Statistical Analysis Plan

MenACWY-TT-100 (C0921004)

Detailed Title:	A phase IIIb, open, multi-center study to evaluate the long-term antibody persistence at 6, 7, 8, 9 and 10 years after the administration of one dose of the meningococcal conjugate vaccine MenACWY-TT versus one dose of Meningitec® vaccine or one dose of the meningococcal polysaccharide vaccine Mencevax® ACWY, and to evaluate the safety and immunogenicity of a booster dose of MenACWY-TT vaccine administered 10 years after primary vaccination of 1-10 year old subjects with MenACWY-TT, Meningitec or Mencevax ACWY.
SAP Version:	Version 4
SAP Date:	26-Jul-2017
Scope:	All data pertaining to the above study.
Coordinating Author:	PPD
Other Author(s):	
Ad hoc Reviewers:	PPD PPD PPD PPD

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LIST OF ABBREVIATIONS

AE	adverse event
ANOVA	analysis of variance
ATP	according-to-protocol
BS	blood sample
BTV	booster total vaccinated
CI	confidence interval
CSR	clinical study report
ESFU	extended safety follow-up
GMT	geometric mean titer
GSK	GlaxoSmithKline
hSBA	serum bactericidal assay against *Neisseria meningitidis* using human complement
hSBA-MenA	serum bactericidal assay against *Neisseria meningitidis* group A using human complement
hSBA-MenC	serum bactericidal assay against *Neisseria meningitidis* group C using human complement
hSBA-MenW-135	serum bactericidal assay against *Neisseria meningitidis* group W-135 using human complement
hSBA-MenY	serum bactericidal assay against *Neisseria meningitidis* group Y using human complement
LL	lower limit of the confidence interval
MedDRA	Medical Dictionary for Regulatory Activities
MenA	*Neisseria meningitidis* group A
MenC	*Neisseria meningitidis* group C
MenW-135	*Neisseria meningitidis* group W-135
MenY	*Neisseria meningitidis* group Y
PHE	Public Health England
rSBA	serum bactericidal assay against *Neisseria meningitidis* using rabbit complement
rSBA-MenA	serum bactericidal assay against *Neisseria meningitidis* group A using rabbit complement
rSBA-MenC	serum bactericidal assay against *Neisseria meningitidis* group C using rabbit complement
rSBA-MenW-135	serum bactericidal assay against *Neisseria meningitidis* group W-135 using rabbit complement
rSBA-MenY	serum bactericidal assay against *Neisseria meningitidis* group Y using rabbit complement
SAE	serious adverse event

SAP	statistical analysis plan
SD	standard deviation
TC	telephone call
TFL	tables, figures, and listings (template annexed to SAP)
UL	upper limit of the confidence interval

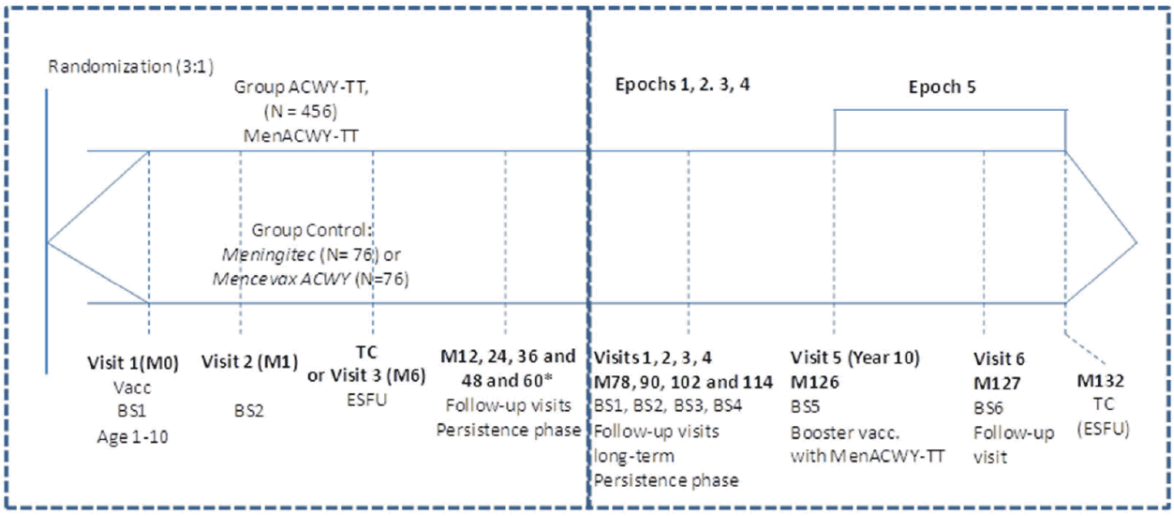
1. DOCUMENT HISTORY

Date	Description	Protocol Version
06-May-2015	Version 1	Final Version 1: 25 Jun 2013
17-Aug-2016	Version 2	Amendment 2, 25 Feb 2016
18-Jan-2017	Version 3: Intervals from primary vacation to perseverance visits in Table 2 changed as follows: 2282-2450 to 2290-2458 2646-2814 to 2655-2823 3010-3178 to 3020-3188 3374-3542 to 3386-3554 3738-3906 to 3751-3919 Revised intervals match windows used by GSK.	Amendment 2, 25 Feb 2016
26-Jul-2017	Version 4: In Section 8.1, vaccination day should be considered as Day 0 (not Day 1) to be consistent with the protocol.	Amendment 2, 25 Feb 2016

2. INTRODUCTION

The complete statistical analysis plan (SAP) and results presentation is divided into 2 parts: the first part detailing the analyses to be performed (known as SAP, current document) and a second part, annex (-es) (tables, figures, and listings [TFLs]) describing the flow and format of TFLs to be annexed to the study report. This SAP covers the planned statistical analysis from Month 78 (Year 6) to Month 126 (Year 10) post primary vaccination, and ending at the Phone Contact (Month 132 or six months post booster vaccination).

3. STUDY DESIGN



BS = blood sample
TC = telephone contact
Vacc = vaccination
ESFU = extended safety follow-up

Experimental Design: Phase IIIB, open-label, multi-centric, single-country study with four parallel groups.

