NCT04142242

Safety and Immunogenicity of a Single Dose of MenACYW Conjugate Vaccine at Least 3 Years Following Initial Vaccination With Either Menomune® Vaccine or MenACYW Conjugate Vaccine in Older Adults

Phase III, randomized, open-label, multi-center study to evaluate the safety and immunogenicity of a single dose of MenACYW conjugate vaccine in subjects who received a dose of Menomune® vaccine or MenACYW conjugate vaccine ≥ 3 years previously, at ≥ 56 years of age. Antibody persistence following the primary dose of either Menomune vaccine or MenACYW conjugate vaccine will also be evaluated.

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	MEQ00066
Development Phase:	III
Sponsor:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, 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:	Single dose of MenACYW conjugate vaccine for adults ≥ 56 years of age 3 years after the primary dose
Version and Date of the SAP Core Body Part:	Version 1.0 30SEP2020

Table of Contents

List of Tables

List of Abbreviations

Ab	Antibody
AE	Adverse Event
AESI	Adverse Event of Special Interest
AR	Adverse Reaction
BL	Blood Sample
CDM	Clinical Data Management
CI	Confidence Interval
CRB	Case Report Book
CRF	Case Report Form
CTL	Clinical Team Leader
CTM	Clinical Trial Manager
CSR	Clinical Study Report
D	Day
D0	Day 0
D30	Day 30
DC	Diary Card
dil	Dilution
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ECL	Electrochemiluminescence
EIA	Enzyme Immunosorbent Assay
EMA	European Medicines Agency
EDC	Electronic Data Capture
FAS	Full Analysis Set
FAS1	Stage I Day 30 Full Analysis Set
FAS2	Stage I Day 6 Full Analysis Set
FAS3	Stage I Full Analysis Set for Persistence
FAS4	Stage II Day 30 Full Analysis Set
FAS5	Stage II Full Analysis Set for Persistence
FDA	Food and Drug Administration
GCI	Global Clinical Immunology
GCP	Good Clinical Practice
GM	Geometric Mean
GMC	Geometric Mean Concentration

GMT	Geometric Mean Titer
hSBA	Serum Bactericidal Assay Using Human Complement
ICH	International Conference on Harmonisation
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IME	Important Medical Event
IND	Investigational New Drug
IU	International Unit
IRT	Interactive Response Technology
LLOQ	Lower Limit of Quantification
LLT	Lowest Level Term
MD	Missing Data
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
mm	Millimeter
NA	Not Applicable
NM	Non-Measurable
PPAS	Per-Protocol Analysis Set
PPAS1	Stage I Day 30 Per-Protocol Analysis Set
PPAS2	Stage I Day 6 Per-Protocol Analysis Set
PPAS3	Stage II Day 30 Per-Protocol Analysis Set
PT	Preferred Term
Q1; Q2; Q3	First Quartile; Second Quartile (Median); Third Quartile
RCDC	Reverse Cumulative Distribution Curve
rSBA	Serum Bactericidal Assay Using Baby Rabbit Complement
SAS	Statistical Analysis System
SAE	Serious Adverse Event
SafAS1	Stage I Safety Analysis Set
SafAS2	Stage II Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class (Primary)
TLF	Table(s), Listing(s), and Figure(s)
ULOQ	Upper Limit of Quantification

1 Introduction

Meningococcal disease is caused by the gram-negative aerobic diplococcus *Neisseria meningitidis*, an exclusively human pathogen. Meningococci colonize the nasopharynx of an estimated 10% to 20% of the population at any given time and are transmitted by respiratory secretions (1). Of note, only a small proportion of carriers develop invasive disease and predicting an individual's risk is difficult.

The 2 most common clinical presentations are meningococcemia or sepsis, which is accompanied by a petechial or purpuric rash in the majority of patients, and purulent meningitis. Despite appropriate treatment, the overall mortality rate for meningococcal disease has been reported to be 7%–19% (2). Approximately 10%–20% of cases result in permanent disabilities such as limb loss, deafness, seizures, or psychomotor retardation.

The epidemiology of meningococcal disease varies by the causative serogroup, with serogroups A, B, C, and W having caused large epidemics worldwide. To help prevent invasive meningococcal disease among those considered at increased risk for the disease, the Advisory Committee on Immunization Practices recommends that travelers who visit or reside in the so-called meningitis belt during the dry season (December–June) receive vaccination with a quadrivalent (serogroups A, C, W, and Y) meningococcal vaccine before travel. In addition, Kingdom of Saudi Arabia visitors travelling to the Hajj and Umrah are required to submit a valid vaccination certificate indicating administration of either a quadrivalent polysaccharide meningococcal vaccine within the last 3 years or a quadrivalent conjugate vaccine within the last 5 years (3). This requirement stems from evidence demonstrating that protection following MenACWY vaccination may wane 3 to 5 years after primary vaccination (4) (5) (6) (7).

The safety and immunogenicity of MenACYW conjugate vaccine when used for primary vaccination of adults ≥ 56 years of age was previously evaluated in two studies (MET44 and MET49). In those studies, MenACYW conjugate vaccine was compared to a quadrivalent plain polysaccharide meningococcal vaccine (Menomune) (8) (9).

The purpose of the current study, MEQ00066, is to further evaluate MenACYW conjugate vaccine in older adults. Specifically, MEQ00066 seeks to assess the safety and immunogenicity of a single dose of MenACYW conjugate vaccine ≥ 3 years after primary vaccination with Menomune vaccine or MenACYW conjugate vaccine in subjects who were ≥ 56 years of age at primary vaccination. [redacted] Antibody persistence 3, 5, or 7 years following a primary dose of either Menomune vaccine or MenACYW conjugate vaccine will also be evaluated. [redacted] titers will be determined using both a serum bactericidal assay using human complement (hSBA) and a serum bactericidal assay using baby rabbit complement (rSBA).

2 Trial Objectives

2.1 Primary Objective

To demonstrate sufficiency of the vaccine seroresponse to meningococcal serogroups A, C, W, and Y following administration of a single dose of MenACYW conjugate vaccine to Group 1 subjects (who received primary vaccination with Menomune vaccine ≥ 3 years earlier at ≥ 56 years of age in Study MET49).

The endpoint for the primary objective is presented in Section 4.1.1.1.

2.2 Secondary Objectives

Secondary Objective 1

- To demonstrate sufficiency of the vaccine seroresponse to meningococcal serogroups A, C, W, and Y following administration of a single dose of MenACYW conjugate vaccine to Group 2 subjects (who received primary vaccination with MenACYW conjugate vaccine ≥ 3 years earlier at ≥ 56 years of age in Study MET49).

Secondary Objective 2

- To describe vaccine seroresponse rates with respect to serogroups A, C, W, and Y in serum specimens collected 6 (window, 5–7) days post-vaccination in approximately 60 subjects from Group 1 (Menomune-primed) and approximately 60 subjects from Group 2 (MenACYW conjugate vaccine-primed).

Secondary Objective 3

- To describe antibody persistence ≥ 3 years after primary vaccination with Menomune vaccine or MenACYW conjugate vaccine for subjects from all groups.

The endpoints for the secondary objectives are presented in Section 4.2.1.1.

2.3 Observational Objective(s)

Immunogenicity

- To describe the immunogenicity of a single dose of MenACYW conjugate vaccine, as assessed by hSBA and rSBA antibody titers, among persons who previously received Menomune vaccine or MenACYW conjugate vaccine ≥ 3 years earlier.
- To describe antibody levels against tetanus toxoid at enrollment (all groups), 2 years after enrollment (Groups 3 and 4), and 30 (window, + 14) days after a single dose of MenACYW conjugate vaccine (Groups 1–4) in Study MEQ00066.

