

**Persistence of bactericidal antibodies following early infant immunisation with a serogroup B meningococcal vaccine and pre-school booster dose immunogenicity**

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## **ABSTRACT**

### **BACKGROUND**

There are currently no data on the persistence of bactericidal antibodies induced by infant immunisation with 4CMenB, the serogroup B meningococcal vaccine recently licensed for use in Europe. The objective of this study was to evaluate serogroup B specific bactericidal antibodies in 40 month old children previously immunized at 2, 4, 6 and 12 months of age.

### **METHODS**

Participants given four infant doses of 4CMenB received a fifth dose of vaccine at 40 months of age. MenB vaccine-naïve participants received 4CMenB at 40 and 42 months (control group). hSBA titres were evaluated at 40 months (baseline) and 1 month after each dose of 4CMenB.

### **FINDINGS**

Prior to a booster dose at 40 months of age 41–76% of 17 participants previously immunized with 4CMenB in infancy has serum bactericidal assay titres  $\geq 4$  against four reference strains. Prior to vaccination in the control group (n= 40) these proportions were similar for strains 44/76-SL (63%) and M10713 (68%) but low for strains NZ98/254 (0%) and 5/99 (3%). A booster dose in the 4CMenB primed participants generated greater increases in hSBA titres than in controls.

### **INTERPRETATION**

As has been observed with other meningococcal vaccines bactericidal antibodies wane following immunisation with 4CMenB administered according to an approved infant immunisation schedule of 2, 4, 6 and 12 months of age, but there was an anamnestic response to a booster dose at 40 months of age. If 4CMenB was introduced into routine immunisation schedules, assessment of the need for a booster dose will require data on the impact of these declining titres on vaccine effectiveness.

## FUNDING

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**INTRODUCTION**

A vaccine against serogroup B meningococcus has recently been licensed for use in Europe and is being considered for licensure in Canada<sup>1</sup>. This vaccine, known as 4CMenB, consists of three recombinant proteins: factor H binding protein (fHbp), neisseria adhesin A (NadA) and neisseria heparin binding antigen (NHBA) combined with detoxified outer membrane vesicles from the strain responsible for an epidemic of serogroup B meningococcal disease in New Zealand (NZ98/254). Clinical trials of 4CMenB have shown it to be immunogenic against reference strains selected to specifically express one of the vaccine antigens<sup>2-6</sup>. On the basis of these trials the approved schedule for infants aged 2 to 5 months is 3 doses given at least one month apart with a booster dose given at 12 to 23 months of age<sup>7</sup>. The persistence of vaccine induced antibodies through childhood following this booster dose is unknown, but is particularly relevant as the incidence of invasive serogroup B meningococcal disease in children aged one to four years is second only to the incidence under one year of age.<sup>8</sup>

This study assessed the persistence of these bactericidal antibodies in 40–44 month-old children who had previously received either 4CMenB or a vaccine containing the recombinant proteins alone (rMenB) at either two, four, six, and twelve months of age or just at twelve months of age.<sup>3</sup> The immunogenicity and reactogenicity of a booster dose given at 40 months of age were also assessed, and 4CMenB vaccine naïve 40 month old children were enrolled to study the immunogenicity and reactogenicity of two doses of 4CMenB given two months apart, as *per* the approved schedule for children two to ten years of age.

## **Methods**

### *Participants*

Participants in the original phase II study were recruited at two sites: 12 at Gloucester Vaccine Evaluation Unit and 135 at the Oxford Vaccine Group, University of Oxford. In the original study children were randomised 2:2:1:1 to receive: 4CMenB at two, four, six and twelve months, rMenB at two, four, six and twelve months, 4CMenB at twelve months or rMenB at twelve months (see figure 1). All 126 participants completing the original study at the Oxford site were invited to take part in this follow-on study, and it was intended to recruit 50 vaccine naïve, age-matched participants as controls. Inclusion criteria were healthy children aged 40–44 months who had completed the original study, or for controls, children at this age who had not previously received a serogroup B meningococcal vaccine. Exclusion criteria were previous meningococcal disease (or household/intimate contact with anyone with meningococcal disease), allergy to vaccine components, severe acute or chronic disease, immune dysfunction, receipt of blood products, planned receipt of non-study vaccines within 30 days of the study vaccines, enrolment in another clinical trial, recent antibiotic use, being a family member of research staff or antipyretic use within 6 hours before enrolment. Written informed consent was obtained from participant's parents or legal guardians. Ethical approval was obtained from Oxfordshire Research Ethics Committee B (09/H0605/89). The study was conducted from January to December 2010.