Control: active control for persistence phase (MenCCRM and MenPS groups), uncontrolled for booster phase (all subjects receive the same booster vaccination [MenACWY-TT vaccine]).

Vaccination Schedule: At Visit 5 (Month 126 after primary vaccination), one dose of MenACWY-TT will be administered to the subjects in all study groups.

Duration of the Study:

Persistence Phase:

- Epoch 001: Persistence Visit 1 [Month 78 (Year 6) after primary vaccination]
- Epoch 002: Persistence Visit 2 [Month 90 (Year 7) after primary vaccination]
- Epoch 003: Persistence Visit 3 [Month 102 (Year 8) after primary vaccination]
- Epoch 004: Persistence Visit 4 [Month 114 (Year 9) after primary vaccination]

Booster Phase:

- Epoch 005: Booster vaccination starting at Visit 5 [Month 126 (Year 10) after primary vaccination] and ending at the phone contact (Month 132 or 6 months after booster vaccination)

Blinding: Open-label

Sampling Schedule: At each study visit a blood sample will be collected from each subject enrolled.

Study Groups:

The following group names will be used for the statistical analyses:

Group Order in Tables	Group Label in Tables	Group Definition for Footnote
1	ACWY<2	Vaccinated with MenACWY-TT in Study MenACWY-TT-027 (108658) and aged <2 years at the time of primary vaccination
2	MenCCRM	Vaccinated with Meningitec® in Study MenACWY-TT-027 (108658)
3	ACWY≥2	Vaccinated with MenACWY-TT in Study MenACWY-TT-027 (108658) and aged ≥2 years at the time of primary vaccination
4	MenPS	Vaccinated with Mencevax® ACWY in Study MenACWY-TT-027 (108658)

4. OBJECTIVES

4.1. Primary Objectives

Long-term persistence phase: 6, 7, 8, 9, and 10 years after primary vaccination with MenACWY-TT, Meningitec, or Mencevax ACWY in Study MenACWY-TT-027:

- To evaluate the long-term persistence of the serum bactericidal (antibody) titers induced by MenACWY-TT vaccine as compared to Meningitec when administered to individuals 1-<2 years of age in terms of the percentage of subjects with *Neisseria meningitidis* group A (MenA), group C (MenC), group W-135 (MenW-135), and group Y (MenY) titers ≥1:8, ≥1:128 and geometric mean titers (GMTs) as measured by a serum bactericidal assay using rabbit complement (rSBA) in those subjects who received MenACWY-TT, and group C (MenC) rSBA titers ≥1:8, ≥1:128 and GMTs in those subjects who received Meningitec.

- To evaluate the long-term persistence of the serum bactericidal (antibody) titers induced by MenACWY-TT vaccine as compared to Mencevax ACWY when administered to individuals 2-10 years of age in terms of the percentage of subjects with *Neisseria meningitidis* group A (MenA), group C (MenC), group W-135 (MenW-135), and group Y (MenY) titers ≥1:8, ≥1:128 and GMTs as measured by rSBA.

4.2. Secondary Objectives

Persistence Phase:

Long–term persistence phase: 6, 7, 8, 9, and 10 years after primary vaccination with MenACWY-TT, Meningitec, or Mencevax ACWY in Study MenACWY-TT-027:

- To evaluate the long-term persistence induced by MenACWY-TT vaccine as compared to Meningitec when administered to individuals 1-<2 years of age in terms of percentage of subjects with serum bactericidal assay using human complement (hSBA) titers ≥1:4, ≥1:8 and GMTs for all 4 groups in those subjects who received MenACWY-TT and for group C (MenC) hSBA titers ≥1:4, ≥1:8 and GMTs in those subjects who received Meningitec.

- To evaluate the long-term persistence induced by MenACWY-TT vaccine as compared to Mencevax ACWY when administered to individuals 2-10 years of age in terms of percentage of subjects with hSBA titers ≥1:4, ≥1:8 and GMTs for all 4 groups.

Booster Phase:

One month after the booster vaccination with MenACWY-TT vaccine administered 10 years after primary vaccination:

- To evaluate the immunogenicity of a booster dose vaccination of MenACWY-TT with respect to the percentage of subjects with rSBA-MenA, rSBA-MenC, rSBA-MenW- 135, and rSBA-MenY antibody titers ≥1:8, ≥1:128 and GMTs.

- To evaluate the immunogenicity of a booster dose vaccination of MenACWY-TT with respect to the percentage of subjects with hSBA-MenA, hSBA-MenC, hSBA-MenW- 135, and hSBA-MenY antibody titers ≥1:4, ≥1:8 and GMTs.

- To evaluate the immunogenicity of a booster dose of MenACWY-TT conjugate vaccine in terms of the percentage of subjects with an rSBA-MenA, rSBA-MenC, rSBA-MenW-135, and rSBA-MenY booster response.*

  *rSBA booster response to meningococcal antigens (A, C, W-135, and Y) is defined as:

  - For initially seronegative subjects (prevaccination rSBA titer <1:8): rSBA antibody titer ≥1:32 1 month after vaccination, and

  - For initially seropositive subjects (prevaccination rSBA titer ≥1:8): at least 4-fold increase in rSBA titers from prevaccination to 1 month after vaccination.

- To evaluate the immunogenicity of a booster dose of MenACWY-TT conjugate vaccine in terms of the percentage of subjects with an hSBA-MenA, hSBA-MenC, hSBA-MenW-135, and hSBA-MenY booster response.*

  *hSBA booster response to meningococcal antigens (A, C, W-135, and Y) is defined as:

  - For initially seronegative subjects (prevaccination hSBA titer <1:4): hSBA antibody titers ≥1:8 1 month after vaccination, and

  - For initially seropositive subjects (hSBA titer ≥1:4): at least 4-fold increase in hSBA titers from prevaccination to 1 month after vaccination.

Secondary Safety Objectives:

Persistence Phase:

- To describe serious adverse events (SAEs) related to vaccination and any event related to lack of vaccine efficacy (ie, meningococcal disease) from the last persistence time point the subject participated in up to each yearly visit in the current study in a retrospective manner.

