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## Introduction

The number of immunizations recommended for children in Europe in the first 2 years of life has increased dramatically over time. Simplifying immunization schedules through the use of combination vaccines reduces painful injections for the infant and has been shown to lead to higher rates of compliance with complex vaccination schedules, while simultaneously protecting against several diseases in a short period of time [1-4]. DTaP5-HB-IPV-Hib (diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus, *Haemophilus influenzae* type *b* (Hib) conjugate, and hepatitis B [recombinant] vaccine; Vaxelis®, MCM Vaccine B. V., Leiden, The Netherlands), is a new hexavalent vaccine developed to provide protection against six childhood infectious diseases: diphtheria, tetanus, pertussis, hepatitis B, polio, and Hib. It is a ready-to-use, preservative-free, fully liquid preparation with the potential to minimize errors related to inadequate reconstitution of Hib. It is a combination of existing antigens from vaccines already licensed in Europe and/or in the United States (Table 1a).

In four phase 3 studies of the vaccine, various primary schedules were studied with coadministration of rotavirus vaccine, pneumococcus 13-valent conjugate vaccine (PCV-13), and the measles, mumps, rubella (MMR) vaccine [5-8]. In some European Union countries, including Ireland, Iceland, Spain, and Greece, the childhood vaccination calendar includes administration of meningococcus group C conjugate (MCC) vaccines with the primary series. In 2011, the United Kingdom (UK) childhood vaccination schedule was an accelerated 3-dose primary series of a pentavalent (diphtheria, tetanus, pertussis [acellular, component DTaP], poliomyelitis [inactivated IPV], and Hib) vaccine at 2, 3, and 4 months of age. The second and third doses were given concomitantly with an MCC vaccine, followed by a booster dose with a combined Hib-MCC vaccine at 12 months of age. PCV-13, a $CRM_{197}$ conjugated vaccine, was also administered concomitantly at 2 and 4 months of age with a booster dose at 12 months of age. The UK schedule changed in June 2013 (after this study had started) with only one dose of MCC vaccine at 3 months of age being recommended,

and again in July 2016, when infant meningococcus group C immunization was discontinued completely.

The present study evaluates the concomitant administration of DTaP5-HB-IPV-Hib with two different MCC vaccines. The primary objective was to describe anti-meningococcus group C seroprotection rates (SPR) in healthy infants aged 5 months following 2 doses of either an MCC-detoxified tetanus toxin vaccine (MCC-TT; NeisVac-C®, Baxter AG, Wien, Austria) or an MCC-*Corynebacterium diphtheriae* $CRM_{197}$ protein vaccine (MCC-CRM; Menjugate®, Novartis Vaccine and Diagnostics, S.R.L., Siena, Italy) given at 3 and 4 months of age concomitantly with second and third doses of DTaP5-HB-IPV-Hib. In addition, primary seroprotection rates after the primary series, geometric mean titers (GMTs), or geometric mean concentrations (GMCs) to the antigens in DTaP5-HB-IPV-Hib, and anti-meningococcus group C seroprotection rates after only one dose of MCC were described in the two study groups, as well as following the Hib-MCC vaccine given in the booster phase. Post-primary and post-booster seroresponses in the groups randomised to receive the two different MCC vaccines at 3 and 4 months of age were compared in a post hoc analysis. Safety data are also reported.

## Materials & Methods

This was a randomised, open-label, multicentre trial evaluating two MCC vaccines when given concomitantly with DTaP5-HB-IPV-Hib (EudraCT 2011-002413-11). The study was conducted at 11 sites in the UK and was carried out in accordance with Good Clinical Practice guidelines under the favourable opinion of the National Research Ethics Service Committee South West – Central Bristol (11/SW/0328) and with UK Medicines and Healthcare Product Regulatory Agency approval.

*Participants and recruitment*

Invitation letters were sent to the parents of children due for their routine immunizations, and parents who expressed an interest in enrolling their child in the study were called to ensure eligibility. Exclusion criteria included participation in another trial involving an investigational compound or device, known immunosuppression, immunodeficiency or other chronic illness, administration of blood products, previous vaccination with antigens being administered as part of the study, or illness relating to these diseases and allergic reactions to any vaccine components.

Eligible infants were either visited in their homes or seen at the hospital or clinical research facility for their visits. Informed consent was obtained from at least one parent before any study procedures commenced.

*Visits and vaccines*

A total of 284 healthy infants aged 46 to 74 days were recruited over a 7-month period and randomised (1:1 based on balanced permuted blocks of randomization ranging in size from 4 to 8 and stratified by site) to receive either the MCC-TT vaccine (MCC-TT group) or the MCC-CRM vaccine (MCC-CRM group). An overview of the visit schedule is provided in Table 1b. The study was divided into two parts: a primary vaccination phase (2 to 5 months of age), and a booster phase (12 to 13 months of age). Regardless of group assignment, all participants were scheduled to receive the following:

Primary phase: DTaP5-HB-IPV-Hib and PCV-13 at 2 months of age; followed by DTaP5-HB-IPV-Hib and an MCC vaccine at 3 months of age; and DTaP5-HB-IPV-Hib, MCC, and PCV-13 vaccines at 4 months of age.

Booster phase: MMR and Hib-MCC vaccines at 12 months of age.

Blood samples were obtained at 2, 4, and 5 months of age during the primary phase, and at 12 and 13 months of age during the booster phase.

*Serological assays*

Serology was performed at three different laboratories as follows.

