Statistical Analysis Plan

Immunogenicity and reactogenicity of concomitantly administered hexavalent and group B meningococcal vaccines in infancy

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Section 1. Introduction

1.1. BACKGROUND AND RATIONALE

Hepatitis B virus (HBV) is a viral illness that results in inflammation of the liver. It causes significant morbidity and mortality worldwide and is the most common chronic viral infection in the world. It is estimated that up to 30% of the world's population has serological evidence of a current or past HBV infection. HBV can manifest either as an acute illness causing nausea, malaise, abdominal pain and jaundice or as an often asymptomatic chronic infection. Chronic HBV infection can lead to liver cirrhosis and hepatocellular carcinoma.

HBV is blood borne and transmitted via exposure to infected blood or bodily fluids contaminated by blood. One of the most common forms of transmission is vertical or perinatal transmission of HBV from infected mothers to neonates. Low and middle-income countries have disproportionally higher rates of HBV thus there is a high prevalence in immigrants to higher-income countries. Of those who develop an acute HBV infection, 95% of babies, 20-30% of children and less than 5% of adults will go on to have a chronic infection. There is no available treatment for an acute HBV infection whilst antivirals such as Tenofovir can improve chronic HBV infections by slowing cirrhosis and reducing the risk of liver cancer.

Given the complications associated with HBV, prevention of transmission is the best method for controlling this infection. Prevention includes avoidance of transmission from infected people via counselling against high risk behaviours, screening of blood products and more stringent infection control in healthcare settings through universal precautions. By far the most effective way of controlling HBV is through vaccination.

The first HBV vaccine was developed in 1982 and is in widespread use. Most vaccines for HBV were developed using recombinant DNA to express a protein (antigen) against hepatitis (HBsAg). HBV vaccines are available in monovalent (single), combination (with Hepatitis A) and multivalent forms (with multiple other vaccines). Routine immunisation of neonates is a common practice worldwide with the WHO recommending a dose of HBV at birth followed by either a 2 or 3 dose schedule. Completion of either of these programmes induces protective antibody levels in up to 95% of infants, children and adolescents.

The burden of HBV in the UK reflects that of other high-income countries. In 2016 there were 453 cases of acute HBV reported and an annual incidence of 0.82 per 100,000 people. During the same period 11,901 cases of HBV were recorded, the remainder being chronic infections. The UK added a vaccine for HBV to the routine childhood immunisation schedule in 2017 as part of a multivalent vaccine. Multivalent vaccines are cost effective on a population level. They reduce the number of needles that need to be administered thus reducing the risk of complications, they minimise the number of vaccine healthcare visits needed and associated costs and they improve compliance and vaccine coverage.

Characteristics of licensed vaccines

1.2. 6 in 1(IH) (Infanrix hexa)

Developed by GlaxoSmithKline, 6 in 1(IH) is a multivalent vaccine that protects against diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenza* type B (Hib) and hepatitis B virus (HBV). It is licensed in Europe and has been widely used internationally with data to support its efficacy and safety. It is available in a powder and suspension for injection form. 6 in 1(IH) has been included in the UK's routine immunisation schedule at 2, 3 and 4 months of age since 2017. It is made up of diphtheria and tetanus toxoid, Bordetella pertussis antigens (pertussis toxoid, filamentous haemagglutinin and pertactin), inactivated poliovirus (type 1 – 3), Hib polysaccharide conjugated to tetanus toxoid as the carrier protein and hepatitis B surface antigen.

1.1.3. 6 in 1(V) (Vaxelis)

One of the other multivalent vaccines that contains Hepatitis B currently licensed in Europe is 6 in 1(V). Developed jointly by Merck/MSD and Sanofi Pasteur, 6 in 1(V) is available as a fully liquid and ready to use injection and protects against the same organisms as 6 in 1(IH) however the structure of some components differs. 6 in 1(V) contains diphtheria and tetanus toxoid, *Bordetella pertussis* antigens (including pertussis toxoid, filamentous haemagglutinin, pertactin and fimbriae types 2 and 3), inactivated poliovirus (including type 1 – 3), Hib polysaccharide conjugated to a meningococcal outer membrane complex (OMPC) and hepatitis B surface antigen. It is the structure of the Hib component that may be relevant to the use of 6 in 1(V) in the UK. 4CMenB is the meningococcal B vaccine currently in use in the UK's routine

immunisation schedule at 2, 4 and 12 months of age. The structure of 4CMenB includes meningococcal outer membrane vesicles which carries with it a theoretical risk of a carrier induced epitopic suppression of the Hib responses of 6 in 1(V) when given concurrently with 4CMenB. This interaction has the potential to lead to the creation of a birth cohort with sub-optimal responses to the Hib antigen of 6 in 1(V) and thus risk a re-emergence of Hib as was seen in the UK in 1999 – 2003.

