

Persistence of immunity following immunisation with a capsular group B meningococcal vaccine in three different toddler schedules: follow-up of a previous randomized-controlled trial

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Competing Interests

M Sadarangani has been an investigator on investigator-initiated research studies funded by Pfizer and his institution received research grants from GlaxoSmithKline Biologicals SA. M D Snape, A Finn, P T Heath, G Bona, S Esposito, J Diez-Domingo, R Prymula act as investigators for clinical vaccine studies from both non-commercial funding bodies and commercial sponsors (i.e. some or all of Novartis Vaccines and Diagnostics S.r.l. [now a member of the GSK group of companies], GlaxoSmithKline Biologicals SA, Janssen, Sanofi-Aventis, Sanofi-Pasteur MSD, MedImmune, Pfizer Vaccines, Alios Bio Pharma and Ablynx NV) conducted on behalf of their institutions as listed in the affiliations. M D Snape participates in advisory boards and speaking engagements for vaccine manufacturers; all payments received are paid to his institution. G Bona declares being paid by Novartis Vaccines and Diagnostics through his institution for lectures. Before October 2014, A Finn undertook paid consultancy and speaking engagements for vaccine manufacturers,

all income was paid to his employers. Owing to his membership of the UK Department of Health's (DH) Joint Committee on Vaccination and Immunization (JCVI), A Finn no longer gives talks or undertakes advisory work for industry, either paid or unpaid. R Prymula, J Diez-Domingo, and S Esposito also undertake consultancy and advisory work and receive speaking honoraria, travel and accommodation reimbursements for several commercial sponsors (i.e. some or all of GlaxoSmithKline Biologicals SA, Pfizer, Novartis Vaccines and Diagnostics, MedImmune. M A Iro has received travel grants from GlaxoSmithKline Biologicals SA for attendance at conferences. The NIHR Oxford Biomedical Research Centre provides salary support for M D Snape, who is a Jenner Investigator. A J Pollard is a Jenner Investigator and James Martin Senior Fellow and has previously conducted research on behalf of Oxford University funded by vaccine manufacturers (Novartis Vaccines and Diagnostics, Pfizer, Sanofi Pasteur. A J Pollard, P T Heath and A Finn do not receive any personal remuneration from vaccine manufacturers. A J Pollard is chair of the UK Department of Health's (DH) Joint Committee on Vaccination and Immunization (JCVI); the views presented in this manuscript do not necessarily represent the views of DH or JCVI. A Oduyungbo is former employee of Novartis Vaccines and Diagnostics; D Toneatto is current employee of GSK group of companies and declares ownership of restricted shares. T Sell and M Voysey declare no competing interests.

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Abstract

Background: One schedule for the capsular group B meningococcal vaccine, 4CMenB, is 2 doses 2 months apart for children 12-23 months of age, with a booster dose 12-24 months later. This study provides the first data on persistence of human serum bactericidal antibody (hSBA) titres up to 4 years of age after initial doses at 12-24 months, and immunogenicity of a booster dose at 48 months of age compared with vaccine-naïve children.

Methods: Children previously immunised with 2 doses of 4CMenB at 12-24 months of age received a booster at four years of age. Vaccine-naïve age-matched toddlers received 2 doses of 4CMenB. hSBA titres against reference strains H44/76, 5/99, NZ98/254 and M10713 were evaluated at baseline and 30 days after one dose of 4CMenB (all), and one month after the second dose (vaccine-naïve recipients).

Results: Of 332 children in the study, 123 had previously received 4CMenB and 209 were vaccine-naïve controls. Prior to the booster, the proportions of participants (comparing previously vaccinated groups with controls) with hSBA titres $\geq 1:5$ were 9-11% vs 1% (H44/76), 84-100% vs 4% (5/99), 0-18% vs 0% (NZ98/254) and 59-60% vs 60% (M10713). After one dose of 4CMenB in previously immunised children the proportions achieving hSBA titres $\geq 1:5$ were 100% (H44/76 and 5/99), 70-100% (NZ98/254) and 90-100% (M10713).

Interpretation: Waning of hSBA titres by 4 years of age occurs following 2 doses of 4CMenB administered at 12-24 months. A pre-school booster would then be necessary to maintain hSBA titres $\geq 1:5$ among those who are at increased risk of disease.