**Serum bactericidal antibody with rabbit complement assay (rSBA).** Meningococcal serogroup C antibody levels were measured at the Vaccine Evaluation Unit, Public Health England, Manchester, UK, using an internationally standardized serum bactericidal antibody assay with baby rabbit complement (rSBA) [9, 10]. rSBA titers were expressed as the reciprocal of the final serum dilution giving $\geqslant$50% killing at 60 minutes as compared with control (heat-inactivated complement, meningococci, and no unknown serum). The lower limit of quantitation (LLOQ) for the rSBA assay was 4. For immunogenicity calculations, values below the LLOQ were replaced by half of the LLOQ (i.e., were assigned a titer of 2).

**Radioimmunoassay for antibodies to Hib capsular polysaccharide (PRP).** A standard Farr technique radioimmunoassay (RIA) was used to detect antibody to Hib capsular polysaccharide [11]. These assays were performed at Pharmaceutical Product Development, Vaccines and Biologics Laboratory Department, Wayne, Pennsylvania, USA.

**Enhanced chemiluminescence assay for antibodies to hepatitis B surface antigen (HBsAg).** Antibody concentrations to hepatitis B were measured with a hepatitis-B–enhanced chemiluminescence assay that detected total antibody to human plasma-derived HBsAg (Pharmaceutical Product Development, Vaccines and Biologics Laboratory Department, Wayne, Pennsylvania, USA) [11,12].

**Micrometabolic inhibition tests for antibodies to diphtheria and poliovirus**. Antibody concentrations to diphtheria toxin and titers to poliovirus types 1, 2, and 3 were measured at Global Clinical Immunology, Sanofi Pasteur Inc., Swiftwater, Pennsylvania, USA, using micrometabolic inhibition tests (see Supplemental Methods).

**Enzyme-linked immunosorbent assays for antibodies to pertussis and tetanus antigens**. Antibody concentrations to pertussis antigens (PT, FHA, PRN, and FIM-2,3) and to tetanus antigen were assessed at Global Clinical Immunology using enzyme-linked immunosorbent assays (see Supplemental Methods).

*Safety evaluation*

Safety measurements in the primary phase of this study included daily measurement of axillary temperatures in the evening from Day 1 (day of vaccination) to Day 5 following each vaccination; daily collection of solicited injection site reactions (from Day 1 to Day 5 following each vaccination; daily collection of solicited systemic adverse events (AEs) from Day 1 to Day 5 following each vaccination; and collection of any unsolicited AEs (i.e., spontaneously reported) from Day 1 to Day 15 following each vaccination.

During the primary and booster phases, all serious AEs (SAEs) were recorded, including death due to any cause, occurring from the time of consent to 14 days (Day 1 to Day 15) following each vaccination, whether or not related to the study vaccines. Any SAE which occurred at any time outside of the follow-up period (Day 1 to Day 15) was also reported if the event was either: (1) a death or (2) an SAE that was considered by an investigator to be possibly, probably, or definitely vaccine-related.

*Statistical analysis*

The sample size of the study was calculated for the primary objective of the study using PASS 2008 software (NCSS, LLC, Kaysville, Utah) based on the binomial distribution. The main immunogenicity analyses were performed on the per protocol set (PPS) which excluded participants with protocol deviations that could potentially interfere with vaccine immunogenicity. Additional intention-to-treat immunogenicity analyses were performed on the full analysis set (FAS), which included all participants with immunogenicity results. The safety evaluation in the post primary series included all randomized participants who

received at least one vaccine during the primary phase of the study and who had safety follow-up data, and in the booster phase, all participants who received at least one vaccine and who had safety follow-up data in that phase.

The SPR to MCC was defined as the proportion of participants in each group with an anti-MCC titer of at least 8. The percent of participants with titers ≥128 dilution was also recorded. It was predefined that it would be considered acceptable if the lower bound of the associated two-sided 95% confidence interval (CI; adjusted for multiplicity) was at least 90% after two doses. For seroconversion rates, 95% CIs were calculated using the exact binomial method [13]; 95% CIs of GMTs were calculated using the t-distribution of the natural log-transformed antibody titers. For the post hoc analysis of seroconversion rates and GMT comparisons between randomized groups (MCC vaccines) were performed using Fisher exact testing and student *t* test after log transformation of individual titers, respectively.

It was predefined that it would be considered acceptable if the lower bound of the Hib SPR two-sided 95% CI (adjusted for multiplicity) was at least 80% after three doses of DTaP5-HB-IPV-Hib. A seroresponse to the pertussis antigens was defined as either any detectable concentration if pre-vaccine concentrations were <LLOQ or any detectable rise in concentration. Statistical analyses were performed using SAS® software version 9.1 (SAS® Institute Inc., Cary, North Carolina, USA).

## Results

*Demographics*

There were no clinically significant demographic differences noted between groups. Of the 284 participants enrolled in the study, 54.6% (155) were male, with a mean age at enrolment of 62.1 days (range 47 to 76 days). The number of participants lost to follow-up or withdrawn over the course of the study was similar in both groups (Figure 1).

*Immunogenicity*

Primary phase

In the primary phase, results for all randomised participants were included in the analysis except those with protocol deviations that interfered with the immunogenicity evaluation (per protocol analysis). These mostly related to difficulties obtaining sufficient blood from the infants and/or scheduling visits within the permitted timelines. Infants in both groups exceeded the predefined acceptability threshold for seroprotection against meningococcus group C for the two groups (Table 2). Seroconversion rates (with titers ≥8 dilution) and GMTs were lower post-dose 1 in the MCC-CRM group (96.4% and 285.0, respectively) than in the MCC-TT group (100% and 1353.0, respectively; $P<0.001$ for both) (Table 2).

