Protocol C0921001

A phase III, open, multi-centre, controlled study to evaluate the long-term antibody persistence at 2, 3, 4, 5 and 6 years after a booster dose of meningococcal serogroup A, C, W-135, Y- tetanus toxoid conjugate vaccine (MenACWY-TT) or *Meningitec*® administered in healthy 5 year old children in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036), who were primed with the same vaccine in study MENACWY-TT-039 (109670) at 12 through 23 months of age

Statistical Analysis Plan
(SAP)

Version: 2.0

Date: 15-FEB-2016

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ATP	According-To-Protocol
CI	Confidence Interval
GMT	Geometric mean antibody titres
hSBA-MenA	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup A (using human complement)
hSBA-MenC	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup C (using human complement)
hSBA-MenW-135	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup W-135 (using human complement)
hSBA-MenY	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup Y (using human complement)
MedDRA	Medical Dictionary for Regulatory Activities
rSBA-MenA	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup A (using rabbit complement)
rSBA-MenC	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup C (using rabbit complement)
rSBA-MenW-135	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup W-135 (using rabbit complement)
rSBA-MenY	Serum bactericidal assay/activity against *Neisseria meningitidis* serogroup Y (using rabbit complement)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TFL	Tables Figures and Listing template annexed to SAP

1. VERSION HISTORY

This Statistical Analysis Plan (SAP) for study C0921001 is based on the protocol dated 15-Feb-2016.

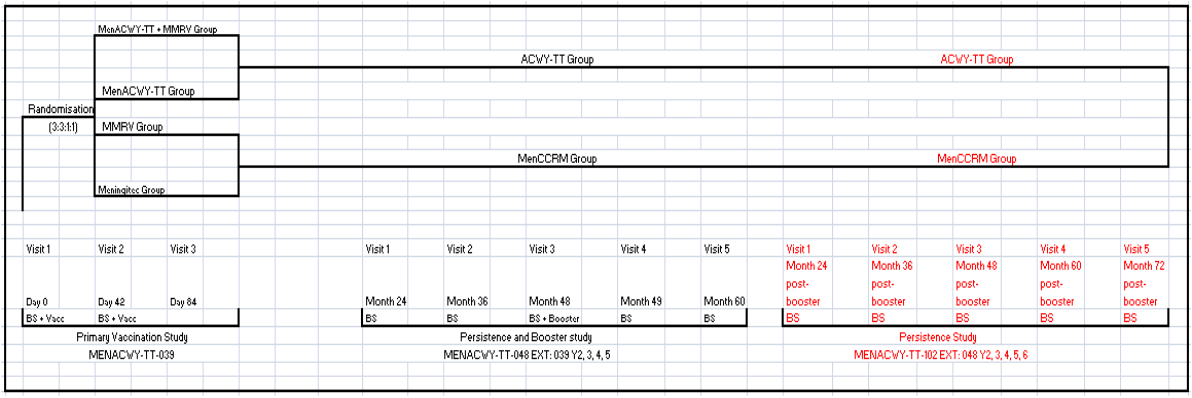
Table 1. Summary of Major Changes in SAP Amendments

Date	Version	Description	Protocol Version
15-JUL-2014	First Version		Version 1 – 26-Mar-2013
27-APR-2015	Amendment 1	1. There are changes in sequence of analysis and number of TFLs planned. 2. Total Enrolled Cohort is updated to be used instead of ATP Total Cohort to reduce number of cohorts defined. 3. Decimal points to be displayed for SD of age are changed from 2 to 1.	Version 1 – 26-Mar-2013
15-FEB-2016	Amendment 2	SAP amended to reflect sponsorship change to Pfizer following the acquisition of the GSK meningococcal vaccine by Pfizer on the 01st October 2015. Typos fixed. Section 6.4: table with elimination codes deleted because these are specific to GSK. Section 7.2.2: 'covariate' changed to 'fixed effect' in description of analysis of variance. Section 7.2.3: the second paragraph says some analyses would be performed only in the ACWY-TT group, but the SAS code include vaccine group as a factor. Text revised to say that the modeling prediction analysis will be performed in both groups for all assays. Section 7.2.3: LSMEANS statement added to Proc Mixed code. Section 9.1: description of tables for persistence time points deleted because all prior persistence analyses have been completed. Section 10: text was added to description of changed criteria to explain the motivation for the changes.	Amendment 1 – 04-Jan-2016

2. INTRODUCTION

The SAP is divided into 2 parts: the first part detailing the analyses to be performed (current document) and a second part, annexes (called TFL) describing the flow and format of tables, figures and listings.