Trial registration: ClinicalTrials.gov (NCT01717638)

Introduction

Neisseria meningitidis causes meningitis and septicaemia¹, with very rapid disease onset, high case-fatality rate^{2, 3} and increased rates of long-term neurological and non-neurological sequelae among survivors⁴⁻¹¹. Conjugate vaccines have been remarkably successful over the last 15 years with near elimination of endemic disease in countries with high coverage group C vaccine programmes¹² and marked impact of group A vaccines in Africa¹³. The majority of disease in many endemic countries is now caused by capsular group B *N. meningitidis* (MenB)¹⁴. A recently licensed vaccine (4CMenB) designed primarily to prevent MenB infection was introduced into the routine infant immunisation schedule in the UK in September 2015¹⁵ and has also been used in a response to hyperendemic MenB disease in the Saguenay-Lac-Saint-Jean region of Quebec, Canada¹⁶. One study suggested that the mean coverage of MenB strains by 4CMenB would be 78% across 5 European countries and 66% in Canada^{17, 18}. The vaccine is highly immunogenic and ecological data on the effectiveness in the UK are awaited. If evidence of substantial impact against disease is observed, use of the vaccine in older children might be considered for catch-up campaigns or outbreaks. Information on duration of protection is therefore needed.

The recommended schedules for 4CMenB include a regime of two doses at least two months apart for children aged 12-23 months, followed by a booster dose 12-23 months thereafter¹⁹. At present there are no data on the persistence of bactericidal antibodies (on which the correlate of protection is based) through to the pre-school period following vaccination of toddlers.

The aims of this follow-on study were to assess: (i) the persistence of human serum bactericidal antibody (hSBA) titres at 4 years of age in children previously immunised with two doses of 4CMenB two months apart in the second year of life, compared with age-matched vaccine-naïve children; (ii) the percentage of children with hSBA titres $\geq 1:5$ following a booster dose of 4CMenB at 4 years of age; (iii) the proportion of vaccine-naïve 4-year old children achieving hSBA titres $\geq 1:5$ following two doses of 4CMenB; (iv) adverse reactions after 4CMenB in 4-year old children.

Methods

Locations

This study (NCT01717638) was conducted at 31 centres in the Czech Republic, Italy, Spain and the UK between November 2012 and October 2013 (Supplementary Table 1).

Participants

This study was part of a larger study, of which the primary outcome was persistence of hSBA titres in infants given 3 priming doses of 4CMenB as infants and a booster dose at 12, 18 or 24 months, reported elsewhere²⁰.

Participants involved in a previous follow-on study were invited to take part in this study. In the previous study three cohorts received two doses of 4CMenB at 12 and 14 months (n=300), 18 and 20 months (n=50), or 24 and 26 months (n=50)²¹. A further 190 vaccine-naïve participants were recruited as age-matched controls (Figure 1). Inclusion criteria: healthy child aged 48-59 months; previous receipt of 2 doses of 4CMenB in the previous study ('follow-on' participants) or no doses of

4CMenB ('vaccine-naive' participants). Exclusion criteria: previously ascertained or suspected disease caused by *N. meningitidis*; household contact and/or intimate exposure to an individual with laboratory-confirmed *N. meningitidis*; previous allergic reaction to any vaccine component; serious chronic or progressive disease; known/suspected immunosuppression; participation in another clinical trial within 90 days prior to enrolment or during the study; family member of research staff.

Vaccine

The investigational vaccine was 4CMenB (Bexsero™, GSK Vaccines). It contains 50 µg each of three proteins - Neisseria heparin binding antigen (NHBA), Neisserial adhesin A (NadA) and factor H binding protein (fHbp) - and 25 µg of outer membrane vesicle (OMV) from *N. meningitidis* strain NZ98/254, plus aluminum hydroxide¹⁹. The vaccine was administered as 0.5 ml intramuscularly.

Procedures

Follow-on participants received one dose of 4CMenB and had blood samples obtained prior to and 30 days post-vaccination. Vaccine-naïve participants received two doses of 4CMenB, two months apart, and had blood samples taken immediately before the first immunisation and one month after each dose.

For 7 days post-vaccination parents recorded adverse events (AEs) and graded their severity. Solicited local AEs were injection site pain, erythema, induration and swelling. Solicited systemic AEs were fever (axillary temperature $\geq 38^{\circ}\text{C}$), change in eating habit, sleepiness, vomiting, diarrhoea, irritability, arthralgia, headache and rash. AEs requiring a physician's visit and use of antipyretic and/or analgesic

medication were recorded. The relationship of AEs to the study vaccine was determined by the study investigators, considering temporal relationship and biological plausibility.

Serum bactericidal antibody

Immunogenicity was assessed by measuring hSBA titres, using human serum as the source of exogenous complement²². Assays were performed at Novartis Vaccines and Diagnostics (GmbH, Marburg, Germany). The fHbp response was assessed with strain H44/76, NadA with strain 5/99, PorA with NZ98/254 and NHBA with M10713.