SPRs and seroresponse rates (SRRs) for, and GMTs of antibodies to the DTaP5-HB-IPV-Hib antigens following the three dose primary series for both study groups are shown in Table 3. Infants in both groups exceeded the predefined acceptability threshold for seroprotection against Hib (Table 3). SPRs or SRRs to all antigens exceeded 90% in both groups. GMTs of antibodies to the DTaP5-HB-IPV-Hib antigens were comparable in the two study groups that received different MCC vaccines with widely overlapping 95% CIs in all cases (Table 3).

Booster phase

A similar per-protocol analysis approach was taken in the booster phase. As in the primary phase, exclusions were mostly related to visit scheduling (numbers analysed are shown in Table 4). As expected, the participants' responses to both Hib (PRP) and meningococcus group C antigens had waned by the time the Hib-MCC booster vaccination was administered (Table 4). This was particularly evident for meningococcus group C bactericidal antibodies in the MCC-CRM group. Responses to both antigens were boosted in both groups, although the GMT values for MCC remained significantly lower in the MCC-CRM than the MCC-TT

primed group (580.8 vs 3257.9; *P*<0.001), and the post-booster GMCs of antibodies to Hib (PRP) did not differ significantly between groups.

*Safety*

Safety data from all participants who received at least 1 study vaccine dose during the primary phase of the study and who had any safety follow-up data collected are shown in Table 5. No significant differences between rates of AEs in the two study groups were observed, and combined data are presented. There were no withdrawals due to AEs. One participant experienced 2 SAEs (severe abdominal pain; inconsolable crying) that occurred 2 days after the second dose of DTaP5-HB-IPV-Hib and the first dose of MCC-CRM; these events spontaneously resolved within 2 days and were considered possibly vaccine-related by the investigator.

## Discussion

This study was conducted primarily to demonstrate the compatibility of this DTaP5-HB-IPV-Hib vaccine with two different MCC vaccines in the infant primary series that were in use in the UK at the time of this study. Although the UK has since ceased to use meningococcus group C vaccines in infants, other European countries continue to do so, although use of a single priming-dose is now more common there. The results of the present study confirm that this hexavalent combination vaccine when given to infants in an accelerated 2-, 3-, and 4-month schedule along with two doses of these TT- and CRM-containing MCC, results both in satisfactory immune responses to antigens within the DTaP5-HB-IPV-Hib vaccine and is associated with high rates of seroprotection against meningococcus group C. In fact, very high seroprotection rates after a single priming dose of either MCC vaccine were also seen. Similarly, high seroprotection rates against meningococcus group C were achieved following second-year boosting, indicating effective priming and excellent levels of direct protection against this disease by the vaccine regimens used.

Immune responses to all the antigens in the DTaP5-HB-IPV-Hib combination vaccine were studied in detail, and high seroprotection rates and seroconversion rates were consistently observed (Table 3). Immunogenicity results for the DTaP5-HB-IPV-Hib antigens did not differ between groups, suggesting that the two different MCC vaccines had no observable effect on the immunogenicity of these antigens. This is especially relevant for Hib, given that previous studies using combination vaccines employing TT or CRM carrier proteins for Hib have shown an inhibition of the Hib response when coadministered with MCC [14-16], thought to be due to carrier protein induced epitopic suppression [17]. In contrast, the Hib component of DTaP5-IPV-Hib-IPV is conjugated to the outer membrane protein complex (OMPC) from *N*. *meningitidis* serogroup B (PRP–OMPC) rather than TT or CRM, and no interference was observed. Thus, any interference between MCC and Hib immunogenicity via carrier-induced epitopic suppression may be avoided.

Because a randomized approach was taken to the allocation of infants to one of the two MCC vaccines, comparisons can be made between these groups despite being a post hoc analysis. The results demonstrate the superior immunogenicity of the MCC-TT vaccine used compared with the MCC-CRM vaccine. However, a previous study comparing different MCC-TT and MCC-CRM vaccines than those used in the present study showed no differences in reactogenicity or immunogenicity profiles [18]. Nevertheless, an open-label study of three MCC vaccines licenced in the UK showed that administering an MCC-TT vaccine at 4 months of age, after receipt of an MCC-CRM vaccine at 3 months of age, resulted in lower GMTs compared with receipt of an MCC-TT or MCC-CRM vaccine at both time points or receipt of the MCC-TT vaccine followed by the MCC-CRM vaccine, suggesting that MCC vaccines with different carrier proteins are not fully interchangeable [19]. Another study found that a single infant MCC-TT priming dose induced a more robust post-booster response than either one or two MCC-CRM priming doses [20]. Thus, the unequal immunogenicity results for the two MCC vaccines in the present study could be of clinical importance, particularly toward the end of the first year of life in settings where invasive

meningococcus group C strains continue to circulate, as MCC-CRM primed infants' responses frequently waned to levels below the putative protective threshold. This observation confirms the greater immunogenicity of prime-boost conjugate schedules using the same protein at both phases [19,21]; in this case, tetanus toxoid.

The safety data summarized in this report are concordant with rates of local and systemic reactions previously reported for acellular-pertussis containing vaccines and conjugate vaccines given according to this accelerated schedule [22-24].

Finally, the results of this study should be taken in the context of evolving understanding of the mechanisms of effectiveness of conjugate vaccines in general, and meningococcal vaccine, in particular. Although direct protection by induction of protective bactericidal concentrations of antibody in infants, the most frequent victims of invasive bacterial disease, has been the cornerstone of vaccine development and licensure, it is now widely appreciated that such protective immune responses induced in infancy are relatively short-lived [25]. Furthermore, disease control appears to occur most reliably and effectively when circulation of invasive bacterial strains is interrupted at the population level [26]. Immunization schedules are changing in response to these new insights and the regimens being tested as part of vaccine development programs in the future are likely also to change as a result.

