



Immunogenicity of a single 4CMenB vaccine booster in adolescents 11 years after childhood immunisation

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ABSTRACT

The clinical development of the meningococcal vaccine, 4CMenB, included 2 doses in vaccine-naïve adolescents, which was considered unlikely to be cost-effective for implementation. Theoretically, priming with 4CMenB in early childhood might drive strong immune responses after only a single booster dose in adolescents and reduce programmatic costs. To address this question, children over 11 years old who took part in previous trials involving the administration of 3–5 doses of 4CMenB at infant/preschool age from 2006 were recruited into a post licensure single-centre trial, and were divided into two groups: those who received their last dose at 12 months old (infant group) and those who received their last dose at 3 years old (infant + preschool group). Naïve age-matched controls were randomised to receive one (adolescent 1 group) or two doses at days 0 and 28 (adolescent 2 group) of 4CMenB. Serum bactericidal antibody (SBA) assays using human complement were performed against three reference strains prior to vaccination, and at 1, 6 and 12 months. Previous vaccination was associated with a higher response to a single booster dose at 11 years of age, one-month post-vaccination, when compared with a single dose in naïve age-matched controls. At day 180, the highest responses were observed in participants in the infant + preschool group against strain 5/99 (GMT 316.1 [CI 158.4 to 630.8]), as compared with naïve adolescents who received two doses (GMTs 84.5 [CI 57.7 to 123.6]). When the last dose was received at 12-months of age, responses to a single adolescent dose were not as robust (GMT 61.1 [CI 14.8 to 252.4] to strain 5/99). This descriptive study indicates that the highest SBA responses after a single dose in adolescence were observed in participants who received a preschool dose, suggesting that B cell memory responses are not sufficiently primed at less than 12 months of age.

Trial registration EudraCT 2017-004732-11, ISRCTN16774163.

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1. Introduction

The group B meningococcal vaccine, 4CMenB, was developed with the intention of protecting all age groups against group B meningococcal disease (MenB) [1]. The vaccine was licensed in 2013 in Europe and North America and in various other jurisdictions, and the UK has included it into the national immunisation programme for infants (at 2, 4 and 12 months of age) since 2015.

Abbreviations: 4CMenB, four component group B meningococcal vaccine (Bexsero®); SBA, serum bactericidal activity.

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During clinical development, the vaccine was evaluated in adolescents, and it was demonstrated that two doses of 4CMenB induced robust serum bactericidal antibody (SBA) responses [2,3]. The vaccine was well tolerated, and no safety concerns were identified [1]. In the UK and in many other countries, control of group C meningococcal disease was obtained with the group C meningococcal glycoconjugate vaccine immunization programs in infants and adolescents. However, the absence of an adolescent 4CMenB immunisation programme leaves this age group vulnerable to this devastating disease [4,5].

An adolescent programme was not initiated in the UK because the vaccines was not cost-effective in this age group (JCVI position statement on use of Bexsero® meningococcal B vaccine in the UK March 2014). However, these assessments were made for 4CMenB

naïve adolescents, who would require two injections at a minimum of a 1-month interval to elicit an acceptable level of protection in all vaccinees. In the future, almost all adolescents in the UK will have received their primary course of 4CMenB vaccination in infancy [6]. No study has yet quantified the immune response in adolescents after a primary course of vaccination with 4CMenB given as babies. While the level of vaccine-induced protective antibody titers >10 years after the last vaccination is expected to be low, these adolescents may have a substantial level of vaccine-induced memory B-cells, which may in turn produce a strong antibody recall response to the vaccine antigens. If this was the case, a single dose adolescent booster could be envisaged, and would provide the potential for additional scenarios to be considered for vaccination. The data to support or refute this hypothesis are not available, because the adolescents enrolled in previous 4CMenB clinical trials were naïve.

In 2006, the first ever infants were vaccinated with 4CMenB in a trial conducted by the Oxford Vaccine Group and Public Health England [7]. Infants received 4CMenB at 2, 4, 6, and 12 months of age. Immunogenicity was demonstrated using serum bactericidal antibody (SBA) assay using human complement against a panel of relevant meningococcal strains. Further studies included priming schedules at 6, 8 and 12 months or just 12 months, as well as a follow-up study investigating the persistence of the SBA response, and the effect induced by a booster injection given at 40 months of age, or 40 and 42 months [8,9]. The children involved in the initial and the follow-up studies have reached adolescence, and the persistence of the protective antibody response and B-cell memory in adolescents around 11 years after infant vaccination can now be assessed.[10].

2. Materials and methods

2.1. Study design and participants

This study is an open-label, descriptive immunogenicity analysis, conducted between 24 March 2018 and 23 January 2020 in the UK (EudraCT 2017-004732-11, ISRCTN, ISRCTN16774163). The first participant was consented, enrolled and vaccinated on 24-03-2018, and the last participant on 29-01-2019 (Visit one). The follow up from the first visit two occurred on 21-04-2018 and the last visit 4 occurred on 23-01-2020. The study protocol was previously described [10]. The protocol and all study documents were reviewed and approved by the institutional clinical trial and regulations governance board, and by Nottingham 2 Research Ethics Committee (reference 17/EM/0466). The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. A full list of inclusion and exclusion criteria is described (Supplementary Text 1). Written informed consent was obtained from parents or legal guardians prior to enrolment, and assent was obtained from all participants (Table 1). These children, born between June and December 2006, received either an infant schedule (infant group; 2–4–6 + 12 months or 6–8–12 months or just 12 months), or an infant schedule followed by a boost at 40 months or 40 and 42 months as described in Table 2 (infant + preschool group). Further dividing of sub-groups according to the exact

schedule is described in supplementary Table 1. The parent studies describing their 4CMenB immunization history included the clinical trials V72P6 [7], V72P6E1 [9], V72P9 [9,11] and V72P9E1[8]. These participants were invited to receive a booster dose of 4CMenB at day 0. Naïve age-matched controls were randomized to receive a single dose (day 0) or two doses of 4CMenB at day 0 and 28. For ethical reasons, the control groups that received a single dose at day 0 was offered a second dose at the end of study (day 365), so they would have received the full adolescent schedule. Computer-generated randomisation lists were prepared by the study statistician using STATA/IC 14.2 for the naïve participants. The participants were randomised 1:1 using simple block randomisation with random block sizes of 2 and 4. The randomisation was implemented using paper envelopes and prepared by an independent team at Oxford Vaccine Group, who was not involved in the running of this trial. The research nurse took the envelope and opened it at visit one, all participants received the vaccine at the first visit. All participants had blood drawn at day 0 (prior to vaccination), day 28, and at or around day 180 (+/- 14 days) and day 365 (+/- 21 days). Clinical staff were not blinded.

