received the study vaccine at Visit 2 around 4 months of age (Group 1 and Group 2) and have any safety data available. Subjects will have their safety analyzed after this dose according to the vaccines they actually received at that visit.

Safety data recorded for a vaccine received out of the protocol design at that visit will be excluded from the analysis (and listed separately).

5.2.3.5 Safety Analysis Set for Vaccination at 4.5 months of Age for Russia Only

The safety analysis set 4 (SafAS4) is defined as those subjects who have received the study vaccine at Visit 3 for Group 3 and Group 4 in Russia at around 4.5 months of age and have any safety data available. All subjects will have their safety

analyzed after this dose according to the vaccines they actually received at that visit.

Safety data recorded for a vaccine received out of the protocol design at that visit will be excluded from the analysis (and listed separately).

5.2.3.6 Safety Analysis Set for Vaccination at 6 Months of Age

The safety analysis set 5 (SafAS5) is defined as those subjects who have received the study vaccine at Visit 3 (or Visit 4 for Group 3 and Group 4) at around 6 months of age and have any safety data available. All subjects will have their safety analyzed after this dose according to the vaccines they actually received at that visit.

Safety data recorded for a vaccine received out of the protocol design at that visit will be excluded from the analysis (and listed separately).

5.2.3.7 Safety Analysis Set for Vaccination at 12 Months of Age

The safety analysis set 6 (SafAS6) is defined as those subjects who have received the study vaccine at Visit 5 (or Visit 6 for Group 3 and Group 4) at around 12 months of age and have any safety data available. All subjects will have their safety analyzed after this dose according to the vaccine they actually received at that visit.

Safety data recorded for a vaccine received out of the protocol design at that visit will be excluded from the analysis (and listed separately).

5.2.4 Populations Used in Analyses

All immunogenicity analyses will be performed on the Per-Protocol Analysis Set (PPAS) including PPAS1 and PPAS2. Additional immunogenicity analyses will be performed for exploratory purposes on the Full Analysis Set (FAS), including FAS1 and FAS2, according to randomization group. All safety analyses will be performed on the Safety Analysis Set (SafAS and SafAS1 – SafAS6).

5.3 Handling of Missing Data and Outliers

5.3.1 Safety

No replacement will be done.

5.3.2 Immunogenicity

Missing data will not be imputed. No test or search for outliers will be performed.

In order to appropriately manage extreme values (undetectable responses < LLOQ and ≥ upper limit of quantitation [ULOQ]), the following computational rule is applied to the values provided in the clinical database for each blood sample drawn for analysis purposes:

- If a value is < LLOQ, then use the computed value LLOQ / 2
- If a value is between ≥ LLOQ and < ULOQ, then use the value
- If a value is ≥ ULOQ, then use the computed value ULOQ

The derived endpoint of fold-rise is computed as follows for extreme values, to minimize the numerator and maximizes the denominator:

- If the baseline computed value is < LLOQ and the post-baseline computed value is < LLOQ then the fold-rise is 1
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is ≥ LLOQ then the fold-rise is post-baseline computed value / baseline computed value
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is < LLOQ then the fold-rise is (LLOQ / 2) / baseline computed value
- If the baseline computed value is < LLOQ and the post-baseline computed value is ≥ LLOQ then the fold-rise is post-baseline computed value / LLOQ

5.3.3 Efficacy

No efficacy data will be collected.

5.4 Interim / Preliminary Analysis

No interim / preliminary analyses are planned.

5.5 Determination of Sample Size and Power Calculation

The sample size of this study was chosen to provide immunogenicity and safety data; it is not intended for the purposes of hypothesis testing. No formal sample size calculations will be performed.

Though there are no statistically powered hypotheses, the overall study cohort (n=525) will provide a probability of approximately 95% of observing any AE with a true incidence of 0.57%. The overall MenACYW conjugate vaccine cohort (n=350) will provide a probability of approximately 95% of observing any AE with a true incidence of 0.85%. In treatment arm with n=200, there is a probability of

approximately 95% of observing any AE with a true incidence of 1.5%. In treatment arm with n=150, there is a probability of approximately 95% of observing any AE with a true incidence of 2%.

In case of unexpected situations or any study hold resulting in an unexpected number of unevaluable subjects, total sample size may be increased to replace withdrawn, or unevaluable subjects.

5.6 Data Review for Statistical Purposes

A blind review of the data is anticipated through the data review process led by Data Management before database lock. The safety of the investigational product will be continuously monitored by the Sponsor. Periodic safety data review will be performed by the Sponsor's SMT.

5.7 Changes in the Conduct of the Trial or Planned Analyses

No significant change occurred during the conduct of the trial not documented in a protocol amendment.

Changes in Planned Analysis:

Due to delay in the release of PRP and rSBA immunogenicity data, the statistical analysis will be performed in 2 steps as follow:

-A first statistical analysis of the safety and immunogenicity data (except PRP and rSBA data) will be done following a database lock with provision. All data included at this stage will be final and won't be modified in later analyses

-A final statistical analysis will be performed including PRP and rSBA data after final database lock

Serostatus cutoff value for measles antigen contained in MMR vaccine was corrected to 255 mIU/mL instead of 225 mIU/mL as wrongly reported in the protocol.

6 References List

1 Newcombe R.G., Two-sided confidence intervals for the single proportion: comparison of seven methods, Statistics in Medicine, (1998) 17, 857-872