NCT Number: NCT02955797

Immunogenicity and Safety of an Investigational Quadrivalent Meningococcal Conjugate Vaccine in Toddlers 12 to 23 Months of Age

Phase III, modified double-blind, randomized, parallel-group, active-controlled, multi-center trial to compare the immunogenicity and describe the safety of a single dose of MenACYW conjugate vaccine to a single dose of a licensed quadrivalent meningococcal serogroups A, C, W-135, and Y tetanus toxoid conjugate vaccine (MenACWY-TT) in toddlers in the European Union who are either meningococcal vaccine naïve or received MenC vaccination during infancy.

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	MET51
Development Phase:	Phase III
Sponsor:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
Investigational Product:	MenACYW conjugate vaccine: Meningococcal Polysaccharide (Serogroups A, C, Y, and W) Tetanus Toxoid Conjugate Vaccine
Form / Route:	Liquid solution / Intramuscular
Indication For This Study:	MenACYW conjugate vaccine as a single dose in toddlers aged 12 to 23 months
Version and Date of the SAP core body part:	Version 1.0, 13APR2018

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List of Abbreviations

AE	adverse event
[redacted]	[redacted]
BL	blood sample
CI	confidence interval
CRF	electronic case report form
D	day
FAS	full analysis set
GM	geometric mean
GMT	geometric mean titers
GMTR	geometric mean titer ratio
hSBA	serum bactericidal assay using human complement
IMD	invasive meningococcal disease
IU	international unit
LLOQ	lower limit of quantitation
MD	missing data
PPAS	per-protocol analysis set
PT	preferred term
RCDC	reverse cumulative distribution curve
rSBA	serum bactericidal assay using baby rabbit complement
SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SOC	system organ class (primary)
ULOQ	upper limit of quantitation

1 Introduction

This trial will evaluate the immunogenicity and safety of a single dose of the quadrivalent Meningococcal Polysaccharide (Serogroups A, C, Y and W) Tetanus Toxoid Conjugate Vaccine (hereafter referred to as MenACYW conjugate vaccine) in toddlers 12 to 23 months of age who are either meningococcal vaccine naïve or have a background of vaccination with monovalent MenC vaccines during infancy.

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 > 65 years of age) against invasive meningococcal disease (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 vaccine, Menactra®, the MenACYW conjugate vaccine is prepared by 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 monovalent MenC conjugate vaccines are made from capsular polysaccharide that has been extracted from cultures of capsular group C *Neisseria meningitidis*. The polysaccharide is linked (conjugated) to a carrier protein, either CRM_{197} (a non-toxic variant of diphtheria toxin) or tetanus toxoid. The conjugation increases the immunogenicity, especially in young children in whom the plain polysaccharide vaccines are less immunogenic. The vaccination schedule and the number of vaccinations vary across the EU countries. Depending on the country, an infant may have received 1 to 2 doses of meningococcal C conjugate vaccine during the first year of life.

The purpose of MET51 is to evaluate the quadrivalent meningococcal conjugate vaccine when used as a single-dose toddler vaccine in individuals who are either meningococcal vaccine naïve or have received one or more doses of monovalent MenC vaccines during infancy. The study will aim to demonstrate non-inferior immunogenicity of MenACYW conjugate vaccine versus Nimenrix® and evaluate the safety of 1 dose of MenACYW conjugate vaccine compared to 1 dose of Nimenrix® in toddlers 12 to 23 months of age who are either meningococcal vaccine naïve or had received monovalent MenC vaccination during infancy.

2 Trial Objectives

2.1 Primary Objectives

1) To demonstrate the non-inferiority of the antibody response to meningococcal serogroups A, C, Y, and W after a single dose of MenACYW conjugate vaccine or Nimenrix® in toddlers who

either are meningococcal vaccine naïve or have received monovalent MenC vaccination during infancy

2) To demonstrate the non-inferiority of the antibody response to meningococcal serogroups A, C, Y, and W after a single dose of MenACYW conjugate vaccine or Nimenrix® in meningococcal vaccine naïve toddlers