2.2. Study objectives

The primary objective was to assess whether a single booster dose of 4CMenB induces protective antibody responses in adolescents who were previously immunized during childhood, measured by the SBA assay. The secondary objectives were to address whether there was persistence of the SBA responses in adolescents in (i) the infant group (ii) the infant + preschool group. Exploratory objectives included (i) characterisation of B-cell memory in adolescents in both the infant group and the infant + preschool group after adolescent vaccination, (ii) characterisation of fever and other adverse outcomes in adolescents following 4CMenB administration and (iii) assessment of whether a booster dose of 4CMenB is more reactogenic in any of the study groups.

2.3. Vaccines

4CMenB is an inactivated vaccine comprising three recombinant Neisserial proteins formulated with Outer Membrane Vesicles (OMVs) from *N. meningitidis* serogroup B strain NZ98/254. It was supplied in pre-filled syringes that deliver a single dose of 0.5 mL, stored between 2 and 8 °C, and administered by intramuscular injection into the deltoid muscle region of the non-dominant upper arm.

2.4. Serum bactericidal assay

At each study visit (day 0, 28, 180 and 365), a maximum of 20 mL of venous blood sample was collected. The SBA was performed using human complement (hSBA) with samples blinded to group allocation (using allocated sample identification numbers) against strains 44/76-SL, NZ98/254 and 5/99 as described previously [12], to indicate responses to the fHbp, PorA and NadA antigens respectively. The hSBA titers were expressed as the reciprocal of the final interpolated dilution that killed at least 50% of bacteria after 60 min. An hSBA titer of ≥ 4 is an accepted threshold for putative protection against serogroup B [13].

2.5. Enumeration of antigen specific memory B cells

The memory B cell responses were measured against matching *N. meningitidis* recombinant proteins (fHbp from strain 44/76-SL, NadA from strain 5/99, PorA from strain NZ98/254 and NHBA from strain M10713). Anti-fHBP, NadA, NHBA and PorA-specific IgA, IgG

Table 1
Contact and recruitment progress.

	Follow on group	Naïve
No responses	15	3
Yes responses	43	62
Excluded	0	18
Yes but changed mind	3	12
Actual visit 1's	40	32

Table 2
Description of study groups, N represents the number of samples available.

Status	Number/age of doses in childhood	Age at Last dose	Adolescent regimen tested	N
infant group	1 (12 M) 3 (6, 8, 12 M) 4 (2, 4, 6, 12 M)	12 months	1	16
infant + preschool group	3 (12, 40, 42 M) 4 (6, 8, 12, 40 M) 5 (2, 4, 6, 12, 40 M)	3 years of age	1	24
Adolescent 1 group (naïve, 1 adolescent dose)	0	–	1	16
Adolescent 2 group (naïve, 2 adolescent doses)	0	–	2 (Day 0 + 28)	16

and IgM memory B-cell responses were measured using triple colour Fluorospot. Cryopreserved PBMCs were recovered and plated at 2×10^5 /well in 96-well plates. The total number of wells varied between participants as all cells recovered for each participant and time points were plated (typically between 12 and 30 wells). Cells were stimulated with 1 µg/mL R848 and 10 ng/mL IL-2 for 72 h at 37 °C. Stimulated PBMCs were harvested and plated at 2×10^5 /well in duplicate on fluorospot plates (X-06G05R17M-10, Mabtech) coated with 2 µg/mL of either fHBP, NadA, NhBA or PorA, and incubated at 37 °C for 16 h. Detection of spots was carried out according to the manufacturer's instructions (Mabtech) and analyzed with the iSpot EliSpot reader (Autoimmun Diagnostika). A positive response is defined as $> =$ to 5 spots per million cells.

2.6. Assessment of reactogenicity

For the first 7 days after each immunization, all adverse events (AEs) observed by the study team or reported by the participant's parent/legal guardian, whether attributed to the study vaccine or not, were recorded (description, date of onset and end date, severity, assessment of relatedness to study vaccine and action taken, including any medication taken to treat an AE). From day 7 to day 28 post vaccination, only illnesses considered serious (serious adverse events) were recorded. Participants were provided with a digital thermometer to record axillary temperature and a plastic ruler to measure local reactions at the vaccination site. Reactions occurring in the first 7 days after immunisation (including day of immunisation, day 0 to day 6) were divided up into: solicited reactions, considered related to study vaccine unless stated otherwise; and unsolicited adverse events, which were assessed for relatedness and graded for severity by a medically qualified member of the study team. Severity of local reactions (redness, induration, swelling) were categorized by diameter as none, $0 \leq 10$ mm, $10 \leq 25$ mm, $25 \leq 50$ mm, $50 \leq 100$ mm and > 100 mm in diameter. Solicited symptoms, and the severity of tenderness and systemic reactions were graded as described in Supplementary Table 2. Prescription medication used between day 7 and 28 post vaccination and the reason for taking it were recorded after each vaccine received. The parents/legal guardians were asked to record school absence due to 4CMenB receipt within 7 days of vaccination or if they would have been absent if vaccination occurred within school term/days. Serious adverse events were reported through participant enrolment within the study.

2.7. Statistical analysis

As a preliminary study to explore the persistence and potential impact of antigen-specific memory B cell responses on an adolescent programme, a formal sample size calculation was not carried out and the sample size in the naïve groups was purely pragmatic. The sample size of the participants previously vaccinated was determined by the number of participants who completed the previous studies in 2006–2009 at Oxford. A pre-specified analysis

included combining the participants who had received an infant schedule with their last dose at 12 months of age (infant group), and those who received an infant schedule followed by a booster at 40 or 42 months of age (infant + preschool group).

Percentage of participants with an hSBA titer ≥ 4 , geometric mean hSBA titers and their 95% confidence intervals were determined at all time points. For SBA analysis, data were reported in the form of percentages, geometric mean titers with 95% confidence intervals, per group and per strain (for the three strains: 44/76-SL, 5/99, NZ98/254). For geometric mean titers, data were log transformed (natural), and confidence intervals calculated on this log scale, prior to transforming back onto the original scale for reporting and interpretation. Confidence intervals for proportions were calculated using the binomial exact method. For the reactogenicity analysis, the frequency and severity of local and systemic solicited vaccine reactions were summarized for the participants overall for each dose of 4CMenB vaccine. The number of participants (denominator) included in each analysis is included in the result tables (The number in the column heading are the number who have endpoint data available, consistent with the consort diagram). A Kruskal-Wallis test was used to evaluate if all study groups were drawn from populations with the same mean value of immunity at each time point and for each target strain. For the solicited AE analysis, the participants missing all diary were excluded. All analysis was done on the modified intention-to-treat basis, i.e. by the arm randomised. There were no deviations on the vaccine schedule received.

