

A phase III observer-blind randomized, controlled study to evaluate the immune response and the correlation with nasopharyngeal carriage after immunization of university students with a quadrivalent meningococcal ACWY glycoconjugate or serogroup B meningococcal vaccine



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ABSTRACT

Background: University students have high rates of pharyngeal carriage of *Neisseria meningitidis*. Interruption of carriage acquisition is an important mechanism of vaccines for inducing herd protection. 4CMenB and MenACWY-CRM vaccines have been shown to be immunogenic against meningococcal serogroups B and ACWY respectively in younger age groups, and also to elicit a modest impact on meningococcal carriage in vaccinated students. However, vaccine responses in university students and the impact of serum bactericidal antibody (SBA) titers on meningococcal carriage are undetermined.

Methods: Immunogenicity of two 4CMenB doses or one MenACWY-CRM dose was measured in university students at Months 2, 4, 6 and 12 post-first vaccination. Immunogenicity of one MenACWY-CRM dose in students with previous meningococcal serogroup C conjugate vaccination was also assessed. Immune responses were measured with an SBA assay using human complement (hSBA) against three reference strains for serogroup B and against one strain for each for serogroups C and Y. Correlations between hSBA titers and meningococcal carriage were analyzed.

Results: All subjects demonstrated robust functional antibody responses to both vaccines at Month 2 and a high proportion maintained protective hSBA titers up to Month 12. At baseline, carriage of disease-associated serogroup B strains and serogroups C and Y were higher in subjects with already-protective hSBA titers. Post-vaccination, while both 4CMenB and MenACWY-CRM elicited robust immunogenicity in students, significant correlations between post-vaccination hSBA titers and carriage of disease-associated serogroups were not observed.

Conclusions: 4CMenB and MenACWY-CRM were both highly immunogenic. There was no correlation between carriage and post-vaccination hSBA titers.

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1. Introduction

Invasive meningococcal disease (IMD) is most common in infants and children, but there is a second peak of incidence in young adults – particularly those in higher education [1,2] – who have relatively high case fatality rates [3,4] and morbidity [5].

While IMD is generally rare (<1 per 100,000 cases per annum in Europe [6,7]), rates of asymptomatic nasopharyngeal carriage of *Neisseria meningitidis* by healthy students living in close quarters are commonly up to 35% [8–12]. This can lead to isolated outbreaks of meningococcal disease, as have recently occurred in US universities [13,14].

In Europe, America and Asia, most IMD cases are due to serogroups B, C, Y [4] and increasingly W [15]. Capsular polysaccharide–protein conjugate vaccines (e.g. against serogroups A, C, W and Y) provide direct protection to vaccinated individuals against IMD. They also have the added effect of reducing acquisition of new carriage to induce herd protection [16–18], although the mechanism is undetermined.

Of the vaccines currently available, MenACWY-CRM (*Menveo*TM, GSK Vaccines, Italy) is a polysaccharide–CRM₁₉₇ conjugate vaccine that has been shown to be immunogenic against serogroups A, C, W and Y, well tolerated in all age groups [19–28], and approved for use in individuals aged 2 months up to at least 55 years. Another vaccine against serogroup B strains (4CMenB; *Bexsero*TM, GSK Vaccines, Italy) is approved in the European Union for use in individuals over 2 months of age and has recently been deployed in the UK infant schedule. 4CMenB includes factor H binding protein (fHbp), *Neisseria* adhesin A (NadA), and Neisserial Heparin Binding Antigen (NHBA) combined with outer membrane vesicles (OMV) from the New Zealand outbreak strain NZ98/254 [19]. 4CMenB is immunogenic with an acceptable safety profile [29] and predicted to be protective against a substantial proportion of serogroup B strains currently circulating worldwide [20,21].

Recently, we reported that vaccination of students with MenACWY-CRM and 4CMenB reduced overall meningococcal carriage over one-year follow-up. Specifically, 4CMenB vaccination was estimated to result in a 27% reduction in carriage of capsular groups BCWY; and MenACWY-CRM was estimated to reduce carriage of serogroups CWY by 36% [24]. Here, we report the immunogenicity endpoints for each vaccine in the same student cohort, including a subset who received a prior dose of a meningococcal serogroup C conjugate vaccine during the UK catch-up vaccination campaigns in the early 2000s [25]. In addition, correlations between serum bactericidal antibody (SBA) titers and carriage of capsular groups BCY in all groups were calculated.

2. Methods

2.1. Subjects

Healthy 18–24 year-old students of both genders were enrolled and randomized 1:1:1 to receive two doses of 4CMenB (4CMenB group), one dose of MenACWY-CRM plus a saline placebo (MenACWY-CRM group), or two doses of a Japanese encephalitis (JE) vaccine (Control group). Inclusion and exclusion criteria have been described previously by Read et al. [24], who reported primary and secondary results of the trial (carriage rates at 1 month and 1 year post-vaccination and safety data). The present report focuses on the secondary immunogenicity outcomes of the same trial registered at ClinicalTrials.gov (NCT01214850). Immunogenicity assessments were planned in the first 200 study participants enrolled in each group at the Sheffield University Hospital site, out of the total of 2968 subjects enrolled in the study.