3. STUDY DESIGN



ACWY-TT Group: Vaccinated and boosted with MenACWY-TT in studies MENACWY-TT-039 (109670) and MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036)
MenCCRM Group: Vaccinated and boosted with *Meningitec* in studies MENACWY-TT-039 (109670) and MENACWY TT-048 EXT: 039 Y2, 3, 4, 5 (112036)
BS: Blood sample
Vacc: Vaccination in study MENACWY-TT-039 (109670)
Booster: Booster vaccination at Month 48 in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036)

- Experimental design: Phase III, open-label, controlled, multi-centric, single-country study with two parallel groups.
- Control: Active control (MenCCRM group).
- Vaccination schedule: No vaccine will be administered during this long-term persistence study.
- Blinding: Study will be conducted in an open manner.
- Sampling schedule: Blood samples will be taken at each of the study visits, ie, at 2 (Visit 1), 3 (Visit 2), 4 (Visit 3), 5 (Visit 4) and 6 (Visit 5) years after booster vaccination in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036).
- Duration of the study: Approximately four years per subject.
  - Epoch 001: Persistence Visit 1 (Year 2 [Month 24] post-booster vaccination)
  - Epoch 002: Persistence Visit 2 (Year 3 [Month 36] post-booster vaccination)

- Epoch 003: Persistence Visit 3 (Year 4 [Month 48] post-booster vaccination)
- Epoch 004: Persistence Visit 4 (Year 5 [Month 60] post-booster vaccination)
- Epoch 005: Persistence Visit 5 (Year 6 [Month 72] post-booster vaccination)

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-TT	Pooled Co-ad and ACWY-TT groups from primary study 109670 (MENACWY-TT-039) who were primed and boosted with the MenACWY-TT vaccine
2	MenCCRM	Pooled MMRV and MenCCRM groups from primary study 109670 (MENACWY-TT-039) who were primed and boosted with the *Meningitec* vaccine

4. OBJECTIVES

4.1. Primary Objective

Immunogenicity

Persistence

At 2, 3, 4, 5, 6 years after booster vaccination of children with MenACWY-TT or *Meningitec*

- To evaluate the persistence of meningococcal antibodies in terms of the percentage of subjects with rSBA antibody titres ≥1:8 for each of the four serogroups.

4.2. Secondary Objective

Immunogenicity

Persistence

At 2, 3, 4, 5, 6 years after booster vaccination of children with MenACWY-TT or *Meningitec*

- To evaluate the persistence of meningococcal antibodies in terms of the percentage of subjects with rSBA titres ≥1:128 and GMTs and hSBA titres ≥1:4 and ≥1:8 and GMTs for each of the four serogroups.

Safety

- To describe serious adverse events (SAEs) related to vaccination and any event related to lack of vaccine efficacy (ie, meningococcal disease) from the subject's last visit in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) up to each yearly visit in the current study in a retrospective manner.

5. ENDPOINTS

5.1. Primary Endpoints

- Persistence of antibodies with respect to components of the investigational vaccine, 2, 3, 4, 5, 6 years after booster vaccination:
  - rSBA-MenA titres ≥1:8.
  - rSBA-MenC titres ≥1:8.
  - rSBA-MenW-135 titres ≥1:8.
  - rSBA-MenY titres ≥1:8.

5.2. Secondary Endpoints

- Persistence of antibodies with respect to components of the investigational vaccine, 2, 3, 4, 5, 6 years after booster vaccination:
  - rSBA-MenA titres ≥1:128 and GMTs.
  - rSBA-MenC titres ≥ 1:128 and GMTs.
  - rSBA-MenW-135 titres ≥ 1:128 and GMTs.
  - rSBA-MenY titres ≥ 1:128 and GMTs.
  - hSBA-MenA titres ≥ 1:4, ≥ 1:8 and GMTs.
  - hSBA-MenC titres ≥ 1:4, ≥ 1:8 and GMTs.
  - hSBA-MenW-135 titres ≥ 1:4, ≥ 1:8 and GMTs.
  - hSBA-MenY titres ≥ 1:4, ≥ 1:8 and GMTs.
- Serious adverse events:
  - Occurrence of serious adverse events related to vaccination and any event related to lack of vaccine efficacy (ie, meningococcal disease) from the subject's last visit in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) up to each yearly visit in the current study in a retrospective manner.

6. STUDY POPULATION

Several cohorts are defined for the purpose of analyses. The definitions of the cohorts are given as below.

6.1. Total Cohort at Month X

The Total Cohort at Month X will include all subjects who received primary vaccination in study MENACWY-TT-039 (109670) and booster vaccination in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) and came back for visit at Month X. The analysis of persistence at Month X will include all vaccinated subjects for whom data concerning persistence endpoint measures are available at Month X.