3. Results

3.1. Demographics

Eighty-three participants involved in the previous trials of 4CMenB were contacted. Forty participants were recruited and enrolled into groups according to their previous vaccination schedule (Table 1 and 2). Naïve participants were recruited through website-based advertising, social media, poster advertisements, dissemination via GP practices, in educational/recreational settings, in schools and school newsletters and direct mail out as described previously [10]. 32 participants were recruited and randomized into the two naïve control groups (Table 2). The baseline characteristics of the participants are described in Table 3.

3.2. Antibody persistence 11 years post infant vaccination with or without a dose at 3 years of age

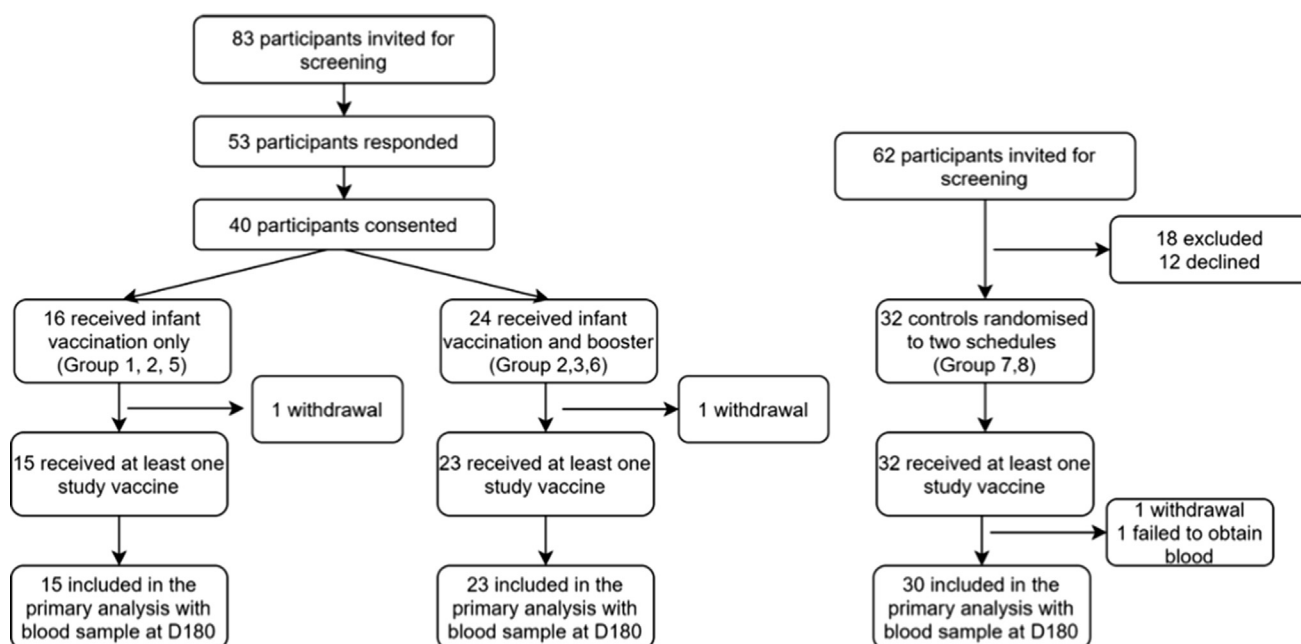
Before the adolescent dose, bactericidal antibodies were detected against strain 5/99 in 4 out of 15 participants in the infant group (26.7%) and 16 out of 23 in the infant + preschool group (69.6%) (Table 4, Figs. 2 and 3). Higher SBA titers were also observed in the latter group (GMT 21.0 [CI 8.43–52.8], as compared with 3.2 [CI 1.3–7.6] in the infant group), supporting a positive impact of the preschool boost. None of the 32 naïve participants

Table 3**Baseline Characteristics of the 72 participants.** The demographics include the number of participants who received at least one dose.

Number/age of doses in childhood N	Children received infant vaccination only (infant group)			Children received infant vaccination and preschool booster (infant + preschool group)			Naïve adolescent controls	
	1 (12 M) [N = 4]	3 (6, 8, 12 M) [N = 6]	4 (2, 4, 6, 12 M) [N = 6]	3 (12, 40, 42 M) [N = 4]	4 (6, 8, 12, 40 M) [N = 9]	5 (2, 4, 6, 12, 40 M) [N = 11]	0 [N = 16]	0 [N = 16]
Age (median and IQR)	11.2 (11.4–11.8)	11.8 (11.4–12.0)	11.5 (11.5–11.8)	11.6 (11.4–11.7)	11.7 (11.4–12.0)	11.6 (11.4–11.9)	12.1 (11.8–12.3)	12.1 (11.7–12.6)
Sex (Number and proportion of male)	3 (75%)	3 (50%)	2 (33.3%)	2 (50%)	2 (22.2%)	5 (45.5%)	5 (31.3%)	10 (62.5%)

Table 4**Serum bactericidal antibody (SBA) responses and titers per group.** Data show the number and percentage of participants with titers $\geq 1:4$ in each group and per strain, and the geometric mean titers (GMTs) and 95% confidence interval (CIs).