The endpoints for the observational immunogenicity objectives are presented in Section 4.3.1

Safety

To describe the safety profile of a single dose of MenACYW conjugate vaccine administered to adults who received Menomune vaccine or MenACYW conjugate vaccine ≥ 3 years earlier (Groups 1 and 2 during Stage I; Groups 3 and 4 during Stage II).

The endpoints for the observational safety objectives are presented in Section 4.3.2.

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

This will be a Phase III, 2-stage, randomized, open-label, multi-center study to evaluate the safety and immunogenicity of a single dose of MenACYW conjugate vaccine ≥ 3 years after a prior dose of either Menomune vaccine or MenACYW conjugate vaccine in subjects ≥ 56 years of age at the time of primary vaccination. Antibody persistence after primary vaccination with Menomune vaccine or MenACYW conjugate vaccine will also be evaluated. The study will be conducted in 2 stages.

Subjects who received Menomune vaccine or MenACYW conjugate vaccine in Study MET49 or Study MET44 are eligible for enrollment in Study MEQ00066.

A planned minimum of 440 subjects who participated in Study MET49 (i.e., 3 years prior to enrollment in the current study) will be randomly assigned to Group 1 or Group 3, or Group 2 or Group 4, depending on the vaccine originally received in Study MET49. These subjects will have antibody persistence assessed, followed by receipt of a single dose of MenACYW conjugate vaccine as per the stages described below.

Stage I:

Group 1: Approximately 180 subjects who received Menomune vaccine in Study MET49 will provide a blood sample (BL) at enrollment in Study MEQ00066 (to assess antibody persistence 3 years after primary vaccination), followed by receipt of a single dose of MenACYW conjugate vaccine (ratio 9:2 [Group 1:Group 3]). The first 60 subjects enrolled in Group 1 will comprise a subset from which an additional BL will be obtained at 6 (window, 5–7) days post-vaccination.

Group 2: Approximately180 subjects who received MenACYW conjugate vaccine in Study MET49 will provide a BL at enrollment in Study MEQ00066 (to assess antibody persistence 3 years after primary vaccination), followed by receipt of a single dose of MenACYW conjugate vaccine (ratio 9:2 [Group 2:Group 4]). The first 60 subjects enrolled in Group 2 will comprise a subset from which an additional BL will be obtained at 6 (window, 5–7) days post-vaccination.

Group 3: Approximately 40 subjects who received Menomune vaccine in Study MET49 will provide a BL at enrollment in Study MEQ00066 for the assessment of antibody persistence 3 years after primary vaccination.

Group 4: Approximately 40 subjects who received MenACYW conjugate vaccine in Study MET49 will provide a BL at enrollment in Study MEQ00066 for the assessment of antibody persistence 3 years after primary vaccination.

A planned total of 120 subjects who participated in Study MET44 (i.e., 6–7 years prior to enrollment in the current study) will provide a BL at enrollment in Study MEQ00066 for the assessment of antibody persistence 6–7 years after primary vaccination with either Menomune vaccine or MenACYW conjugate vaccine. These subjects will not receive a vaccination with MenACYW conjugate vaccine at enrollment. The subjects will be grouped as follows:

Group 5: 60 subjects who received Menomune vaccine in Study MET44

Group 6: 60 subjects who received MenACYW conjugate vaccine in Study MET44

Stage II (2 years after enrollment)

Group 3: The approximately 40 subjects who were randomly assigned to this group at the time of initial enrollment in Study MEQ00066 will provide an additional BL (to assess antibody persistence at 5 years), followed by receipt of a single dose of MenACYW conjugate vaccine.

Group 4: The approximately 40 subjects who were randomly assigned to this group at the time of initial enrollment in Study MEQ00066 will provide an additional BL (to assess antibody persistence at 5 years), followed by receipt of a single dose of MenACYW conjugate vaccine.

The study design is summarized in Table 3.1

Table 3.1: MEQ00066 Study Design

Stage	Group	Vaccine Received in MET49	Vaccine Received in MET44	Antibody Persistence	Will Receive MenACYW Conjugate Vaccine in MEQ00066	Planned Sample Size
I	**1**	Menomune		3 years	Yes	180
	2	MenACYW		3 years	Yes	180
	3	Menomune		3 years	At Stage II	40
	4	MenACYW		3 years	At Stage II	40
	5		Menomune	7 years	No	60
	6		MenACYW	7 years	No	60
II	**3**	Menomune		5 years	Yes	See Stage I
	4	MenACYW		5 years	Yes	See Stage I

3.2 Trial Plan

Eligible subjects who are 59 years of age and older form studies MET49 and MET44 will be identified and recruited. Subjects will sign and date an informed consent form (ICF) before any procedure or vaccination associated with the trial is performed.

Vaccination

- Subjects in Groups 1 and 2 will receive a single dose of MenACYW conjugate vaccine at Visit 1.
- Subjects in Groups 3 and 4 will receive a single dose of MenACYW conjugate vaccine 2 years after the enrollment visit (i.e., approximately 5 years after having received primary vaccination in Study MET49).
- Subjects in Groups 5 and 6 will not receive a vaccination.

Blood Sampling

- All subjects (Groups 1–6) will provide a BL at enrollment (Visit 1) to be tested for antibody persistence 3 years (Groups 1–4), or 6–7 years (Groups 5 and 6) after having received primary vaccination in Study MET49 or Study MET44, respectively
- Subjects in Groups 1 and 2 will provide a BL 30 (window, + 14) days after receiving a single dose of MenACYW conjugate vaccine at Visit 1. A subset of subjects, approximately 60 in Group 1 and 60 in Group 2, will also provide a BL 6 (window, 5–7) days following vaccination.
- Subjects in Groups 3 and 4 will provide 2 additional BLs after enrollment: 1 at 2 years (window, + 44 days) after the enrollment visit prior to vaccination with a single dose of MenACYW conjugate vaccine and 1 at 30 (window, + 14) days post-vaccination.

Handling of Low Serum Bactericidal Antibody Levels (Groups 5 and 6 Only)

- It is possible that some subjects in Groups 5 and 6 may have hSBA titers < 1:8 (the putative level of protection against invasive meningococcal disease) to 1 or more of vaccine serogroups (A, C, W, or Y). Investigators will be informed of and notify all subjects in Groups 5 and 6 whose hSBA titers are < 1:8.

Handling of Low Serum Antitetanus Antibody Levels (All Groups)

- Regardless of group assignment, it is possible that some subjects may have serum antitetanus antibody titers < 0.1 IU/mL (the putative level of protection against tetanus disease). Investigators will be informed of and notify all subjects whose antitetanus antibody titers are < 0.1 IU/mL.

Collection of Safety Data

Collection of safety data for subjects who are revaccinated with MenACYW conjugate vaccine (i.e., subjects in Groups 1 and 2 during Stage I, and in Groups 3 and 4 during Stage II).

Staff will observe subjects for 30 minutes following vaccination and will record the occurrence of any immediate unsolicited systemic AEs. Subjects will record information about solicited

injection site and systemic reactions from Day 0 through Day 7 after vaccination. Information on unsolicited AEs, including medically attended adverse events (MAAEs), adverse events of special interest (AESIs), and SAEs, will be collected from Day 0 through ~Day 30 after vaccination.

Collection of safety data for subjects in Groups 3 and 4 after Visit 1 and Groups 5 and 6 during Stage I.

Safety follow-up for subjects in Groups 3 and 4 after Visit 1 and Group 5 and Group 6 will be limited to the collection of SAEs considered by the Investigator to be related to study procedures (e.g., blood sampling).