Statistical analysis

The percentage of children at each blood sampling time point with hSBA titres $\geq 1:5$ and associated 2-sided 95% exact Clopper-Pearson confidence intervals (CIs) were calculated for each indicator strain. hSBA geometric mean titres (GMTs) were calculated and geometric mean ratios (GMRs) by comparison of post-immunisation and pre-immunisation values. GMT and GMR with associated 95% CIs were computed by taking anti-logs. Sample size for the follow-on participants was determined by the number of participants in the previous study whose parents were willing to take part. Though the primary aim was descriptive, the secondary aim in the vaccine-naïve cohort was to demonstrate a “sufficient” immune response following two doses of the vaccine, pre-defined as $>70\%$ of participants with hSBA $\geq 1:5$. Power calculations were done assuming the actual percentage would be 80%, so 162 participants were required in the vaccine-naïve cohort to obtain 79% power (5% alpha) to demonstrate a sufficient immune response against NZ98/254,

providing >99.9% power for strains H44/76 and 5/99. Assuming a 15% drop-out rate, enrolment of 190 participants in the vaccine-naïve control group was required.

Ethics

Written, informed consent was obtained from the parents or legal guardians of participants. The study was implemented according to ICH. Ethical approval was obtained from independent review committees at all study centres.

Results

Study population

Of 304 children invited, 123 were recruited into the follow-on cohort, of whom 100 received their first dose of 4CMenB at 12 months (group 1), 11 at 18 months (group 2) and 12 at 24 months (group 3) – 122/123 (99%) completed the study (Figure 1). The vaccine-naïve cohort (group 4) included 209 children, of whom 190 (91%) completed the study. The demographics of the groups were broadly similar (Table 1).

Immunogenicity

Persistence of hSBA titre $\geq 1:5$ at 4 years of age in the follow-on cohort was 9-11% against H44/76, 84-100% against 5/99, 0-18% against NZ98/254 and 59-60% against M10713 (Figure 2). In the vaccine-naïve cohort, 0-5% had hSBA titres $\geq 1:5$ against H44/76, 5/99 and NZ98/254 pre-vaccination, and 60% against M10713 (Figure 2). The hSBA GMTs were <5 in all groups for H44/76 and NZ98/254, but were ≥ 5 for 5/99 and M10713 (Figure 3). hSBA GMTs in the vaccine-naïve cohort

before the first dose were similar to the follow-on cohorts at the same time point, except strain 5/99 (1.15 vs 23-69) (Figure 3).

After the (3rd) dose of 4CMenB at 4 years of age, 100% of participants had an hSBA titre $\geq 1:5$ against H44/76 and 5/99, 70-100% against NZ98/254, and 90-100% against M10713; 24-90% reached this after their first dose in the vaccine-naïve cohort (Figure 2). Against H44/76, 5/99 and NZ98/254, $\geq 90\%$ of participants in all follow-on groups (with the exception of group 2 (primary doses at 18 and 20 months of age) against strain NZ98/254) had ≥ 4 -fold rise in hSBA titre after a single booster dose (Supplementary Figure 1). For strain M10713, 56%-67% had a 4-fold rise in hSBA titre after the booster. A single dose of 4CMenB at 4 years of age in vaccine-naïve children resulted in a 4-fold rise in hSBA titre in 63% against H44/76, 86% against 5/99, 17% against NZ98/254 and 21% against M10713. A second dose in this previously unvaccinated cohort resulted in 91-100% achieving an hSBA titre $\geq 1:5$, and a 4-fold rise from baseline in 51-100% of participants, depending on the target strain (Supplementary Figure 1). In previously vaccinated individuals, this third dose resulted in hSBA GMTs similar to or higher than the GMTs achieved 1 month after the 2nd dose (Figure 3). After 2 doses in the vaccine-naïve group, GMTs were lower or similar to the post-booster dose values in the follow-on groups (Figure 3). The GMRs comparing pre- to post-booster vaccine responses in the follow-on groups were highest for strain H44/76 (67-133) and lowest for strain M10713 (5.24-7.35) (Table 2). In the vaccine-naïve cohort, GMRs were similar or higher after two doses compared with post-booster in the follow-on groups, with highest GMRs observed with strain 5/99 (GMR=299) and lowest with M10713 (GMR=5.12).

Reactogenicity

The most commonly reported local AE was injection site pain, occurring in 114/121 (94%) of the follow-on cohort overall after the booster dose, and in 185/205 (90%) of the vaccine-naïve cohort after the first dose and 157/194 (81%) after the second dose (Table 3). Sleepiness and irritability were the most common systemic AEs overall, occurring after 200/519 (39%) and 188/518 (36%) doses, respectively (Table 3). Fever $\geq 38.0^{\circ}\text{C}$ occurred in 25/121 (21%) of the follow-on cohort after a single dose, and in 20/204 (10%) of the vaccine-naïve cohort after the first dose and 16/189 (8%) after the 2nd dose (Table 3). Fever treatment was given after 73/518 (14%) vaccine doses overall, preventive therapy before 49/517 (9%) doses and medical attention for fever was sought in 9/517 (2%) cases (Table 3).