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

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

- who have received the primary and booster vaccination with MenACWY-TT or *Meningitec* vaccine in studies MENACWY-TT-039 (109670) and MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036), respectively.
- who have available assay results for at least one tested antigen at Month X.
- who have not received a meningococcal polysaccharide or a meningococcal polysaccharide conjugate vaccine not planned in protocol MENACWY-TT-102 EXT: 048 Y2, 3, 4, 5, 6 (200088) before Month X.
- who do not have a history of meningococcal serogroup A, C, W-135, and Y disease prior to Month X.
- who comply with the blood sampling intervals defined in the protocol for Month X (see Section 5.5 of the protocol).
- who do not have an immune compromising medical condition.
- who have not received any immunosuppressant(s) or other immune-modifying drug(s), immunoglobulins, any blood products, investigational drugs, and/or investigational vaccines.
- who were not excluded from the Booster ATP cohort for immunogenicity or ATP cohort for persistence at Month 60 in the MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) study, 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. All the previous elimination will be evaluated on case-by-case basis by the clinician to check for validity at that particular time point.

6.3. 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/persistence has been used. More specifically the analyses on the pre and post booster time points will be based on the booster ATP cohort for immunogenicity defined in MENACWY-TT-048 while the analyses on Month 12 time point will be based on the ATP cohort for persistence at Month 60 in MENACWY-TT-048 and the analysis on Month 24, 36, 48, 60, 72 time points will be based on the ATP cohort for persistence at Month 24, 36, 48, 60, 72 in MENACWY-TT-102, respectively.

6.4. 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.

For the analysis of persistence, presenting pre and post booster data from study -048 and all time points from study -102, this will include all subjects with immunogenicity result available at respective time point. More specifically, this will include subjects with immunogenicity results available at each time point and belonging to corresponding Total Vaccinated Cohort at Month 60 (study -048) or Total Cohort (studies -048, -and -102) regardless their enrolment in study -102.

7. STATISTICAL METHODS

7.1. Analysis of Demographics/Baseline Characteristics

- Demographic characteristics of each study cohort will be tabulated: age (in months, at each study visit, with the range and standard deviation), gender, and geographic ancestry.

- Months since the booster vaccination at the corresponding persistence time point will be calculated by group.

- The distribution of subjects enrolled at each study visit among the study sites will be tabulated as a whole and per group and reason for not attending a visit at each study visit among all subjects who participated in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) will be summarized.

7.2. Analysis of Persistence

Persistence data will be analyzed at Month 24, Month 36, Month 48, Month 60 and Month 72 as soon as results for all individual subjects at each time point are available.

The analysis of persistence will be based on the Adapted ATP cohort.

If for any vaccine group, at any time point, the percentage of subjects with serological results excluded for the corresponding ATP cohort is higher than 5%, a second analysis based on the ***Total Enrolled Cohort*** will be performed to complement the ATP analysis.

7.2.1. Within Group Analysis

For each vaccine group, at each blood sampling time point, for each antigen assessed:

- Geometric mean titres (GMT) with 95% CIs will be tabulated.
- Percentages of subjects with titres above the proposed cut-offs with exact 95% CIs will be calculated.
- The distribution of antibody titres will be tabulated and also presented using reverse cumulative curves.

7.2.2. Between Group Analysis

An exploratory evaluation of the differences in the immune response at approximately 24, 36, 48, 60 and 72 months after the booster vaccination will be performed in terms of:

- Differences in the percentage of subjects with rSBA-MenC titres ≥1:8 and ≥1:128 between the ACWY-TT group and the MenCCRM group, with their standardized asymptotic 95% CIs.
- Differences in the percentage of subjects with hSBA-MenC titres ≥1:4 and ≥1:8 between the ACWY-TT group and the MenCCRM group, with their standardized asymptotic 95% CIs.
- Ratio of GMTs between the ACWY-TT group and the MenCCRM group, with their standardized asymptotic 95% CIs for rSBA-MenC antibody titres and hSBA-MenC antibody titres. This will be performed using an Analysis of Variance (ANOVA) model on the logarithm_{10} transformation of the titres using the vaccine group as fixed effect.

7.2.3. Modeling Prediction

In order to complement the descriptive analyses of observed persistence per time point and evaluate the bias that may have occurred due to the loss to follow-up after the vaccination, longitudinal analysis will be performed at the last persistence time point at Month 72.

This analysis will be performed for both the rSBA and hSBA assays in both groups.