Table 3.2: Study Procedures – Groups 1 and 2, Subjects Without a Day 6 Visit

Visit/Contact	**Visit 1**	**Telephone Call**	**Visit 2**
Study timelines (days)	**D0**	**D08 (Visit 1 + 8 days)**	**D30 (Visit 1 + 30 days)**
Time windows (days)	**--**	+ 2 days	+ 14 days
Informed consent form	X		
Inclusion/exclusion criteria	X		
Collect subject number from Study MET49	X		
Collection of demographic data	X		
Urine pregnancy test (if applicable)	X		
Medical history	X		
Physical examination[a]	X		
Review of temporary contraindications for blood sampling[b]			X
Randomization/allocation of subject number via IRT	X		
Blood sampling (BL) 10 mL[c]	BL0001		BL0002
Vaccination[d]	X		
Immediate surveillance (30 minutes)	X		
DC provided	X		
Telephone call		X[e]	
Recording of solicited injection site and systemic reactions	D0 to D07		
Recording of unsolicited AEs, including MAAEs[f]	To be reported throughout the study period		
DC reviewed and collected			X
Reporting of SAEs, including AESIs	To be reported throughout the study period		
Collection of reportable concomitant medications	X		X
Termination of study			X

AE: adverse event; AESI: adverse event of special interest; D: day; DC: diary card; IRT; interactive response technology; MA: memory aid; MAAE: medically attended adverse event; SAE: serious adverse event

[a] Temperature needs to be measured and recorded in source documents.

[b] 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 Visit 1). 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.

[c] A pre-vaccination blood sample will be collected from all subjects at Day 0. A post-vaccination blood sample will be collected from all Group 1 and Group 2 subjects 30 (window, + 14) days following vaccination.

[d] Subjects in Groups 1 and 2 will receive 1 dose of MenACYW conjugate vaccine.

[e] This call is made 8 to10 days after the vaccinations on Day 0. If Day 8 (+2 days) 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 SAE not yet reported, and will remind the subject to continue using the DC up to Visit 2, to bring the DC to the study center at Visit 2, and confirm the date and time of Visit 2.

[f] MAAEs that occur between Visit 1 (Day 0) and Visit 2 will be recorded as unsolicited AEs.

Table 3.3: Study Procedures – Groups 1 and 2: Subjects With a Day 6 Visit

Visit Number	**Visit 1**	**Visit 2**	**Telephone Call**	**Visit 3**
Study Timelines (Days)	**D0**	**D06 (Visit 1 + 6 days)**	**D08 (Visit 1 + 8 days)**	**D30 (Visit 1 + 30 days)**
Time Windows (Days)	**--**	± 1 day	+ 2 days	+ 14 days
Informed consent	X			
Inclusion/exclusion criteria	X			
Collect subject number from Study MET49	X			
Collection of demographic data	X			
Urine pregnancy test (if applicable)	X			
Medical history	X			
Physical examination[a]	X			
Review of temporary contraindications for blood sampling[b]		X		X
Randomization/allocation of subject number via IRT	X			
Blood sampling (BL) – 10 mL[c]	BL0001	BL0002[d]		BL0003
Vaccination[e]	X			
Immediate surveillance (30 minutes)	X			
DC provided	X			
Telephone call[f]			X	
Recording of solicited injection site and systemic reactions	D0 to D07			
Recording of unsolicited AEs, including MAAEs[g]	To be reported throughout the study period			
DC reviewed and collected				X
Reporting of SAEs, including AESIs	To be reported throughout the study period			
Collection of reportable concomitant medications	X	X		X
Termination of study				X

AE: adverse event; AESI: adverse event of special interest; D: day; DC: diary card; IRT: interactive response technology; MAAE: medically attended adverse event; SAE: serious adverse event

[a] Temperature needs to be measured and recorded in source documents.

[b] Should a subject receive oral or injectable antibiotic therapy within 3 days prior to the second or third 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 (6 [window, 5–7] days after vaccination at Visit 1 and 30 [window, + 14] days after vaccination at Visit 1). 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.

[c] A pre-vaccination blood sample will be collected from all subjects at Day 0. A post-vaccination blood sample will be collected from all subjects in the Group 1 and Group 2 subset approximately 6 (window, 5–7) days and 30 (window, + 14) days following vaccination.

[d] A planned total of 120 subjects (the first 60 subjects in Group 1 and the first 60 subjects in Group 2).

[e] Subjects in Groups 1 and 2 will receive 1 dose of MenACYW conjugate vaccine.

[f] This call is made 8 to 10 days after the vaccinations on Day 0. If Day 8 (+2 days) 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 SAE not yet reported, and will remind the subject to continue using the DC up to Visit 3, to bring the DC to the study center at Visit 3, and confirm the date and time of Visit 3.

[g] MAAEs that occur between Visit 1 (Day 0) and Visit 3 will be recorded as unsolicited AEs

Table 3.4: Study Procedures – Groups 3 and 4

Visit/Contact	**Visit 1**	**Telephone Call**		**Visit 2**	**Telephone Call**	**Visit 3**
Study timelines (days)	**D0**	**Visit 1 + 8 days**		**Visit 1 + 2 years**	**Visit 2 + 8 days**	**Visit 2 + 30 days**
Time windows (days)	--	+ 2 days		+ 44 days	+ 2 days	+ 14 days
Informed consent form	X					
Inclusion/exclusion criteria	X			X		
Collect subject number from Study MET49	X					
Collection of demographic data	X					
Urine pregnancy test (if applicable)	X			X		
Medical history	X			X		
Physical examination[a]	X			X		
Review of temporary contraindications for blood sampling[b]				X		X
Randomization/allocation of subject number via IRT	X					
Blood sampling (BL) 10 mL[c]	BL0001			BL0002		BL0003
Immediate surveillance after blood sampling (15 minutes) for the occurrence of a syncopal episode	X					
MA provided to collect any SAEs related to blood sampling performed at Visit 1	X					
Vaccination[d]				X		
Immediate surveillance (30 minutes)				X		
DC provided				X		
Telephone call		X[e]			X[f]	
Recording of solicited injection site and systemic reactions				Visit 2 + 7 days		
Recording of unsolicited AEs, including MAAEs[g]				Visit 2 through Visit 3		
DC reviewed and collected						X

Periodic follow-up during inactive phase of study (i.e., at least 1 call every 3 months)			X			
Reporting of SAEs, including AESIs	X			To be reported throughout the study period		
Collection of reportable concomitant medications	X			X		X
Termination of study						X

AE: adverse event; AESI: adverse event of special interest; D: day; DC: diary card; IRT: interactive response technology; MA: memory aid; MAAE: medically attended adverse event; SAE: serious adverse event

[a] Temperature needs to be measured and recorded in source documents.

[b] Should a subject receive oral or injectable antibiotic therapy within 3 days prior to the second or third 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 [window, + 14] days after vaccination at Visit 2). 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.

[c] A blood sample will be collected from all subjects at Visit 1, at Visit 2 prior to vaccination, and at Visit 3 approximately 30 (window, + 14) days after vaccination.

[d] Subjects will receive 1 dose of MenACYW conjugate vaccine.

[e] This call is made 8 to10 days after blood sampling at Visit 1. If Visit 1 + 8 (window, +2) days 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 in the first 3 days after blood sampling.

[f] This call is made 8 to10 days after the vaccinations at Visit 2. If Visit 2 + 8 (window, +2) days 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 SAE not yet reported, and will remind the subject to continue using the DC up to Visit 3, to bring the DC to the study center at Visit 3, and confirm the date and time of Visit 3.

[g] MAAEs will be recorded as unsolicited AEs.

Table 3.5: Study Procedures – Groups 5 and 6

Visit/Contact	Visit 1	Telephone Call
Study timelines (days)	**D0**	**D08** **Visit 1 + 8 days**
Time windows (days)	--	+ 2 days
Informed consent form	X	
Inclusion/exclusion criteria	X	
Collect subject number from Study MET44	X	
Collection of demographic data	X	
Medical history	X	
Physical examination[a]	X	
Allocation of subject number via IRT	X	
Blood sampling (BL) 10 mL	BL0001	
Immediate surveillance after blood sampling (15 minutes) for the occurrence of a syncopal episode	X	
MA provided to collect any SAEs related to blood sampling performed at Visit 1	X	
Telephone call[b]		X
Reporting of SAEs related to study procedures	X	
Collection of reportable concomitant medications	X	
Termination of study	X	

IRT: interactive response technology; SAE: serious adverse event

[a] Temperature needs to be measured and recorded in source documents.

