

Statistical Analysis Plan (SAP) for LionMenB

FULL/LONG TITLE OF THE STUDY	Early Protection against meningococcal disease B in infants
SHORT STUDY TITLE / ACRONYM	LION MenB (earLy protectIO n agaiNst Meningococcal B disease in infants)
SAP VERSION NUMBER AND DATE	Version 1.0 04/07/2024
Study Type / Phase:	Phase IV randomisation study of different Men B vaccine primary immunisation schedules in UK infants Protocol V7.0, 06 Dec 2022
IRAS Number:	265199
Clinical Studies registry number (ISRCTN Number / Clinical trials.gov Number) :	52318758
JRES (sponsor) Reference Number	2020.0027

List of Abbreviations:

AE	Adverse event
AESI	Adverse event of special interest
SAE	Serious adverse event
PCV13	Pneumococcal conjugate vaccine, 13 valent
4CMenB	4 component MenB protein vaccine, (Bexsero®)
PO	Primary objective
SO	Secondary objective

1. Introduction

1.1. Trial Details

Neisseria meningitidis serogroup B (MenB) causes the majority of meningococcal disease in the UK where infants have the highest incidence. The 4CMenB vaccine (Bexsero®) can protect infants against MenB after at least two priming doses. The UK schedule currently offers a two-dose priming regime at 2 and 4 months with a booster at 12 months.

An earlier schedule may prevent more cases of MenB. This study aims to compare the immune responses of UK infants who receive their routine immunisations alongside two different 4CMenB primary immunisation schedules. Full rationale can be found in the protocol.

1.2. Person responsible for the statistical analysis

Nick Andrews, Statistics Unit, UK Health Security Agency (UKHSA). The analysis may be performed under supervision of the responsible statistician.

1.3. Changes to the analysis plan

A final version of this plan will be produced prior to provision of data to the statistician. Any additional analysis or deviation(s) from this analysis plan will be documented and the reasons given in appendix 1 of this document.

2. Trial Design Summary

This is a multicentre, open label, phase IV study at several participating NHS sites in England. Term, healthy infants (born at ≥ 37 weeks gestation), aged ≥ 56 days to ≤ 70 days are randomised to one of two 4CMen B schedules:

- Group 1: 4CMenB Bexsero® at 2, 3 and 12 months of age; PCV13 at 4 months of age
- Group 2: 4CMenB Bexsero® at 2, 4 and 12 months of age; PCV13 at 3 months of age

All other routine immunisations are received alongside the above allocation, as per the UK vaccination schedule. Infants remain in the study from recruitment to around 13 months of age with blood sample schedule as below. After each vaccination visit, parents/caregivers complete a 7 day paper diary for collection of solicited AEs (temperature, redness, swelling, tenderness, feeding, activity, irritability, persistent crying, diarrhoea, vomiting). All other unsolicited AEs are collected at each visit by study staff. SAEs and AESIs are collected from the time of consent to last visit. AESIs for this study were: Febrile seizures, arthritis.

2.1. Study Visit Schedule

Simplified study visit procedures for groups 1 and 2 are shown below, for full study procedures see protocol.

Table 1. Study Procedures - Group 1 and 2

Visit Number	1	2	3	4	5	6
Age	2 months	3 months	4 months	5 months	12 months	13 months
Study Timeline	Day 0	V1 + 28 days	V2 +28 days	V3 + 28-days		V5 +28 days
Visit window		+14 days	+ 14 days	+ 14 days	+21 days	+ 21 days
Group 1	4CMenB DTaP/IPV/ Hib/HepB Rotavirus	4CMenB DTaP/IPV/ Hib/HepB Rotavirus	PCV13 DTaP/IPV/ Hib/HepB  Blood Sample 1		4CMenB PCV13 MCC-TT/ Hib-TT/MMR  Blood Sample 2	 Blood Sample 3
Group 2	4CMenB DtaP/IPV/ Hib/HepB Rotavirus	PCV13 DtaP/IPV/ Hib/HepB Rotavirus	4CmenB DtaP/IPV/ Hib/HepB	 Blood Sample 1	4CMenB PCV13 MCC-TT/ Hib-TT/MMR  Blood Sample 2	 Blood Sample 3

2.2. Randomisation and blinding

Participants are randomised in a 1:1 ratio between groups 1 and 2. A computerised block randomisation list is produced for each site, placed inside an opaque envelope with corresponding participant number. At recruitment, each participant is allocated, in order of inclusion, the next available participant number from the randomisation. Prior to first vaccinations, the local study team will open the envelope and reveal the group number allocation and so vaccination schedule to be followed.