Booster Phase:

- To evaluate the safety and reactogenicity of a booster vaccination dose of MenACWY-TT conjugate vaccine.

5. ENDPOINTS

5.1. Primary Endpoint

Immunogenicity with respect to the components of the investigational vaccine 6, 7, 8, 9, and 10 years after primary vaccination in Study MenACWY-TT-027:

- rSBA-MenA, rSBA-MenC, rSBA-MenW-135, and rSBA-MenY antibody titers ≥1:8, ≥1:128 and GMTs.

5.2. Secondary Endpoint(s)

Persistence Phase:

Immunogenicity with respect to the components of the investigational vaccine 6, 7, 8, 9, and 10 years after primary vaccination in Study MenACWY-TT-027:

- hSBA-MenA, hSBA-MenC, hSBA-MenW-135, and hSBA-MenY antibody titers ≥1:4, ≥1:8 and GMTs.

Booster Phase:

Immunogenicity with respect to the components of the investigational vaccine 1 month after booster vaccination at 10 years after primary vaccination:

- rSBA-MenA, rSBA-MenC, rSBA-MenW-135, and rSBA-MenY antibody titers ≥1:8, ≥1:128 and GMTs and rSBA booster response.

- hSBA-MenA, hSBA-MenC, hSBA-MenW-135, and hSBA-MenY antibody titers ≥1:4, ≥1:8 and GMTs and hSBA booster response.

Safety and Reactogenicity

Persistence Phase:

- Occurrence of SAEs related to vaccination and any event related to lack of vaccine efficacy (ie, meningococcal disease) since the last persistent time point the subject participated in up to each yearly visit in the current study in a retrospective manner.

Booster Phase:

- Occurrence of solicited local and general events on Days 0-3 following the booster vaccination.
- Occurrence of unsolicited adverse events (AEs) up to 31 days following booster vaccination.
- Occurrence of all SAEs, and new-onset chronic illness(es) (eg, autoimmune disorders, asthma, type 1 diabetes, and allergies) from administration of the vaccine dose until study end.

6. STUDY POPULATION

Several cohorts are defined for the purpose of analysis.

6.1. Persistence Cohorts

The requirements for the persistence cohorts are summarized in Table 1. Details are included in Section 6.1.1 through Section 6.1.4.

Table 1. Summary Description of Cohorts Associated with Persistence Analyses

Total Enrolled	Total Enrolled - Persistence	Total Cohort at Month X	ATP Cohort for Persistence MenC at Month X	ATP Cohort for Persistence MenAWY at Month X
Enrolled in study regardless of visit when enrolled.	Any titer at any time point.	All MenACWY-TT-027 vaccinated subjects who return for Month X	MenC result at Month X available	MenA, MenW-135, or MenY result at Month X available
	Enrollment or lack of enrollment in MenACWY-TT-100 is not exclusionary	Persistence data available	Eligible for MenACWY-TT-027	Eligible for MenACWY-TT-027

Table 1. Summary Description of Cohorts Associated with Persistence Analyses

Total Enrolled	Total Enrolled - Persistence	Total Cohort at Month X	ATP Cohort for Persistence MenC at Month X	ATP Cohort for Persistence MenAWY at Month X
			Vaccinated in MenACWY-TT-027 with assigned vaccine	Vaccinated in MenACWY-TT-027 with assigned vaccine
			No unplanned meningococcal vaccines before Month X	No unplanned meningococcal vaccines before Month X, except MenC vaccine in response to suboptimal response in MenC from MenACWY- TT-027
			No meningococcal history before Month X	No meningococcal history before Month X
			Comply with blood sampling interval for Month X	Comply with blood sampling interval for Month X
			No immuno-compromising medical condition	No immuno-compromising medical condition
			No immuno-suppressants, etc.	No immuno-suppressants, etc.
			Not excluded from ATP in MenACWY-TT-027 and not excluded from previous ATP persistence cohorts, unless reason for exclusion is either missed windows or no immunogenicity results.	Not excluded from ATP in MenACWY-TT-027 and not excluded from previous ATP persistence cohorts, unless reason for exclusion is either (1) missed windows or (2) no immunogenicity results or (3)re-vaccination with monovalent MenC due to suboptimal MenC response in MenACWY-TT-027

Table 1. Summary Description of Cohorts Associated with Persistence Analyses

Total Enrolled	Total Enrolled - Persistence	Total Cohort at Month X	ATP Cohort for Persistence MenC at Month X	ATP Cohort for Persistence MenAWY at Month X
			Medications/products/ vaccines and/or no intercurrent medical conditions that may lead to the exclusion of a subject according to Protocol sections 6.5.2 and 6.6	Medications/ products/vaccines and/or no intercurrent medical conditions that may lead to the exclusion of a subject according to Protocol sections 6.5.2 and 6.6

Persistence cohorts are described in more detail in Section 6.1.1, Section 6.1.2, Section 6.1.3, and Section 6.1.4.

6.1.1. Total Cohort at Month X

The total cohort at Month X will include all vaccinated subjects from the vaccination stage of Study MenACWY-TT-027 (108658) who return for the Month X follow-up. The analysis of persistence will include all vaccinated subjects for whom data concerning persistence endpoint measures are available.

6.1.2. According-To-Protocol (ATP) Cohort for Persistence at Month X

Two ATP cohorts for persistence at Month X are defined, one for MenC and one for MenAWY. The requirements for both cohorts are the same except that membership in the MenC (or MenAWY) cohort requires at least one MenC (or MenAWY) titer.

The ATP cohort for persistence at Month X will include all evaluable subjects:

- who were eligible in Study MenACWY-TT-027 (108658).
- who received the primary vaccination with MenACWY-TT, Meningitec, or Mencevax ACWY during Study MenACWY-TT-027 (108658) according to their vaccine group.
- who have available assay results for at least 1 of 3 antigens (MenA or MenW-135 or MenY) or for MenC (for subjects who did not receive MenC vaccine because they were non-responders during the persistence phase of Study MenACWY-TT-027 please see Section 7.1 of the SAP) at Month X.