[b] This call is made 8 to10 days after blood sampling at Visit 1. If Visit 1 + 8 (window, +2) days 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 in the first 3 days after blood sampling.

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

4.1.1 Immunogenicity

4.1.1.1 Immunogenicity Endpoints

The primary endpoint for the evaluation of immunogenicity is:

- Vaccine seroresponse against meningococcal serogroups A, C, W, and Y as measured by hSBA at baseline (D0, pre-vaccination) and 30 (window, +14) days after vaccination in Group 1 (Menomune-primed) subjects.

4.1.1.2 Immunogenicity Assessment Methods

Antibodies to Meningococcal Antigens (hSBA Method)

Functional meningococcal antibody activity against serogroups A, C, W, and Y will be measured in a serum bactericidal assay (SBA) utilizing human complement. 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.

The hSBA testing will be performed in Global Clinical Immunology (GCI), at Sanofi Pasteur, Swiftwater, PA or at a qualified contract laboratory for GCI.

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:

- Vaccine seroresponse to meningococcal serogroups A, C, W, and Y as measured by hSBA at baseline (D0, pre-vaccination) and 30 (window, +14) days after vaccination in Group 2 (MenACYW conjugate vaccine-primed) subjects.
- Vaccine seroresponse 6 (window, 5–7) days after vaccination as measured by hSBA in approximately 60 subjects from Group 1 and approximately 60 subjects from Group 2.
- During Stage I, antibody persistence after primary vaccination with Menomune vaccine or MenACYW conjugate vaccine as measured by hSBA and rSBA will be evaluated at Day 0 in Groups 1-4 (3 years after primary vaccination in Study MET49) and in Groups 5 and 6 (6-7 years after primary vaccination in Study MET44). Two years later, during Stage II, antibody persistence will be evaluated again in Groups 3 and 4 (5 years after primary vaccination in Study MET49).

4.2.1.2 Immunogenicity Assessment Methods

The assay methods to be used are summarized below. Laboratory technicians conducting the immunogenicity assays will be blinded to the group to which each subject is randomized / assigned.

Antibodies to Meningococcal Antigens (hSBA Method)

The immunogenicity assessment method for the secondary endpoints for hSBA is the same as that presented in Section 4.1.1.2.

Antibodies to Meningococcal Antigens (rSBA Method)

Functional meningococcal antibody activity against serogroups A, C, W, and Y will be measured in an rSBA. 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 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 (average number of bacteria in each control well after incubation) colony-forming unit. The LLOQ for this assay is a titer of 1:4.

4.3 Observational Endpoints and Assessment Methods

4.3.1 Immunogenicity

4.3.1.1 Immunogenicity Endpoints

Antibody titers (hSBA and rSBA) will be measured for each meningococcal serogroup for the following groups of subjects:

- For all subjects on Day 0
- For a subset of subjects in Groups 1 and 2, on Day 6 (window, Days 5–7) following vaccination
- For subjects in Group 3 and 4, on Day 0 (Visit 1) + 2 years
- For all subjects who receive a single dose of MenACYW conjugate vaccine, 30 (window, + 14) days following vaccination

Tetanus toxoid is contained in the investigational vaccine as a carrier protein. Therefore, BLs will also be tested to assess:

- Antibody concentrations against tetanus toxoid at Day 0 (all groups), 2 years after enrollment (Groups 3 and 4), and 30 (window, + 14) days after the administration of a single dose of MenACYW conjugate vaccine in Study MEQ00066 (Groups 1-4)

4.3.1.2 Immunogenicity Assessment Methods

Antibodies to Meningococcal Antigens (hSBA Method)

The immunogenicity assessment method for the secondary endpoints for hSBA is the same as that presented in Section 4.1.1.2.

Antibodies to Meningococcal Antigens (rSBA Method)

The immunogenicity assessment method for the secondary endpoints for rSBA is the same as that presented in Section 4.2.1.2.

Antibodies to Tetanus Toxin

Anti-tetanus antibodies will be measured by ECL (Electrochemiluminescence).

The 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, tetanus toxoid, and 4 pertussis antigens: pertussis toxin, filamentous haemagglutinin, fimbriae and pertactin.

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. For this study, only tetanus results will be calculated.

4.3.2 Safety

4.3.2.1 Safety Definitions

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

Adverse Event:

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:

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[a]
- Requires inpatient hospitalization or prolongation of existing hospitalization[b]
- Results in persistent or significant disability / incapacity[c]
- 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 adverse reactions (AR).

(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)

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[a] The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

[b] All medical events leading to hospitalizations will be recorded and reported as SAEs, with the exception of: hospitalization planned before inclusion into the study or outpatient treatment with no hospitalization.

[c] "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

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:

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 (e.g., injection site pain or headache occurring between Day 0 and Day 7 post-vaccination). 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.

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 headache between Day 0 and Day 7 is a solicited reaction (i.e., prelisted in the protocol and CRB), then a headache starting on Day 7 is a solicited reaction, whereas headache starting on Day 8 post-vaccination is an unsolicited AE. Unsolicited AEs include both SAEs and non-serious unsolicited AEs.

Medically Attended AE (MAAE)

An MAAE is a new onset or a worsening of a condition that prompts the subject to seek unplanned medical advice at a physician's office or emergency department. A physician contact made over the phone or by email will be considered a physician office visit for the purpose of MAAE collection. This definition excludes pre-planned medical office visits for routine medical care, as well as follow-up visits of chronic conditions with an onset prior to entry in the study. An AE discovered during a planned routine visit (e.g., upper respiratory tract infection, otitis) will be collected as an MAAE.

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 headache, 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 does not occur 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, and relationship to vaccination of any unsolicited systemic AEs reported in the 30 minutes after vaccination.
- Occurrence, time to 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 CRB) injection site reactions occurring from Day 0 through Day 7 after vaccination.
- Occurrence, time to 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 CRB) systemic reactions occurring from Day 0 through Day 7 after vaccination.
- Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, relationship to vaccination (for systemic AEs only), and whether the event led to early termination from the study, of unsolicited AEs occurring from Day 0 through ~Day 30 after vaccination. MAAEs will be collected as unsolicited non-serious AEs from Day 0 through ~Day 30 after vaccination.
- Occurrence, nature (MedDRA preferred term), time to 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 study.

4.3.2.3 Safety Assessment Methods

At each visit, the Investigator or a delegate will perform a directed physical examination, if indicated, based on interim history and will ask the subject 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 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, according to the procedures described in Section 10 of the protocol.

4.3.2.3.2 Reactogenicity (Solicited Reactions from Day 0 to Day 7 after Vaccination)

After vaccination, subjects in Groups 1 to 4 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., Day 0 through Day 7) 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 subject 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

Subjects will be contacted by telephone 8 days after vaccination 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 present, respectively, the injection site reactions and systemic reactions that are prelisted in the diary cards and CRB, together with the intensity scales.