### *Procedures*

Participants who had previously received four doses of 4CMenB or rMenB had blood samples taken before and 30 days after a booster dose of the respective vaccines (Figure 1). Participants previously given a single dose of 4CMenB or rMenB in the original study had two doses of the

respective vaccine administered approximately 60 days apart; control participants received two doses of 4CMenB. These participants had blood samples taken at enrolment and 30 days after each immunisation.

Both vaccines were manufactured and supplied by Novartis Vaccine and Diagnostics and contained 50 µg each of NadA allele 3, NHBA-GNA1030 fusion protein (containing NHBA peptide variant 2) and GNA2091-fHbp fusion protein (containing fHbp variant 1.1) as well as 1.5mg aluminium hydroxide and 10mM histidine. 4CMenB also contained 25µg of detoxified outer membrane vesicles from *N. meningitidis* strain NZ 98/254 (expressing the immunodominant antigen PorA serosubtype P1.4). The vaccines were 0.5ml in volume and were administered by intramuscular injection into the deltoid area of the non-dominant arm. Participants also received a combination diphtheria-toxoid, tetanus-toxoid, acellular pertussis and inactivated polio vaccine (Repevax, Sanofi Pasteur MSD, Maidenhead, UK) and a combination measles, mumps and rubella vaccine (MMRVaxPro, Sanofi Pasteur-MSD) at the final study visit; these vaccines were administered to keep participants immunised according to the United Kingdom routine schedule and did not form part of the study evaluation.

#### *Immunogenicity evaluation*

Sera were analysed at the Vaccine Evaluation Unit, Health Protection Agency, Manchester UK for serum bactericidal activity using a human complement source as previously described.<sup>9</sup> Laboratory staff were blinded to participant group. The correlate of protection was a serum bactericidal antibody titre  $\geq 1:4$ . As previously described,<sup>3</sup> reference serum bactericidal antibody strains were used to assess the immunogenicity of specific vaccine components: fHbp was assessed by strain 44/76-SL, NadA by strain 5/99 and PorA (the immunodominant antigen in the outer membrane vesicle) by NZ98/254 (Table 1). A novel strain (M10713) was used to

assess the immunogenicity of NHBA as this expresses NHBA cross-reactive to that contained in the vaccine (peptide 10) but is mismatched for the vaccine components fHbp (subvariant 2.24) and PorA (16-3) and does not contain the gene for NadA. Therefore, in accordance with the principle underlying the use of the existing reference strains, any increase in serum bactericidal antibody titres against M10713 following immunisation may be attributed to the NHBA component of the vaccine. Immunogenicity against additional strains (M00 242922, M01 240101, M01 240364) was assessed to evaluate the impact of antigenic variation and expression on susceptibility to vaccine-induced antibodies (Table 1). Strain M01-240355 was specifically selected as a strain likely to be relatively resistant to vaccine-induced bactericidal antibodies as it was mismatched for all vaccine antigens.

#### *Reactogenicity evaluation*

Each day for the week after immunisation parents recorded their child's axillary temperature, local reactions at the site of immunisation (pain, erythema, swelling and induration), solicited systemic reactions (fever, irritability, vomiting, diarrhoea, sleepiness, anorexia arthralgia, headache and rash) and the use of analgesic and antipyretic medication. reactions were classified as severe if tenderness prevented daily activity, local erythema and induration were >50 mm diameter or the child's post immunisation fever was  $\geq 40^{\circ}\text{C}$ .

*Outcome measures* The primary immunogenicity objective was to assess the persistence of bactericidal antibodies at 40 months of age in children who previously received four doses of rMenB or 4CMenB at two, four, six and twelve months of age. The primary safety objective was to assess the safety and tolerability of one or two booster doses of these vaccines administered at 40 months of age.