There is also the possibility that the combination of the OMPC from 6 in 1(V) and 4CMenB could cause increased systemic and local vaccine adverse reactions when co-administered (as compared to adverse reactions that can occur using the existing schedule). In the absence of any evidence to show that these concerns are unfounded, it is possible that the use of 6 in 1(V) in the UK immunisation schedule would be seen as inappropriate. This could limit the flexibility of vaccine procurement for the UK government.

We are conducting a head-to-head unblinded open randomised trial comparing the immunogenicity and reactogenicity at 5 and 13 months of both licensed DTaP-Hib-IPV-HepB vaccines when administered at 2, 3 and 4 months of age alongside the current UK vaccination schedule (including 4CMenB).

Section 2. Study Methods

2.1 Trial Design

This study is an open label, non-inferiority 1:1 randomised clinical trial conducted by the UKPVG sites led by the Oxford Vaccine Group (OVG). The study initially intended to enrol 240 healthy infants residing in the UK aged between 8 and 13 weeks. There are 6 visits in total throughout this study including two blood tests. These visits take place in participant's homes or a suitable, convenient location. Participants will be involved in the study for approximately 11 months (from enrolment at 8 weeks minimum to the last blood test at 13 months of age).

2.2 Sample size

The initial sample calculation was based on the following assumptions:

1. The non-inferiority margin is 0.5 fold-difference between the GMC in the 6 in 1(V) arm and that in the 6 in 1(IH) arm (reference) or -0.3 absolute difference of GMC on log scale (base 10).

2. The standard deviation of GMC on log scale (base 10) is 0.72

3. The true difference of GMC on log scale (base 10) is 0.

Based on the above assumptions, the intent was to recruit 104 infants for each arm to achieve 85% of power at two-sided 5% significance level. The attrition rate was expected to be approximately 10%. To incorporate further allowances for protocol violations and unexpected dropouts, the initial target sample size was 240 infants (120 per arm).

The protective threshold for Hib is 0.15 µg/ml. (13) Assuming the proportion of infant with anti-PRP IgG concentrations at 5 months of age≥ 0.15 µg/ml is 91%, this trial would have had >70% of power with two-sided 5% significance level to claim the 6 in 1(V) is non-inferior to 6 in 1(IH) at a margin of 10%.

After reviewing the disruption on clinical activities by COVID-19 from March 2020 onwards, and the urgency to obtain the data for policy making in the UK, the study team decided to evaluate the study power based on the recruitment up to the time this was paused for the COVID-19

pandemic, and decided to increase the type I error from two-sided 5% (one-sided 2.5%) to one-sided 5%, which is a common type I error rate in non-inferiority trials. At this stage the study had recruited 194 participants. From these there was approximately 172 participants with blood samples likely to be available for primary endpoints in the ITT analysis. Based on the original assumptions and the 5% type I error, the current study power is 85%. Since the current study recruitment has achieved the planned power based on the adjusted type I error, the study CI, sponsor and funder group decided not to resume recruiting and continue with a sample size of 194 participants.

2.4 Objectives and outcome measures

Primary and secondary objectives are reported in Table 1, alongside the outcome measures and timepoint of evaluation for each measure.

Table 1 – Objectives, outcome measures, and timepoints

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objective Compare the immunogenicity of the Hib component of 6 in 1(IH) and 6 in 1(V) when co-administered with 4CMenB in the UK routine immunisation schedule at 5 months of age.	Measurement of anti-PRP (Hib) IgG concentrations at 5 months of age as measured by ELISA.	At 5 months of age (at least 4 weeks after administration of the last dose of either 6 in 1(IH) and 6 in 1(V) primary immunisations).

Secondary Objectives Compare the anti-PRP (Hib) IgG concentrations at 13 months of age (1 month after administration of Hib-MenC at 12 months of age) in participants primed with 6 in 1(IH) and 6 in 1(V).	Assess the anti-PRP (Hib) IgG concentrations at 13 months of age as measured by ELISA.	At 13 months of age (at least 4 weeks after the Hib-MenC vaccination at 12 months of age).
The immunogenicity of the other antigens in the routine immunisation schedule incorporating either 6 in 1(IH) or 6 in 1(V).	Assess the IgG concentrations at 5 and 13 months of: a.Diphtheria-toxoid b.Tetanus toxoid c.Hepatitis B virus d.Vaccine-serotype pneumococcal capsule antigens e.Pertussis antigens f.Poliovirus neutralising antibodies Assess serum bactericidal titres at 5 and 13 months of age of: a. 3 reference serogroup B meningococcal strains b. Serogroup C meningococcus	At 5 and 13 months of age (approximately 4 weeks after completion of the primary routine immunisations and approximately 4 weeks after the booster doses in the routine immunisation schedule).
The reactogenicity of 6 in 1(IH) and 6 in 1(V) when administered in the routine UK immunisation schedule.	Solicited local and systemic adverse events within 5 days of immunisations	In the 5 days post immunisation in participant diaries