This analysis will include all results from pre- and post-booster (Month 48 and Month 49 in study MENACWY-TT-048 (112036)) for subjects belonging to the Booster ATP cohort for immunogenicity, Month 12 (Month 60 in study MENACWY-TT-048 (112036)) for subjects belonging to ATP cohort for persistence at Month 60, Month 24 up to Month 72 (in study MENACWY-TT-102 (200088)) for subjects belonging to ATP cohort for persistence at Month 24 up to Month 72.

A longitudinal model taking into account the group and all available immunogenicity time points from pre-booster timepoint (in study 048) until the last timepoint Month 72 (in study MENACWY-TT-102 (200088)) will be fitted. This model will be primarily aimed at evaluating the selection effect in the group and the time points will be considered as categorical.

For a specific assay, the model will include assay results from a time point provided that these were part of the ATP cohort for that time point. Results below cut-off will be set at half the value of the cut-off. The model will be fitted via the proc mixed procedure according to the following code:

```
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;
  Lsmeans group*time;
RUN;
```

7.2.4. Analysis of Safety

At each persistence time point all reported SAEs related to vaccination and any event related to lack of vaccine efficacy from the subject's last visit in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) will be described in detail in a retrospective manner.

8. STATISTICAL CALCULATIONS

8.1. Derived and Transformed Data

Immunogenicity

- 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 for the considered parameter.

- The cut-off value is defined by the laboratory before the analysis and is described in Section 5.7.3 of the protocol.

- A seronegative subject is a subject whose titre is below the cut-off value.

- A seropositive subject is a subject whose titre is greater than or equal to the cut-off value.

- The Geometric Mean Titres (GMTs) calculations are performed by taking the anti-$\log_{10}$ of the mean of the $\log_{10}$ titre transformations. Antibody titres below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation.

Safety

At each persistence time point, all reported SAEs related to vaccination and any event related to lack of vaccine efficacy from the subject's last visit in study MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) will be described in detail in a retrospective manner.

8.2. Data Presentation Description

The following decimal description from the decision rules will be used for the demography, immunogenicity and safety.

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
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

8.3. Methodology for Computing Confidence Intervals

- 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, 1934.[1]
- The standardized asymptotic 95% CI for the group difference in proportions will be based on the method 6 described in paper by Newcombe, 1998.[2]
- The 95% CI for geometric mean titres (GMTs) will be obtained within each group separately. The 95% CI for the mean of log-transformed titre 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 titre.
- The 95% CIs of the group GMT ratios will be computed using an ANOVA model on the logarithm10 transformation of the titres. The ANOVA model will include the vaccine group as fixed effect.

9. CONDUCT OF ANALYSES

9.1. Sequence of Analyses

The analysis will be done by epoch and in a stepwise fashion:

- Analysis of persistence data at Months 24, 36, 48 and 60 will be conducted as soon as the results for all individual subjects are available at each time point.

These analyses will be done on a validated and frozen database. The data at Months 24, 36, 48, 60 will be as clean as possible.

- A final analysis will be done at Month 72 which will include all analyses described in Section 7. This final analysis will be reported in a clinical study report (CSR) after Month 72 results are available.

9.2. Statistical Considerations for Interim Analyses

All immunogenicity analyses will be conducted on final data and therefore no statistical adjustment for interim analyses is required.

10. CHANGES FROM PLANNED ANALYSES

Please note that the changes from planned analysis to be included in CSR will be identified during the CSR review.

- Two criteria for evaluating subjects to be part of ATP cohort for persistence at Month X have been changed:
    - The criteria ‘who have not received a meningococcal polysaccharide or a meningococcal polysaccharide conjugate vaccine not planned in protocol MENACWY-TT-48 EXT: 039 Y2, 3, 4, 5 (112036) before Month X’ has been changed to ‘who have not received a meningococcal polysaccharide or a meningococcal polysaccharide conjugate vaccine not planned in protocol MENACWY-TT-102 EXT: 048 Y2, 3, 4, 5, 6 (200088) before Month X’, ie, the protocol number was changed.
    - The criteria ‘who were not excluded from the Booster ATP for immunogenicity or ATP cohort for persistence at Month 60 in the MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) study, 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’ has been changed to ‘who were not excluded from the Booster ATP for immunogenicity or ATP cohort for persistence at Month 60 in the MENACWY-TT-048 EXT: 039 Y2, 3, 4, 5 (112036) study, 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. All the previous elimination will be evaluated on case-by-case basis by the clinician.

- The sequence of analysis is changed. There will be a single CSR presenting all the persistence analyses from Month 24 to Month 72 after Month 72 data is available.

- ***The concept of Adapted ATP cohorts and Total Enrolled Cohort has been added and are explained in detail.***

11. REFERENCES

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

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