Table 4.1: Solicited Injection Site Reactions: Terminology, Definitions, and Intensity Scales

CRB term (MedDRA lowest level term [LLT])	Injection site pain	Injection site erythema	Injection site swelling
Diary card term	Pain	Redness	Swelling
Definition	Pain either present spontaneously or when the injection site is touched or injected limb is 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[a]	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.	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm

[a] For the subjective reaction of pain, subjects 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 4.2: Solicited Systemic Reactions: Terminology, Definitions, and Intensity Scales

CRB term (MedDRA lowest level term [LLT])	Fever	Headache	Malaise	Myalgia
Diary card term	Temperature	Headache	Feeling unwell	Muscle aches and pains
Definition	Elevation of temperature to ≥°38.0°C (≥ 100.4°F)	Pain or discomfort in the head or scalp. Does not include migraine.	General ill feeling. Malaise is a generalized feeling of discomfort, illness, or lack of well-being that can be associated with a disease state. It can be accompanied by a sensation of exhaustion or inadequate energy to accomplish usual activities.	Muscle aches and pains are common and can involve more than one muscle at the same time. Muscle pain can also involve the soft tissues that surround muscles. These structures, which are often referred to as connective tissues, include ligaments, tendons, and fascia (thick bands of tendons). Does not apply to muscle pain at the injection site which should be reported as injection site pain.
Intensity scale[a]	Grade 1: ≥ 38.0°C to ≤ 38.4°C, **or** ≥ 100.4°F to ≤ 101.1°F	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 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 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.

CRB term (MedDRA lowest level term [LLT])	**Fever**	**Headache**	**Malaise**	**Myalgia**
Diary card term	Temperature	Headache	Feeling unwell	Muscle aches and pains
	Grade 2: ≥ 38.5°C to ≤ 38.9°C, **or** ≥ 101.2°F to ≤ 102.0°F	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 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 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: ≥ 39.0°C **or** ≥ 102.1°F	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.	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.	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.

[a] For all reactions but fever, subjects 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:

Subjects 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 oral. 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, subjects will be instructed to record any other medical events that may occur during the 30-day period after vaccination. Space will be provided in the diary card for this purpose.

Information on SAEs will be collected and assessed throughout the study, from inclusion until 30 days after vaccination. 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).

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

- Start and stop dates[a]
- 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 (see Table 4.1 and Table 4.2).

  All other unsolicited AEs will be classified according to the following intensity scale:

  - Grade 1: A type of AE 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 AE 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.

[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.

  - Grade 3: A type of AE 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", as described in Section 4.3.2.7.

- Action taken for each AE (e.g., medication)

  The action(s) taken by the subject 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

- 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

4.3.2.4 Medically-Attended Adverse Events (MAAEs)

MAAE information will be collected throughout the study. MAAEs that occur from D0 to the D30 (+ 14 days) visit will be recorded as unsolicited AEs on the diary card as part of all unsolicited AEs collected for this post-vaccination period. An MAAE that occurs within the study period but meets the definition of an SAE should be reported only on the SAE Reporting Form, and not on the MAAE page of the CRF.

The Investigator will assess the causal relationship between the MAAE and the investigational or study product as either "Not related" or "Related," as described in Section 4.3.2.7.

4.3.2.5 Serious Adverse Events

Information on SAEs will be collected and assessed throughout the trial, from inclusion until 6 months after vaccination.

Any SAE occurring at any time during the trial will be reported by the Investigator through the electronic data capture (EDC) system and according to the completion guidelines provided by the Sponsor. 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 (eg, outcome, medical history, results of investigations, copy of hospitalization reports. The Investigator will assess the causal relationship between the SAE and the investigational product as either "Not related" or "Related", as described in protocol.

See protocol for further details on SAE reporting.

4.3.2.6 Adverse Events of Special Interest

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

These events have been listed as AESIs on the basis of the feedback received from the European Union regulators for other studies (conducted in younger age groups). 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 protocol. Further instructions on the data collection for these events and the relevant definitions will be provided in the Operating Guidelines.

4.3.2.7 Assessment of Causality

The Investigator will assess the ***causal relationship*** between each unsolicited systemic AE and vaccination 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 Immunogenicity

4.4.1.1 Computed Values for Analysis

To appropriately manage extreme values (< LLOQ and ≥ ULOQ) for analysis purposes, the following computational rule will be 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

To appropriately manage replicate values for analysis purposes, the individual GM (geometric mean) of all values will be computed for each BL after managing extreme values as described as above.

4.4.1.2 Seroresponse

4.4.1.2.1 Seroresponse as Defined in MEQ00066

An hSBA vaccine seroresponse for serogroups A, C, W, and Y is defined as a post-vaccination titer ≥ 1:16 for subjects with pre-vaccination titer < 1:8, or a post-vaccination titer ≥ 4 times the pre-vaccination titer for subjects with a pre-vaccination titer ≥ 1:8.

An 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.

4.4.1.2.2 Seroresponse as Defined in MET49

hSBA and rSBA vaccine seroresponse were defined in MET49 as presented in Section 4.4.1.2.1.

4.4.1.2.3 Seroresponse as Defined in MET44

Both an hSBA and rSBA vaccine seroresponse for serogroups A, C, W, and Y were defined as a post-vaccination titer ≥ 1:8 for subjects with pre-vaccination titer < 1:8, or a post-vaccination titer ≥ 4 times the pre-vaccination titer for subjects with a pre-vaccination titer ≥ 1:8. These definitions were based on the latest regulatory recommendations at the time of MET44.

4.4.1.3 Seroprotection

4.4.1.3.1 Serogroups A, C, W and Y

hSBA vaccine seroprotection for serogroups A, C, W, and Y is defined as an hSBA titer ≥1:8.

4.4.1.3.2 Tetanus

Tetanus seroprotective antibody level is defined as ≥ 0.1 IU/mL tetanus toxoid.

4.4.1.4 Fold-rise

The derived endpoint fold-rise is driven by both baseline and post-baseline computed values and will be computed using the FDA definition 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

If the computed value is ≥ 4-fold rise, then the derived ≥ 4-fold rise indicator will be “Yes” for that test, otherwise ≥ 4-fold rise will be "No."

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

4.4.2 Safety

4.4.2.1 Solicited Reactions

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 (e.g., injection site pain or headache occurring between Day 0 and Day 7 post-vaccination). 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.

4.4.2.1.1 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 measurable injection site reactions (Erythema/Swelling):

Grade 1: ≥ 25 to ≤ 50 mm

Grade 2: ≥ 51 to ≤ 100 mm

Grade 3: > 100 mm

For measurable systemic reactions (Fever):

Grade 1: ≥ 38.0°C to ≤ 38.4°C **or** ≥ 100.4°F to ≤ 101.1°F

Grade 2: ≥ 38.5°C to ≤ 38.9°C **or** ≥ 101.2°F to ≤ 102.0°F

Grade 3: ≥ 39.0°C **or** ≥ 102.1°F

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

1) Solicited reactions (except fever/pyrexia) 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 value that is 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, the daily intensities will correspond to the 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 that a reaction that is too large to measure (non measurable, "NM") is Grade 3. Note: The maximum intensity during 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.

4.4.2.1.2 Maximum Overall Intensity

Maximum overall intensity is derived from the daily intensities computed as described in Section 4.4.2.1.1 and is calculated as the maximum of the daily intensities over the period considered.

4.4.2.1.3 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.

4.4.2.1.4 Time of Onset

Time of onset is derived from the daily intensities computed as described in Section 4.4.2.1.1. 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.

Categories for time to onset are as follows

- D0-D3

- D4-D7

4.4.2.1.5 Number of Days of Occurrence

Number of days of occurrence over the period considered is derived from the daily intensities computed as described Section 4.4.2.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. Number of days of occurrence during the solicited period with a specified intensity may also be derived.

Categories for number of days of occurrence during the solicited period are as follows

- 1-3 days
- 4-7 days
- 8 days

4.4.2.1.6 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 missing data [MD]), the overall number of days of occurrence will be considered as Missing.

Categories for overall number of days of occurrence are as follows

- 1-3 days
- 4-7 days
- ≥ 8 days
- Missing

4.4.2.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed as described in Section 4.4.2.1.1 and the maximum intensity during the ongoing period. The Investigator's ongoing flag is not used because the measurement would determine the ongoing status of the reaction. Note: The intensity could be considered None (not a reaction) in the analysis despite being considered a reaction by the Investigator (e.g., fever > 37.5°C but < 38°C). 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.