There were three reported serious AEs, all resulting in hospitalisation and occurring in the vaccine-naïve cohort. None were considered vaccine-related. One child developed croup 60 days after the first dose; another child had a head injury with concussion, contusion and periorbital haematoma 23 days after the first dose; a third child required IV fluids for gastroenteritis and dehydration three days after the second dose of vaccine. There was one withdrawal (parental decision) due to an AE – a child in the vaccine-naïve cohort with moderate injection site pain after the first vaccination.

Interpretation

This is the first study to describe waning of bactericidal antibodies in children two years or more following immunisation with two dose regimens of 4CMenB in the second year of life, suggesting the need for a booster dose to optimise protection

during early school years for children at elevated risk of disease. This schedule is currently recommended in the UK for 12-23 month-old children at high risk of meningococcal disease²³ and has been used in Quebec, Canada¹⁶. A single booster dose at 4 years of age was sufficient to boost hSBA titres to protective levels in the majority of previously vaccinated children, and two doses in vaccine-naïve children provided similar protection, supporting the currently licensed schedule in this age group.

One previous study in Europe found variable waning of bactericidal antibodies in the first 12 months after two doses of 4CMenB given at 12 and 14 months or 13 and 15 months²⁴. After 12 months, 56-75% had hSBA titres $\geq 1:5$ against H44/76, 94-97% against 5/99 and 6-18% against NZ98/254. With our data this suggests that waning of bactericidal antibody against fHbp most commonly occurs >12 months post-vaccination, whereas waning of anti-PorA antibodies occurs almost entirely within 12 months in this age group. Little waning of anti-NadA antibodies was seen up to 26 months post-vaccination. This was unlikely due to ongoing boosting by natural exposure, since only 4% of 4 year old vaccine-naïve children had hSBA $\geq 1:5$ against strain 5/99. Persistence of the vaccine-induced anti-NHBA response was poor as similar proportions of the vaccine-naïve and follow-on cohorts had bactericidal antibodies against strain M10713 in this study. In the only other published study of antibody persistence in this age group, hSBA titres $\geq 1:4$ occurred in 0-38% (depending on target strain) of three-year old UK children after one dose of 4CMenB at 12 months of age – confirming the need for two doses in this age group²⁵. Similar patterns of antigen-dependent differential waning of bactericidal antibodies have been reported after two doses of 4CMenB at 40 months of age^{26, 27}.

Similar persistence of antibodies at four years of age has been described in children after three infant doses and a booster in the second year of life²⁰. This suggests that 3 doses in infants plus a booster is broadly equivalent to two doses in the second year of life with respect to antibody persistence to four years. However, this latter regime provides no protection to young infants who have the highest incidence of disease²⁸. While further doses after four years may be required in individuals at elevated risk of disease, incidence rates in immunocompetent children aged ≥ 5 years remain very low, until a small increase in adolescence in some populations²⁸.

Our data suggest that antibody persistence following two doses at 24 months is similar to immunisation at 12 or 18 months, although the numbers were small. At present, boosters are not recommended for children receiving their first dose beyond 23 months of age²³. In combination with the finding of significant waning of bactericidal antibodies in children aged 3 years who received two vaccine doses by 5 years of age^{26, 27}, these results suggest children receiving two doses of 4CMenB at 2-3 years of age should receive further boosters at 4-5 years if ongoing protection is required. Persistence data are needed for 4-10 year-old children.

The most common local AE in this study was injection site pain, with rates similar to those found in previous studies of 4CMenB in 3-year and 5-year old children²⁵⁻²⁷.

The rate of post-immunisation fever was also similar to previous data²⁵.

Limitations

The major limitation was the low proportion of participants in the follow-on cohort from those who completed the previous study (42% in group 1, 22% in group 2, and 24% in group 3). There is therefore the potential for selection bias as participants who tolerated the previous vaccinations better are more likely to take part. The group sizes for those receiving priming doses at 18 and 24 months of age were small, making comparisons between the follow-on groups difficult – within this limitation, there were no significant differences between the groups. Further studies would be required to explore differences between schedules in the second year of life.

Conclusion

Two doses of 4CMenB given at 12-24 months prime the immune system against the vaccine antigens so that there is a booster effect following a single dose 2 years later. This regime appears equivalent to 3 infant doses plus a single booster dose at 12 months in protecting children from 1 to 4 years of age, but lacks the earlier protection provided by the infant schedule. The rates at which serum antibody titres to the different vaccine antigens wane vary widely, although the implications of this for vaccine effectiveness is yet to be established. Children receiving their first doses at 2-3 years of age may require further booster doses if ongoing direct protection beyond 4-5 years is required, as may be the case for those in high risk groups, although further data in larger cohorts are required to confirm this.

Bexsero is a trademark of the GSK group of companies.