Section 3. Analysis – general considerations

The primary analysis will be conducted on the modified intent-to-treat basis, i.e. anyone who received at least one dose of study vaccine with the study endpoint available will be included in this analysis. The per-protocol analysis will be considered as a sensitivity analysis to rule out the effect of non-compliance. The non-compliance is defined as a visit outside the pre-specified window or missing doses of vaccination (Table 2). Windows of inclusion will be relaxed to allow +/-7 days difference to the visit schedule for visits 1, 2, 3, 4, and 6, and +/-14 days difference to the visit schedule for visit 6.

Table 2 – Trial visit schedules

Group	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Age of participant	2 months	3 months	4 months	5 months	12 months	13 months
Visit windows	8 – 13 weeks of age	28-42 days after visit 1	28-42 days after visit 2	28-42 days after visit 3	12 months of age (+28 days)	28-42 days after visit 5
Relaxed window	N/A	21-49 days after visit 1	21-49 days after visit 2	21-49 days after visit 3	350-406 days of age	21-49 days after visit 5
Visit description	Enrolment Vaccination	Vaccination	Vaccination	Blood sampling	Vaccination	Blood sampling

The analysis on primary outcome of the 5-month anti-PRP Hib IgG concentrations and secondary outcomes of safety and other 5-month antigens will be conducted once the 5-month data are available for all participants, while the trial will continue to follow up all participants

until 13 months of age. The final analysis for the other outcomes will be carried out once the 13-month data are available for all participants.

The concentration of IgG is expected to be positively skewed and thus mathematical transformations (log10) will be applied, where appropriate, in order to render a normal distribution. Values below the lower limit detectable by the assay will be imputed a value half the lower limit of detection prior to log transformation. Distributional assumptions will be assessed graphically and if these assumptions are violated alternative analysis methods (e.g. non-parametric) or alternative transformations will be used. For assays that have an upper limit of detection, the value of the upper limit will be used, and the data will be transferred to categorical data for analysis.

Continuous variables that follow an approximately normal distribution will be summarised using means and standard deviations. Skewed continuous variables will be summarised using medians and inter-quartile ranges if there is no appropriate transformation to render a normal distribution. Categorical variables will be summarised using frequencies and percentages.

Baseline characteristics will be summarised for each group to describe the study population. No formal statistical comparisons of baseline characteristics between randomised groups will be conducted. Patient throughput from census, enrolment, through randomisation, follow up and analysis will be presented in a CONSORT flow diagram. This will contain the numbers of participants randomly assigned to each group, receiving different doses of study vaccine, completing the study and analysed for the primary outcome. It will also include a breakdown of reasons for withdrawal.

A P value lower than 0.05 (one-sided) will be considered to be significant for the non-inferiority analysis of the primary objective. For superiority tests, a 2-sided P value less than 0.05 will be considered significant.

Section 4. Analysis

4.1 Outcome Definitions and Analysis Methods

4.1.1 Primary Endpoint

The primary outcome is anti-PRP Hib IgG concentrations at 5 months of age as measured by ELISA. The geometric mean concentrations (GMC) of anti-PRP IgG will be compared between 6 in 1(IH) and 6 in 1(V) under the hypothesis:

$$H_0: GMC_{6\text{ in }1(V)} / GMC_{6\text{ in }1\text{-}(IH)} \leq 0.5 \text{ or } log_{10} GMC_{6\text{ in }1(V)} - log_{10} GMC_{6\text{ in }1(IH)} \leq -0.3;$$

$$H_1: GMC_{6\text{ in }1(V)} / GMC_{6\text{ in }1(IH)} > 0.5 \text{ or } log_{10} GMC_{6\text{ in }1(V)} - log_{10} GMC_{6\text{ in }1(IH)} > -0.3.$$

The GMC will be transferred using logarithmic transformations (base 10) to render a normal distribution.

We will test the above hypothesis using Student's t-test on log_{10}GMC. The mean difference of log_{10}GMC will be presented with the one-sided 95% confidence interval (CI). The difference will be calculated as the mean of log_{10}GMC in 6 in 1(V) arm compared to that in the 6 in 1(IH) arm. We will claim 6 in 1(V) is non-inferior to 6 in 1(IH) if the lower CI of log_{10}GMC difference lies above -0.3. If a non-inferiority of 6 in 1(V) to 6 in 1(IH) is claimed, the Student's t-test will also be used to test superiority and two-sided 95% CI will be presented. If the data cannot be rendered normal after a log transformation, Wilcoxon rank sum tests will be used in place of the Student's t-test.