2.2. Vaccines

Vaccines administered in the study were: 4CMenB, MenACWY-CRM, and a JE vaccine (inactivated, adsorbed; *Ixiaro*, Valneva Austria, lot number JEV09L38A). 4CMenB (lot number 090101V) was supplied as a liquid suspension in a pre-filled, single-dose syringe.

MenACWY-CRM (lot number M10076) was prepared immediately prior to administration by mixing the lyophilized MenA-CRM component with the liquid MenCWY-CRM component. Subjects in the MenACWY-CRM group also received one dose of a saline placebo containing 1.5 mg aluminum hydroxide at a subsequent visit. The JE vaccine was supplied as a liquid suspension in a pre-filled, single-dose syringe.

All vaccines were administered as 0.5 mL doses injected intramuscularly into the deltoid region by designated unblinded staff who did not participate in evaluations.

2.3. Immunogenicity

Blood samples (≤ 20 mL) were obtained immediately prior to the first vaccination (Day 1) and at subsequent visits at Months 2, 4, 6 and 12 post-first injection (Fig. 1).

Immunogenicity of 4CMenB or MenACWY-CRM were assessed by SBA assay using human complement (hSBA) against three reference strains for serogroup B (selected to assess responses specific to vaccine antigens: 44/76-SL for fHbp, 5/99 for NadA, and NZ98/254 for PorA [an immunodominant protein of OMVs]) and against a strain each for serogroups C and Y. Table 1 presents genotypic and phenotypic information regarding the indicator strains for serogroups B, C and Y. Carriage of serogroups A and W was undetectable or low in this study (as expected [30]) and immunogenicity against them was not assessed.

Data for serogroup B were expressed as the percentage of subjects with hSBA titers ≥ 4 (previously defined as a serologic correlate indicative of protection) and hSBA geometric mean titers (GMTs) [26]. For serogroups C and Y, the percentage of subjects with hSBA titers ≥ 8 , hSBA GMTs, and the percentage of subjects with seroresponse at Month 2 (one month post-second injection; Fig. 1) were assessed. Seroresponse was defined as an increase from hSBA titers <4 pre-vaccination to ≥ 8 post-vaccination, or as a ≥ 4 -fold increase for subjects with pre-vaccination hSBA titers ≥ 4 .

Analyses were conducted on the immunogenicity subset of the pre-specified modified intention-to-treat (MITT) population, which included subjects having received a study vaccination, provided at least one evaluable serum sample and had results for at least one serogroup beyond baseline. The subjects and timepoints evaluated varied with the serogroup analyzed. In the 4CMenB group, immunogenicity against serogroup B and Y strains was assessed for all subjects, while immunogenicity against serogroup C was assessed in a random subset of 50 subjects. In the MenACWY-CRM group, immunogenicity was evaluated for all subjects for serogroups C and Y, and in a random subset of 50 subjects for serogroup B strains. In the Control group, a random subset of 50 subjects was generated for immunogenicity assessments against serogroup B and C. Immunogenicity against serogroup Y was assessed for all subjects in each group, as higher rates of carriage were expected.

Immunogenicity of one MenACWY-CRM dose against serogroups C and Y was evaluated in subjects with documented evidence (from a physician) of previous vaccination with meningococcal serogroup C glycoconjugate vaccine.

The effects of 4CMenB and MenACWY-CRM on *N. meningitidis* carriage were recently reported [24] and new acquisition of carriage was defined as the post-vaccination detection of an *N. meningitidis* isolate in a subject who had lacked it at baseline. Similarly, displacement of carriage was defined as the post-vaccination loss of an *N. meningitidis* isolate in a subject. In the present study, correlations between meningococcal carriage (as assessed in [24]) and pre- and post-vaccination hSBA titers were investigated. Accordingly, the percentage of subjects with carriage of potentially virulent serogroup B sequence type strains (identified as those causing disease in the UK in 2006–2010) was evaluated. Subjects