2.2 Secondary Objectives

1) To compare the antibody responses (geometric mean titers [GMTs]) to meningococcal serogroups A, C, Y, and W after a dose of MenACYW conjugate vaccine or Nimenrix® as measured by serum bactericidal assay using human complement (hSBA) in toddlers who either are meningococcal vaccine naïve or have received monovalent MenC vaccination during infancy
2) To compare the antibody responses (GMTs) to meningococcal serogroups A, C, Y, and W after a dose of MenACYW conjugate vaccine or Nimenrix® as measured by hSBA in meningococcal vaccine naïve toddlers
3) To compare the antibody responses (GMTs) to meningococcal serogroups A, C, Y, and W after a dose of MenACYW conjugate vaccine or Nimenrix® as measured by hSBA in toddlers who received monovalent MenC vaccination during infancy

2.3 Observational Objectives

Immunogenicity

- To describe the antibody response to meningococcal serogroups A, C, Y, and W before and 30 days (+14 days) after a dose of MenACYW conjugate vaccine or Nimenrix® in terms of serum bactericidal assay using baby rabbit complement (rSBA) titers ≥1:8 and ≥ 1:128 in toddlers in a subset of subjects per group:
    - Group 1 and Group 2: 100 subjects each
    - Group 3: 50 subjects in each subgroup (MenC-TT or MenC-CRM primed subjects)
    - Group 4: 25 subjects in each subgroup (MenC-TT or MenC-CRM primed subjects)
- To describe the antibody response to meningococcal serogroups A, C, Y, and W before and 30 days (+14 days) after vaccination with MenACYW conjugate vaccine in toddlers
- To describe the antibody responses to the meningococcal serogroups A, C, Y, and W before and 30 days (+14 days) after vaccination with Nimenrix® in toddlers
- To describe the antibody responses to the meningococcal serogroups A, C, Y, and W before and 30 days (+14 days) after vaccination with MenACYW conjugate vaccine or Nimenrix® in toddlers who received monovalent MenC vaccine conjugated to the tetanus toxoid carrier protein during infancy
- To describe the antibody responses to the meningococcal serogroups A, C, Y, and W before and 30 days (+14 days) after vaccination with MenACYW conjugate vaccine or Nimenrix® in

toddlers who received monovalent MenC vaccine conjugated to the CRM_{197} protein carrier during infancy

Safety

To evaluate the safety profile of MenACYW conjugate vaccine and Nimenrix®.

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

This was a Phase III, modified double-blind, randomized, parallel-group, active-controlled, multi-center trial to compare the immunogenicity and describe the safety of a single dose of MenACYW conjugate vaccine to a single dose of a licensed quadrivalent meningococcal polysaccharide groups A, C, W-135, and Y conjugate vaccine, Nimenrix®, in toddlers (12 to 23 months of age) in the EU who are either meningococcal vaccine naïve or received monovalent MenC vaccination during infancy.

Approximately 918 healthy toddlers aged 12 to 23 months were to be enrolled and randomized as follows depending on the meningococcal background:

Meningococcal Vaccine-Naïve Subjects: 612 subjects were to be randomized in a 1:1 ratio to the following 2 groups:

- Group 1: MenACYW conjugate vaccine (n=306)
- Group 2: Nimenrix® (n=306)

MenC-Primed Subjects: 306 subjects were to be randomized in a 2:1 ratio to the following 2 groups:

- Group 3: MenACYW conjugate vaccine (n=204)
- Group 4: Nimenrix® (n=102)

Enrollment of MenC-primed subjects was stratified by the type of primed vaccine, MenC-TT (NeisVac-C®) or MenC-CRM (Menjugate®, Meningitec®), considering that at least 25% and a maximum of 50% of subjects had been primed with MenC-CRM as described in Table 3.1 below:

Table 3.1: MenC priming strategy

Priming	Group 3 MenACYW conjugate vaccine	Group 4 Nimenrix®
MenC-TT	102 - 152*	51 - 76*
MenC-CRM	52* - 102	26* - 51

Total	204	102

*Sample size corresponding to 25% of subjects primed with MenC-CRM

All subjects were to provide blood samples for immunogenicity assessment at baseline (pre-vaccination) and at 30 to 44 days post-vaccination.

Solicited adverse event (AE) information were to be collected for 7 days after vaccination, unsolicited AE information were to be collected from Visit (V) 01 (Day [D] 0) to V02 (D30 [+14 days]), and serious adverse event (SAE) information, [redacted] were to be collected throughout the trial.

3.2 Trial Plan

Eligible subjects were identified and recruited.

A schedule of assessments and study vaccinations is provided in Table 3.2.

Vaccination

All subjects were to receive a single dose of either MenACYW conjugate vaccine or Nimenrix® on D0 (V01).