- who only received a meningococcal vaccine planned in Study MenACWY-TT-027 (108658) before Month X (with the exception of subjects who received the MenC vaccine because they were suboptimal responders during the persistence phase of Study MenACWY-TT-027).

- who did not have a history of meningococcal disease prior to Month X.

- who complied with the blood sampling intervals defined in the protocol for Month X (see Table 5 of the protocol and Table 2).

- who did not have an immune compromising medical condition.

- who did not receive any immunosuppressant(s) or other immune-modifying drug(s), immunoglobulins, any blood products, investigational drugs, and/or investigational vaccines within 30 days of the persistence blood sample.

- who were not excluded from the ATP cohort in the primary study (MenACWY-TT-027 [108658]) and from the previous ATP persistence cohorts, unless the reason for exclusion was either non-compliance with the protocol-defined serum sampling windows or a lack of availability of immunogenicity results at a previous time point.

Table 2. Intervals from Primary Vaccination in TT-027 to Persistence Visits

Visit	Interval in Days
78 months	2290-2458
90 months	2655-2823
102 months	3020-3188
114 months	3386-3554
126 months	3751-3919

6.1.3. Adapted ATP Cohort

When presenting different time points, the adapted ATP cohort will be used to denote that for each time point, the corresponding ATP cohort for immunogenicity has been used.

More specifically:

- the analyses on the pre and post primary time points will be based on the ATP cohort for immunogenicity defined in MenACWY-TT-027.

- the analyses on Months 12, 24, 36, 48, and 60 time points will be based on the ATP cohort for persistence in MenACWY-TT-028, -029, -030, -031, and -032 respectively.

- the analyses on Months 78, 90, 102, 114, and 126 time points are based on the ATP cohort for persistence at Months 78, 90, 102, 114, and 126 in MenACWY-TT-100, respectively.

6.1.4. Total Enrolled Cohort

The total enrolled cohort will include all the subjects enrolled in the study irrespective of the visit at which they are enrolled. Lack of enrollment in MenACWY-TT-100 is not exclusionary.

The total enrolled – persistence cohort will include all subjects with any immunogenicity result available at any time point, which means data from Studies MenACWY-TT-027/-028/-029/-030/-031/-032 and all time points from Study -100. More specifically, this cohort will include subjects with immunogenicity results available at any time point who also belong to the corresponding total vaccinated cohort (Study MenACWY-TT-027) or total cohort (Studies MenACWY-TT-028, -029, -030, -031, -032, and -100) regardless of their enrollment in Study MenACWY-TT-100.

6.2. Booster Cohort

The requirements for the persistence cohorts are summarized in Table 3. Details are included in Section 6.2.1 through Section 6.2.4.

Table 3. Summary Description of Cohorts Associated with Booster Analyses

Booster Total Vaccinated Cohort	Booster Total Vaccinated for Immunogenicity Cohort	Booster ATP for Safety Cohort	Booster ATP Immunogenicity MenC Cohort	Booster ATP Immunogenicity MenAWY Cohort
Vaccinated in MenACWY-TT-027	Vaccinated in MenACWY-TT-027	Vaccinated in MenACWY-TT-027 with assigned vaccination	Vaccinated in MenACWY-TT-027 with assigned vaccination	Vaccinated in MenACWY-TT-027 with assigned vaccination
Received booster dose	Received booster dose	Received booster dose	Received booster dose	Received booster dose
		Met all inclusion/exclusion for MenACWY-TT-100	Met all inclusion/exclusion for MenACWY-TT-100	Met all inclusion/exclusion for MenACWY-TT-100
	At least 1 postbooster titer available	Have not received a vaccine not specified or forbidden in protocol	Have not received a vaccine not specified or forbidden in protocol	Have not received a vaccine not specified or forbidden in protocol
		Admin site of booster vaccination known	Admin site of booster vaccination known	Admin site of booster vaccination known

Table 3. Summary Description of Cohorts Associated with Booster Analyses

Booster Total Vaccinated Cohort	Booster Total Vaccinated for Immunogenicity Cohort	Booster ATP for Safety Cohort	Booster ATP Immunogenicity MenC Cohort	Booster ATP Immunogenicity MenAWY Cohort
		Not excluded from ATP cohort for Year 10, unless reason was (1) missing window or (2) no immuno results at Year 10	Not excluded from ATP cohort for Year 10, unless reason was (1) missing window or (2) no immuno results at Year 10	Not excluded from ATP cohort for Year 10, unless reason was (1) missing window or (2) no immuno results at Year 10
			Comply with protocol procedures	Comply with protocol procedures
			No elimination criteria during the study.	No elimination criteria during the study.
			Member of booster ATP for safety cohort	Member of booster ATP for safety cohort
			MenC titer available post booster	MenA, W-135, or Y titer available
			No unforeseen vaccinations from 30 days before booster until the postvaccination bleed	No unforeseen vaccinations from 30 days before booster until the postvaccination bleed
			No MenC or MenACWY conjugate vaccine between MenACWY-TT-027 vaccination and booster due to suboptimal MenC response	No MenACWY conjugate vaccine between MenACWY-TT-027 vaccination and booster due to suboptimal response
				Receiving monovalent MenC conjugate due to suboptimal response is not exclusionary
			Interval for inclusion is 21-48 days from vaccination to post bleed	Interval for inclusion is 21-48 days from vaccination to post bleed
			Medications/products/vaccines and/or no intercurrent medical conditions that may lead to the exclusion of a subject according to Protocol sections 6.5.2 and 6.6	Medications/products/vaccines and/or no intercurrent medical conditions that may lead to the exclusion of a subject according to Protocol sections 6.5.2 and 6.6

Persistence cohorts are described in more detail in Section 6.2.1, Section 6.2.2, Section 6.2.3, and Section 6.2.4.

6.2.1. Booster Total Vaccinated Cohort

The booster total vaccinated (BTV) cohort for safety will include all vaccinated subjects in the primary vaccination Study MenACWY-TT-027 (108658) with a documented booster vaccine administration.

For the analysis of immunogenicity after the booster vaccination the BTV cohort will include all subjects for whom data concerning post–booster immunogenicity endpoint measures are available.