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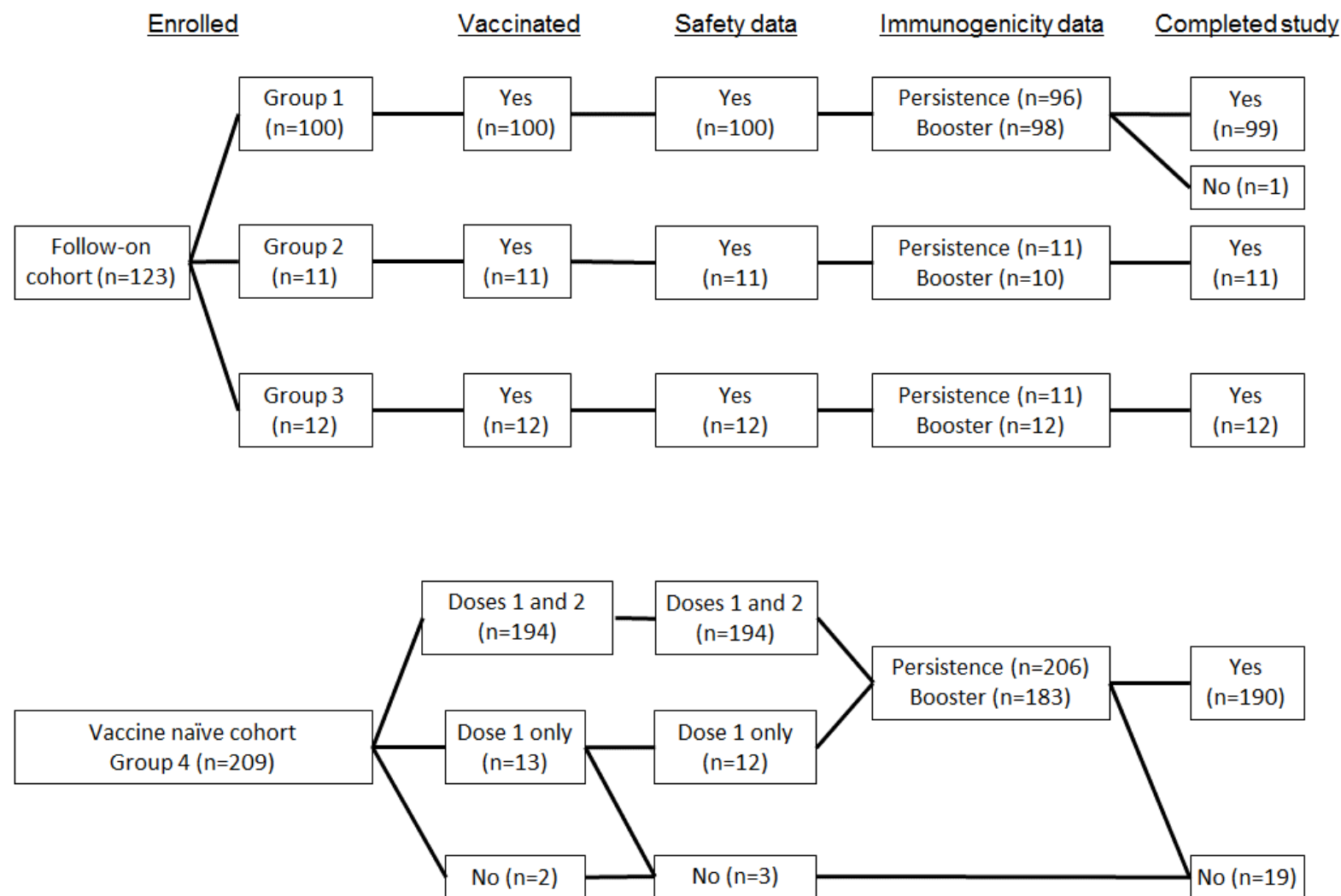
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<http://ecdc.europa.eu/en/publications/Publications/Surveillance%20of%20IBD%20in%20Europe%202012.pdf>. Accessed on 08 Oct 2016.