Blood Sampling

All subjects were to provide a pre-vaccination blood sample at V01 (D0) and a post-vaccination sample at V02 (30 to 44 days after vaccination at V01).

Collection of safety data

All subjects were to be observed for 30 minutes after vaccination and any unsolicited systemic AEs occurring during that time were to be recorded as immediate unsolicited systemic AEs in the electronic case report form (CRF)

The subjects' parent/legally acceptable representative were to record information in a diary card (DC) about solicited reactions from D0 to D07 after vaccination and unsolicited AEs only from D0 to V02 (D30 + 14 days). SAEs were reported throughout the duration of the trial.

In addition, the subject's parent/legally acceptable representative was asked to notify the site immediately about any potential SAEs at any time during the trial.

Staff contacted the subject's parent/legally acceptable representative by telephone on D08 (+2 days) to identify the occurrence of any SAE not yet reported and to remind them to complete the DC up to V02 and to bring it back to V02 (D30 + 14 days).

The completed DC was reviewed with the subject's parent/legally acceptable representative at V02 and collected at V02 (D30 + 14 days).

Table 3.2: Study procedures

Phase III Trial, 2 Visits (V), 1 Telephone Call (TC), 1 Vaccination, 2 Blood Samples (BLs), 30 to 44 Days Duration per Subject

Visit/Contact	V01	TC	V02
Trial timelines (days)	D0	D08	D30
Time windows (days)	-	+2 days	+14 days
Informed consent form	X		
Inclusion/exclusion criteria*	X		
Collection of demographic data	X		
Medical history	X		
Collection of MenC vaccination history†	X		
Physical examination	X		
Review of temporary contraindications for blood sampling‡			X
Randomization/allocation of subject number	X		
Blood sample, 5 mL§	BL1		BL2
Study Vaccination**	X		
Immediate surveillance (30 minutes)	X		
DC provided	X		
TC		X††	
Recording of solicited injection site & systemic reactions	D0 to D07		
Recording of unsolicited AEs	D0 to V02		
Reporting of SAEs [redacted]	To be reported throughout the study period		
DC reviewed and collected			X
Collection of reportable concomitant medications	X		X
Termination record			X

*Temperature needs to be measured by oral, rectal, or axillary route (axillary preferred) and recorded in the source document.

†Documentation of MenC vaccination during infancy will be collected for subjects who will be randomized to Group 3 or Group 4.

‡ 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 (30 to 44 days after vaccination at D0). If postponement would result in the sample collection falling outside of the appropriate 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.

§ Blood sample at V01 will be drawn before administration of the vaccine

** Subjects will receive 1 dose of MenACYW conjugate vaccine or Nimenrix®.

†† This call is made 8 to 10 days after the vaccination at V01. If D08 (+2 days) falls on a weekend or holiday, the TC may be made on the following business day. During this TC, the staff will find out whether the subject experienced any SAE, [redacted], not yet reported, and will remind the subject's parent/legally acceptable representative to continue using the DC up to V02, to bring the DC to the study center at V02, and confirm the date and time of V02 (D30 + 14 days).

[redacted]

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

See Section 9.1 of the protocol.

4.2 Secondary Endpoints and Assessment Methods

See Section 9.2 of the protocol.

4.3 Observational Endpoints and Assessment Methods

See Section 9.3 of the protocol.

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.

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

1) Solicited reactions (except Fever) with an investigator presence recorded as "No" and with all daily records missing then all daily intensities will be derived as None.
2) For a temperature partially missing after decimal point, the data will be analyzed replacing "MD" (missing data) by zero. For example, a "39.MD" daily temperature will be considered as "39.0°C" at the time of analysis.
3) 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 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 Overall Intensity

Maximum overall intensity is derived from the daily intensities computed as described in Section 4.4.1.1.1 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 on the period considered:

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

Subjects 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.

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 Occurrence

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 stop date of the reaction after the end of the solicited period. The overall number of days of occurrence is:

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

If the stop date is missing or incomplete (contains MD), 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.If the last daily intensity of the solicited period is at least Grade 1 and maximum intensity on the ongoing period is also at least Grade 1, then the reaction is considered ongoing. In any other cases the reaction will not be considered as ongoing.

4.4.1.2 Unsolicited Non-serious 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 should be included in the listing "Unsolicited non-serious adverse events not included in the safety analysis."