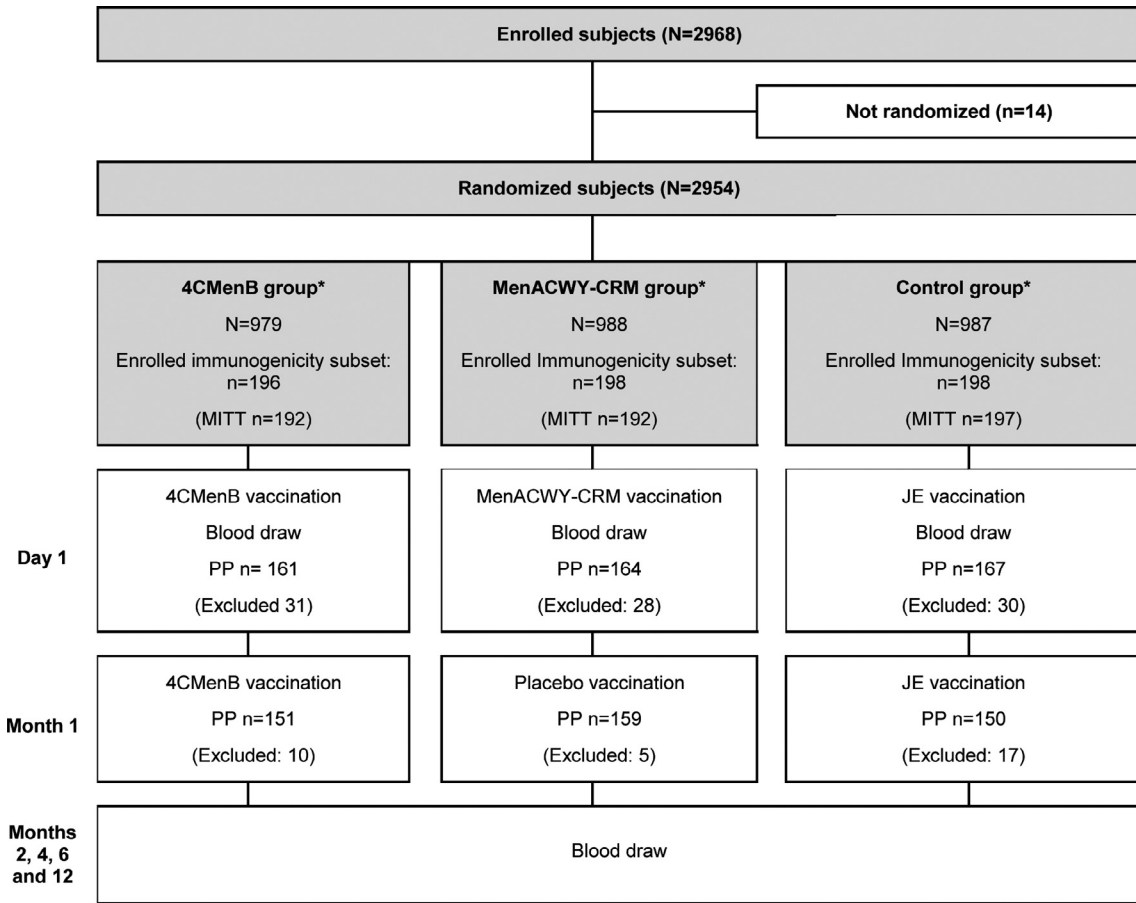


Fig. 1. Subject disposition flowchart (modified intention-to-treat (MITT) immunogenicity subset; per protocol (PP)).

Table 1

4CMenB vaccine antigen genotypic and phenotypic characteristics of serogroups B, C and Y reference strains.

Serogroup (test strain)	Vaccine antigen molecular typing ^a			PorA match	MATS relative potency ^b		
	fHbp	NHBA	NadA		fHbp	NHBA	NadA
Serogroup B							
5/99	2.p0023	20	+	No	<LLOQ	0.179	1.089
44/76-SL	1.p0001	3	–	No	1.006	0.352	<LLOQ
NZ98/254	1.p0014	2	–	Yes	0.044	0.847	<LLOQ
Serogroup C (C11)	2.22	15	+	No	<LLOQ	0.420	0.028
Serogroup Y (860800)	2.23	9	–	No	<LLOQ	0.068	<LLOQ

LLOQ: Lower Limit of Quantitation.

^a The fHbp variant/subvariant, NHBA peptide and presence (“+”) of the *nadA* gene are shown for each strain. The fHbp nomenclature used to describe the different variants and sub-variants of fHbp use a prefix (1, 2, or 3) that defines the variant and a suffix (e.g. 16) that defines the subvariant (or “peptide”) and distinguishes each by as little as a single amino acid change and is consistent with the nomenclature used in the *Neisseria* PubMLST database (<http://pubmlst.org/neisseria/>). The 4CMenB vaccine includes fHbp subvariant 1.1, NHBA subvariant 1.2, NadA subvariant 3.1, and PorA subtype P1.4.

^b Meningococcal Antigen Typing System (MATS) Relative Potency (RP) values are expressed relative to the reference strain for each antigen. The positive bactericidal thresholds are as follows: fHBP = 0.021, NHBA = 0.29 and NadA = 0.009.³¹ Numbers in bold indicate a relative potency above the positive bactericidal threshold. The clinical significance of the MATS RP for the non-serogroup B strains is unknown.

were then stratified by pre-vaccination titers <4 or ≥4 against serogroup B test strains. The percentages of subjects with carriage of serogroups C and Y were also evaluated and stratified by pre-vaccination titers <8 or ≥8 against each serogroup. Carriage rates were assessed as described previously [24].

2.4. Statistical analyses

Analyses were performed on per protocol data. No formal statistical hypotheses were tested and all hSBA results were analyzed descriptively. The percentage of subjects with hSBA titers above the pre-specified thresholds, and with seroresponse against sero-

groups C and Y were calculated by group and timepoint, with associated 95% Clopper-Pearson confidence intervals (CIs). The hSBA GMTs and two-sided 95% CIs were calculated by exponentiating the corresponding log-transformed means (or mean differences from baseline in log-transformed titers) and their 95% CIs for the log-transformed means obtained from a two-way analysis of variance, accounting for vaccine group. Titers below detection limit were set to half the limit.

To explore correlations between hSBA titers following 4CMenB and MenACWY-CRM vaccinations and persistence of carriage of *N. meningitidis*, hSBA results and the percentage of seropositive subjects both with and without pharyngeal carriage for each sero-

group/4CMenB reference strain were calculated with 95% CIs. Chi-square or Fisher's exact tests were performed to assess differences between vaccine groups.