6.2.2. Booster ATP Cohort for Safety

The booster ATP cohort for safety will include all subjects:

- who met all inclusion criteria and no exclusion criteria for the study.
- who received a dose of study vaccine MenACWY-TT, Meningitec, or Mencevax ACWY in the primary study (MenACWY-TT-027) according to their vaccine group.
- who did not receive a vaccine not specified or forbidden in the protocol (subjects who received a vaccine not foreseen by the study protocol from 30 days before until 30 days after the study vaccine dose will be eliminated from the ATP cohort for safety if the vaccine not foreseen by the protocol was administered before the postvaccination blood sample).
- who received a booster dose of study vaccine MenACWY-TT in the booster epoch.
- for whom the administration site of the vaccine is known.
- who were not excluded from either ATP cohort for persistence at Month 126 (ie, MenC or MenAWY), unless the reason for exclusion was either non-compliance with the protocol-defined serum sampling windows or a lack of availability of immunogenicity results at Month 126 (Year 10, prebooster vaccination).

6.2.3. Booster ATP Cohort for Immunogenicity – MenC

The booster ATP cohort for immunogenicity – MenC will include all subjects who:

- are evaluable (ie, those meeting all eligibility criteria, complying with the procedures defined in the protocol and with no exclusion criteria during the study).
- are from the booster ATP cohort for safety.
- have assay results available for antibodies against MenC antigen for the blood sample taken 1 month post-vaccination, and

- were not administered a vaccine not foreseen by the study protocol from 30 days before the booster administration until the post-vaccination blood sample, and
- have not received a monovalent MenC conjugate vaccine or MenACWY conjugate vaccine because of suboptimal response to *N meningitidis* group C between the vaccination in the primary study (MenACWY-TT-027 [108568]) and the booster vaccine in this study, and
- have an interval between Visit 5 and Visit 6 of 21 to 48 days, inclusive.

6.2.4. Booster ATP Cohort for Immunogenicity –MenAWY

The booster ATP cohort for immunogenicity – MenAWY cohort will include all subjects who:

- are evaluable (ie, those meeting all eligibility criteria, complying with the procedures defined in the protocol and with no exclusion criteria during the study).
- are from the booster ATP cohort for safety.
- have assay results available for antibodies against at least 1 of the 3 antigens (MenA or MenW-135 or MenY) for the blood sample taken 1 month after vaccination, and
- were not administered a vaccine not foreseen by the study protocol from 30 days before the booster administration until the post-vaccination blood sample and
- have not received a MenACWY conjugate vaccine because of sub-optimal response between the vaccination in the primary study (MenACWY-TT-027 [108568]) and the booster vaccine in this study (if a subject previously received monovalent MenC conjugate vaccine because of suboptimal response to *N meningitidis* group C after vaccination in the primary study MenACWY-TT-027 (108568), the subject can still be included in the booster ATP cohort for immunogenicity – MenAWY), and
- have an interval between Visit 5 and Visit 6 of 21 to 48 days, inclusive.

7. STATISTICAL METHODS

7.1. Persistence Analyses

7.1.1. Analysis of Demographics/Baseline Characteristics

Demographic characteristics of subjects in each study cohort will be tabulated: age (in years) at Month X (with the range and standard deviation), sex, and geographic ancestry.

The distribution of subjects enrolled in this study among the study sites will be tabulated as a whole and per group. The reasons for not enrolling will be summarized for all subjects who were vaccinated in the primary study (MenACWY-TT-027 [108658]).

7.1.2. Analysis of Persistence

The persistence analyses will be based on the adapted ATP cohort. If for any vaccine group, at any time point, the percentage of subjects who have serological results but are otherwise excluded for the corresponding ATP cohort is higher than 10%, a second analysis based on the total enrolled cohort will be performed to complement the ATP analysis. The percentage of subjects with excluded serological results will compiled separately for the MenC ATP persistence cohort and the MenAWY ATP persistence cohort.

Different laboratories performed the rSBAs and hSBAs at different time points. rSBAs were performed either by GSK or Public Health England (PHE) (see Table 4). hSBAs were performed either by GSK or Neomed (Neomed was a GSK facility until it was spun off as an independent company) (see Table 5). Furthermore, GSK did not perform hSBAs for 2-<6 year subjects. The consequences will be described in Section 7.1.2.1, Section 7.1.2.2, and Section 7.1.3.

Table 4. Description of Laboratories Performing rSBAs by Time Point

		GSK		PHE	
Study MenACWY-	**Time Point**	**1-<2 years**	**2-<11 years**	**1-<2 years**	**2-<11 years**
TT-027	Baseline (Month 0)	✓	✓		
	Month 1	✓	✓		
TT-028	Year 1	✓	✓		
TT-029	Year 2	✓	✓		
TT-030	Year 3	✓	✓		
TT-031	Year 4	✓	✓	✓	✓
TT-032	Year 5			✓	✓
TT-100	Month 78 (Year 6)			✓	✓
	Month 90 (Year 7)			✓	✓
	Month 102 (Year 8)			✓	✓
	Month 114 (Year 9)			✓	✓
	Month 126 (Year 10)			✓	✓
	Month 127			✓	✓

Abbreviations: rSBA = serum bactericidal assay against *Neisseria meningitidis* using rabbit complement.

Table 5. Description of Laboratories Performing hSBAs by Time Point

		GSK		Neomed	
Study MenACWY-	**Time Point**	**1-<2 years**	**6-<11 years**	**1-<2 years**	**2-<11 years**
TT-027	Baseline (Month 0)	✓	✓		
	Month 1	✓	✓		
TT-028	Year 1	✓	✓		
TT-029	Year 2	✓			
TT-030	Year 3	✓			
TT-031	Year 4	✓			
TT-032	Year 5	✓			
TT-100	Month 78 (Year 6)			✓	✓
	Month 90 (Year 7)			✓	✓
	Month 102 (Year 8)			✓	✓
	Month 114 (Year 9)			✓	✓
	Month 126 (Year 10)			✓	✓
	Month 127			✓	✓

Abbreviations: hSBA = serum bactericidal assay against *Neisseria meningitidis* using human complement.