4.4.1.2.2 Intensity

Intensity for unsolicited non-serious adverse event (AE) will be derived according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

If the unsolicited non-serious 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 than the intensity scales defined in the protocol for that measurable injection site or systemic reaction.

Intensity for the other unsolicited non-serious 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 non-serious AE is derived from the visit numbers provided in the clinical database and is calculated as follows:

- If an unsolicited non-serious AE has a non-missing visit number, the visit number should be used to determine the last vaccination before the unsolicited non-serious AE

If the visit number is missing, then the start date should be used to determine the last vaccination before the unsolicited non-serious AE

4.4.1.2.4 Time of Onset

Time of onset is derived from the start date of the unsolicited non-serious AE provided in the clinical database and the date of last vaccination:

- start date of the unsolicited non-serious AE – date of previous vaccination

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

The unsolicited non-serious AEs will be analyzed "Within 30 days", 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 vaccination indicated by the visit number, so will be included in these tables.

Note: Unsolicited non-serious 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 stop dates of the unsolicited non-serious AE provided in the clinical database:

- stop date of unsolicited non-serious AE - start date of unsolicited non-serious AE + 1.

The duration should be considered as missing only if one or both of the start and stop dates of the unsolicited non-serious AE is missing or partially missing.

4.4.1.3 SAEs

4.4.1.3.1 Last Vaccination

Last vaccination before an SAE is derived from the last visit numbers provided in the clinical database and is calculated as follows:

- If an SAE has a non-missing visit number, the visit number should be used to determine the last vaccination before the SAE
- If the visit number is missing, then the start date should be used to determine the last vaccination before the SAE

4.4.1.3.2 Time of Onset

Time of onset will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.1.2.4.

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

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

An SAE with missing time of onset will be considered to have occurred just after the vaccination indicated by the visit number, so will be included in these tables.

Note: SAEs that occurred before vaccination (negative time of onset) will not be included in analysis, but will be listed separately.

4.4.1.3.3 Duration

Duration will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.1.2.5.

4.4.1.4 Other Safety Endpoints

4.4.1.4.1 Pregnancy

Not applicable for this study.

4.4.1.4.2 Action Taken

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

4.4.1.4.3 Seriousness

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

4.4.1.4.4 Outcome

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

4.4.1.4.5 Causality

This information will be summarized as collected. Missing causality (relationship) will be handled as described in Section 5.3.1.2.

4.4.1.4.6 AEs Leading to Study Discontinuation

A flag is available in the clinical database for all AEs in order to identify AEs leading to discontinuation.

In general, the items that are counted are:

- Disposition table: A subject who has, on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" checked
- Safety overview table: A subject who has either on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" checked or lists an AE on an AE page (solicited, unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated
- System Organ Class/Preferred Term (SOC/PT) table: An event (solicited, unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated

4.4.2 Immunogenicity

4.4.2.1 Computed Values for Analysis

In order to appropriately manage extreme values (< 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 BL 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 Fold-rise

The derived endpoint fold-rise is driven by both baseline and post-baseline computed values and is computed as follows:

- Calculate the fold-rise of values as the ratio of post-baseline computed value divided by baseline computed value

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

4.4.2.3 hSBA Vaccine Seroresponse

The derived seroresponse indicator for hSBA will be "Yes" if

- hSBA titer is < 1:8 at baseline with a post-baseline hSBA titer ≥ 1:16
- or hSBA titer is ≥ 1:8 at baseline with a ≥ 4-fold increase at post-baseline

4.4.2.4 rSBA Vaccine Seroresponse

The derived seroresponse indicator for rSBA will be "Yes" if

- rSBA titer is < 1:8 at baseline with a post-baseline rSBA titer ≥ 1:32
- or rSBA titer is ≥ 1:8 at baseline with a ≥ 4-fold increase at post-baseline

4.4.2.5 Efficacy

Not applicable

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 months at the time of inclusion.

4.4.3.2 Duration of a Subject in the Trial

The duration of a subject in the study is computed as follows:

Maximum (date of last visit, date of termination) – (date of Visit 1 of that subject) +1.

4.4.3.3 Duration of the Study

The duration of the study is computed as follows:

Maximum of all subjects (date of last visit, date of termination) – Minimum of all subjects (date of Visit 1) + 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. 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 (seroresponse, ≥ 4 fold rise, cutoff)**	Number and percentage (95% CIs) of subjects.
	Continuous data (titer / concentration)	Log_{10}: Mean and standard deviation. Anti-Log_{10} (work on Log10 distribution, and anti-Log_{10} applied): Geometric mean (GM), 95% CI of the GM. Graphical representation by Reverse Cumulative Distribution Curve (RCDC).