Secondary objectives were assessment of the persistence of bactericidal antibodies following immunisation with one dose of 4CMenB or rMenB at 12 months of age, increases in serum bactericidal antibody titres following dose(s) of these vaccines at 40 months of age and the bactericidal antibody response and adverse reactions following a two dose 'catch-up' immunisation schedule of 4CMenB administered at 40 and 42 months of age.

### *Statistical analysis*

The percentages of children in each study group with serum bactericidal antibody titres  $\geq 1:4$  were calculated along with two sided 95% Clopper–Pearson confidence intervals (C.I.). Similarly, serum bactericidal antibody geometric mean titres were calculated and 95% C.I. determined by exponentiating (base 10) the corresponding means and 95% C.I. of the log<sub>10</sub> serum bactericidal antibody titre. Geometric mean ratios of post-immunisation serum bactericidal antibody geometric mean titres to baseline (40 months) and 95% C.I. were also calculated. Serum bactericidal antibody titres below the lower limit of detection of two were allocated a value of one for these calculations.

The primary population for immunogenicity analysis was the intention to treat analysis, consisting of all participants who were randomised and received a dose of either vaccine and provided at least one evaluable serum sample. Safety analysis was conducted on a population consisting of all participants who received a dose of either vaccine and provided post-baseline safety data.

The sample size for the follow-on participants was determined by the number of participants completing the original study at the Oxford site. All comparisons were descriptive, however it was calculated that if the percentage of participants with serum bactericidal antibody titres  $\geq$



1:4 was 40% in a follow-on group and 5% in the naïve group a sample size of 30 participants in the follow-on group and 50 participants in the control group would be 95% powered to show superiority.

## **Results**

Of the 126 participants completing the original study at the Oxford site 70 were enrolled into this follow-on study, of whom 68 were included in the intention to treat analysis. In addition 43 MenB vaccine naïve participants were enrolled, of whom 40 were included in the intention to treat analysis (Table 2). The demographic characteristics of the participants were similar across all groups. The median age at enrolment was 41 months (range 40 to 44), 50% were male (compared with 57% in the original study) and 92% of participants were Caucasian (compared with 94% in the original study).

### *Immunogenicity results: Primary Objective.*

Waning of antibodies was observed by 40 months of age following vaccination in early infancy with either vaccine. At least 65% of participants in the 4CMenB246-12 group had serum bactericidal antibody titres  $\geq 1:4$  at 40 months of age for strains 44/76-SL (evaluating fHbp), 5/99 (NadA) and M10713 (NHBA) compared with 41% for NZ98/254 (PorA) (Table 3 and Supplementary Figure 1). Among participants receiving rMenB in the same schedule (rMenB246-12), at least 43% showed serum bactericidal antibody titres  $\geq 1:4$  for these strains except for NZ98/254 (PorA) (3%). As expected, the percentage of participants with serum bactericidal antibody titres  $\geq 1:4$  for strain M01-240355 was low regardless of vaccine received.

### *Immunogenicity results: Secondary Objectives*

By 40 months of age 25% and 38% of children receiving 4CMenB at 12 months (4CMenB-12) had serum bactericidal antibody titres  $\geq 1:4$  for strain M10713 (NHBA) & 44/76-SL (fHbp) respectively, whereas for strains 5/99 (NadA) and NZ98/254 (PorA) these proportions were 0% (Table 3 and Supplementary Figure 1).

Administration of a booster dose of 4CMenB at 40 months of age resulted in 86-100% of 4CMenB primed participants achieving serum bactericidal antibody titres  $\geq 1:4$  for all strains except M01-240355.