5/99 SBA titer	Infant (N = 15)	Infant + preschool (N = 23)	Adolescent 1 (N = 16)	Adolescent 2 (N = 16)
Before adolescent dose				
% SBA titer ≥ 4 (95% CIs)	26.67% (1.32–52.02)	69.57% (49.22–89.91)	0.00% (0.00–0.00)	0.00% (0.00–0.00)
GMT (95% CIs)	3.17 (1.32–7.62)	20.99 (8.34–52.81)	1.04 (0.96–1.14)	1.04 (0.96–1.14)
D28 after adolescent dose				
% SBA titer ≥ 4	92.86% (77.43–100.00)	100.00% (100.00–100.00)	87.50% (69.30–100.00)	93.33% (79.03–100.00)
GMT (95% CIs)	148.50 (45.77–481.81)	1,609.25 (927.57–2,791.89)	22.63 (9.41–54.39)	40.32 (22.18–73.28)
D180 after adolescent dose				
% SBA titer ≥ 4	73.33% (47.98–98.68)	95.65% (86.64–100.00)	46.67% (18.07–75.26)	100.00% (100.00–100.00)
GMT (95% CIs)	61.11 (14.79–252.44)	316.12 (158.42–630.84)	2.76 (1.89–4.05)	84.45 (57.70–123.59)
D365 after adolescent dose				
% SBA titer ≥ 4	69.23% (40.20–98.26)	100.00% (100.00–100.00)	25.00% (1.17–48.83)	100.00% (100.00–100.00)
GMT (95% CIs)	35.60 (11.65–108.79)	299.68 (170.14–527.85)	2.18 (1.40–3.40)	57.53 (45.53–72.69)
NZ98/254 SBA titer				
Before adolescent dose				
% SBA titer ≥ 4 (95% CIs)	0.00% (0.00–0.00)	17.39% (0.63–34.15)	0.00% (0.00–0.00)	0.00% (0.00–0.00)
GMT (95% CIs)	1.05 (0.96–1.15)	1.77 (1.10–2.87)	1.00 (1.00–1.00)	1.09 (0.97–1.22)
D28 after adolescent dose				
% SBA titer ≥ 4	78.57% (53.99–100.00)	91.30% (78.85–100.00)	81.25% (59.77–100.00)	66.67% (39.64–93.69)
GMT (95% CIs)	7.25 (3.92–13.38)	17.51 (10.78–28.46)	8.35 (4.21–16.57)	6.65 (3.10–14.28)
D180 after adolescent dose				
% SBA titer ≥ 4	26.67% (1.32–52.02)	69.57% (49.22–89.91)	33.33% (6.31–60.36)	53.33% (24.74–81.93)
GMT (95% CIs)	2.09 (1.20–3.65)	5.25 (3.17–8.68)	2.30 (1.35–3.91)	4.19 (2.16–8.11)
D365 after adolescent dose				
% SBA titer ≥ 4	30.77% (1.74–59.80)	45.45% (22.86–68.05)	25.00% (1.17–48.83)	30.77% (1.74–59.80)
GMT (95% CIs)	1.80 (1.17–2.75)	3.31 (2.06–5.31)	1.61 (1.14–2.27)	2.35 (1.37–4.03)

**Fig. 1. Consort diagram.** Between 24th March 2018 and 29th January 2019, the trial recruited 72 participants. Seventy participants received at least one dose of 4CMenB and were included in the safety analysis. The number of participants in the primary outcome analysis is 68 after excluding 3 withdrawals and a participant with no blood sample taken at day 180.