This is an unblinded study; both study teams and participants are aware of their allocation. However, laboratory staff testing the vaccine responses will be blinded to group allocation.

2.3. Supply of data

Data is entered from the paper source at sites into a secure, online, password access eCRF (MACRO) by delegated site staff. Data is cleaned, locked and then downloaded from this database and provided to the study statistician in Excel/CSV format for upload into STATA.

3. Sample Size

We propose that a comparison of 2 schedules with respect to doses of 4CMenB will require 60 infants in each arm. Based on data from a recent study (Gossger et al. 2012) the standard deviation of the GMT responses to the 3 antigens is expected to be around 1.0 log_e units.

Therefore, in order to detect a 1.49 fold difference between groups at 80% power with 5% significance, and anticipating a 10% drop-out rate, we aimed to recruit 110 infants in each group, 220 in total. See protocol for further details.

4. Objectives

4.1. Primary objectives

PO1: To compare the immunological responses of UK infants at 28 days after the second dose (in blood sample 1) between two different primary immunisation schedules of 4CMenB (2, 3 vs. 2, 4 months of age).

4.2. Secondary objectives

SO1: To compare the persistence of immunological responses to 4CMenB at 12 months chronological age after two different primary immunisation schedules of 4CMenB (2, 3 vs. 2, 4 months of age).

SO2: To compare the immunological responses one month after the 12-month 4CMenB booster in infants who received two different primary immunisation schedules of 4CMenB (2, 3 vs. 2, 4 months of age).

SO3: To compare the persistence of immunological responses to PCV13 at 12 months chronological age after two different primary immunisation schedules of PCV13 (3 vs. 4 months of age).

SO4: To compare the immunological responses to the booster dose of PCV13 given at 12 months in infants who received two different primary immunisation schedules of PCV13 (3 vs. 4 months of age).

4.3. Safety objectives

1. To describe the safety of 4CMenB when given concomitantly with routine vaccines at 2 and 3 months of age **(Group 1)**.
2. To describe the safety of 4CMenB when given concomitantly with routine vaccines at 2 and 4 months of age **(Group 2)**.
3. To describe the safety of PCV13 when given concomitantly with routine vaccines at 3 months of age **(Group 2)**.
4. To describe the safety of PCV13 when given concomitantly with routine vaccines at 4 months of age **(Group 1)**.
5. To describe the safety of 4CMenB booster when given concomitantly with routine vaccines at 12 months of age **(Group 1 and 2)**.
6. To describe the safety of PCV13 booster when given concomitantly with routine vaccines at 12 months of age **(Group 1 and 2)**.

5. Outcome Measures

5.1. Primary end points

Objective	Outcome Measures
To compare the immunological responses of UK infants between two different primary immunisation schedules of 4CMenB (2 and 3 versus 2 and 4 months of age).	1. Antibody titres against relevant 4CMenB antigens (fHbp, NadA and PorA) measured by serum bactericidal assay using human complement (hSBA) 4 weeks after 2nd dose of 4CMenB (blood sample 1 at 4 months of age for Group 1 versus 5 months of age for Group 2). 2. Antibody titres $\geq 1:4$ against relevant 4CMenB antigens (fHbp, NadA and PorA) measured by hSBA assessed at 4 weeks after 2nd dose of 4CMenB (blood sample 1 at 4 months of age for Group 1 versus 5 months of age for Group 2).