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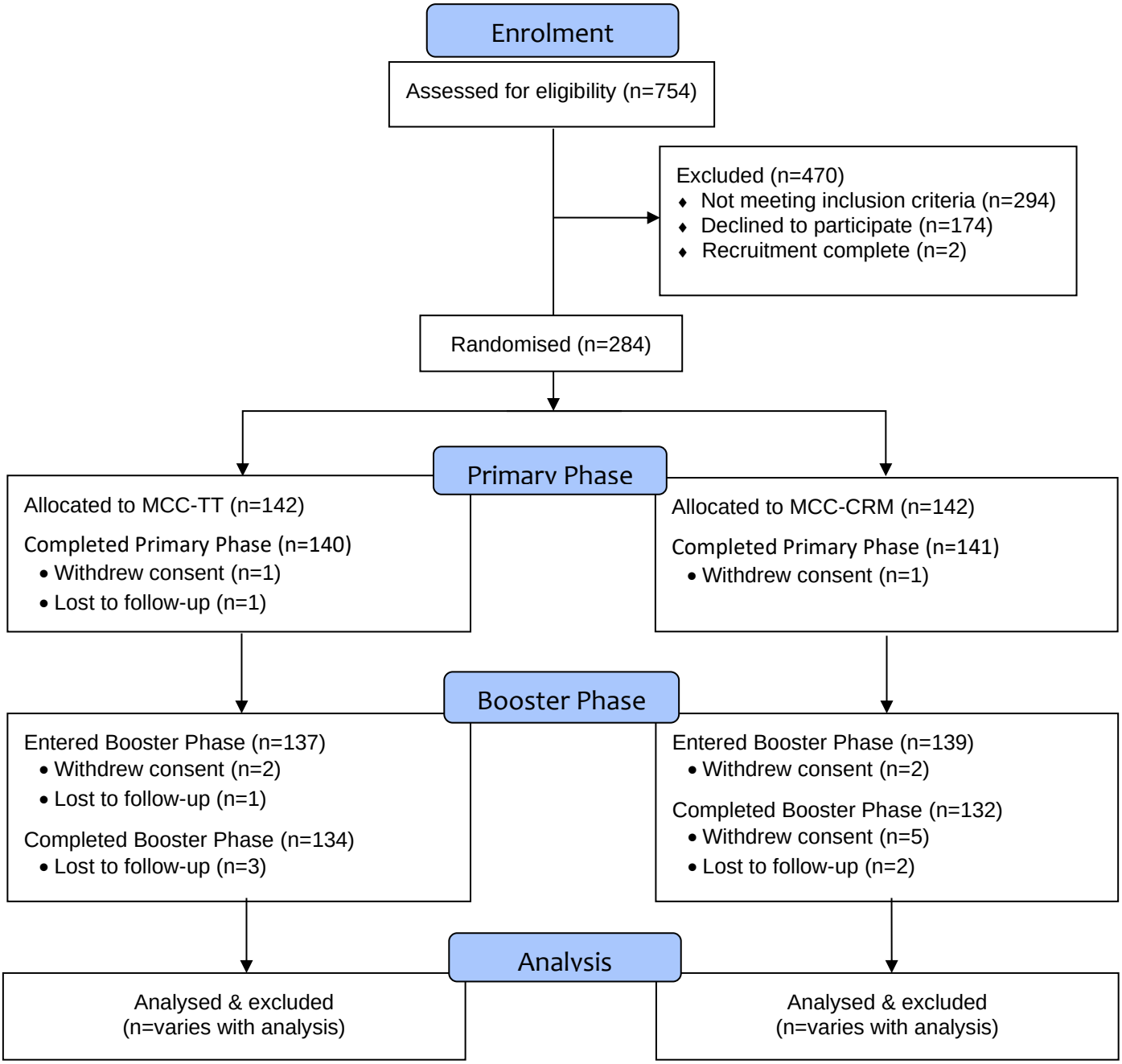
## FIGURE AND TABLES

**Figure 1:** Participant disposition



343

344 **Table 1**

345 Vaccine details (A) and schedule of vaccine administration and blood sampling (B).

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| **A. Vaccines administered** | | |
|---|---|---|
| **Target disease** | **Antigen (s)** | **DTaP5-HB-IPV-Hib[a,b]** |
| Diphtheria | D | 15 Lf |
| Tetanus | T | 5 Lf |
| Pertussis | Pertussis toxin (PT) | 20 µg |
| | Filamentous haemagglutinin (FHA) | 20 µg |
| | Pertactin (PRN) | 3 µg |
| | Fimbriae types 2&3 (FIM-2,3) | 5 µg |
| Polio | Type 1 (Mahoney) | 40 D-antigen units |
| | Type 2 (MEF-1) | 8 D-antigen units |
| | Type 3 (Saukett) | 32 D-antigen units |
| Haemophilus influenzae type b | polyribosylribitol phosphate (PRP), outer membrane protein complex (OMPC) from *N. meningitidis* serogroup B (OMPC) | PRP 3 µg<br>OMPC 50 µg |
| Hepatitis B | HBsAg | 10 µg |
| (Adjuvant) | Aluminium | 319 µg |
| [a]Lot C3146B<br>[b]Other licensed vaccines were used in the study, all were given as 0.5 mL intramuscular doses: MCC-CRM (Novartis Vaccine or Diagnostics lots 382011 & BA4559A) or MCC-TT (Baxter AG, lot VNS1L05A), PCV-13 (Pfizer Inc. lots F54378 & G29716), MMR (Merck & Co. Inc., Kenilworth, NJ, USA lots H010594 & H010453), Hib-MCC (GlaxoSmithKline, lot A76CA209A). | | |