7.1.2.1. Within Vaccine Group Analysis

Descriptive statistics will be compiled for Month 78 (Year 6) through Month 126 (Year 10) for each antibody obtained via rSBA and via hSBA. For each vaccine group, at each blood sampling time point, for each antigen assessed:

- GMTs with 95% CIs will be tabulated.
- Percentages of subjects with titers above the proposed cutoffs with exact 95% CIs will be calculated.
- The distribution of antibody titers will be tabulated and also presented using reverse cumulative distribution curves.

7.1.2.2. Between Vaccine Group Analysis

Exploratory comparisons of the differences between ACWY≥2 and MenPS will be compiled for Month 78 (Year 6) through Month 126 (Year 10) for each antibody obtained via rSBA and via hSBA. However, exploratory comparisons of the differences between ACWY<2 and MenCCRM will be compiled Month 78 (Year 6) through Month 126 (Year 10) only for MenC (via rSBA and via hSBA) because MenCCRM does not contain components for A, W-135, or Y.

Exploratory evaluations of the differences in the immune response will be performed in terms of:

- Differences in the percentage of subjects with rSBA titers ≥1:8 and ≥1:128 and hSBA titers ≥1:4 and ≥1:8 between the ACWY≥2 and MenPS (primed) groups, with their standardized asymptotic 95% CIs for the 4 serological groups.

- Differences in the percentage of subjects with rSBA-MenC titers ≥1:8 and ≥1:128 and hSBA-MenC titers ≥1:4 and ≥1:8 between the ACWY<2 and MenCCRM (primed) groups with their standardized asymptotic 95% CIs.

- Ratio of GMTs between the ACWY≥2 and MenPS vaccine groups for the 4 serological groups and the ratio of MenC GMTs between the ACWY<2 and MenCCRM (primed) vaccine groups, with their 95% CIs. Both rSBA and hSBA will be analyzed. This will be performed using an analysis of variance (ANOVA) model on the logarithm10 transformation of the titers using the vaccine group as covariate. In each pairwise comparison, the ANOVA model will use only data from the 2 groups being compared. The ANOVA for ratio of GMTs between the ACWY≥2 and MenPS groups will also include age strata 2-<6 and 6-<11 years of age at primary vaccination.

7.1.3. Modeling Prediction

In order to complement the descriptive analyses of observed persistence per time point, longitudinal analyses will be performed at Month 126, which is the last persistence time point before booster vaccination.

Except as noted below, these longitudinal analyses will include all titers from:

- Pre- and post-primary analyses (Month 0 and Month 1 in Study MenACWY-TT-027) for subjects belonging in the ATP cohort for immunogenicity,

- Months 12, 24, 36, 48, and 60 (in Studies MenACWY-TT-028, 029 , 030, 031, and 032 respectively) for subjects belonging in the ATP cohort for persistence in corresponding studies and

- Months 78, 90, 102, 114, and 126 (in Study MenACWY-TT-100) for subjects belonging in the ATP cohort for persistence at Month 78 up to Month 126, respectively.

Age group is nearly confounded with vaccine group. Subjects 1-<2 years are assigned only to ACWY<2 and MenCCRM vaccine groups, while subjects 2-<11 years are assigned only to ACWY≥2 and MenPS vaccine groups. Therefore the longitudinal analyses will be performed separately in the 2 age strata.

Titers below the cutoff will be set at half the value of the cutoff. The time points will be considered as categorical. The model will be fitted via the proc mixed procedure according to the following code, depending on the age cohort:

```
* for 1-<2 year subjects;

*Model 1 – Repeated model on all available time points;

PROC MIXED data=sero;

  CLASS group time;

  MODEL log_val = group | time;

  Repeated time / TYPE=UN SUBJECT=pid;

RUN;

*for 2-<11 year cohort;

PROC MIXED data=sero;

  CLASS group time age_cat;

  MODEL log_val = group | time age_cat;

  Repeated time / TYPE=UN SUBJECT=pid;

RUN;
```

where 'age_cat' will be defined as '2-<6 yr' and '6-<11 yr'.

The exceptions and limitations to the longitudinal analyses are:

- rSBA titers at Year 4 will be limited to PHE titers, even though GSK also obtained some Year 4 titers, because PHE performed all subsequent rSBAs.
- Only MenC titers will be analyzed in the 1-<2 year age group because MenCCRM does not have an A, W-135, or Y component.
- hSBA analyses for the 2-<11 year age group will be limited to Month 78 (Year 6) through Month 126 (Year 10) time points because there are no hSBA titers for 2-<6 year subjects (see Table 5).

Inspection of the observed rSBA geometric means from Month 0 to Year 5 in TT-032 suggests that PHE titers are about 1 order of magnitude lower than GSK titers. In spite of the apparent change in titers all time points will be retained in the modelling prediction. The modeling prediction method specifies that time point is a categorical variable, so the abrupt change starting Year 4 will be captured in the model. Interpretation of the model's predicted values should keep the change in laboratories in mind.

7.1.4. Analysis of Safety (Persistence Epoch)

At each persistence time point, all reported SAEs related to vaccination and any event related to lack of vaccine efficacy since the last persistent time point the subject participated in up to each persistence visit in the current study will be described in detail in a retrospective manner.

Listings will be compiled for

- All SAEs
- AEs/SAEs leading to withdrawal
- SAEs related to study vaccination or any event related to lack of vaccine efficacy
- SAEs related to study participation
- Intercurrent medical conditions

for the time interval from last persistence time point subject participated in up to Month 126.

7.2. Post-booster Analyses

7.2.1. Analysis of Demographics/Baseline Characteristics

Demographic characteristics of subjects in each study cohort will be tabulated: age (in years) at booster vaccination (with the range and standard deviation), sex, and geographic ancestry.

The distribution of subjects among the study sites will be tabulated as a whole and per group.

7.2.2. Analysis of Postbooster Immunogenicity

The analysis of postbooster immunogenicity for MenC will be based on the booster ATP cohort for immunogenicity - MenC.