The confidence interval (CI) for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (1)). For immunogenicity results, assuming that Log_{10} transformation of the titers follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log_{10} (titers) 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 GMs and their 95% CI.

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Primary Objectives

Two co-primary objectives will be evaluated.

5.1.1.1 Hypotheses

5.1.1.1.1 Hypothesis for Co-primary Objective 1

Co-primary hypothesis 1: Non-inferiority testing after 1 dose of MenACYW conjugate vaccine or Nimenrix® in toddlers who either are meningococcal vaccine naïve or have received monovalent MenC vaccination during infancy

The percentages of subjects who achieve an hSBA titer ≥ 1:8 for meningococcal serogroups A, C, Y, and W in toddlers who received MenACYW conjugate vaccine (Group 1 and Group 3) are non-inferior to the corresponding percentages in toddlers who received Nimenrix® (Group 2 and Group 4) 30 days post administration.

Null hypothesis (H_0): $p_{(Men)} - p_{(Nim)} \leq -10\%$
Alternative hypothesis (H_1): $p_{(Men)} - p_{(Nim)} > -10\%$

where $p_{(Men)}$ and $p_{(Nim)}$ are the percentages of subjects who achieve an hSBA titer ≥ 1:8 in the MenACYW vaccine group and the Nimenrix® group, respectively.

5.1.1.1.2 Hypothesis for Co-primary Objective 2

Co-primary hypothesis 2: Non-inferiority testing after 1 dose of MenACYW conjugate vaccine or Nimenrix® in meningococcal vaccine naïve toddlers

Thirty days after the administration of MenACYW conjugate vaccine or Nimenrix®, the percentages of subjects who achieve an hSBA titer ≥ 1:8 for meningococcal serogroups A, C, Y, and W in Group 1 are non-inferior to the corresponding percentages in Group 2.

Null hypothesis (H_0): $p_{(G1)} - p_{(G2)} \leq -10\%$

Alternative hypothesis (H_1): $p_{(G1)} - p_{(G2)} > -10\%$

where $p_{(G1)}$ and $p_{(G2)}$ are the percentages of subjects who achieve an hSBA titer ≥ 1:8 in Group 1 and Group 2, respectively.

5.1.1.2 Statistical Methods

5.1.1.2.1 Statistical Method for Co-primary Objective 1

Each of the serogroups A, C, Y, and W will be tested separately. If the lower limit of the 2-sided 95% confidence interval (CI) of the difference between the 2 percentages is > -10%, the inferiority assumption will be rejected.

For the 4 non-inferiority hypotheses using the response rates (percentages of subjects who achieve an hSBA titer ≥ 1:8), the 95% CI will be stratified on the priming status (meningococcal vaccine naïve or primed monovalent MenC vaccination during infancy) and calculated using the Wald method (normal approximation). Weighted average of the difference over strata will be calculated using the Minimal Risk weights with the null variance method (2).

The 100(1-α)% two-sided CIs for the difference in proportions between two groups using the Minimum Risk Weights with the null variance are calculated as follows:

$$\hat{\delta}_w \pm Z_{\alpha/2}\sqrt{\left\{\sum_{i=1}^{s} w_i^2 \hat{V}_0(\hat{\delta}_i)\right\}}$$

where i=1,2, s=2, $\hat{\delta}_w = \sum_{i=1}^{s} w_i \hat{\delta}_i = \sum_{i=1}^{s} w_i (\hat{P}_{iB} - \hat{P}_{iA})$, $\hat{P}_{iA}, \hat{P}_{iB}$ are the observed response rates for MenACYW vaccine group and the Nimenrix® group respectively at stratum i, $Z_{\alpha/2}$ is the 100(1-

α/2)th percentile of the standard normal distribution, and w_i are the weight assigned at stratum i, and

$\hat{V}_0(\hat{\delta}_i) = \frac{\tilde{P}_{iA}(1-\tilde{P}_{iA})}{n_{iA}} + \frac{\tilde{P}_{iB}(1-\tilde{P}_{iB})}{n_{iB}}$ is the null variance, where $\tilde{P}_{iA}, \tilde{P}_{iB}$ are the constrained maximum likelihood estimates (MLEs) of response rates of MenACYW and Nimenrix at stratum I, and n_{iA}, n_{iB} are the sample sizes for MenACYW and Nimenrix at each stratum.