4.4.2.2 Unsolicited Non-serious AEs

4.4.2.2.1 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."

4.4.2.2.2 Intensity

Intensity for unsolicited non-serious AEs 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 (PT) is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule as the intensity scales defined in Section 4.4.2.1.1 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.

4.4.2.2.3 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

4.4.2.2.4 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.

Unsolicited non-serious AEs will be analyzed "Within 30 days," which corresponds to AEs with a time of onset between D0 and D30 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 beyond 30 days, will not be included in analysis; but will be listed separately.

Time of onset will be displayed as follows:

- D0-D3
- D4-D7
- D8-D14
- ≥ D15
- Missing

4.4.2.2.5 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 the start and / or stop date of the unsolicited non-serious AE is missing or partially missing.

Duration will be displayed by period as following:

- 1-3 days
- 4-7 days
- 8-14 days
- ≥ 15 days
- Missing

4.4.2.3 SAEs

4.4.2.3.1 Last Vaccination

Last vaccination before an SAE is derived from the 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

4.4.2.3.2 Time of Onset

Time of onset will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.2.2.4.

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

- Within 30 days
- During the study (i.e., all SAEs that 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.

4.4.2.3.3 Duration

Duration will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.2.2.5.

4.4.2.4 Medically-Attended Adverse Events

MAAEs that occur within 30 days after vaccination(s) will be collected as unsolicited AEs. Unsolicited non-serious AEs that occur within the unsolicited period (within 30 days after vaccination[s]) and have action taken of "Health care provider contact" will be summarized and presented as MAAEs.

4.4.2.4.1 Last Vaccination

Last vaccination will be derived using the same methodology as used for unsolicited non-serious AEs described in Section 4.4.2.2.3.

4.4.2.4.2 Time of Onset

Time of onset will be computed using the same methodology as used for unsolicited non-serious AEs described in Section 4.4.2.2.4.

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

- Within 30 days after vaccination(s)

An MAAE 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 the safety analysis tables mentioned above.

4.4.2.4.3 Duration

Duration will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.2.2.5

4.4.2.5 Other Safety Endpoints

4.4.2.5.1 Pregnancy Testing

A urine human chorionic gonadotropin pregnancy test supplied by Sanofi Pasteur will be used to test females of child-bearing potential for pregnancy prior to vaccination. Subjects will not

participate in the study if the initial pregnancy test was positive. For the purposes of the study, female subjects ≥ 60 years of age are defined as not of childbearing potential.

4.4.2.5.2 Action Taken

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

The action(s) taken by the subject 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

4.4.2.5.3 Seriousness

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

4.4.2.5.4 Outcome

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

4.4.2.5.5 Causality

This information will be summarized as collected. An adverse reaction (AR) is defined as an unsolicited non-serious AE or an SAE considered causally related to the vaccine. Missing causality (relationship) will be handled as described in Section 5.3.1.2.

4.4.2.5.6 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
- 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

4.4.2.5.7 AEs of Special Interest (AESIs)

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 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.

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.

AESIs were to be collected throughout the study. The AESIs that will be retrieved for analysis in this trial are presented in Section 4.3.2.6.

4.4.2.5.8 Medically Attended AE (MAAE)

An MAAE is a new onset or a worsening of a condition that prompts the subject to seek unplanned medical advice at a physician's office or emergency department. A physician contact made over the phone or by email will be considered a physician office visit for the purpose of MAAE collection. This definition excludes pre-planned medical office visits for routine medical care, as well as follow-up visits of chronic conditions with an onset prior to entry in the study. An AE discovered during a planned routine visit (e.g., upper respiratory tract infection, otitis) will be collected as an MAAE.

4.4.3 Derived Other Variables

4.4.3.1 Time since Primary Vaccination in Either MET44 or MET49

Time in years since primed vaccines is computed as follows: (Date of Vaccination in MEQ00066 – Date of Vaccination in MET44, MET49 + 1) / 365.25

4.4.3.2 Age for Demographics

The age of a subject in the study is the calendar age as defined in the protocol.

4.4.3.3 Age Subgroup for Demographics

Age subgroups for Groups 1 and 2 are as follows:

- 59 through 64 years
- ≥ 65 years

4.4.3.4 Race for Subgroup Analyses

Race for subgroup analyses will be categorized as:

- White
- Asian
- Black or African American
- Other

Note: The original race categories collected in CRF will still be used for demographics analyses. The American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Mixed origin, Not Reported and Unknown will be classified into "Other" stratum for subgroup analysis purposes only.

4.4.3.5 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.

The calculation should be conducted for:

- Groups 1 and 2 at the end of stage I
- Groups 5 and 6 at the end of stage I
- Groups 3 and 4 at the end of stage II

4.4.3.6 Duration of the Study

The duration of the study is computed in days as follows:

Maximum of all subjects (date of last visit, date of termination) – minimum for all subjects (date of Visit 1) +1.

The calculation should be conducted for:

- Groups 1 and 2 at the end of stage I
- Groups 5 and 6 at the end of stage I
- Groups 3 and 4 at the end of stage II

4.4.3.7 Protocol-Prohibited Therapy, Medications or Vaccines

In general, the "prohibited" variable is not derived. All concomitant medications are reviewed by the clinical team and the value of the "prohibited" variable is determined before database lock by the clinical team.

Only two categories of reportable medications will be considered for protocol-prohibited/restricted therapy, medications or vaccines:

Category 2:

- Influenza vaccines administered within 14 days before or after vaccination with the study vaccine
- Other vaccines not included as study vaccines (e.g., oral poliovirus, yellow fever, Japanese encephalitis, or other routine/non-routine vaccines not described in the protocol) within the 28 days (4 weeks) preceding or after vaccination with the study vaccine
- Immune globulins, blood or blood-derived products and immunosuppressive therapy, as described in the protocol.

Category 3:

- Antibiotics that the subject received within the 72 hours preceding each visit for blood sampling related to assessment of the study vaccine

Receipt of the above protocol-prohibited therapy, medications, or vaccines at or around the time of specific visits may impact safety and/or immunogenicity analyses.

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).

For descriptive purposes, the following statistics in Table 5.1 will be presented. The confidence intervals (CI) for the single proportions will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (10), ie, using the inverse of the beta integral with SAS®).

For immunogenicity results, assuming that Log10 transformation of the titers' data follow 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 GMs and their 95% CI.

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 / data)**	Log10: Mean and standard deviation. Anti-Log10 (work on Log10 distribution, and anti-Log10 applied): Geometric mean, 95% CI of the GM, quartiles, minimum, and maximum. Graphical representation by reverse cumulative distribution curves (RCDCs).

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Primary Objective

5.1.1.1 Hypotheses

Thirty days after the administration of MenACYW conjugate vaccine, the sufficiency of the vaccine seroresponse as assessed by the percentages of subjects who achieve an hSBA vaccine seroresponse* for meningococcal serogroups A, C, W, and Y, in Group 1 will be tested.

Vaccine seroresponse will be considered sufficient if the lower limit of the 1-sided 97.5% confidence interval (CI) for the percentage of subjects with an hSBA vaccine seroresponse against serogroups A, C, W and Y is greater than 40%.

This is equivalent to testing H0: $p \leq 0.40$ against H1: $p > 0.40$, where p is the observed proportion of subjects with hSBA vaccine seroresponse against serogroups A, C, W and Y. The 1-sided 97.5% CI for the single proportion will be calculated using the exact method (Clopper-Pearson method).

*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 at least 4-fold greater than the pre-vaccination titer.

5.1.1.2 Statistical Methods

The CI for the single proportion was calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (10), i.e., using the inverse of the beta integral with SAS®).