3. Results

3.1. Demographics

A total of 592 subjects were included in this immunogenicity investigation, and 585 subjects meeting entry criteria were included in the MITT population. Exclusions were primarily due to missed vaccinations or blood draw at Month 2. The students' mean age was 20.2 years, 53% of participants were female, and the majority were Caucasian. Demographic characteristics at baseline were well matched across groups (Table 2).

3.2. Immune responses against serogroup B test strains

At baseline, high percentages of subjects across groups had hSBA titers ≥ 4 against all three serogroup B test strains (Fig. 2A): 44/76-SL (fHbp: 62–71%), 5/99 (NadA: 45–60%), and NZ98/254 (PorA: 38–57%). By Month 2, 99–100% subjects in the 4CMenB group had hSBA titers ≥ 4 against all the serogroup B test strains,

and a high proportion of these subjects maintained titers ≥ 4 up to Month 12 (85–97%). In the MenACWY-CRM and Control groups, the percentage of subjects with hSBA titers ≥ 4 against serogroup B test strains remained within the same ranges at baseline and each post-vaccination timepoint (Fig. 2A).

Post-vaccination, increases in hSBA GMTs against serogroup B test strains were consistent with the increased percentage of subjects with hSBA titers ≥ 4 against serogroup B test strains in each group (Fig. 2B). Namely, one month after the second vaccination in the 4CMenB group (Month 2), there was an increase in hSBA GMTs relative to baseline (16- to 66-fold). Over the subsequent ten months, while hSBA GMTs decreased (Fig. 2B), they were still elevated compared to the level associated with seroprotection (≥ 4). In contrast, hSBA GMTs in the MenACWY-CRM and Control groups remained at baseline level for each of the serogroup B reference strains at all post-vaccination timepoints.

3.3. Immune responses against serogroups C and Y

At baseline (Day 1; Fig. 3), the majority of subjects across study groups had hSBA titers ≥ 8 against serogroups C (80–90%) and Y (72–78%; see Fig. 3A and B). Post-vaccination, a high percentage of subjects in all groups maintained hSBA titers ≥ 8 against ser-

Table 2

Demographics and baseline characteristics of subjects included in the modified intention-to-treat (MITT) immunogenicity subset.

	4CMenB N = 192	MenACWY-CRM N = 192	Control N = 197	Total N = 581
Age (Years \pm SD)	20.2 \pm 1.7	20.2 \pm 1.5	20.2 \pm 1.7	20.2 \pm 1.6
Males, n (%)	91 (47)	98 (51)	86 (44)	275 (47)
Ethnic origin, n (%)				
Asian	10 (5)	11 (6)	11 (6)	32 (6)
Black	3 (2)	5 (3)	2 (1)	10 (2)
Caucasian	177 (92)	165 (86)	170 (86)	512 (88)
Hispanic	1 (<1)	2 (1)	1 (<1)	4 (<1)
Other	1 (<1)	9 (5)	13 (7)	23 (4)
Weight (kg \pm SD)	68.8 \pm 13.1	68.6 \pm 14.4	67.5 \pm 13.6	68.3 \pm 13.7
Height (cm \pm SD)	172.7 \pm 9.3	173.6 \pm 10.1	171.7 \pm 9.7	172.7 \pm 9.7