The Minimum Risk method computes the weights w_i as follows:

$$w_i = \frac{\beta_i}{\sum_{k=1}^{s} V_k^{-1}} - \left(\frac{\alpha_i V_i^{-1}}{\sum_{k=1}^{s} V_k^{-1} + \sum_{k=1}^{s} \alpha_k \delta_k V_k^{-1}} \right) \left(\frac{\sum_{k=1}^{s} \delta_k \beta_k}{\sum_{k=1}^{s} V_k^{-1}} \right)$$

where $\alpha_i = \delta_i \sum_{k=1}^{s} V_k^{-1} - \sum_{k=1}^{s} \delta_k V_k^{-1}$ and $\beta_i = V_i^{-1}\left(1 + \alpha_i \sum_{k=1}^{s} f_k \delta_k\right)$, $V_i = V(\hat{\delta}_i)$, and f_i is the fraction of subjects in the target population that belong in stratum i ($\sum_{i=1}^{s} f_i = 1$).

For two strata, with observed values for $V_i, f_i, and \delta_i$ the formula reduces to the following:

$$\hat{w}_1 = \frac{\hat{V}_2 + \hat{f}_1(\hat{\delta}_1 - \hat{\delta}_2)^2}{\hat{V}_1 + \hat{V}_2 + (\hat{\delta}_1 - \hat{\delta}_2)^2}, \quad \hat{w}_2 = 1 - \hat{w}_1.$$

The overall non-inferiority of this objective will be demonstrated if all 4 individual null hypotheses are rejected.

5.1.1.2.2 Statistical Method for Co-primary Objective 2

Each of the serogroups A, C, Y, and W will be tested separately. If the lower limit of the 2-sided 95% CI of the difference between the 2 percentages is > -10%, the inferiority assumption will be rejected.

For the 4 non-inferiority hypotheses using the response rates (percentages of subjects who achieve an hSBA titer ≥ 1:8), the CI of the difference in proportions will be computed using the Wilson Score method without continuity correction (3).

Let $\hat{\theta} = p_1 - p_2$, then $L = \hat{\theta} - \delta$ and $U = \hat{\theta} + \varepsilon$ are respectively the lower and the upper limits of the CI, where:

$$\delta = Z_{0.025}\sqrt{\left\{\frac{l_1(1-l_1)}{n_1} + \frac{u_2(1-u_2)}{n_2}\right\}}$$

$$\varepsilon = Z_{0.025}\sqrt{\left\{\frac{l_2(1-l_2)}{n_2}+\frac{u_1(1-u_1)}{n_1}\right\}}$$

l_1 and u_1 are calculated from the CI of the single proportion in Group 1 given by:

$$\frac{(2n_1p_1 + Z^2_{0.025} \pm Z_{0.025}\sqrt{(Z^2_{0.025} + 4n_1p_1(1-p_1))})}{2(n_1 + Z^2_{0.025})}$$

l_2 and u_2 are calculated from the CI of the single proportion in Group 2 given by:

$$\frac{(2n_2p_2 + Z^2_{0.025} \pm Z_{0.025}\sqrt{(Z^2_{0.025} + 4n_2p_2(1-p_2))})}{2(n_2 + Z^2_{0.025})}$$

where $Z_{0.025}$ is the upper 97.5th percentile of the standard normal distribution.

The overall non-inferiority of this objective will be demonstrated if all 4 individual null hypotheses are rejected.

To conclude, non-inferiority in toddlers who either are meningococcal vaccine-naïve or have received monovalent MenC vaccination during infancy and non-inferiority in meningococcal vaccine-naïve subjects have to be demonstrated.

5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

5.1.2.1 Hypotheses

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

5.1.2.2 Statistical Methods

5.1.2.2.1 Statistical Method for Secondary Objective 1

Thirty days after the administration of MenACYW conjugate vaccine or Nimenrix® in toddlers who either are meningococcal vaccine naïve or have received monovalent MenC vaccination during infancy, the hSBA geometric mean titer ratio (GMTR) between MenACYW conjugate vaccine or Nimenrix® will be calculated, and 95% CI will be provided. The 95% CI of the ratio of post-vaccination GMTs will be stratified on the priming vaccination status (meningococcal vaccine naïve or primed monovalent MenC vaccination) and calculated using an analysis of variance (ANOVA) model of $\log_{10}$-transformed titers.