5.2. Secondary end points

Objective	Outcome Measures
To compare the persistence of immunological responses to 4CMenB at 12 months chronological age after two different primary immunisation schedules of 4CMenB (2 and 3 versus 2 and 4 months of age).	1. Antibody titres against relevant 4CMenB antigens (fHbp, NadA and PorA) measured by hSBA at 12 months of chronological age (blood sample 2, pre-booster). 2. Antibody titres $\geq 1:4$ against relevant 4CMenB antigens (fHbp, NadA and PorA) measured by hSBA at 12 months of chronological age (blood sample 2, pre booster).
To compare the immunological responses one month after the 12-month 4CMenB booster in infants who received two different primary immunisation schedules of 4CMenB (2 and 3 versus 2 and 4 months of age).	1. Antibody titres against relevant 4CMenB antigens (fHbp, NadA and PorA) measured by hSBA at 13 months of chronological age (blood sample 3, 4 weeks post-booster). 2. Antibody titres $\geq 1:4$ against relevant 4CMenB antigens (fHbp, NadA and PorA) measured by hSBA at 13 months of chronological age (blood sample 3, 4 weeks post booster).
To compare the persistence of immunological responses to PCV13 at 12 months chronological age after two different primary immunisation schedules of PCV13 (3 versus 4 months of age).	1. Serotype specific antibody concentrations against PCV13 serotypes at 12 months of age (blood sample 2, pre PCV13 booster). 2. Antibody concentrations $\geq 0.35 \mu\text{g/ml}$ against PCV13 serotypes at 12 months of age (blood sample 2, pre PCV13 booster).
To compare the immunological responses to the booster dose of PCV13 given at 12 months in infants who received two different primary immunisation schedules of PCV13 (3 versus 4 months of age).	1. Serotype specific antibody concentrations against PCV13 serotypes at 13 months of age (blood sample 3, 4 weeks post PCV13 booster). 2. Serotype-specific antibody concentrations $\geq 0.35 \mu\text{g/ml}$ against PCV13 serotypes at 13 months of age (blood sample 3, 4 weeks post PCV13 booster).

5.3. Observational end points: Safety data

Objective	Outcome Measures
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To describe the safety of 4CMenB when given concomitantly with routine vaccines at 2 and 3 months of age (Group 1). To describe the safety of 4CMenB when given concomitantly with routine vaccines at 2 and 4 months of age (Group 2). To describe the safety of PCV13 when given concomitantly with routine vaccines at 3 months of age (Group 2). To describe the safety of PCV13 when given concomitantly with routine vaccines at 4 months of age (Group 1). To describe the safety of 4CMenB booster when given concomitantly with routine vaccines at 12 months of age (Group 1 and 2). To describe the safety of PCV13 booster when given concomitantly with routine vaccines at 12 months of age (Group 1 and 2).	1. Occurrence, nature, time of onset, duration, intensity, action taken and whether the event led to early termination from the study, of any solicited AEs within 7 days after each vaccination(s). 2. Occurrence, nature, time of onset, duration, intensity, action taken and whether the event led to early termination from the study, of any unsolicited AEs within 28 days after each vaccination(s). 3. Occurrence, nature, time of onset, duration, intensity, action taken and whether the event led to early termination from the study, of any medically attended AEs for the whole duration of the study. 4. Occurrence, nature, time of onset, duration, intensity, action taken, relationship to vaccination, outcome and whether the event led to early termination from the study, of any SAEs and AESIs for the whole duration of the study.
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6. Analysis sets, missing data, censored data, significance level

6.1. Analysis sets

Data will be analysed using a modified intention to treat (mITT) analysis: all infants vaccinated, and with a blood sample taken and with antibody results available for the relevant end point will be included and analysed according to their randomised group. The mITT analysis will include blood samples taken outside the recommended timing.

Per protocol analysis (whereby all vaccinated infants with at least one post vaccination blood sample taken and with no major protocol deviations) may also be performed if major protocol deviations are recorded in more than 5% of subjects. This will be specified in the report.