The analysis of postbooster immunogenicity for MenA, MenW-135, and MenY will be based on the booster ATP cohort for immunogenicity - MenAWY.

If, for any vaccine group, the percentage of subjects who have at least 1 serological result but are also excluded from the booster ATP cohort is higher than 10%, a second analysis based on the booster total vaccinated cohort will be performed to complement the booster ATP analysis.

7.2.2.1. Within Group Analysis

For each vaccine group, at each blood sampling time point (Month 126 and Month 127), for each antigen assessed:

- GMTs with 95% CIs will be tabulated.
- Percentages of subjects with titers above proposed cutoffs and vaccine response with 95% CIs will be calculated.
- The antibody titers will be tabulated and also presented using reverse cumulative distribution curves.

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7.2.3. Analysis of Post-booster Safety

The safety analysis will be performed on the booster total vaccinated cohort. If more than 10% of the enrolled subjects are excluded from the booster ATP cohort for safety, all analyses will be performed on the booster ATP cohort for safety to support the analyses of the booster total vaccinated cohort.

For each vaccine group, after the booster vaccination administered at Visit 5:

The number and percentages of subjects with at least 1 local event (solicited event and unsolicited AE), with at least 1 general event (solicited event and unsolicited AE) and with any event during the 4-day (Days 0-3) follow-up period will be tabulated with exact 95% CI. The same calculations will be performed for events rated as Grade 3 and for events related to vaccination.

The numbers and percentages of subjects reporting each solicited local (any grade, Grade 3, medical advice) and general (any grade, Grade 3, related, Grade 3 and related, medical advice) event during the 4-day follow-up period (Days 0-3) after vaccination and their exact 95% CIs will be tabulated. Occurrence of fever will also be reported to 0.5°C cumulative increment as well as the percentage of subjects with oral temperature >39.5°C.

The verbatim reports of unsolicited AEs will be reviewed by a clinical development manager. The signs and events will be coded according to the MedDRA Dictionary for Adverse Reaction Terminology. The percentages of subjects with unsolicited AEs within 31 days after vaccination (Days 0-30) and the exact 95% CIs will be tabulated by vaccine group and by MedDRA preferred term. Similar tabulations will be done for Grade 3 unsolicited AEs, for unsolicited AEs possibly related to vaccination and for Grade 3 unsolicited AEs possibly related to vaccination.

The number and percentage of subjects who reported SAEs and new-onset chronic illness from administration of the booster vaccine dose until the end of the study will be tabulated with exact 95% CI.

SAEs and large injection site reactions will be described in detail.

The percentage of subjects using concomitant medication (any medication, any antipyretic/analgesic, any antipyretic/analgesic taken prophylactically, respectively) during the 4-day and 31-day follow-up periods (Days 0-3 and Days 0-30, respectively) after vaccination will be summarized.

Listings will be compiled for:

- Solicited local events
- Solicited general events

for the time interval from the booster vaccination until Day 4 after booster

- Pregnancies

for the time interval from the booster vaccination until Day 31 after booster

- All SAEs
- AEs/SAEs leading to withdrawal
- SAEs related to study vaccination or any event related to lack of vaccine efficacy
- SAEs related to study participation
- All unsolicited AEs

for the time interval from the booster vaccination until the end of study follow-up.

8. STATISTICAL CALCULATIONS

8.1. Derived and Transformed Data

- Age in years will be calculated as (date minus date of birth)/365.25, rounded down to nearest integer.
- Duration from primary vaccination to any persistence time point will use day of primary vaccination as Day 0. Duration from booster will use day of booster vaccination as Day 0.
- Activity date is defined as the date associated with any activity performed at indicated visit.
- For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.
- The cutoff values for the assays are defined by the laboratory before the analysis and are described in Section 5.7.3 of the protocol.

- A seronegative subject is a subject whose titer is below the cutoff value.
- A seropositive subject is a subject whose titer is greater than or equal to the cutoff value.
- GMTs are calculated by taking the antilog of the mean of the log titer transformations. Antibody titers below the cutoff value of the assay will be given an arbitrary value of half the cutoff value for the purpose of the GMT calculation.
- rSBA vaccine response for groups A, C, W-135, and Y after the booster dose is defined as:
  - For initially seronegative subjects (pre-vaccination titer <1:8): rSBA antibody titers ≥1:32 1 month after vaccination, and
  - For initially seropositive subjects (pre-vaccination titer ≥1:8): a 4-fold increase in rSBA antibody titers 1 month after vaccination.
- hSBA vaccine response for groups A, C, W-135, and Y after the booster dose is defined as:
  - For initially seronegative subjects (pre-vaccination hSBA titer <1:4): hSBA antibody titers ≥1:8 1 month after vaccination, and
  - For initially seropositive subjects (pre-vaccination hSBA titer ≥1:4): a 4-fold increase in hSBA antibody titers 1 month after vaccination.
- For subjects who received MenC vaccine because they were suboptimal responders during the persistence phase of the MenACWY-TT-027 study, all subsequent persistence results for MenC will be replaced by half the cutoff in order to reflect the immunogenicity of the subjects, had they not been revaccinated. This includes Month 126. However, there will be no adjustment to postbooster MenC titers.
- For a given subject and the analysis of solicited events within 4 days after vaccination, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited events based on the total vaccinated cohort will include only vaccinated subjects for doses with documented safety data (ie, event screen completed). More specifically the following rules will be used:
  - Subjects who documented the absence of a solicited event after 1 dose will be considered not having that event after that dose.

- Subjects who documented the presence of a solicited event and fully or partially recorded daily measurement over the solicited period will be included in the summaries at that dose and classified according to their maximum observed daily recording over the solicited period.

- Subjects who documented the presence of a solicited event after 1 dose without having recorded any daily measurement will be assigned to the lowest intensity category at that dose (ie, 37.5°C for fever or Grade 1 for other events).

- Doses without event sheets documented will be excluded.

- All solicited local injection site events will be assigned a causal relationship to vaccination. Causal relationships of solicited general events were determined by the investigator.