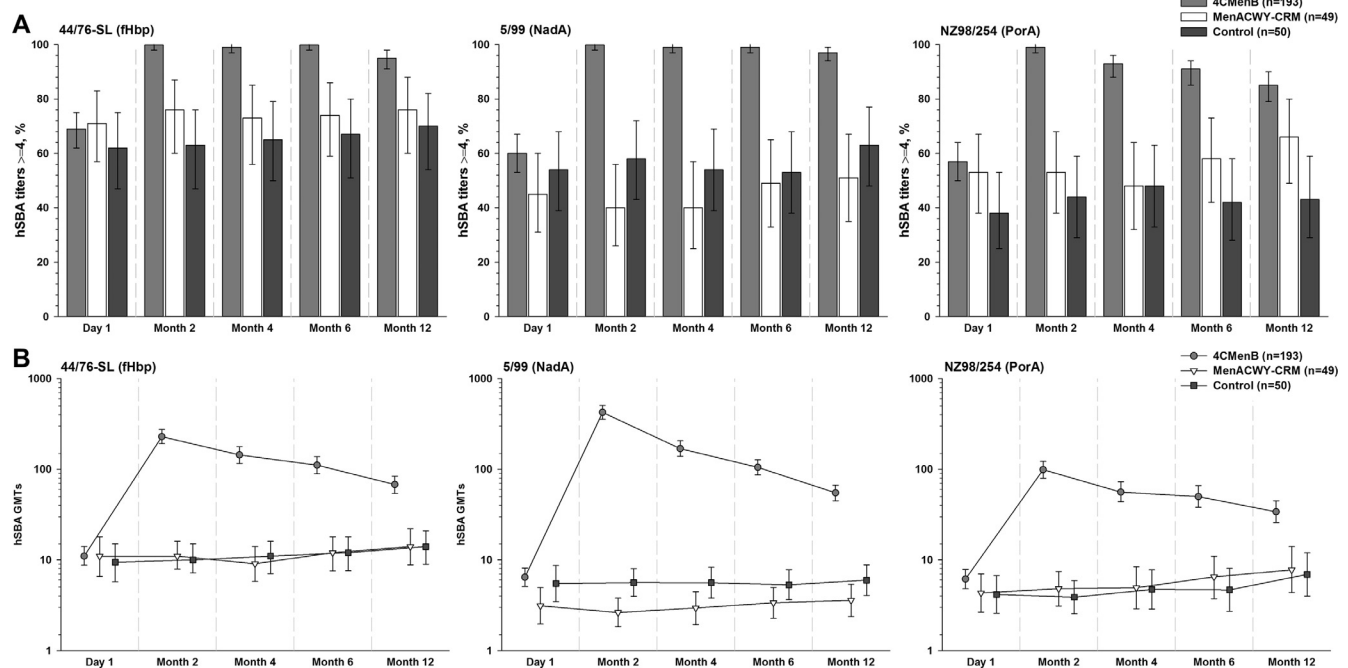


Fig. 2. Immunogenicity against serogroup B test strains. (A) Percentage of subjects (95% CI) with hSBA titers ≥ 4 and (B) hSBA GMTs against serogroup B test strains for fHbp (44/76-SL), NadA (5/99), and PorA (NZ98/254) at Day 1 (baseline) and Months 2, 4, 6 and 12 (1, 3, 5, and 11 months following the second vaccination, respectively), by group (modified intention-to-treat (MITT) immunogenicity subset; hSBA – serum bactericidal antibody assay with human complement; GMT – geometric mean titers).