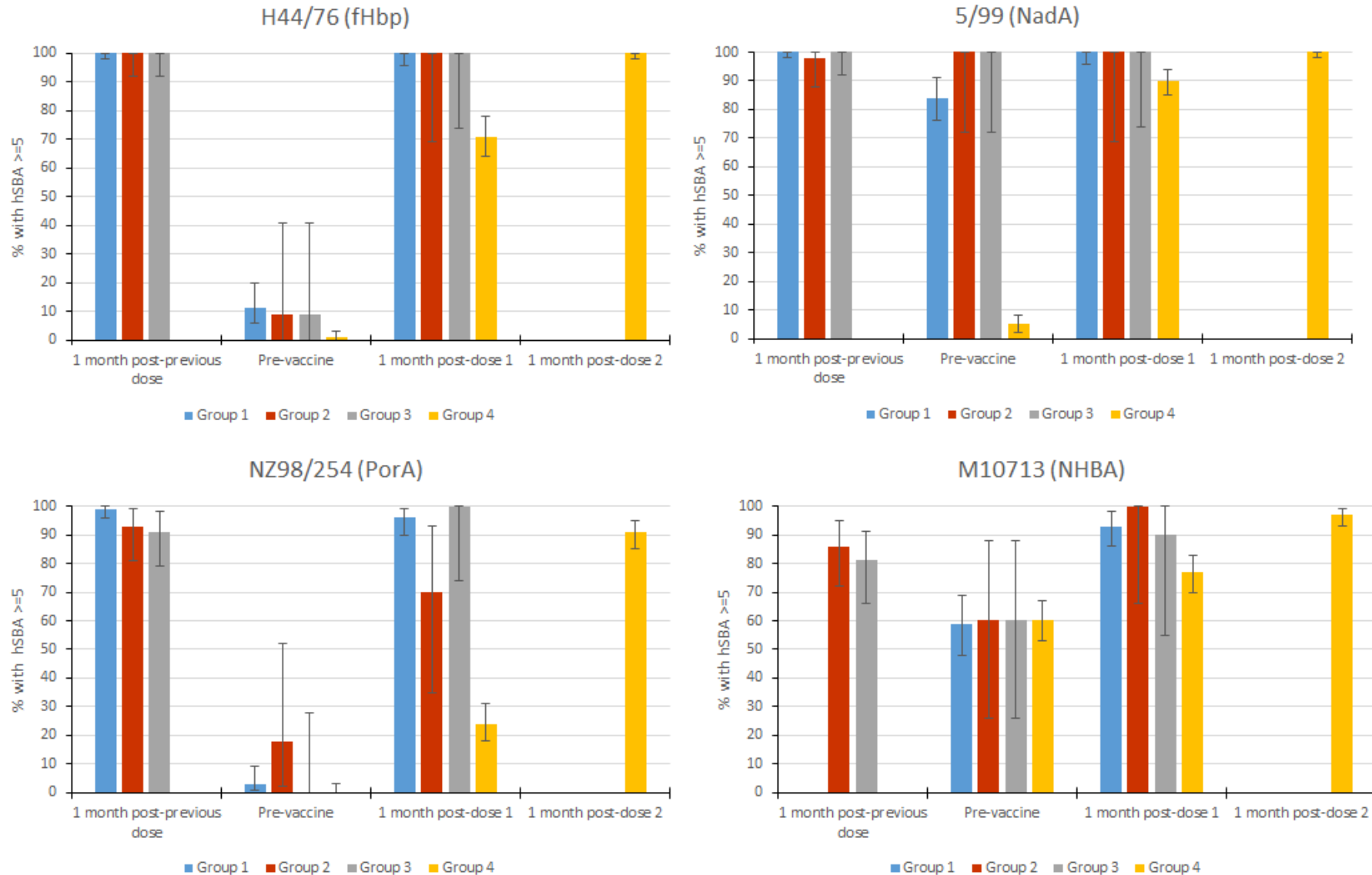
Figures

Figure 1. Participant flow diagram



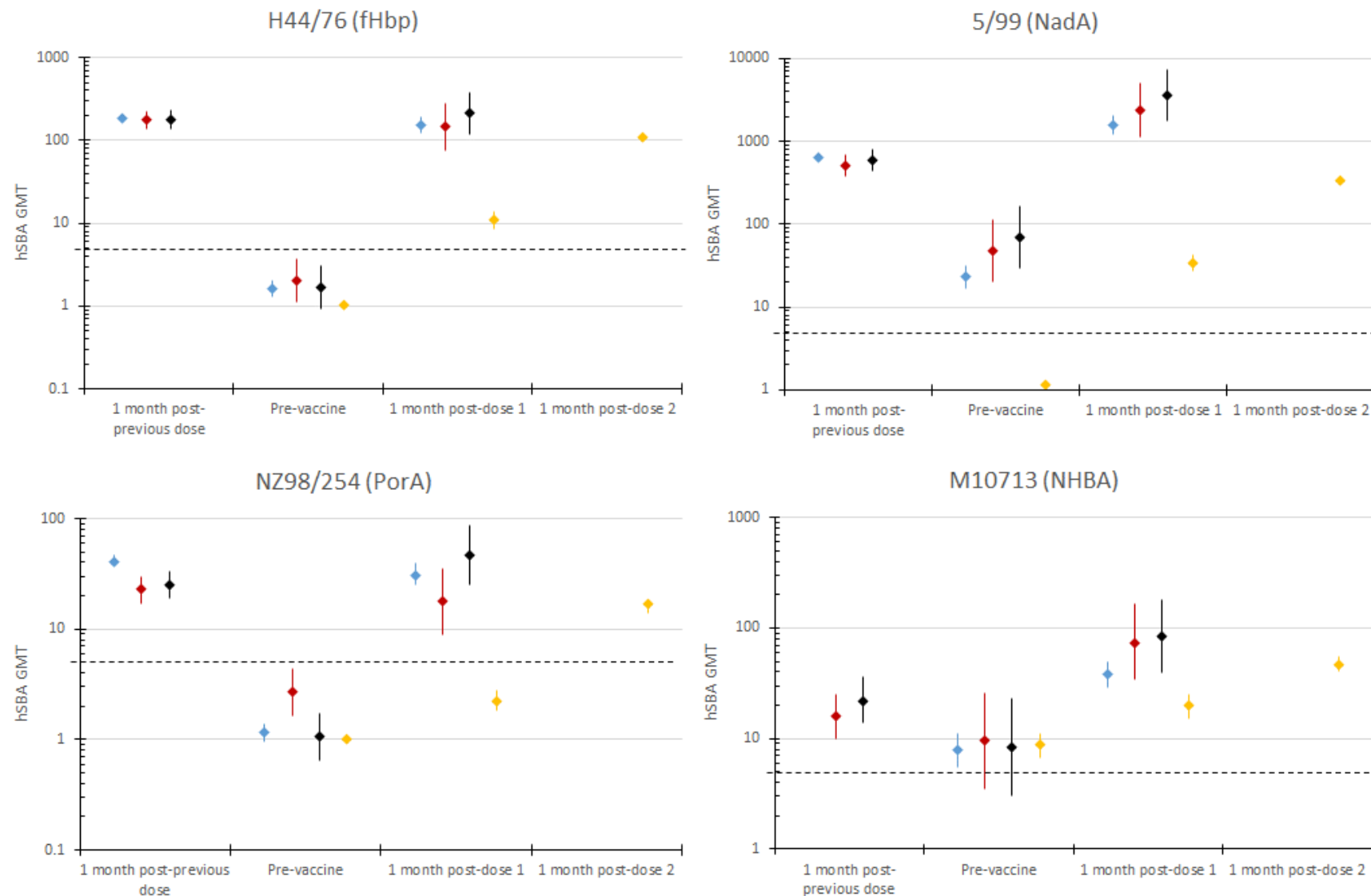
The follow-on cohort all received two previous doses of 4CMenB at 12 and 14 months (Group 1), 18 and 20 months (Group 2) or 24 and 26 months (Group 3). The vaccine naïve cohort had not previously received any 4CMenB doses. All children received one dose at 4 years of age, the vaccine naïve cohort given an additional dose two months later. Blood for persistence analysis was taken prior to any doses at four years of age and for booster analysis 30 days after each dose. Safety data were collected after each dose.

Figure 2. Proportion of participants with human serum bactericidal antibody (hSBA) titre $\geq 1:5$ at each time point against each strain



Proportion of participants with hSBA titre $\geq 1:5$ at 1 month post-previous dose (Groups 1, 2 and 3 only) based on data from Snape *et al.*²¹ Time points from this study include pre-vaccine (before first dose, all groups), 1 month post-dose 1 (all groups) and 1 month post-dose 2 (Group 4 only). Bars represent overall percentage of subjects achieving an hSBA titre $\geq 1:5$, with error bars representing 95% confidence intervals. Data shown separately for the four indicator strains H44/76 (fHbp response), 5/99 (NadA response), NZ98/254 (PorA response) and M10713 (NHBA response). hSBA, human serum bactericidal antibody.