In serogroup B meningococcal vaccine-naïve children (Control Group) administration of two doses of 4CMenB two months apart resulted in 89-100% achieving serum bactericidal antibody titres  $\geq 1:4$  for all strains except M01-240355 (69%). More than half of these children had serum bactericidal antibody titres  $>1:4$  to strains 44/76-SL (fHbp), M10713 (NHBA), M01-240101 and M01-240364 at 40 months, with serum bactericidal antibody geometric mean titres similar to those in 4CMenB246-12. The geometric rise in serum bactericidal antibody titres for all these strains following a 4CMenB booster dose was higher in the 4CMenB246-12 group than controls, as determined by non-overlapping confidence intervals (Table 4). The serum bactericidal antibody geometric mean titres for all timepoints in this and the original study are displayed in Supplementary Figure 2.

### *Reactogenicity Results*

The majority of recipients of both rMenB and 4CMenB developed local pain and erythema at the injection site, but few reactions were severe (Supplementary Figures 3 and 4). Few participants reported fever.

One serious adverse event was experienced by a control group participant who developed otitis media 12 days after vaccination with 4CMenB followed by cervical lymphadenitis, requiring hospitalisation for IV antibiotics. This was considered unrelated to vaccination.

## **Discussion**

4CMenB was licensed for use in Europe in January 2013, with approved dosage schedules including the 2, 4, 6, 12 month and 40, 42 month schedules evaluated in this study. In contrast to the single dose of 4CMenB given at 12 months of age to study children in the 4CMenB12 group, the licensed indication for children 1 to 2 years of age is 2 doses of 4CMenB at least 2 months apart, with a booster dose given 1 to 2 years later.

Of particular interest is the waning of antibodies observed in children immunised with the 2, 4, 6 and 12 month schedule, which could potentially be used for routine immunisation with this vaccine. Experience with other meningococcal vaccines has shown that waning of bactericidal antibody titres was associated with a decline in vaccine effectiveness following infant immunisation with serogroup C meningococcal conjugate vaccines<sup>10</sup>, adolescent immunisation with an investigational outer membrane vesicle vaccine in Norway<sup>11</sup> and infant immunisation with the New Zealand outer membrane vesicle vaccine.<sup>12 13</sup> Predicting the potential impact of a decline in bactericidal antibodies on vaccine effectiveness following immunisation with 4CMenB is less straightforward. The serogroup C meningococcal conjugate and outer membrane vesicle vaccines generate an immune response primarily directed against a single antigen (the capsular polysaccharide and PorA, respectively), whereas 4CMenB is a multicomponent vaccine which aims to induce antibodies against each component. The immunogenicity of 4CMenB antigens can therefore only be assessed by use of multiple meningococcal strains, and there is considerable inter-strain variation in the rate

of decline of serum bactericidal antibody titres. It is unclear whether this reflects true differences in the persistence efficacy for each vaccine component, or if this simply reflects different susceptibilities of the strains to killing in the serum bactericidal antibody assay. However, it does raise the possibility that post-immunisation susceptibility to meningococcal infection could develop at different rates for different strains, depending on whether they are expressing proteins recognised by ‘persistent’ or ‘waning’ antibodies. It is also notable that the bactericidal antibody concentrations against strains 44/76-SL (fHbp) and M10713 (NHBA) were similar in children previously immunised with 4CMenB and those who were vaccine naïve. Although this may suggest the acquisition of natural immunity against these strains (evident in over two-thirds of the vaccine-naïve cohort), these children had a relatively poor increase in bactericidal antibodies following a single dose of 4CMenB when compared to the vaccine-primed children. This suggests previous immunisation with 4CMenB has resulted in effective ‘priming’ of vaccine recipient’s immune systems, but whether this means they have any greater protection against serogroup B meningococcal disease than ‘unprimed’ children with the same serum bactericidal titres remains to be seen.

A related issue is the proportion of serogroup B meningococcal strains likely to be prevented by immunisation with 4CMenB. Since the use of serum bactericidal antibody assay for a very large panel of strains is not practical for technical reasons, attempts to predict coverage of meningococcal vaccines in development using surrogate assays more suitable for high throughput and standardisation have been used. One example of this is the Meningococcal Antigen Typing System developed by Novartis Vaccines.<sup>14</sup> The latter assay evaluates a representative panel of meningococcal strains to correlate the strength of binding (‘relative potency’) of vaccine antigen-specific IgG to the presence or absence of strain-specific bactericidal activity on the serum bactericidal antibody assay (performed using pooled post-