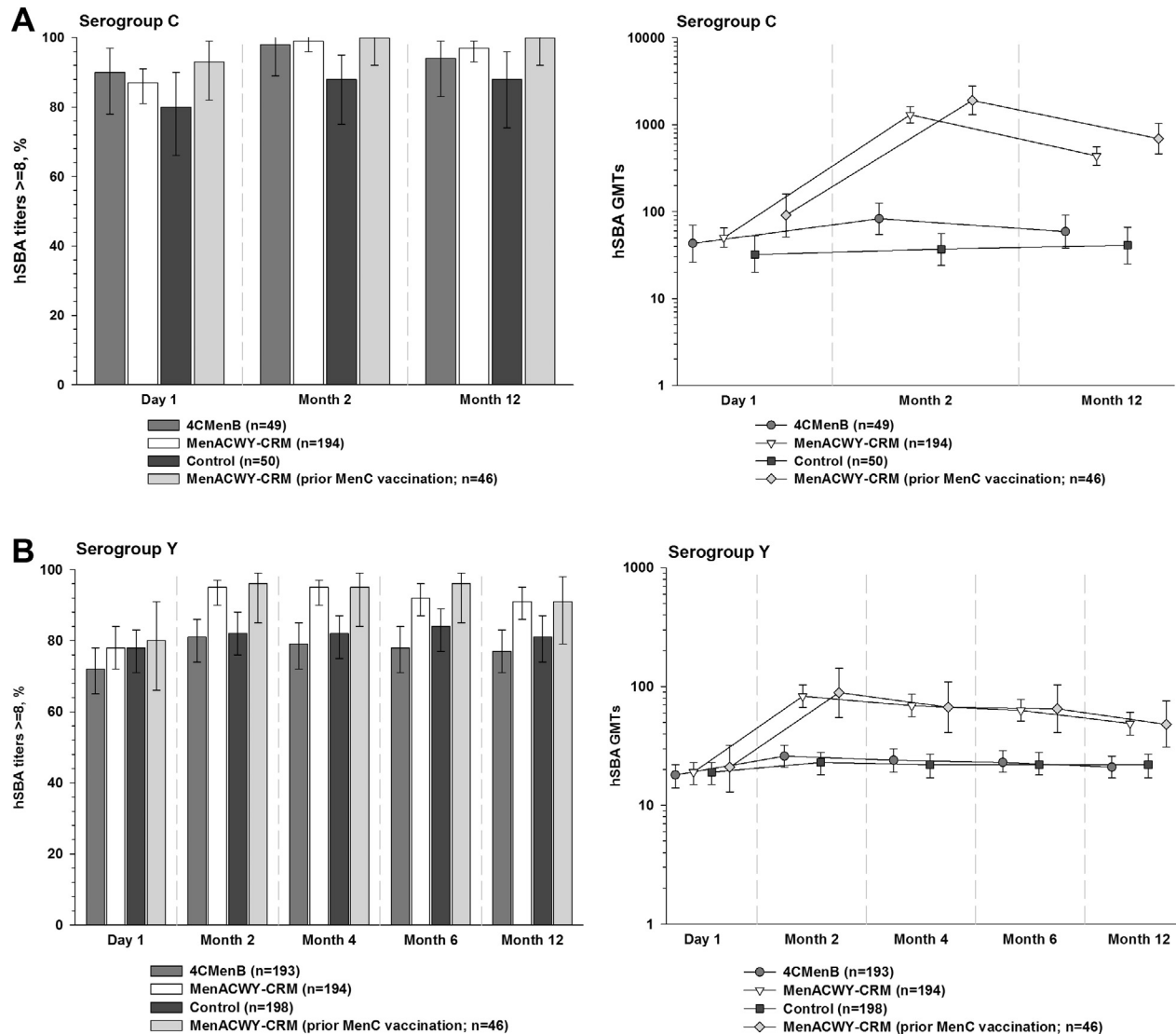


Fig. 3. Immunogenicity against serogroups C and Y. (A) Percentage of subjects (95% CI) with hSBA titers ≥ 8 and hSBA GMTs (95% CI) against serogroup C at Day 1 (baseline) and Months 2 and 12 (1 and 11 months following the second vaccination/placebo injection, respectively), by group and in those subjects in the MenACWY-CRM group with documentation of prior meningococcal serogroup C vaccination. (B) Percentage of subjects (95% CI) with hSBA titers ≥ 8 and hSBA GMTs (95% CI) against serogroup Y at Day 1 (baseline) and Months 2, 4, 6, and 12 (1, 3, 5, and 11 months following the second vaccination/placebo injection, respectively), by group and in those subjects in the MenACWY-CRM group with documentation of prior meningococcal serogroup C vaccination. (modified intention-to-treat (MITT) immunogenicity subset; hSBA – serum bactericidal antibody assay with human complement; GMT – geometric mean titers).

ogroup C (Fig. 3A). The percentage of subjects with hSBA titers ≥ 8 against serogroup Y remained under 85% at each post-vaccination timepoint, in all groups except the ones receiving MenACWY-CRM (Fig. 3B).

Baseline GMTs against serogroups C and Y were similar across groups (Fig. 3). At Month 2, however, while hSBA GMTs against serogroup C and Y strains in the Control group remained at baseline levels, the MenACWY-CRM group showed a 26- and 4-fold increase in GMTs against serogroups C and Y, respectively, at Month 2 after a single dose of MenACWY-CRM.

One month after completing the 4CMenB vaccine course (at Month 2), 2- and 1.5-fold increases in hSBA GMTs against serogroups C and Y, respectively, were observed in the 4CMenB group.

3.4. Immune responses against serogroups C and Y in prior recipients of meningococcal C vaccine

At baseline, 93% and 80% of subjects in the MenACWY-CRM group with prior record of a MenC vaccination had hSBA titers ≥ 8 against serogroups C and Y, respectively. Two months after receiving a single MenACWY-CRM vaccination in this study, 100% and 96% of subjects achieved hSBA titers ≥ 8 against serogroups C and Y, respectively. There was also high antibody persistence with 100% and 91% subjects still maintaining hSBA titers ≥ 8 at 11 months post-vaccination against serogroups C and Y, respectively (see Fig. 3). hSBA GMTs in the MenACWY (with prior MenC) and Control groups were similar at baseline. At Month 2, hSBA

GMTs in the Control group did not change, while hSBA GMTs against serogroups C and Y in the MenACWY group increased by 8 and 4-fold, respectively (Fig. 3). At Month 2, 147 of 181 subjects achieved a seroresponse against serogroup C (81% [95% CI: 75–87%]); whereas seroresponse rates against serogroup Y were modest (44% [95% CI: 37–52%]). In contrast, the 4CMenB and Control groups showed low seroresponse rates against serogroups C (8% [95% CI: 2–20%] and 4% [95% CI: 1–14%], respectively) and Y (12% [95% CI: 8–17%] and 7% [95% CI: 4–12%], respectively).

3.5. Carriage of *N. meningitidis* and seroresponses

At baseline, carriage rates for potentially virulent serogroup B strains, and for serogroups C and Y were higher in subjects with hSBA titers above the pre-specified threshold for each serogroup (Table 3). Contrastingly in 4CMenB group, post-vaccination at Month 12, there was a trend of subjects with baseline hSBA < 4 to be associated with higher rates of carriage when compared to subjects with hSBA ≥ 4. Additionally, serogroup-specific immune responses among MenACWY-CRM recipients appeared to differ ($p < 0.001$, post hoc analysis) depending on whether or not they

were carrying a serogroup Y strain at baseline. Specifically, at Month 2, following one vaccination with MenACWY-CRM, 76 of 174 non-carriers (44% [95% CI: 36–51%]) achieved a 4-fold increase in hSBA titers for serogroup Y (seroresponse) compared with 0 of 7 carriers (0% [95% CI: 0–41%]).

The immune responses elicited against serogroups C and Y after one dose of MenACWY-CRM persisted up to Month 12 post-vaccination. No clear correlation between carriage rates and post-vaccination antibodies was shown against 4CMenB reference strains or serogroups C and Y.

4. Discussion

This study showed that robust immune responses against serogroup B reference strains were elicited with 4CMenB, and against serogroups C and Y with MenACWY-CRM, from one month and for up to at least 11 months post-vaccination of students. There was no clear correlation between changes in carriage rates and post-vaccination bactericidal antibody levels.

The immunogenicity results following two doses of 4CMenB in the present study are consistent with a previous study in high

Table 3
Percentage of subjects with carriage of ‘disease-associated’ capsular group B sequence types, stratified by pre-vaccination hSBA titer <4 and ≥4 against serogroup B test strains at baseline (Day 1), 1 month post-first vaccination (Month 1), and 1, 3, 5, and 11 months post-second vaccination/injection (Months 2, 4, 6 and 12, respectively) in the 4CMenB and control groups and the percentage of subjects with carriage of serogroups CWY strains stratified by pre-vaccination hSBA titers <8 and ≥8 against serogroup C or serogroup Y at baseline (Day 1), 1 month post-first vaccination (Month 1), and 1, 3, 5, and 11 months post-second vaccination/injection (Months 2, 4, 6 and 12, respectively) in the MenACWY-CRM and control groups.