Figure 3. hSBA geometric mean titre (GMT) at each time point against each strain



hSBA GMT at 1 month post-previous dose (Groups 1, 2 and 3 only) based on data from Snape *et al.*²¹. Time points from this study include pre-vaccine (before first dose, all groups), 1 month post-dose 1 (all groups) and 1 month post-dose 2 (Group 4 only). Points represent overall GMT, with error bars representing 95% confidence intervals. Data shown separately for the four indicator strains H44/76 (fHbp response), 5/99 (NadA response), NZ98/254 (PorA response) and M10713 (NHBA response). hSBA titre of 1:5 shown as horizontal dashed line on each graph to enable comparison as different y-axis scale on each graph. hSBA, human serum bactericidal antibody; GMT, geometric mean titre.

Tables

Table 1. Participant demographics

	Follow-on cohort			Vaccine-naïve cohort
	Group 1	Group 2	Group 3	Group 4
n	100	11	12	209
Ages at previous 4CMenB doses (months)	12, 14	18, 20	24, 26	None
Number of doses in this study	1	1	1	2 (2 months apart)
Age at first dose in this study (months), mean \pm SD	51.7 \pm 3.3	53.4 \pm 4.3	56.8 \pm 1.5	53.7 \pm 3.6
Male sex, n (%)	50 (50)	5 (45)	8 (67)	110 (53)
Caucasian, n (%)	97 (97)	8 (73)	7 (58)	193 (92)
Weight (kg), mean \pm SD	18.1 \pm 2.4 ^a	18.7 \pm 2.4 ^b	18.8 \pm 1.8 ^c	18.1 \pm 2.5 ^d
Height (cm), mean \pm SD	106 \pm 4	107 \pm 4	108 \pm 6	107 \pm 5 ^e

a: n=91; b: n=9; c: n=11; d: n=189; e: n=205

hSBA, human serum bactericidal antibody; SD, standard deviation.