5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

5.1.2.1 Hypotheses

5.1.2.1.1 Secondary Objective 1

Thirty days after the administration of MenACYW conjugate vaccine, the sufficiency of the vaccine seroresponse, as assessed by the percentages of subjects who achieve an hSBA vaccine seroresponse* for meningococcal serogroups A, C, W, and Y, in Group 2 will be tested.

Vaccine seroresponse will be considered sufficient if the lower limit of the 1-sided 97.5% interval (CI) for the percentage of subjects with an hSBA seroresponse against serogroups A, C, W and Y is greater than 40%.

This is equivalent to testing H0: $p \leq 0.40$ against H1: $p > 0.40$, where p is the observed proportion of subjects with hSBA seroresponse against serogroups A, C, W and Y. The 1 sided 97.5% CI for the single proportion will be calculated using the exact method (Clopper-Pearson method).

*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 at least 4-fold greater than the pre-vaccination titer.

5.1.2.1.2 Secondary Objective 2

No hypotheses will be tested.

5.1.2.1.3 Secondary Objective 3

No hypotheses will be tested.

5.1.2.2 Statistical Methods

5.1.2.2.1 Secondary Objective 1

The CIs for the single proportions will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (10), i.e., using the inverse of the beta integral with SAS®).

5.1.2.2.2 Secondary Objective 2

The proportions of subjects in a planned nonrandom subset (approximately 60 subjects from Group 1 and approximately 60 subjects from Group 2) with vaccine seroresponse for meningococcal serogroups A, C, W, and Y at 6 (window, 5–7) days following vaccination will be determined; 95% CIs of point estimates will be calculated assuming proportions follow a binomial distribution using the Exact method.

5.1.2.2.3 Secondary Objective 3

For GMTs, the 95% CIs of point estimates will be calculated using the normal approximation assuming antibody titers/concentrations are log normally distributed. For proportions, 95% CIs of point estimates will be calculated assuming proportions follow a binomial distribution using the Exact method.

Categorical variables will be summarized and presented by frequency counts, proportion percentages, and confidence intervals (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 (6).

RCDC figures and kinetic curves will be provided for the antibody titers against meningococcal serogroups contained in MenACYW conjugate vaccine.

Descriptive analyses on A, C, W, and Y serogroups will include:

- hSBA and rSBA GMTs and corresponding 95% CIs
- hSBA and rSBA titer distribution and RCDCs

- Percentages of subjects with hSBA titer ≥ 1:4 and ≥ 1:8 and corresponding 95% CIs
- Percentages of subjects with rSBA titer ≥ 1:8 and ≥ 1:128 and corresponding 95% CIs

5.1.3 Statistical Methods for Observational Objective(s)

Immunogenicity

All analyses will be descriptive; no hypotheses will be tested.

For GMTs and GMCs, 95% CIs of point estimates will be calculated using the normal approximation assuming antibody titers/concentrations are log normally distributed. For proportions, 95% CIs of point estimates will be calculated assuming proportions follow a binomial distribution using the Exact method.

Descriptive analyses on A, C, W, and Y serogroups will include:

- hSBA and rSBA GMTs and corresponding 95% CIs
- hSBA and rSBA titer distribution and RCDCs
- Percentages of subjects with hSBA titer ≥ 1:4 and ≥ 1:8 and corresponding 95% CIs
- Percentages of subjects with rSBA titer ≥ 1:8 and ≥ 1:128 and corresponding 95% CIs
- Proportion with at least a 4-fold increase in hSBA and rSBA antibody titer compared to baseline (Group 1 and Group 2 during Stage I and Group 3 and Group 4 during Stage II)
    - An hSBA vaccine seroresponse for serogroups A, C, W, and Y is defined as a post-vaccination titer ≥ 1:16 for subjects with pre-vaccination titer < 1:8, or a post-vaccination titer ≥ 4 times the pre-vaccination titer for subjects with a pre-vaccination titer ≥ 1:8.
    - An 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.
- Ratio of the GMT post-vaccination/GMT pre-vaccination and the associated 95% CI of the ratio (at Day 30 for Group 1 and Group 2 and at Day 6 in the subset of Group 1 and Group 2 [during Stage I], and at Day 30 for Group 3 and Group 4 [during Stage II]).

Descriptive analyses on anti-tetanus antibody concentrations will include:

- Geometric mean concentrations (GMCs) at Day 0 (all groups), 2 years after enrollment (Groups 3 and 4), and 30 (window, + 14) days after the administration of a single dose of MenACYW conjugate vaccine in Study MEQ00066 (Groups 1–4)
- Proportion of subjects achieving seroprotective antibody levels ≥ 0.01 IU (international units) /mL, ≥ 0.1 IU/mL, and ≥ 1.0 IU/mL to tetanus toxoid 30 (window, + 14) days after the administration of a single dose of MenACYW conjugate vaccine in Study MEQ00066 (Groups 1–4)
- RCDCs and kinetic curves

Safety

All analyses will be descriptive; no hypotheses will be tested.

Safety results will be described for subjects revaccinated with MenACYW conjugate vaccine (i.e., Groups 1–4). The main parameters for the safety endpoints will be described by 95% CIs (based on the Clopper-Pearson method).

The frequency and percentage of subjects who had solicited injection site and systemic reactions and their 95% CIs will be provided. These events will be tabulated by 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, action taken).

Unsolicited AEs will be collected, coded, and summarized by MedDRA system organ class and PT. For each unsolicited AE, the number of subjects with at least one 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 in the endpoints.

Immediate reactions, SAEs, and any event that leads to subject withdrawal from the study will be tabulated separately.

Table 5.2: Statistical Analyses for Safety Observational Objective at Stage I (Groups 1 and 2) and Stage II (Groups 3 and 4)

Safety Events	Time and Group	Description
Immediate unsolicited non-serious systemic AE	Within 30 minutes after vaccination for all subjects in Groups 1 – 4	Percentage of subjects that have the event, MedDRA terms, intensity, relationship to vaccine, study discontinuation
Solicited injection site reactions	Within 7 days after vaccination for all subjects in Groups 1 – 4	Percentage of subjects that have the event, onset, duration, intensity, action taken, study discontinuation, temperature collection routes
Solicited systemic reactions	Within 7 days after vaccination for all subjects in Groups 1 – 4	
Unsolicited non-serious AEs/ARs	Within 30 days after vaccination for all subjects in Groups 1 – 4	Percentage of subjects that have the event, MedDRA terms, onset, duration, intensity, relationship, action taken, study discontinuation
AEs leading to study discontinuation	Within 30 days after vaccination for all subjects in Groups 1 – 4	Percentage of subjects that have the event, MedDRA terms, time of onset, duration, intensity, relationship, action taken, study discontinuation
SAEs (including AESIs)	Within 7 days after vaccination for all subjects in Groups 1 – 4 Within 30 days after vaccination for all subjects in Groups 1 – 4	Percentage of subjects that have the event, MedDRA terms, onset, duration, relationship, seriousness criteria, outcome, study discontinuation
MAAEs	Within 30 days after vaccination for all subjects in Groups 1 – 4	Percentage of subjects that have the event, MedDRA terms, onset, duration, relationship, seriousness criteria, outcome, study discontinuation

5.2 Analysis Sets

5.2.1 Stage I Analysis Sets

5.2.1.1 Full Analysis Set

The Stage I Day 30 FAS (FAS1) is defined as subjects in Groups 1 and 2 who received the study vaccine and have a valid serology result for at least 1 serogroup from a BL provided 30 (window, +14) days post-vaccination.

The Stage I Day 6 FAS (FAS2) is defined as the subset of Group 1 and 2 subjects who received the study vaccine and have a valid serology result for at least 1 serogroup from a BL provided 6 (window, 5–7) days post-vaccination.