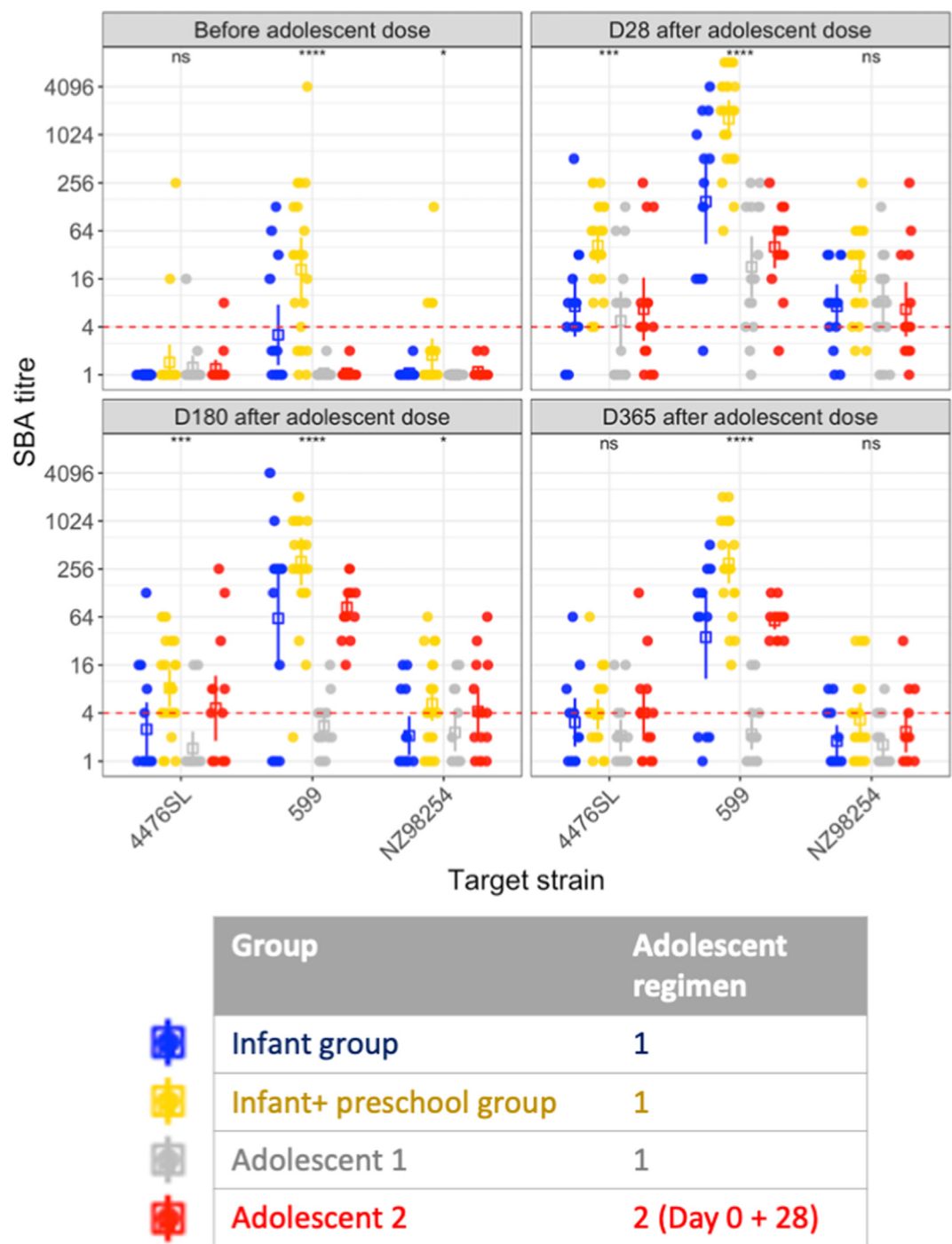


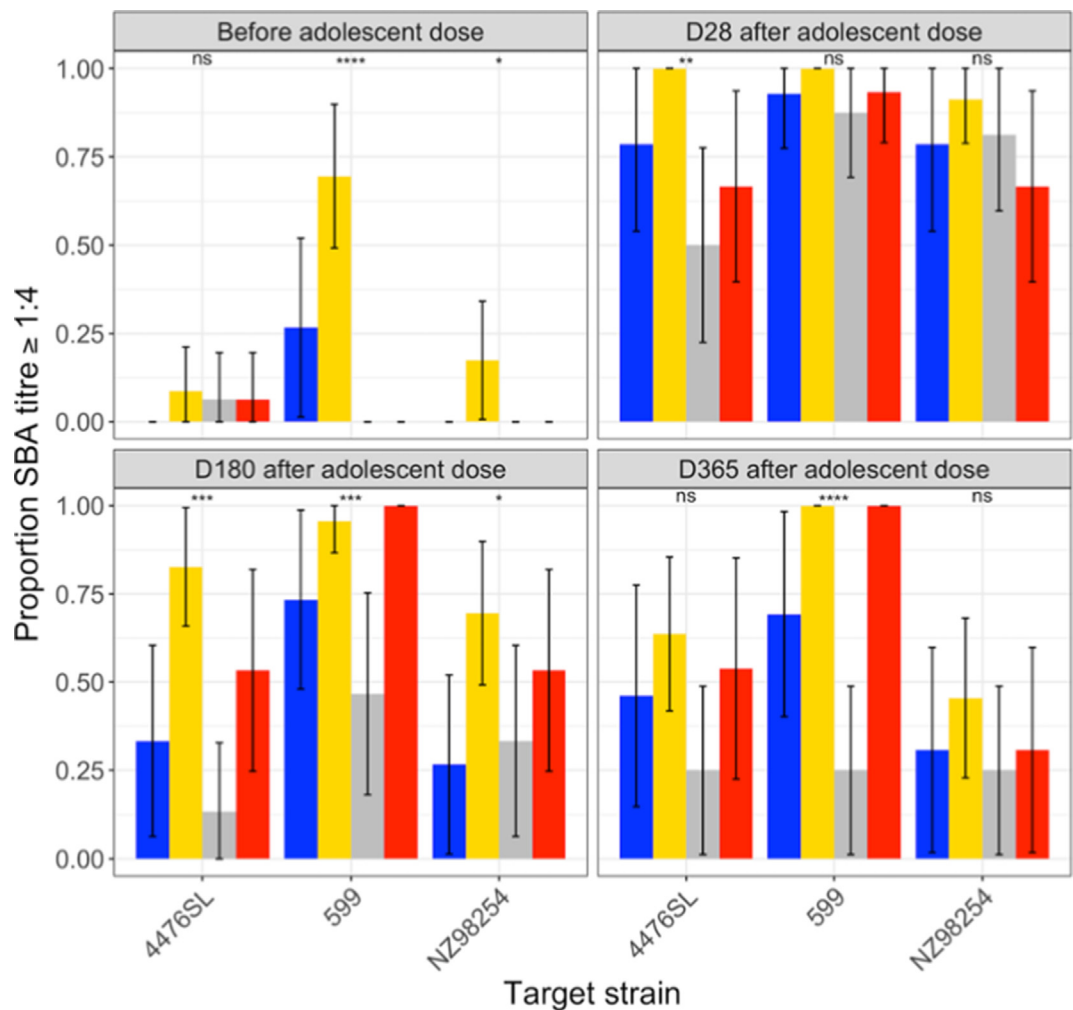
Fig. 2. Individual SBA titers at the four time points for the combined groups. Individual data are shown (dots), as well as geometric means and 95% confidence intervals for each group. The dotted red line indicates SBA titer $\geq 1:4$. The legend indicates the previous vaccination status: previously vaccinated participants who received their last dose at 12 months of age (infant group), previously vaccinated participants who received 4CMenB as infant + preschool dose(s) at 3-year-old (infant + preschool group). The naïve participants received either a single adolescent dose at day 0, or two doses at days 0 and 28. These groups are thus expected to be similar prior to adolescent vaccination and D28 (post one dose). ns: $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

had SBA titers $\geq 1:4$ against this strain before the adolescent dose (Table 4). SBA responses $\geq 1:4$ were rarely detected against strains 44/76-SL (0 to 8.7%) and NZ98/254 (0 to 17.4%) in any of the groups (Table 4, Figs. 2 and 3). Participants who received two doses of 4CMenB in pre-school years (at 40 and 42 months old) had the highest SBA titers prior to adolescent boosting against all three strains (Supplementary Figs. 1 and 2, group 3). Therefore, priming with 4CMenB in early childhood induced a more persistent response to the NadA antigen (expressed on strain 5/99) in those

in the infant + preschool group, that was still detectable at 11 years of age. However, persistence of SBA against the other strains was poor.

3.3. Impact of previous vaccination on the SBA responses induced by a single dose at 11 years of age

All participants received 4CMenB at day 0. The SBA response was measured 28 days later against the three strains (Table 4,

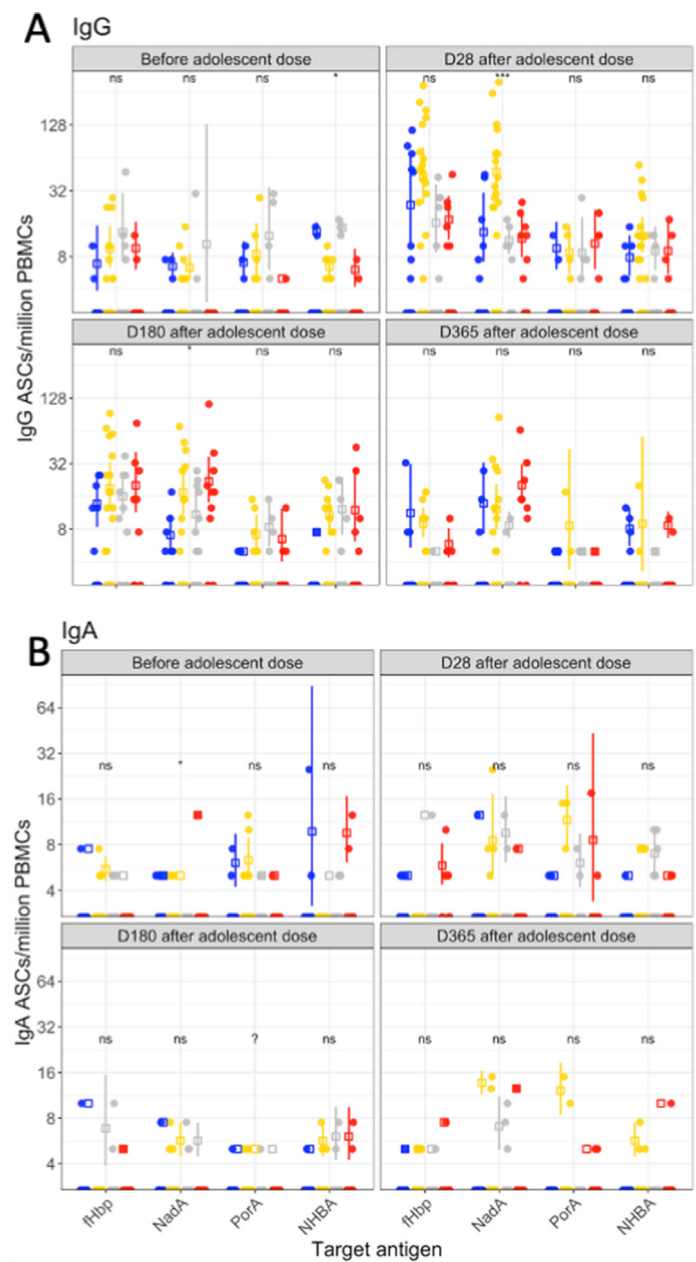


Group	Adolescent regimen
Infant group	1
Infant+ preschool group	1
Adolescent 1	1
Adolescent 2	2 (Day 0 + 28)