- For the analysis, oral/axillary/tympanic temperatures will be coded as follows:

Grade	Temperature
0	<37.5°C
1	≥37.5°C - ≤38.5°C
2	>38.5°C - ≤39.5°C
3	>39.5°C

When temperature is measured by the rectal route, the corresponding oral temperature will be derived by subtracting 0.5°C from the temperature recorded. The above intensity grade will be applied to the derived oral temperature.

- Since subjects with fever will be counted once for each reported route, some subjects may be counted more than once.

- Intensity scales for other solicited general and local events are described in Section 8.2.3.2.1 of the protocol.

- For analysis of unsolicited AEs, such as SAEs or AEs by primary MedDRA term, and for the analysis of concomitant medications, all vaccinated subjects will be considered. Subjects who did not report the AE or the concomitant medication will be considered as subjects without the AE or the concomitant medication respectively.

- The day of onset for any SAEs reported from last persistence time point to Month 126 will be calculated as number of days since primary vaccination in Study MenACWY-TT-027.

- The way the percentage of subjects will be derived will depend on the event analysed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for Deriving % per Vaccine Group
Solicited general event	All subjects with at least 1 solicited general event documented as either present or absent (ie, event screen completed).
Solicited local event	All subjects with at least 1 solicited local event documented as either present or absent (ie, event screen completed).
Unsolicited AE	All subjects with study vaccine administered
Concomitant medication	All subjects with study vaccine administered

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8.3. Data Presentation Description

The following decimal description will be used for the demography, reactogenicity, immunogenicity, and persistence analyses.

Display Table	Parameters	Number of Decimal Digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	1
rSBA-MenA	GMT	1
rSBA-MenC	GMT	1
rSBA-MenW-135	GMT	1

Display Table	Parameters	Number of Decimal Digits
rSBA-MenY	GMT	1
hSBA-MenA	GMT	1
hSBA-MenC	GMT	1
hSBA-MenW-135	GMT	1
hSBA-MenY	GMT	1
Immunogenicity	Ratio of GMT	2
All summaries	% of count, including LL & UL of CI	1
All summaries	% of difference, including LL & UL of CI	2

Abbreviations: CI = confidence interval; GMT = geometric mean titer; hSBA-MenA = serum bactericidal assay against *Neisseria meningitidis* group A using human complement; hSBA-MenC = serum bactericidal assay against *Neisseria meningitidis* group C using human complement; hSBA-MenW-135 = serum bactericidal assay against *Neisseria meningitidis* group W-135 using human complement; hSBA-MenY = serum bactericidal assay against *Neisseria meningitidis* group Y using human complement; LL = lower limit of the confidence interval; rSBA-MenA = serum bactericidal assay against *Neisseria meningitidis* group A using rabbit complement; rSBA-MenC = serum bactericidal assay against *Neisseria meningitidis* group C using rabbit complement; rSBA-MenW-135 = serum bactericidal assay against *Neisseria meningitidis* group W-135 using rabbit complement; rSBA-MenY = serum bactericidal assay against *Neisseria meningitidis* group Y using rabbit complement; SD = standard deviation; UL = upper limit of the confidence interval.

8.3.1. Methodology for Computing CI

- All CI computed will be two-sided 95% CI.
- The exact 95% CIs for a proportion within a group will be based on the method by Clopper and Pearson [Clopper and Pearson, 1934].[1]
- The standardized asymptotic 95% CI for the group difference in proportions will be based on method 6 described in paper by Newcombe [R Newcombe, 1998].[2]
- The 95% CI for GMTs will be obtained within each vaccine group separately. The 95% CI for the mean of log-transformed titer will be first obtained assuming that log-transformed values were normally distributed with unknown variance. The 95% CI for the GMTs will be then obtained by exponential transformation of the 95% CI for the mean of log-transformed titer.
- The 95% CIs of the group GMT ratios will be computed using an ANOVA model on the logarithm$_{10}$ transformation of the titers. The ANOVA model will include the vaccine group as a covariate. Age strata will also be included as a covariate when comparing ACWY>2 to MenPS. In each pairwise comparison, the ANOVA model will use only data from the 2 groups being compared.

9. CONDUCT OF ANALYSES

9.1. Sequence of Analyses

The persistence analysis will be done in stepwise fashion after the persistence time points for Months 78, 90, 102, and 114 as soon as results are available. The persistence analyses will be performed on as clean as possible data.

The final analysis of immunogenicity, safety, and reactogenicity will be performed on fully cleaned data after the extended safety follow-up (ESFU) time point as soon as results are available for Visit 5, Visit 6, and Visit 7.

The booster analyses will be performed on a validated and frozen database.

The final report containing all persistence, post-booster, and ESFU endpoints will be performed on fully cleaned data.

9.2. Statistical Considerations for Interim Analyses

No statistical adjustment for interim analyses is required.

10. CHANGES FROM PLANNED ANALYSES

Please note that the changes from planned analyses to be included in the clinical study report (CSR) will be identified during the CSR review.

- The adapted ATP cohort and total enrolled cohort have been added and are explained in detail.

- Analysis of persistence for all the antigens MenA, MenC, MenW-135, and MenY will be done using the ATP cohort for persistence at Month X which will include those subjects who received a booster dose of MenC vaccine because they were non-responders after the primary vaccination in Study MenACWY-TT-027. A decision was made to include the results of the subjects who were administered a booster dose of MenC vaccine, since the removal of the results of these subjects from the analysis for persistence of MenC would result in the over estimation of the persistence of MenC.

- The new statement is “who have available assay results for at least 1 of 3 antigens (MenA or MenW-135 or MenY) at Month X.” has been modified to “who have available assay results for at least 1 of 3 antigens (MenA or MenW-135 or MenY) or for MenC (for subjects who had not received MenC vaccine because they were non-responders during the persistence phase of Study MenACWY-TT-027 please see Section 7.1 of the SAP) at Month X”.

- For subjects who received MenC vaccine because they were suboptimal responders during the persistence phase of the MenACWY-TT-027 study, the persistence results for MenC will be replaced by half the cutoff in order to reflect the immunogenicity of the subjects, had they not been revaccinated.

11. REFERENCES

[1] Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika.* 1934; 26:404-13.

[2] Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Stat Med*. 1998; 17:873-90.