5.1.2.2.2 Statistical Method for Secondary Objective 2

Thirty days after the administration of MenACYW conjugate vaccine or Nimenrix® in meningococcal vaccine naïve toddlers, the hSBA GMTR between MenACYW conjugate vaccine or Nimenrix® will be calculated, and 95% CI will be provided.

5.1.2.2.3 Statistical Method for Secondary Objective 3

Thirty days after the administration of MenACYW conjugate vaccine or Nimenrix® in toddlers who received monovalent MenC vaccination during infancy, the hSBA GMTR between MenACYW conjugate vaccine or Nimenrix® will be calculated, and 95% CI will be provided.

5.1.3 Statistical Methods for Observational Objectives

5.1.3.1 Hypotheses

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

5.1.3.2 Statistical Methods

Immunogenicity
Descriptive statistics will be provided for the antibody titers against meningococcal serogroups contained in MenACYW conjugate vaccine and Nimenrix®. 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 percentages (1).

For GMTs, 95% CIs of point estimates will be calculated using normal approximation assuming they are log-normally distributed.

Reverse cumulative distribution curve (RCDC) figures will be provided for the antibody titers against meningococcal serogroups contained in MenACYW conjugate vaccine and Nimenrix® treatment groups.

In summary, descriptive analyses on A, C, Y, and W serogroups will include but not be limited to:

- hSBA GMT and 95% CI at D0 and D30
- hSBA titer distribution and RCDC
- Percentage of subjects with hSBA titer ≥ 1:4 and ≥ 1:8 and 95% CI at D0 and D30
- Percentage of subjects with hSBA titer ≥4-fold rise from pre-vaccination to post-vaccination, and 95% CI

- hSBA vaccine seroresponse[1] rate and 95% CI
- rSBA[2] GMT and 95% CI at D0 and D30
- rSBA titer distribution and RCDC
- Percentage of subjects with rSBA titer ≥ 4-fold rise from pre-vaccination to post-vaccination, and 95% CI
- rSBA vaccine seroresponse[3] rate and 95% CI
- Percentage of subjects with rSBA titers ≥ 1:8 and ≥ 1:128 and 95% CI at D0 and D30

Safety

For this trial, the safety data will be assessed by applying descriptive statistical methods, supplemented by the calculation of CIs to aid interpretation. The exact binomial distribution (Clopper-Pearson method) for proportions will be used in the calculation of the 95% CIs of events.

The frequency and percentage of subjects who had solicited injection site reactions and solicited systemic reactions and their 95% CIs will be provided. These events will be tabulated by the type of reactions and intensity for each study group. These events will also be summarized by other categories specified in the endpoints (e.g. time of onset, number of days of occurrence, actions taken).

Unsolicited AEs will be collected, coded, and summarized by MedDRA system organ class and preferred term. For each unsolicited AE, the number of subjects with at least 1 instance of that event will be reported. Unsolicited AEs will also be tabulated by intensity and relatedness of study vaccine and by other categories specified by the endpoints.
Immediate reactions, SAEs, [redacted], and any event that leads to subject withdrawal from the study will be tabulated separately.
Safety tables will be described for subjects who are either meningococcal vaccine naïve or had received monovalent MenC vaccination during infancy (i.e. Group 1 & 3 and Group 2 & 4) and for subjects in all study groups (i.e. based on MenC priming vaccination background).

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1 hSBA vaccine seroresponse 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 at least 4-fold greater that the pre-vaccination titer.

2 In a subset of subjects per group: 100 subjects each in Group 1 and Group 2; 50 subjects in each subgroup (MenC-TT or MenC-CRM primed subjects) in Group 3; 25 subjects in each subgroup (MenC-TT or MenC-CRM primed subjects) in Group 4

3 rSBA vaccine seroresponse is defined as a post-vaccination titer ≥ 1:32 for subjects with pre-vaccination rSBA titer < 1:8, or a post-vaccination titer≥ 4 times the pre-vaccination titer for subjects with pre-vaccination rSBA titer ≥ 1:8.

5.1.4 Complementary outputs

Additional analyses by gender will be provided in Appendix 15 of the CSR.