The Stage I FAS for persistence (FAS3) includes subjects in Groups 1, 2, 3, 4, 5 and 6 who have a valid serology result for at least 1 serogroup from a pre-vaccination BL (3-year or 6–7-year antibody persistence).

5.2.1.2 Per-Protocol Analysis Sets

5.2.1.2.1 Day 30 Per-Protocol Analysis Set
Groups 1 and 2

The Stage I Day 30 Per-Protocol Analysis Set (PPAS1) is a subset of Stage I Day 30 FAS (FAS1). Subjects with at least 1 of the following relevant protocol deviations will be excluded from the PPAS1:

- Subject did not meet all protocol-specified inclusion criteria or met at least 1 of the protocol-specified exclusion criteria
- Subject did not receive MenACYW conjugate vaccine according to randomization
- 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 a post-dose serology sample in the pre-specified time window at 30 (window, + 14) days post-vaccination
- Subject received a protocol-prohibited therapy/medication/vaccine that might impact antibody response to the study vaccine
- Subjects received study vaccine deemed unacceptable for use (e.g., because of a temperature excursion).
- Subject's post-vaccination serology sample did not produce a valid test result for any antigen (i.e., results for all antigens are missing)
- Any other deviation identified during conduct of the study and judged by the clinical team during data review as having a potential impact on the assessment of immunogenicity

5.2.1.2.2 Day 6 Per-Protocol Analysis Set
Groups 1 and 2

The Day 6 PPAS (PPAS2) is a subset of Day 6 FAS (FAS2). Subjects with at least 1 of the following relevant protocol deviations will be excluded from the Day 6 PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least 1 of the protocol-specified exclusion criteria
- Subject did not receive MenACYW conjugate vaccine according to randomization
- 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 a post-dose serology sample in the pre-specified time window at 6 (window, 5 to 7) days post-vaccination
- Subject received a protocol-prohibited therapy/medication/vaccine that might impact antibody response to the study vaccine
- Subjects received study vaccine deemed unacceptable for use (e.g., because of a temperature excursion).
- Subject's post-vaccination serology sample did not produce a valid test result for any antigen (i.e., results for all antigens are missing)
- Any other deviation identified during conduct of the study and judged by the clinical team during data review as having a potential impact on the assessment of immunogenicity.

5.2.1.3 Safety Analysis Set (SafAS1)
Groups 1 and 2

The Stage I Analysis Set (SafAS1) is defined as those subjects who have received the study vaccine and have any safety data available. Safety will be analyzed according to the vaccine actually received by the subject.

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

5.2.2 Stage II Analysis Sets (Groups 3 and 4)

5.2.2.1 Full Analysis Sets

The Stage II Day 30 FAS (FAS4) is defined as subjects in Groups 3 and 4 who received the study vaccine and have a valid serology result for at least 1 serogroup from a BL provided 30 (window, +14) days post-vaccination.

The Stage II FAS for persistence (FAS5) includes subjects in Groups 3 and 4 who have a valid serology result for at least 1 serogroup from a pre-vaccination BL (5-year antibody persistence).

5.2.2.2 Day 30 Per-Protocol Analysis Set

The Stage II Day 30 PPAS (PPAS3) is a subset of the Stage II Day 30 FAS (FAS4). Subjects with at least 1 of the following relevant protocol deviations will be excluded from the Stage II Day 30 PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least 1 of the protocol-specified exclusion criteria
- Subject did not receive MenACYW conjugate vaccine according to randomization
- 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 a post-dose serology sample in the pre-specified time window at 30 (window, + 14) days post-vaccination
- Subject received a protocol-prohibited therapy/medication/vaccine that might impact antibody response to the study vaccine
- Subjects received study vaccine deemed unacceptable for use (e.g., because of a temperature excursion).
- Subject's post-vaccination serology sample did not produce a valid test result for any antigen (i.e., results for all antigens are missing)
- Any other deviation identified during conduct of the study and judged by the clinical team during data review as having a potential impact on the assessment of immunogenicity

5.2.2.3 Analysis Set (SafAS2)

The Stage II Analysis Set (SafAS2) is defined as those subjects who have received the study vaccine and have any safety data available. Safety will be analyzed according to the vaccine actually received by the subject.

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

5.2.2.4 Enrolled Subjects

An enrolled subject is defined as a subject for whom a CRF has been created.

5.2.2.5 Randomized Subjects

A randomized subject is a subject for whom an injection vaccine group has been randomly allocated.

5.2.3 Populations Used in Analyses

5.2.3.1 Stage I

The primary immunogenicity analyses will be performed on the Stage I Day 30 PPAS (PPAS1) and will be confirmed on the Stage I Day 30 FAS (FAS1). In the FAS1, subjects will be analyzed by the vaccine group to which they were randomized.

The secondary immunogenicity analyses (Day 6 subset) will be performed on the Day 6 PPAS (PPAS2) and Day 6 FAS (FAS2).

The secondary antibody persistence analyses will be performed on the FAS3.

The safety analysis will be performed on the Stage I Analysis Set (SafAS1). Subjects will be analyzed according to the vaccine they actually received.

5.2.3.2 Stage II

Immunogenicity analyses will be performed on the Stage II Day 30 PPAS (PPAS3) and confirmed on the Stage II Day 30 FAS (FAS4).

The secondary antibody persistence analyses will be performed on FAS5.

The safety analysis will be performed on the SafAS2. Subjects will be analyzed according to the vaccine they actually received.

5.2.4 Subgroup Analysis

Subgroup analyses by age group (59 through 64 years of age and ≥ 65 years), gender (female and male), and race (White, Asian, Black or African American, and Other) will be provided in Appendix 15 of the CSR.

5.3 Handling of Missing Data and Outliers

5.3.1 Safety

No replacement will be done. In all 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 4.4.2.1.1.

5.3.1.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.4.2.1.1. 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 search for outliers will be performed.

5.4 Interim / Preliminary Analysis

Relevant analyses will be completed for subjects in Groups 1, 2, 3, 4, 5, and 6 after completion of Stage I. After collecting all planned data for Groups 1, 2, 3, 4, 5, and 6 after completion of Stage I, the study database will be cleaned and locked. All statistical analyses planned for these groups will be conducted, and a final study report will be written.

After the post-revaccination safety and immunogenicity data for Group 3 and Group 4 are collected during Stage II, the database will be updated to include the newly collected data, which will be cleaned, and the database will be relocked. Analysis of the new data will be conducted and an addendum to the clinical study report will be written.

5.5 Determination of Sample Size and Power Calculation

A total of 560 subjects are planned to be enrolled:

- Group 1 (subjects who received Menomune vaccine in Study MET49) will receive a single dose of MenACYW conjugate vaccine at enrollment in Study MEQ00066: n=180
- Group 2 (subjects who received MenACYW conjugate vaccine in Study MET49) will receive a single dose of MenACYW conjugate vaccine at enrollment in Study MEQ00066: n=180
- Group 3 (subjects who received Menomune vaccine in Study MET49) will receive a single dose of MenACYW conjugate vaccine 2 years after enrollment in Study MEQ00066: n=40
- Group 4 (subjects who received MenACYW conjugate vaccine in Study MET49) will receive a single dose of MenACYW conjugate vaccine 2 years after enrollment in Study MEQ00066: n =40
- Group 5 (subjects who previously received Menomune vaccine in Study MET44): n=60
- Group 6 (subjects who previously received MenACYW conjugate vaccine in Study MET44): n=60

For the Primary Objective and Secondary Objective 1: For Group 1 and Group 2, a sample size of 120 achieves at least 90.0% power to detect that the lower bound of the one-sided 97.5% CI is greater than 0.40 (proportion under the null hypothesis) using a 1-sided exact test with a significance level (alpha) of 0.025.

For all other groups (i.e, Groups 3–6), descriptive statistics will be calculated and presented in this study; hence, no sample size or study power was calculated for these groups.

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

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

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