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| **B. Vaccine administration and blood sampling schedule** | | | | | | |
|---|---|---|---|---|---|---|
| **Phase** | **Primary phase** | | | | **Booster phase** | |
| **Visit** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** |
| Age | 2 months | 3 months | 4 months | 5 months | 12 months | 13 months |
| DTaP5-HB-IPV-HIB | X | X | X | | | |
| MCC-TT (Group 1) | | X | X | | | |
| MCC-CRM (Group 2) | | X | X | | | |
| PCV-13 | X | | X | | X | |
| Hib-MCC | | | | | X | |
| MMR | | | | | X | |
| Blood draw | X | | X | X | X | X |
| CRM: *Corynebacterium diphtheriae* $CRM_{197}$; Hib: *Haemophilus influenzae* type b; MCC: meningococcus group C conjugate; MMR: measles, mumps, rubella; PCV: pneumococcal conjugate vaccine; TT: tetanus toxoid.<br>DTaP5-HB-IPV-HIB, Vaxelis®, MCM Vaccine B. V., Leiden, The Netherlands; MCC-TT, NeisVac-C®, Baxter AG, Wien, Austria; MCC-CRM, Menjugate®, Novartis Vaccine and Diagnostics, S.R.L., Siena, Italy; PCV-13, Prevenar 13®, Pfizer Inc, England; Hib-MCC, Menitorix®, GlaxoSmithKline, Belgium, MMR, M-M-RVAXPRO®, Merck & Co. Inc., Merck Manufacturing Division, USA. | | | | | | |

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353 **Table 2**

354 Summary of MCC serum bactericidal antibody responses per dose, per protocol set, primary
355 phase (N=236).

| | MCC-TT (N=125) | | MCC-CRM (N=111) | | Total (N=236) | | *P* value |
|---|---|---|---|---|---|---|---|
| | Observed response | | Observed response | | Observed response | | |
| **Endpoint** | **p/n** | **[95% CI]** | **p/n** | **[95% CI]** | **p/n** | **[95% CI]** | |
| **Post-dose 1 of MCC vaccine (at around 3 months of age; 28 to 44 days after Visit 1)** | | | | | | | |
| % with titers ≥8 dil | 102/102 | 100.0 [96.4, 100] | 81/84 | 96.4 [89.9, 99.3] | 183/186 | 98.4 [95.4, 99.7] | NS[b] |
| % with titers ≥128 dil | 100/102 | 98.0 [93.1, 99.8] | 71/84 | 84.5 [75.0, 91.5] | 171/186 | 91.9 [87.0, 95.4] | <0.001[b] |
| GMT<br><br>n missing | | 1353.0 [1058.4, 1729.6]<br>23 | | 285.0 [201.5, 403.1]<br>27 | | 669.6 [530.2, 845.6]<br>50 | <0.001[c] |
| **Post-dose 2 of MCC vaccine (at around 4 months of age; 28 to 44 days after Visit 2)** | | | | | | | |
| % with titers ≥8 dil | 121/121 | 100.0 [97.0, 100] | 108/109 | 99.1 [95.0, 100] | 229/230 | 99.6 [97.6, 100] | NS[b] |
| % with titers ≥128 dil | 120/121 | 99.2 [95.5, 100] | 108/109 | 99.1 [95.0, 100] | 228/230 | 99.1 [96.9, 99.9] | NS[b] |
| GMT<br><br>n missing | | 2024.7 [1689.8, 2425.9]<br>4 | | 1077.4 [847.5, 1369.8]<br>2 | | 1501.5 [1288.8, 1749.3]<br>6 | <0.001[c] |

CI: confidence interval; CRM: *Corynebacterium diphtheriae* $CRM_{197}$; dil: dilution; GMT: geometric mean titer; MCC: meningococcus group C conjugate; n: number of participants included in the analysis; NS: not significant; p: number of participants with the response; TT: tetanus toxoid.
[b]*P* value: Fisher exact test.
[c]*P* value: Student *t* test on log-transformed data.

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**Table 3**

Summary of DTaP5-HB-IPV-HIB antibody responses post-dose 3 (at around 4 months of age; 28 to 44 days after Visit 2), per protocol analysis, primary phase (N=236).