6.1.1. Major protocol deviation definitions

A major protocol deviation will be defined as:

- Incorrect vaccination schedule given per randomisation group for either 4CMenB or PCV13 vaccines – any samples after this deviation occurs will be excluded from a per protocol analysis
- Incorrect number of doses of vaccine given for either 4CMenB or PCV13 – any blood samples after this deviation occurs will be excluded from a per protocol analysis

- Vaccine doses given at more than 12 weeks before or after the intended age for either 4CMenB or PCV13 vaccines – any samples after this deviation occurs will be excluded from a per protocol analysis
- Post vaccination blood sample (blood samples 1 and 3) obtained within 14 days of vaccination date or more than 56 days after vaccination date. This sample will be excluded from a per protocol analysis
- Pre booster blood sample (blood sample 2) obtained before 11 months of age or after 15 months of age. This sample will be excluded from a per protocol analysis

6.2. Missing and censored data

Reasons for all missing and censored data will be reported but missing data will not be imputed. Missing data (e.g. from withdrawals, non-compliers) is assumed missing at random. Infants with missing results (e.g. unable to obtain or insufficient blood sample) at the different time-points (e.g. post-primary immunisation, pre-booster and post-booster) will not be included in the analysis for that particular time-point. Spurious data will be checked to source records and investigated, but included if no cause is identified.

6.3. Significance level and confidence intervals

2-sided 95% confidence intervals will be reported. Where comparisons are made between groups a 5% significance level will be used.

7. Interim Analysis

No interim analysis is planned as all vaccines used in this trial are licensed for routine use in infants.

8. Statistical Methods

8.1. Statistical package

Stata version 17 or 18 will be used for analysis with Excel and GraphPad Prism as required.

8.2. Descriptive analysis

At the end of the study, a flowchart will summarise the number of infants approached, consented, recruited, assigned to the different study arms, receiving the intended vaccines, completing the study protocol and analysed for the primary outcome, as recommended by the CONSORT statement (<http://www.consort-statement.org/>).

Baseline data comparing the two trial arms will be summarised in a table format and will compare median gestational age at birth, age at first vaccination, gender, recruiting study site, birth weight, underlying medical conditions, and relevant concomitant medications or vaccines. Proportion of infants receiving vaccinations and blood tests within protocol specified windows will be summarised for each group.

8.3. Immunogenicity analysis

8.3.1. Primary end-point analysis

For each vaccination schedule, GMTs of the hSBA titres to the three analysed 4CMenB antigens (fHbp, NadA and PorA) will be calculated with 95% confidence intervals. Schedules will be compared using unpaired t-tests on log-transformed titres or the Kruskal Wallis test if log-titres are not normally distributed. Proportions with titres $\geq 1:4$ will also be calculated with exact binomial 95% confidence intervals and compared between groups by Fisher's exact test. Significance will be at a 5% level with no adjustment for multiple comparisons since whilst there are three antigens there are only two groups being compared.

8.3.2. Secondary end-point analysis

This will be done in the same way as the primary end-point analysis described in 8.3.1. In addition, the geometric mean fold change between time points will be calculated with 95% confidence intervals.

8.3.3 Exploratory analysis

Multivariable normal errors regression models on logged titres will be used to adjust for interval to blood from vaccination and other demographic factors (e.g. gestation, age at first vaccination, sex) to assess whether any differences between groups are explained by these factors.

8.4. Safety analysis

Descriptive summary in table form of solicited AEs at each visit for the two vaccination schedules will be reported along with proportions and numbers of Grade 3 AEs. SAEs and AESIs will be summarised and proportion in each schedule group reported. MAAEs frequency and disease category will be reported by vaccine schedule group across the whole duration of the study.

9. Reports

The results of the analysis will be written up in a statistical analysis report. This report can then be used to assist in the production of papers for publication and the main study report. It may also be included as an addendum to the main report. The statistician who analysed the data should see any further reports based on the statistical analysis report.

10. Signatures and approvals

Signature of trial Statistician:	Signature of Chief Investigator:
	
Name: Nick Andrews Date : 04 / July / 2024	Name: Paul Heath Date : 04 / July / 2024

11. Appendix 1: Version Changes

Change	Version and date	Reason