	Pre-vaccination hSBA titer <4		Pre-vaccination hSBA titer ≥4	
	4CMenB n/N (%; 95% CI)	Control n/N (%; 95% CI)	4CMenB n/N (%; 95% CI)	Control n/N (%; 95% CI)
Serogroup B test strain				
<i>44/76-SL (fHbp)</i>				
Day 1	0/60 (0; 0–6)	0/19 (0; 0–18)	13/133 (10; 5–16)	2/31 (6; 1–21)
Month 1	2/60 (3; 0–12)	0/18 (0; 0–19)	7/130 (5; 2–11)	3/30 (10; 2–27%)
Month 2	4/60 (7; 2–16)	0/18 (0; 0–19)	10/130 (8; 4–14)	2/30 (7; 1–22)
Month 4	2/56 (4; 0–12)	0/17 (0; 0–20)	11/120 (9; 5–16)	4/29 (14; 4–32)
Month 6	4/56 (7; 2–17)	0/17 (0; 0–20)	6/115 (5; 2–11)	3/28 (11; 2–28)
Month 12	6/56 (11; 4–22)	0/16 (0; 0–21)	6/123 (5; 2–10)	2/30 (7; 1–22)
<i>5/99 (NadA)</i>				
Day 1	4/77 (5; 1–13)	2/23 (9; 1–28%)	9/116 (8; 4–14)	0/27 (0; 0–13)
Month 1	3/77 (4; 1–11)	2/23 (9; 1–29)	6/113 (5; 2–11)	1/26 (4; 0.1–20)
Month 2	5/77 (6; 2–15)	0/22 (0; 0–15)	9/113 (8; 4–15)	2/26 (8; 1–25)
Month 4	3/71 (4; 1–12)	2/21 (10; 1–30)	10/105 (10; 5–17)	2/25 (8; 1–26)
Month 6	2/68 (3; 0–10)	2/21 (10; 1–30)	8/103 (8; 3–15)	1/24 (4; 0–21)
Month 12	5/71 (7; 2–16)	1/20 (5; 0–25)	7/108 (6; 3–13)	1/26 (4; 0.1–20)
<i>NZ98/254 (PorA)</i>				
Day 1	2/83 (2; 0–8)	2/31 (6; 1–21)	11/110 (10; 5–7)	0/19 (0; 0–18)
Month 1	3/81 (4; 1–10)	2/30 (7; 1–22)	6/109 (6; 2–12)	1/18 (6; 0–27)
Month 2	3/81 (4; 1–10)	0/30 (0; 0–12)	11/109 (10; 5–17)	2/18 (11; 1–35)
Month 4	2/75 (3; 0–9)	1/29 (3; 0.1–18)	11/101 (11; 6–19)	3/17 (18; 4–43)
Month 6	2/70 (3; 0–10)	1/29 (3; 0.1–18)	8/101 (8; 3–15)	2/16 (13; 2–38)
Month 12	8/75 (11; 5–20)	1/28 (4; 0.1–18)	4/104 (4; 1–10)	1/18 (6; 0–27)
	Pre-vaccination hSBA titer <8		Pre-vaccination hSBA titer ≥8	
	MenACWY-CRM n/N (%; 95% CI)	Control n/N (%; 95% CI)	MenACWY-CRM n/N (%; 95% CI)	Control n/N (%; 95% CI)
Serogroup C				
Day 1	0/26 (0; 0–13)	0/10 (0; 0–31)	9/168 (5; 2–10)	3/40 (8; 2–20)
Month 1	0/24 (0; 0–14)	0/9 (0; 0–34)	9/161 (6; 3–10)	4/40 (10; 3–24)
Month 2	1/24 (4; 0–21)	0/8 (0; 0–37)	6/158 (4; 1–8)	3/40 (8; 2–20)
Month 4	1/21 (5; 0–24)	0/7 (0; 0–41)	9/146 (6; 3–11)	5/37 (14; 5–29)
Month 6	0/22 (0; 0–15)	0/7 (0; 0–41)	12/144 (8; 4–14)	3/33 (9; 2–24)
Month 12	3/21 (14; 3–36)	0/7 (0; 0–41)	3/142 (2; 0–6)	1/35 (3; 0.1–15)
Serogroup Y				
Day 1	0/42 (0; 0–8)	0/44 (0; 0–8)	9/152 (6; 3–11)	9/154 (6; 3–11)
Month 1	0/39 (0; 0–9)	1/40 (3; 0.1–13)	9/146 (6; 3–11)	8/151 (5; 2–10)
Month 2	0/39 (0; 0–9)	0/40 (0; 0–9)	7/143 (5; 2–10)	8/149 (5; 2–10)
Month 4	0/36 (0; 0–10)	1/37 (3; 0.1–14)	10/131 (8; 4–14)	10/139 (7; 4–13)
Month 6	0/36 (3; 0.1–15)	2/35 (6; 1–19)	12/130 (9; 5–16)	7/134 (5; 2–10)
Month 12	1/34 (3; 0.1–15)	2/35 (6; 1–19)	5/129 (4; 1–9)	4/135 (3; 1–7)