| | | MCC-TT (N=125) | | MCC-CRM (N=111) | | Total (N=236) | |
|---|---|---|---|---|---|---|---|
| | | Observed response | | Observed response | | Observed response | |
| **Antigen** | **Endpoint** | **p/n or n** | **[95% CI]** | **p/n or n** | **[95% CI]** | **p/n or n** | **[95% CI]** |
| **PRP** | % with conc ≥0.15 µg/mL | 91/93 | 97.8 [92.4, 99.7] | 82/82 | 100.0 [95.6, 100.0] | 173/175 | 98.9 [95.9, 99.9] |
| | GMC (µg/mL) | | 6.44 [4.70, 8.83] | | 8.21 [6.08, 11.09] | | 7.22 [5.81, 8.97] |
| **HBsAg** | % with conc ≥10 mIU/mL | 90/93 | 96.8 [90.9, 99.3] | 79/82 | 96.3 [89.7, 99.2] | 169/175 | 96.6 [92.7, 98.7] |
| | GMC (mIU/mL) | | 195.1 [150.7, 252.7] | | 247.7 [186.3, 329.3] | | 218.2 [180.4, 264.0] |
| **Diphtheria** | % with conc ≥0.01 IU/mL | 125/125 | 100.0 [97.1, 100.0] | 104/104 | 100.0 [96.5, 100.0] | 229/229 | 100.0 [98.4, 100] |
| | % with conc ≥0.1 IU/mL | 85/125 | 68.0 [59.1, 76.1] | 77/104 | 74.0 [64.5, 82.1] | 162/229 | 70.7 [64.4, 76.5] |
| | GMC (IU/mL) | | 0.198 [0.165, 0.237] | | 0.220 [0.181, 0.268] | | 0.208 [0.182, 0.237] |
| **Tetanus** | % with conc ≥0.01 IU/mL | 122/122 | 100.0 [97.0, 100.0] | 105/105 | 100.0 [96.5, 100.0] | 227/227 | 100.0 [98.4, 100] |
| | % with conc ≥0.1 IU/mL | 122/122 | 100.0 [97.0, 100.0] | 105/105 | 100.0 [96.5, 100.0] | 227/227 | 100.0 [98.4, 100] |
| | GMC (IU/mL) | | 1.03 [0.90, 1.17] | | 0.95 [0.82, 1.10] | | 0.99 [0.90, 1.09] |
| **Pertussis PT** | % with seroresponse [2] | 99/100 | 99.0 [94.6, 100.0] | 75/75 | 100.0 [95.2, 100.0] | 174/175 | 99.4 [96.9, 100.0] |
| | GMC (EU/mL) | 112 | 131.5 [117.2, 147.6] | 89 | 133.3 [118.3, 150.2] | 201 | 132.3 [121.8, 143.7] |
| **Pertussis FHA** | % with seroresponse [2] | 91/100 | 91.0 [83.6, 95.8] | 67/74 | 90.5 [81.5, 96.1] | 158/174 | 90.8 [85.5, 94.7] |
| | GMC (EU/mL) | 112 | 50.4 [44.8, 56.6] | 88 | 50.1 [43.7, 57.4] | 200 | 50.2 [46.0, 54.9] |
| **Pertussis PRN** | % with seroresponse [2] | 95/100 | 95.0 [88.7, 98.4] | 66/73 | 90.4 [81.2, 96.1] | 161/173 | 93.1 [88.2, 96.4] |
| | GMC (EU/mL) | 112 | 90.4 [73.2, 111.7] | 87 | 106.8 [83.7, 136.3] | 199 | 97.2 [83.0, 114.0] |
| **Pertussis FIM-2,3** | % with seroresponse [2] | 96/100 | 96.0 [90.1, 98.9] | 72/75 | 96.0 [88.8, 99.2] | 168/175 | 96.0 [91.9, 98.4] |
| | GMC (EU/mL) | 112 | 401.7 [339.4, 475.5] | 89 | 441.7 [363.2, 537.2] | 201 | 419.0 [369.0, 475.6] |
| **Poliovirus Type 1** | % with titers ≥8 | 114/114 | 100.0 [96.8, 100.0] | 95/95 | 100.0 [96.2, 100.0] | 209/209 | 100.0 [98.3, 100] |
| | GMT | | 214.0 [164.9, 277.7] | | 257.9 [193.8, 343.1] | | 232.9 [192.4, 282.0] |
| **Poliovirus Type 2** | % with titers ≥8 | 106/106 | 100.0 [96.6, 100.0] | 89/89 | 100.0 [95.9, 100.0] | 195/195 | 100.0 [98.1, 100] |
| | GMT (dil) | | 385.2 [288.2, 514.9] | | 400.6 [290.6, 552.3] | | 392.2 [316.8, 485.5] |
| **Poliovirus Type 3** | % with titers ≥8 | 90/90 | 100.0 [96.0, 100.0] | 74/74 | 100.0 [95.1, 100.0] | 164/164 | 100.0 [97.8, 100.0] |
| | GMT (dil) | | 502.2 [370.2, 681.4] | | 405.1 [284.9, 576.0] | | 455.8 [362.6, 573.1] |

CI: confidence interval; CRM: *Corynebacterium diphtheriae* $CRM_{197}$; dil: dilution; EU: ELISA units FHA: filamentous haemagglutinin; FIM-= fimbriae; GMC: geometric mean concentration; GMT: geometric mean titer; HBsAg: hepatitis B surface antigen; IU: international units; MCC: meningococcus group C conjugate; n: number of participants included in the analysis; p: number of participants with the response; PRN: pertactin; PRP: polyribosylribitol phosphate; PT: pertussis toxin; TT: tetanus toxoid.

[2] Pertussis seroresponse was defined as: (1) if the pre-vaccination antibody concentration was < lower limit of quantification (LLOQ), then the post-vaccination antibody concentration was to be ≥ LLOQ; (2) if the prevaccination antibody concentration was ≥ LLOQ, then the post-vaccination antibody concentration was to be ≥ pre-immunization levels.

## Table 4

SPRs and GMTs for Hib and meningococcus group C before and 1 month after the Hib-MCC vaccine booster at 12 months, per protocol analysis, booster phase (N=222).[a]