Immunogenicity analyses:

- hSBA GMTs and 95% CI at each time point – Per-Protocol Analysis Set
- Vaccine seroresponse against meningococcal serogroups A, C, Y, and W measured by hSBA and 95% CI – Per-Protocol Analysis Set
- rSBA GMTs and 95% CI at each time point – Per-Protocol Analysis Set
- Vaccine seroresponse against meningococcal serogroups A, C, Y, and W measured by rSBA and 95% CI – Per-Protocol Analysis Set

Safety analyses:

Safety overview after vaccine injection –Safety Analysis Set

Some demographics tables will be also described by MenC primed background.

5.2 Analysis Sets

Three analysis sets will be used: the Full Analysis Set (FAS), the Per-Protocol Analysis Set (PPAS), and the Safety Analysis Set (SafAS).

5.2.1 Full Analysis Set

The FAS is defined as the subset of subjects who received at least 1 dose of the study vaccine and had a valid post-vaccination serology result. All subjects will be analyzed according to the treatment group to which they were randomized.

5.2.2 Per-Protocol Analysis Set

The PPAS is a subset of the FAS. The subjects presenting with at least one of the following relevant protocol deviations will be excluded from the PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not receive vaccine
- 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
- Subject did not provide post-dose serology sample in the proper time window or a post-dose serology sample was not drawn. The time windows are defined as D30 to D44 post-vaccination.

- Subject received a protocol-prohibited Category 2 or Category 3 therapy/medication/vaccine
- Subject's serology sample did not produce a valid test result , i.e. results equal to 'NR' or missing for all 4 serogroups (at post-vaccination)
- Subject had other protocol deviations that affected the subject's immune response, as determined by the clinical team before locking the database

5.2.3 Safety Analysis Set

The 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 according to the vaccine they actually received. If the vaccine received by a subject does not correspond to any study group, the subject will be excluded from the SafAS. The corresponding safety data will be presented in separate listings.

5.2.4 Populations Used in Analyses

All immunogenicity analyses will be performed on the PPAS. Additional immunogenicity analyses will be performed for exploratory purposes on the FAS. In the FAS, subjects will be analyzed by the vaccine group to which they were randomized.

The safety analyses will be performed on the SafAS. Subjects will be analyzed according to the vaccine they actually received.

5.3 Handling of Missing Data and Outliers

5.3.1 Safety

No replacement will be done. In subject listings, partial and missing data will be clearly indicated as missing.

5.3.1.1 Immediate

For unsolicited non-serious systemic AEs, a missing response to the "Immediate" field is assumed to have occurred after the 30-minute surveillance period and will not be imputed.

For SAEs, missing or partially missing elapsed time from last vaccination recorded if within 24 hours will remain missing and not be imputed. Such SAEs will not be considered as immediate.

5.3.1.2 Causality

Missing causality (relationship) for unsolicited non-serious AEs and SAEs will be considered at the time of analysis as related to vaccination.

5.3.1.3 Measurements

Partially missing temperatures will be handled as described in Section 4.4.1.1.1

5.3.1.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.4.1.1.1. For unsolicited non-serious AEs, missing intensities will remain missing and will not be imputed.

5.3.1.5 Start Date and Stop Date

Missing or partially missing start dates for unsolicited AEs will remain missing and not be imputed. If either the start or stop date is missing or partially missing, the time of onset will be considered to be missing. Nevertheless unsolicited AEs with missing time of onset will be included in analyses according to the visit collected.

Missing or partially missing stop dates for AEs (solicited reactions and unsolicited AEs) will remain missing and not be imputed.

5.3.1.6 Action Taken

Missing actions taken will remain missing and not be imputed.

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 ≥ [ULOQ]), the following computational rule is applied to the values provided in the clinical database for each BL 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

5.4 Interim / Preliminary Analysis

No interim / preliminary analyses are planned.

5.5 Determination of Sample Size and Power Calculation

[redacted]

[redacted]

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]				[redacted]

[redacted]

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]				[redacted]

[redacted]

5.6 Data Review for Statistical Purposes

A review of the data has been anticipated through the data review process led by data management before database lock. This review of the data included a statistical review.

5.7 Changes in the Conduct of the Trial or Planned Analyses

Not applicable.

6 References List

1 Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17(8):857-72.

2 Mehrotra DV, Railkar R. Minimum risk weights for comparing treatments in stratified binomial trials. Stat Med 2000;19:811-25.

3 Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med. 1998; 17(8):873-90.