school students (aged 11–17 years), which showed that one month after two doses of 4CMenB nearly all subjects reached hSBA titers ≥ 4 against serogroup B reference strains [27]; however, baseline titers were noticeably high. While the percentage of subjects in the present study with baseline hSBA titers ≥ 4 against serogroup B reference strains was even higher than that reported by Santolaya et al., the percentages were still lower than pre-vaccination levels previously reported in UK adults [31]. These differences likely reflect anthropological and geographic differences (UK/Chile) and/or different carriage rates, which can act as a priming event [28,32]. Irrespective of baseline immune status, all but one subject in the 4CMenB group of the present study had hSBA titers ≥ 4 against the serogroup B strains one month after the two-dose series, and at least 85% of subjects in this group maintained hSBA titers ≥ 4 against each of the serogroup B strains over the subsequent 10 months. As expected, the percentage of subjects seropositive against serogroup B strains did not increase over baseline in either the MenACWY-CRM or Control groups at any post-vaccination timepoint.

The results of the study also demonstrated that one dose of MenACWY-CRM elicited robust immune responses against serogroups C and Y from Month 2 up to Month 12 post-vaccination. This effect appeared even more pronounced in subjects who had previously received a meningococcal serogroup C conjugate vaccination. Similar immunogenicity results following one dose of MenACWY-CRM have been reported in adolescents in other countries [27,33–35]. However, it is important to note that the Month 2 analysis point in this study is unusual for investigations of MenACWY-CRM immunogenicity, which typically measure responses one month post-vaccination. This discrepancy should be considered when comparing the MenACWY-CRM results with other studies.

Population-scale meningococcal serogroup C conjugate vaccination provides benefit not only through direct protection, but also through the reduction of transmission [36]. Consistent with this, our recent carriage analysis of 4CMenB and MenACWY-CRM [24] demonstrated that 4CMenB has a broad–albeit modest–impact on carriage of meningococcal strains, particularly serogroup Y, over the course of one year follow-up [24]. The current study suggests that hSBA cannot be used as a surrogate marker of carriage-reduction efficacy of meningococcal vaccines or that the threshold that disrupts carriage is different to that which elicits serological protection against meningococcal disease [26]. Indeed, it has been shown that greater concentrations of pneumococcal antibody is required to impact carriage compared to that required to confer immunity [37]. Ultimately, it is possible that mucosal or other non-hSBA immune responses to 4CMenB components are more critical for disrupting meningococcal carriage [36,38].

We found that carriage of serogroups by participants influenced their serologic responses. There was a higher rate of serogroup B carriage among subjects with hSBA titers ≥ 4 against serogroup B strains at baseline, compared to subjects with titers < 4 at baseline. Similarly, there was a trend for higher carriage of serogroups C and Y among subjects with hSBA titers ≥ 8 at baseline, compared to subjects with titers < 8 at baseline. While there was no clear association between carriage rates for serogroups B and C and post-vaccination hSBA levels against these strains, there was evidence of a decrease in immune response to the MenACWY-CRM vaccine in subjects with baseline carriage of serogroup Y. This lowered immune response, whilst based on a small sample, emulates previous reports of hyporesponsive effects to the pneumococcal conjugate vaccination in infants and children carrying pneumococcus at the time of immunization [39–41]. Nevertheless, irrespective of baseline hSBA titers or carriage status, two months after one dose of MenACWY-CRM, 95% subjects in the MenACWY-CRM group achieved hSBA titers ≥ 8 against serogroup Y.

In summary, two doses of 4CMenB or one dose of MenACWY-CRM elicited robust immune responses in university students. No correlations between pharyngeal carriage and post-vaccination hSBA titers were observed.

Trademark

Bexsero and *Menveo* are trademarks of the GSK group of companies. *Ixiaro* is a trademark of Valneva SE.

Conflicts of interest

AK, EY, DT, and MM were permanent employees of Novartis group companies at the time of the study and are now employees of GSK group companies. PD was a permanent employee of Novartis Vaccines and Diagnostics, Inc. during study conduct and data analysis and interpretation (but prior to acquisition by GSK group companies). All other authors acted as chief or principal investigators for this Novartis-sponsored trial conducted on behalf of their respective NHS Trusts and/or Universities, but received no personal payments from Novartis for study conduct. RCR has received speaker fees and travel assistance from Novartis to attend a meeting, outside the submitted work. RB, JF, XB and KN have performed contract research on behalf of Public Health England (formerly the Health Protection Agency) for Baxter, GSK, Novartis, Pfizer, Sanofi Pasteur MSD, outside the submitted work.

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Author contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

All authors reviewed and commented critically drafts of the manuscript for important intellectual content and gave final approval to submit for publication. MMC, PD, and RR contributed to the study conception and design. RCR and RB conducted the study/were investigators. MMC, JF, XB, KN, RB, AK and RB contributed to acquisition of data. HW, DT, MMC and PD contributed to the analysis and interpretation of data. HW provided statistical expertise.

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