| | | MCC-TT (N=111) | | MCC-CRM (N=111) | | Total (N=222) | | |
|---|---|---|---|---|---|---|---|---|
| | | Observed response | | Observed response | | Observed response | | |
| **Antigen** | **Endpoint** | **p/n** | **[95% CI]** | **p/n** | **[95% CI]** | **p/n** | **[95% CI]** | ***P* value** |
| **Hib (PRP)** | **Pre-Hib-MCC vaccine** | | | | | | | |
| | % with conc ≥0.15 µg/mL | 77/82 | 93.9 [86.3, 98.0] | 83/87 | 95.4 [88.6, 98.7] | 160/169 | 94.7 [90.1, 97.5] | |
| | % with conc ≥1.0 µg/mL | 45/82 | 54.9 [43.5, 65.9] | 49/87 | 56.3 [45.3, 66.9] | 94/169 | 55.6 [47.8, 63.2] | |
| | GMC (µg/mL) | | 1.09 [0.81, 1.45] | | 1.18 [0.90, 1.55] | | 1.14 [0.93, 1.38] | |
| | **Post-Hib-MCC vaccine** | | | | | | | |
| | % with conc ≥0.15 µg/mL | 110/110 | 100.0 [96.7, 100.0] | 106/106 | 100.0 [96.6, 100.0] | 216/216 | 100 [98.3, 100] | |
| | % with conc ≥1.0 µg/mL | 109/110 | 99.1 [95.0, 100] | 106/106 | 100.0 [96.6, 100.0] | 215/216 | 99.5 [97.4, 100.0] | |
| | GMC (µg/mL) | | 100.19 [81.05, 123.86] | | 121.00 [101.11, 144.80] | | 109.91 [95.66, 126.28] | |
| **MCC** | **Pre-Hib-MCC vaccine** | | | | | | | |
| | % with titer ≥8 dil | 74/89 | 83.1 [73.7, 90.2] | 38/94 | 40.4 [30.4, 51.0] | 112/183 | 61.2 [53.7, 68.3] | <0.001* |
| | % with titer ≥128 dil | 36/89 | 40.4 [30.2, 51.4] | 15/94 | 16.0 [9.2, 25.0] | 51/183 | 27.9 [21.5, 35.0] | <0.001* |
| | GMT (dil) | | 50.3 [34.4, 73.4] | | 8.7 [5.9, 12.9] | | 20.5 [15.2, 27.5] | <0.001** |
| | **Post-Hib-MCC vaccine** | | | | | | | |
| | % with titer ≥8 dil | 109/109 | 100 [96.7, 100] | 107/110 | 97.3 [92.2, 99.4] | 216/219 | 98.6 [96.0, 99.7] | NS* |
| | % with titer ≥128 dil | 108/109 | 99.1 [95.0, 100] | 105/110 | 95.5 [89.7, 98.5] | 213/219 | 97.3 [94.1, 99.0] | NS* |
| | GMT (dil) | | 3257.9 [2597.4, 4086.3] | | 580.8 [432.7, 779.5] | | 1370.1 [1102.4, 1702.9] | <0.001** |

CI: confidence interval; CRM: *Corynebacterium diphtheriae* $CRM_{197}$; dil: dilution; GMC: geometric mean concentration; GMT: geometric mean titer; Hib: *Haemophilus influenzae* type b; MCC: meningococcus group C conjugate; n: number of participants included in the analysis; NS: not significant; p: number of participants with the response; PRP: polyribosylribitol phosphate; SPR: seroprotection rate; TT: tetanus toxoid.

[a]Prevaccination values were obtained prior to vaccination on the same day that the vaccine was administered.

**P* value: Fisher exact test. ***P* value: Student *t* test on log-transformed data.

**Table 5**

Safety data collected during the primary phase of the study (Day 1 through 15 days after last vaccination), safety set (N=284).

| | Total (N=284) | |
|---|---|---|
| | **n** | **(%)** |
| Number (%) of participants: | | |
| With no AE | 6 | (2.1) |
| With ≥1 AEs | 278 | (97.9) |
| ≥1 vaccine-related AE | 277 | (97.5) |
| ISR (day 1 to day 15) | 253 | (89.1) |
| ISR at DTaP5-HB-IPV-HIB site (day 1 to day 15) | 250 | (88.0) |
| Solicited ISR (day 1 to day 5) | 250 | (88.0) |
| Injection site erythema | 193 | (68.0) |
| Injection site pain | 184 | (64.8) |
| Injection site swelling | 140 | (49.3) |
| Unsolicited ISR (day 1 to day 15) | 25 | (8.8) |
| ISR at MCC site (day 1 to day 15) | 197 | (69.4) |
| Solicited ISR (day 1 to day 5) | 196 | (69.0) |
| Injection site erythema | 145 | (51.1) |
| Injection site pain | 124 | (43.7) |
| Injection site swelling | 91 | (32.0) |
| Unsolicited ISR (day 1 to day 15) | 8 | (2.8) |
| Systemic AE (day 1 to day 15) | 274 | (96.5) |
| Solicited systemic AE (day 1 to day 5) | 270 | (95.1) |
| Unsolicited systemic AE (day 1 to day 15) | 128 | (45.1) |
| Vaccine-related systemic AE[a] | 272 | (95.8) |
| Solicited systemic AE (day 1 to day 5) | 270 | (95.1) |
| Crying | 236 | (83.1) |
| Decreased appetite | 181 | (63.7) |
| Irritability | 240 | (84.5) |
| Pyrexia | 31 | (10.9) |
| Somnolence | 226 | (79.6) |
| Vomiting | 126 | (44.4) |
| Unsolicited systemic AE (day 1 to day 15) | 75 | (26.4) |
| SAE (day 1 to day 15) | 10 | (3.5) |
| Vaccine-related SAE | 1 | (0.4) |
| Death | 0 | (0.0) |
| Withdrawn due to AE[b] | 0 | (0.0) |
| Withdrawn due to vaccine-related SAE[a,b] | 0 | (0.0) |

(S)AE: (serious) adverse event; ISR: injection site reaction; MCC: meningococcus group C conjugate; N: number vaccinated.
[a]Determined by the investigator to be related to the vaccine.
[b]Study medication withdrawn.

## Supplemental Methods

### Micrometabolic Inhibition Tests for Antibodies to Diphtheria

Serial dilutions of human sera were mixed with diphtheria challenge toxin and incubated with Vero cells that were sensitive to the toxin. Neutralizing antibodies specific to diphtheria toxin contained in the serum samples bound to and neutralized the toxin. The neutralized toxin did not affect cellular viability, therefore, the cultured cells continued to metabolize and release carbon dioxide ($CO_2$), reducing the potential of hydrogen (pH) of the culture medium. Cell survival correlated with the change in the colour of the pH indicator (phenol red to yellow at pH 7.0) contained in the medium. In the absence of neutralizing antibodies, the challenge toxin reduced cellular metabolism and $CO_2$ production, therefore, the pH did not decrease and a colour change was not detected.