The proportion of infant with anti-PRP Hib IgG concentrations at 5 months of age≥ 0.15 µg/ml will be calculated with the corresponding 95% Yates's continuity-corrected CIs for 6 in 1(IH) and 6 in 1(V) arms. Although the study is not powered based on the proportion of protection, the difference between the two arms will be reported with one-sided 95% Yates's continuity-corrected CIs, and the non-inferiority margin for proportion of protection is -10%. Fisher's Exact tests will be performed and reported with one-sided 95% confidence intervals where expected values are low.

The definitions of the analysis population can be found in section 3 for both primary and sensitivity analyses. Further sensitivity analyses will be carried out using multiple regression to adjust for potential covariates, including gender, age in days at first dose, and maternal pertussis immunisation history (Y/N).

4.1.2 Secondary Endpoints

1. Anti-PRP (Hib) IgG concentrations at 13 months of age as measured by ELISA.

2. IgG concentrations at 5 and 13 months of:

    a. Diphtheria-toxoid

    b. Tetanus toxoid

    c. Hepatitis B virus

    d. Vaccine-serotype pneumococcal capsule antigens

    e. Pertussis antigens

    f. Poliovirus neutralising antibodies

3. Serum bactericidal titres at 5 and 13 months of age of:

    a. 3 reference serogroup B meningococcal strains

    b. Serogroup C meningococcus

The analysis population for each endpoint will follow Section 3. The analysis of GMC for all the secondary outcomes will follow 5.1.1, except that we will adjust for maternal history of pertussis vaccination for the main analyses of pertussis antigens related endpoints. For assays with pre-defined correlates of protection, the data will be dichotomised into binary variables for reporting protection (Table 3). For assays with no consensus on the cut-off to define protection, we will not dichotomise the data into binary variables for reporting. In the situation that those assays also have an upper and/or lower limit of detection (Section 3), the trial statistician will discuss with the chief investigator to define cut-offs to transform the data.

Table 3 – Trial visit schedules

Vaccine antigen		Assay	Level required for protection†
Haemophilus influenzae b (Hib)		ELISA (anti-PRP IgG)	≥0.15 µg/ml (short term) ≥1.0 µg/ml (long term)
Diphtheria		ELISA (IgG to toxoid)	≥0.1 IU/ml
Tetanus		ELISA (IgG to toxoid)	≥0.1 IU/ml
Hepatitis B		ELISA (anti-HBs IgG)	≥10 IU/ml
Group B meningococcus (MenB):	44/76-SL 5/99 NZ98/254	SBA (human complement)	≥4 hSBA titre
Group C meningococcus (MenC)		SBA (rabbit complement)	≥8 rSBA titre
Polio		Neutralisation	≥1/8 titre
Pneumococcus		ELISA	≥0.35 µg/ml

†References:

Davis, K., Pinto, M. V., Andrews, N. J., Goldblatt, D., Borrow, R., Findlow, H., ... & Snape, M. D. (2021). Immunogenicity of the UK group B meningococcal vaccine (4CMenB) schedule against groups B and C meningococcal strains (Sched3): outcomes of a multicentre, open-label, randomised controlled trial. *The Lancet Infectious Diseases*.

Plotkin, S. A. (2010). Correlates of protection induced by vaccination. Clinical and vaccine immunology, 17(7), 1055-1065.

4. Solicited local and systemic adverse events within 5 days of immunisations.

The local adverse events include: Erythema, induration, swelling, and tenderness (pain) at the injection site. Systemic adverse events include: change in feeding/eating habit, drowsiness, vomiting, diarrhoea, lethargy, malaise, excessive crying and temperature. The severity grading can be found in the protocol. Solicited AEs were self-reported using e-Diary or paper diary for the first 5 days after each vaccination visit, and the data will be summarised using frequencies and proportions for each day of the first 5 days after the administration of each vaccine after visits 1, 2 and 3. The local AEs will be reported separately by injection sites.

4.2 Missing Data

The level and pattern of the missing data in the baseline variables and outcomes will be reported. The potential causes of any missing data will be investigated and documented as far

as possible. Any missing data will be dealt with using methods appropriate to the conjectured missing mechanism and level of missing.

4.3 Deviations from the SAP

Deviations from the SAP will be discussed with the Lead Statistician, and reported in the final statistical report.

Appendix A: Amendment history

Amendment No.	SAP Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2.0	02.07.21	N.G.Marchevsky	Additional of details of relaxed windows around visit schedules; addition of non-parametric tests for non-normal data; correlates of protection details for the study antigens; table formatting.