Fig. 3. Proportion of participants with a SBA titer $\geq 1:4$ at the four time points for the combined groups. The bars represent the proportions of participants with a SBA titer $\geq 1:4$ and 95% confidence intervals, illustrated by the lines, for each group. The legend indicates the previous vaccination status, age of the last dose for the previously vaccinated (12 months, infant group, or 3 years old, infant + preschool group) and the regimen tested (1 dose at day 0 for all, except for the naïve participant group who received the licensed adolescent schedule (2 doses at one month interval)). ns: $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

Figs. 2 and 3). For all three strains, SBA titers and the proportion of participants with a hSBA titer ≥ 4 were similar or higher in participants vaccinated in the infant group or the infant + preschool group when compared with naïve participants (Table 4, Figs. 2 and 3); For strain 5/99, geometric mean titers (GMTs) were higher in previously vaccinated (GMTs 148.5 [CI 45.8–481.8] in the infant group, 1,609.2 [CI 927.6–2791.9] in the infant + preschool group)

than in the naïve groups (GMTs 22.6 [CI 9.4–54.4] and 40.3 [CI 22.2–73.3]). For strain 44/76-SL, GMTs were low in all groups (GMT 7.2 [CI 3.1–16.9] and 42.0 [CI 25.2–69.9] in the infant group and the infant + preschool group, respectively), but percent $\geq 1:4$ were higher in previously vaccinated (78.6% [53.9–100%] and 100% in the infant group and infant + preschool group, respectively) than in the naïve groups (50.0% [22.5–77.5%] and 66.7%







Group	Adolescent regimen
 Infant group	1
 Infant+ preschool group	1
 Adolescent 1	1
 Adolescent 2	2 (Day 0 + 28)

Fig. 4. IgG (A) and IgA (B) memory B cell counts at the four time points in each of the combined group. Individual antigen secreting cells (ASCs) are shown (dots), as well as geometric means and 95% confidence interval against the antigens indicated in the X axis, for each group as indicated for Figs. 2 and 3. Note the difference in scale for the IgA panel.?: not possible to compute, ns: $p > 0.05$ *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$.

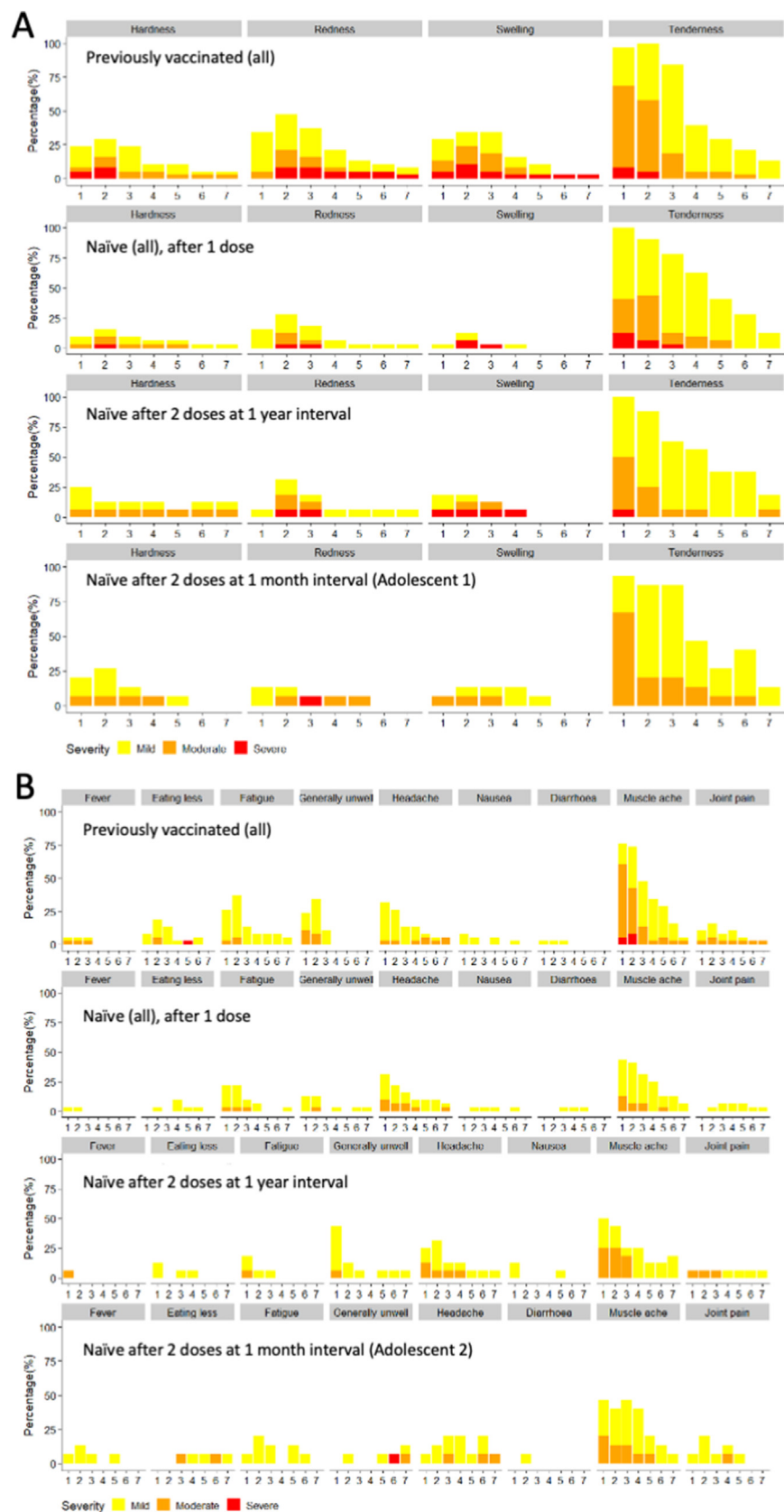


Fig. 5. Severity distribution of solicited local (A) and systemic (B) AEs by combined groups. Previously primed participants are indicated in the top panel. The second row depicts the reactogenicity of naïve participants after their first injection (Prime). The last two rows depict the reactogenicity in naïve participants after their second injection in adolescence, with a year interval (G7-Boost) or a month interval (G8-Boost).