Results were reported in international unit (IU)/mL by inclusion of the World Health Organization (WHO) International Standard for Diphtheria Antitoxin in the assay. The lower limit of quantitation (LLOQ) was 0.005 IU/mL.

### Micrometabolic Inhibition Tests for Antibodies to Poliovirus

Serial dilutions of sera were mixed with challenge poliovirus and incubated with cultured Vero cells that were sensitive to poliovirus. Specific neutralizing antibodies contained in the sera bound to and neutralized the challenge poliovirus. The neutralized poliovirus did not affect cellular viability, and these cells continued to metabolize and release $CO_2$, reducing the pH of the culture medium. Cell survival correlated with the change in the pH indicator (phenol red to yellow at pH 7.0) contained in the medium. In the absence of neutralizing antibodies, the challenge poliovirus reduced cellular metabolism and $CO_2$ production; therefore, the pH did not decrease and a colour change was not detected. The poliovirus micrometabolic inhibition test measured the functional serum antibody response to poliovirus by utilizing Vero cells (African green monkey kidney cells) and wild type poliovirus strains 1, 2, and 3 (Mahoney, MEF-1, and Saukett, respectively) as the challenge virus. The Kärber method* was used to determine the serum dilution that neutralized 50% of the challenge virus.

Results were expressed as titers (1:dil). The LLOQ for polio is 4 and the upper limit of quantitation (ULOQ) is 65536 (1:dil).

### Enzyme-linked Immunosorbent Assays for Antibodies to Pertussis

Purified pertussis antigen (PT, FHA, PRN or FIM-2,3) was adsorbed to the wells of a microtiter plate. Diluted serum samples (test samples, reference standards and quality-control samples) were incubated in the wells. Specific pertussis antibodies in the serum samples bound to the immobilized pertussis antigen to form antigen-antibody complexes. Unbound antibodies were washed from the wells, and enzyme-conjugated anti-human immunoglobulin G was added. The enzyme conjugate bound to the antigen-antibody complex. Excess conjugate was washed away and a specific colorimetric substrate was added. Bound enzyme catalysed a hydrolytic reaction causing colour development. The intensity of the generated colour was proportional to the amount of specific antibody bound to the wells. The results were read on a spectrophotometer (ELISA plate reader). A reference standard serum assayed on each plate was used to calculate the amount of specific PT, FHA, PRN, or FIM antibody in the test samples in ELISA unit (EU)/mL by comparison to the reference standard curves.

The LLOQ for PT, PRN, and FIM was 4 EU/mL and for FHA was 3 EU/mL.

### Enzyme-linked Immunosorbent Assays for Antibodies to Tetanus

Purified tetanus antigen was adsorbed to the wells of a microtiter plate. Diluted serum samples (test samples, reference standard, and quality-control samples) were incubated in the wells. Specific antibodies in the serum samples bound to the immobilized antigen. Unbound antibodies were washed from the wells and enzyme-conjugated anti-human immunoglobulin G was added. The enzyme conjugate bound to the antigen-antibody complex. Excess conjugate was washed away and a specific colorimetric substrate was added. Bound enzyme catalysed a hydrolytic reaction which caused colour development. The intensity of the generated colour was proportional to the amount of specific antibody bound to the wells. The results were read on a spectrophotometer (ELISA plate reader). A reference standard assayed on each plate, WHO human standard lot TE3, was used to calculate the

amount of specific anti-tetanus antibody in the units assigned by the reference standard (IU/mL of serum).

The LLOQ for tetanus is 0.01 IU/mL.

*Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. *World J Virol* 2016;5(2):85–6.

**Conflicts of Interest:**

**Jennifer L Oliver** received a grant for manuscript preparation from Sanofi Pasteur MSD

**Christine Sadorge:** was an employee of Sanofi Pasteur MSD at the time the study was conducted.

**Florence Boisnard** was an employee of Sanofi Pasteur MSD at the time the study was conducted.

**Matthew D Snape** reports a grant for conducting this study from Sanofi-Pasteur; and grants for conducting other studies from GlaxoSmithKline, Janssen, Medimmune, Novavax, MCM, and Pfizer.

**Richard Tomlinson** reports that his institution received grants for conducting this study from Sanofi Pasteur MSD

**Rebecca Mann** reports that her institution received grants for conducting this study from Sanofi Pasteur MSD

**Peter Rudd** reports that his institution received grants for conducting this study from Sanofi Pasteur MSD

**Shyam Bhakthavalsala** reports that his institution received grants for conducting this study from Sanofi Pasteur MSD

**Saul Faust** reports that his institution received grants for conducting this study from Sanofi Pasteur MSD and for conducting other studies from Pfizer, Sanofi, GSK, Novartis, Alios, J&J, and Merck; and his institution received fees for participating as a symposium speaker from Pfizer, and for advisory board participation from AstraZeneca/MedImmune, Sanofi, Pfizer, Sequerius, Sandoz, and Merck.

**Paul Heath** reports that his institution received a grant for conducting this study from Sanofi Pasteur MSD and grants for conducting other studies from GlaxoSmithKline, Janssen, Medimmune, Novavax, and Pfizer.

**Stephen Hughes** has received fees for advisory board participation from Sanofi

**Ray Borrow** performs contract research on behalf of Public Health England for GSK, Pfizer and Sanofi Pasteur.

**Stephane Thomas** was an employee of Sanofi Pasteur MSD at the time the study was conducted.

**Adam Finn** reports that his institution received grants for conducting this study from Sanofi Pasteur MSD and for conducting other studies from GSK.

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