immunisation serum obtained at 13 months of age). Regional reference laboratories can then calculate the percentage of local invasive strains where the relative potency is above this 'positive bactericidal threshold' for one or more of fHbp, NadA or NHBA (or where the PorA variant is homologous to the outer membrane vesicle component of 4CMenB). Using this technique the Meningococcal Antigen Typing System predicts vaccine 'coverage' of 78% of invasive MenB strains in Europe,<sup>15</sup> 76% in Australia<sup>16</sup> and 66% in Canada.<sup>17</sup> Provisional data from the African meningitis belt predict 100% coverage for nine serogroup X meningococcal strains<sup>18</sup>. The demonstration of waning bactericidal antibodies in this study suggests that pooled sera obtained two to three years after immunisation would kill a smaller proportion of strains in the assay than that obtained at thirteen months (one month after immunisation).

The above issues address the generation of direct protection by immunisation with 4CMenB, however the impact of this vaccine will be determined at least as much by the presence or absence of herd immunity, which has been especially important for other meningococcal vaccines to ensure sustained protection in the face of waning immunity. Studies are currently underway to see if 4CMenB is likely to influence nasopharyngeal carriage and hence induce herd immunity by reducing circulation of the organism (NCT01214850); if not the maintenance of adequate levels of bactericidal antibodies to provide direct protection may be even more critical than for conjugate vaccines. If immunisation with 4CMenB does influence nasopharyngeal carriage, then it will be important to monitor the population of 'carried' strains to assess for the potential emergence of strains either lacking the vaccine antigens or expressing these at low levels, as this could herald the emergence of meningococci less susceptible to prevention by 4CMenB.

### *Limitations*

There were a number of limitations in this study, including the relatively small numbers of participants and the fact that 45% of participants from the original study who were eligible to take part in this follow-on study did not do so. There is a potential for bias, in that children who tolerated the vaccine better in the original study may have been more likely to participate in this follow-on study. Furthermore, the control group under-recruited. Follow on studies are currently being conducted from the larger phase IIb and III studies (NCT00944034, NCT00847145) and will provide additional data on antibody persistence and tolerability. There is as yet no information on persistence after mid-childhood booster doses; these data will become available soon as children from this study will also be revisited when they reach five years of age (NCT01027351).

### *Conclusions*

In conclusion, consistent with other vaccines against meningococcal disease a waning of serum bactericidal antibody titres is observed after infant immunisation with 4CMenB. A booster dose in pre-school years is well tolerated. If 4CMenB were to be introduced into a routine immunisation schedule, measures such as adequate disease surveillance would be important to determine whether waning of antibodies might influence the effectiveness of an immunisation campaign against this bacterium.

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#### Author's contributions

MDS and PS prepared the first draft of the text of this article, and revised it in response to comments from all co-authors. PS prepared graphs and the majority of figures. The study protocol was prepared by PD and DT in collaboration with MDS, AJP and TJ. The study organisation, conduct and oversight was performed by TJ, HR, SK, NG, MDS and AJP (chief investigator). Data analysis was performed by HJ and independently verified by LMY. All authors approved the final version of the manuscript for publication.

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#### Conflict of Interest

MDS and AJP have conducted clinical trials on behalf of the University of Oxford, sponsored by Novartis Vaccines and Diagnostics, Pfizer, GlaxoSmithKline and Sanofi-Pasteur MSD. AJP and MDS do not receive personal payments from vaccine manufacturers; grants for support of educational activities are paid to an educational/ administrative fund held by the

Department of Paediatrics, University of Oxford. MDS and TMJ have received assistance to attend scientific meetings from Novartis Vaccines, Pfizer and GlaxoSmithKline. MDS has spent a period of secondment at Novartis Vaccines. HJ, PMD and DT are employees of Novartis Vaccines and Diagnostics. HR, PS, SK, NG and LY have no interests to declare. AJP chairs the European Medicines Agency's Scientific Advisory Group on Vaccines and is a member of the UK Department of Health's Joint Committee on Vaccination and Immunisation Meningococcal subcommittee.





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