[39.6–93.7%]). SBA responses against strain NZ98/254 were low in all groups (GMT 7.2 [3.9–13.4] and 17.5 [10.8–28.5] in the infant group and infant + preschool group, respectively, and 8.3 [4.2–16.6] and 6.6 [3.1–14.3] in the two naïve groups), and the percentage of participants with titers $\geq 1:4$ were no higher in previously vaccinated (78.6% [54.0–100.0%] and 91.3% [78.9–100.0%] in the infants and the infant + preschool group respectively) than in the naïve groups (81.2% [59.8–100.0%] and 66.7% [39.6–93.7%]).

3.4. Those immunised in infancy who also received a preschool booster have the most robust SBA responses induced by a single dose in adolescence

One of the two groups of naïve participants received the full adolescent course of two 4CMenB doses one month apart (group labelled in red in Figs. 2 and 3), while the other naïve group comprised participants who received a single adolescent dose at day 0. The second dose in naïve participants increased the proportion of participants with a titer $\geq 1:4$ as compared with only one dose at day 180 strain against 44/76-SL (53.3% [24.7–81.9%] after two doses and 13.3% [0.0–32.8%] after one dose) as well as against strain 5/99 (100% after two doses and 46.7% [18.1–75.3] after one dose), as expected (Table 4, Figure 2 and 3, D180). The difference for strain NZ98/254 was smaller (53.3% [24.7–81.9] after two doses and 33.3% [6.3–60.4%] after one dose).

Among those who had been vaccinated in early childhood, the highest SBA titers against strains 44/76SL and 5/99 at day 180 were observed in the infant + preschool group (GMTs 8.2 [4.8–14.2] against strain 44/76-SL and 316.1 [CI 158.4–630.8] against strain 5/99). The responses induced in the infant only group was also high against strain 5/99 (GMT 61.1 [CI 14.8–252.4]), but were lower against the two other strains (GMT 2.5 [1.2–5.4] against strains 44/76-SL and NZ98/254). While SBA GMTs after a single adolescent dose in participants in the infant group were higher (GMT 2.5 [1.2–5.4] against strain 44/76-SL and 61.1 [14.8–252.4] against 5/99) than responses observed in naïve participants (GMT 1.45 [0.9–2.3] against 44/76-SL and 2.78 [1.9–4.1] against 5/99, Table 4), they were still lower than in naïve participants who received the 2-dose adolescent schedule (GMT 4.6 [1.9–11.4] against 44/76-SL and 57.5 [45.5–72.7] against strain 5/99).

At day 365, the SBA titers to strains 4476-SL and NZ98/254 were low in all groups (Table 4, Figure 2, D365), with the lowest proportion of participants retaining titers ≥ 4 (25% [1.2–48.8%]) observed in the naïve adolescents who received a single dose (Table 4, Fig. 3 and Supplementary Figs. 1 and 2).

3.5. Memory B cell responses in adolescence and impact of the childhood vaccination

The IgG, IgA and IgM memory B cell numbers against the individual antigens fHbp, NadA, PorA and NHBA were enumerated in all participants at the same timepoints: before the adolescent dose, 28 days after a single adolescent dose, and at 6 months and 12 months after one or two adolescent doses (Fig. 4). Prior to the adolescent dose, 29.6% (8/27) of naïve participants had low levels of circulating IgG memory B cells against one or more antigens, and 45.5% (15/33) of previously vaccinated participants had detectable memory (Fig. 4A, before adolescent dose).

The IgG memory B cell numbers increased 28 days after 4CMenB immunisation with 90.6% (28/31) of the previously vaccinated participants having measurable memory B cells responses to one or more antigens, compared with 65.2% (15/23) of the naïve participants. Memory B-cell counts were higher against fHbp and NadA than the other antigens (Fig. 4A, D28), particularly in the infant + preschool group (Fig. 4 and Supplementary Fig. 3).

The IgG memory B cell responses then decreased over the year follow up, with no notable difference between groups at days 180 and 365 (Fig. 4). There were little to no changes in IgA and IgM B cell responses in the participants during the course of the trial (Fig. 4B and supplementary Fig. 3).

3.6. 4CMenB reactogenicity in pre-vaccinated and naïve adolescents

The safety analysis is presented in three groups: 1. The adolescent booster in previously vaccinated participants (all); 2. First dose in naïve participants (priming, all); and 3. Second dose in naïve participants after a month interval (Adolescent 2), and after a year interval (Adolescent 1 received a second dose of 4CMenB at the end of the study for ethical reason). The vaccine induced expected local and systemic adverse reactions as previously described, with most participants reporting tenderness and muscle ache on day 1, decreasing thereafter (Fig. 5). Almost all the AEs were settled by day 7 except tenderness and myalgia. The details of grading of local and systematic AEs are shown in Supplementary text. There were 8 mild unsolicited AEs recorded in the 7 days after vaccination, including 3 related to vaccination. None of the 8 unsolicited AEs were judged serious. There was a trend for more reactogenicity in the groups that were primed during childhood (infant and infant + preschool groups) as compared with naïve participants, but no formal statistical assessment has been made due to the low number of participants in the study. Five serious adverse events were reported, none related to the study vaccine (Supplementary Table 2).

4. Discussion

In the near future almost all UK children will have had priming with 4CMenB in early childhood. In this study we investigated whether a single booster dose of the group B meningococcal vaccine, 4CMenB (Bexsero®) for adolescents primed during early childhood could generate protective SBA titers against this deadly disease when compared with vaccination of naïve individuals. We show that bactericidal antibodies do rise with a single teenage booster. Though the sample size was too small to assess statistical significance, the adolescent booster in previously vaccinated generally induced better responses than a single dose in naïve individuals, and initial responses and persistence were better when a preschool dose had been previously administered after an infant schedule.

No safety concern was raised with up to six doses of this vaccine with the last dose administered at 11 years. In this study, adolescent participants received a fourth, fifth or sixth dose of vaccine, and while the sample size is small, the reactogenicity is consistent with previous reports. The safety of up to three doses of 4CMenB in children has been demonstrated in the UK three years after the inclusion of this vaccine in the routine immunisation program [14]. No safety concern was raised after a combined three million doses were administered to 1.3 million children. Moreover, the tolerability profile of two and three doses of 4CMenB in adolescents and young adults was reported previously, and no safety concern was raised [15,16].

Before the adolescent dose, persistent protective SBA responses could be detected in a proportion of adolescents who were vaccinated during childhood against one of the three indicator strains (5/99). The highest baseline protective SBA responses to this strain were observed in participants who had previously received their last dose when they were 3 years of age. The relatively sustained bactericidal activity against strain 5/99, and slower decline when compared to other indicator strains has been described previously up to seven years post 4CMenB [16], one hypothesis being because

NadA is over-expressed in strain 5/99 and induces especially robust responses. Nevertheless, the contribution of NadA to the predicted 4CMenB vaccine coverage is currently low, as shown in Australia [17], England and Wales [18] and Italy [19], due to the low proportion of strains with NadA [17,19].

After the adolescent dose in previously primed teenagers, the strongest responses were seen in those who had been primed in infancy and then boosted at three years of age. These robust responses, especially to strain 5/99 may be linked with a better memory response elicited at a later age for the last dose received [20–22]. Previously, Perret *et al.* found that the strongest maintenance of MenC SBA antibodies occurred among children who were immunised at over six years of age in the UK, again suggesting age of final vaccination might be critical in driving sustained immune protection [23]. In children and adolescents, a capsular group C (MenC) meningococcal conjugate vaccine was shown to elicit better persistence of IgG with age of vaccination, including against the carrier protein [20,24]. MenACWY-TT vaccine induced higher B cell responses and memory in children vaccinated at 3–5 years of age than was observed among children vaccinated at 12–15 months of age [25]. It would also be interesting to understand if there is an advantage for those subjects who received 3 or 4 doses as infants as compared with those who received only one dose at 12 months.

The SBA titers elicited in both primed and naïve adolescents by a single dose at 11 years of age against the other target strains, 4476-SL and NZ98/254, were not as strong as against strain 5/99 (mostly below 1:256 at day 28), and declined substantially by six months (below 1:64 at days 180 and 365). Similarly, in infants, the SBA titers decline six months after two doses of 4CMenB, especially to the PorA target strain (here represented by strain NZ98/254). Conversely in the large phase III study in adolescents, higher SBA titers and a higher proportion of participants with titers ≥ 4 were observed at six months post second dose than the titers and proportions observed in the present study [15]. It is not clear whether the differences noted in the current study relate to the small sample size or differences in responses in Chilean and UK adolescents. A decline of SBA responses against these strains was observed in healthy adults just four months after the second dose of 4CMenB [26]. Previously, the OMV vaccine used during the epidemic in Norway induced 87% efficacy, but the efficacy declined to 57% after 29 months [27], and the SBA titers induced by the OMV vaccine used in New Zealand against strain NZ98/254 also declined within the first year following vaccination (reviewed in [28]). More recently, 4CMenB was used to tackle an outbreak in a University in the US. Analysis of SBA responses in the vaccinated teenagers revealed that seven months after two doses of 4CMenB, protective titers were observed in less than 40% for some of the strains tested (including the local outbreak strain) [29], although these were not the indicator strains studied herein.

Memory B cell responses to 4CMenB have not been investigated in adolescents previously. In infants, a two-dose 4CMenB schedule at six and eight months induced an increase of specific memory B cell responses to all four antigens as compared with pre-vaccination levels [30]. Here, an increase in memory B cells responses against all four antigens was observed at day 28 after a single adolescent dose of 4CMenB in both previously vaccinated and naïve adolescents. The increase appeared weaker against PorA (presented in the OMV component of 4CMenB) as compared with the other three components of the vaccine. This agrees with reports that OMV vaccines are poor inducers of memory B-cell responses [31] and antibody persistence [32], and that the OMV component in 4CMenB induces shorter antibody persistence as compared with NadA [8,16,33].

The main weakness of this study is the modest number of participants who were available to participate from the previous early phase studies in 2006. Therefore, the study was not powered for formal statistical analysis. Blood samples were not collected one-month post second dose in naïve participants, thus limiting the comparisons in peak antibody response between the two-dose schedule in naïve participants and one-dose schedule in previously primed participants. Comparison of persistence of SBA responses however was evaluated at six and 12 months. While the study was not blinded to clinical staff and participants recruited from previous studies, the laboratory staff processing the samples were blinded.

5. Conclusions

The 4CMenB infant vaccine programme was introduced in the UK in September 2015. An adolescent programme, requiring administration of a two-dose schedule for naïve adolescents, was not initiated because it was unlikely to be cost-effective at this age. This study provides the first data on the potential of a single vaccination in the second decade of life to provide protection of adolescents against group B meningococcal disease by boosting the immune response induced during infant vaccination. Although the sample size is small, the results suggest that there was poor persistence of SBA responses prior to the adolescent dosing, that boosting adolescents with a single dose, following a 4CMenB schedule in infancy did not appear to induce strong or persistent protective responses. These results suggest that B cell memory responses may not be adequately primed at less than 12 months of age. However, children who received priming in infancy plus a preschool booster have improved responses to a single dose of 4CMenB in the second decade of life.

Contributors.

AJP conceived the study question. AJP, CR, MDS, KF, RB contributed to the study design. CD and RB led the experimental work, performed by CD, AL, LSR, LB, EC, AH, HC, HH, including acquisition of data. KD and KF led the clinical work including acquisition of data. XL and DO'C conducted all statistical analysis. CR wrote the first draft of the manuscript. All authors contributed to subsequent drafts, interpretation of the findings, revising it critically and approved the final version.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data sharing the authors commit to making the relevant anonymised patient level data available on reasonable request.

Conflict of interest statement: AJP is Chair of UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI), and is a member of the WHO's SAGE. The views expressed in this article do not necessarily represent the views of DHSC, JCVI, or WHO. The University of Oxford has entered into a partnership with AstraZeneca on coronavirus vaccine development. AJP, CR and CD are named inventors on a patent in the field of meningococcal vaccines. AJP waives all his rights on any patent. MDS is, or has recently been, an investigator on behalf of the University of Oxford on clinical research related to vaccines that is funded by vaccine manufacturers including Pfizer, GlaxoSmithKline, Medimmune, Novavax, Janssen, AstraZeneca and MCM vaccines. RB & AH perform contract research on behalf of UK HSA for GSK, Pfizer and Sanofi Pasteur.

LB, AL, LSR, LC, KD, KF, XL, DO'C, HC and HH declare no conflict.

The views expressed in this publication are those of the authors.

All authors attest they meet the ICMJE criteria for authorship

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2022.04.085>.

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