Table 2. Geometric mean ratios (GMRs) of serum bactericidal antibody titres

	Follow-on cohort			Vaccine-naïve cohort	
Strain	Group 1	Group 2	Group 3	Group 4 – Dose 1	Group 4 – Dose 2
H44/76, GMR (95% CI)	99 (79, 125) <i>n</i> =95	67 (34, 135) <i>n</i> =10	133 (68, 258) <i>n</i> =11	10 (8.2, 13) <i>n</i> =175	105 (94, 116) <i>n</i> =175
5/99, GMR (95% CI)	70 (57, 86) <i>n</i> =92	51 (27, 95) <i>n</i> =10	55 (30, 99) <i>n</i> =11	29 (23, 37) <i>n</i> =168	299 (256, 350) <i>n</i> =172
NZ98/254, GMR (95% CI)	27 (21, 36) <i>n</i> =93	5.96 (2.7, 13) <i>n</i> =10	38 (18, 81) <i>n</i> =11	2.25 (1.84, 2.75) <i>n</i> =173	17 (14, 19) <i>n</i> =174
M10713, GMR (95% CI)	5.24 (3.91, 7.02) <i>n</i> =88	7.06 (2.92, 17) <i>n</i> =9	7.35 (3.03, 18) <i>n</i> =9	2 (1.62, 2.46) <i>n</i> =158	5.12 (3.95, 6.65) <i>n</i> =161

GMR represents the ratio of geometric mean titre (GMT) post-immunisation to pre-immunisation. Post-immunisation GMT was measured 1 month after each dose of vaccine and pre-immunisation GMT prior to any doses given in this study. 95% confidence intervals (CIs) were computed by taking the anti-log of the mean and the lower and upper limits of the 95% CI. Data shown separately for the four indicator strains H44/76 (fHbp response), 5/99 (NadA response), NZ98/254 (PorA response) and M10713 (NHBA response). GMR, geometric mean ratios; n, participants with available results.

Table 3. Number and percentage of participants with solicited local and systemic adverse events up to day 7 post-vaccination for each dose (day of vaccination = day 1)

	Follow-on cohort			Vaccine-naïve cohort – Group 4	
	Group 1	Group 2	Group 3	Dose 1	Dose 2
Local symptoms					
Pain – any	94/99 (95%)	9/10 (90%)	11/12 (92%)	185/205 (90%)	157/194 (81%)
Severe¹	19/99 (19%)	1/10 (10%)	1/12 (8%)	27/205 (13%)	21/194 (11%)
Erythema²					
≥25mm	21/99 (21%)	3/10 (30%)	0/12	43/204 (21%)	34/194 (18%)
>50mm	9/99 (9%)	1/10 (10%)	0/12	9/204 (4%)	19/194 (10%)
>100mm	2/99 (2%)	0/10	0/12	1/204 (<1%)	0/194
Induration²					
≥25mm	9/99 (9%)	1/10 (10%)	0/12	26/204 (13%)	20/194 (10%)
>50mm	1/99 (1%)	0/10	0/12	3/204 (1%)	4/194 (2%)
>100mm	1/99 (1%)	0/10	0/12	0/204	0/194
Swelling²					
≥25mm	20/99 (20%)	2/10 (20%)	4/12 (33%)	30/204 (15%)	24/194 (12%)
>50mm	2/99 (2%)	0/10	1/12 (8%)	4/204 (2%)	4/194 (2%)
>100mm	0/99	0/10	0/12	1/204 (<1%)	0/194
Systemic symptoms					
Change in eating habits – any	42/99 (42%)	0/10	3/12 (25%)	49/203 (24%)	43/194 (22%)
Severe³	2/99 (2%)	0/10	1/12 (8%)	3/203 (1%)	2/194 (1%)
Sleepiness – any	52/99 (53%)	3/10 (30%)	3/12 (25%)	74/205 (36%) ⁷	67/193 (35%)
Severe¹	3/99 (3%)	0/10	0/12	5/205 (2%)	2/193 (1%)
Vomiting – any	6/99 (6%)	2/10 (20%)	1/12 (8%)	8/205 (4%)	6/194 (3%)
Severe⁴	0/99	0/10	0/12	0/205	0/194
Diarrhoea – any	5/99 (5%)	0/10	2/12 (17%)	11/204 (5%)	8/193 (4%)
Severe⁵	0/99	0/10	0/12	1/204 (<1%)	0/193
Irritability – any	53/99 (54%)	4/10 (40%)	5/12 (42%)	67/204 (33%)	58/193 (30%) ⁷
Severe¹	6/99 (6%)	0/10	0/12	8/204 (4%)	5/193 (3%)
Headache – any	20/99 (20%)	2/10 (20%)	4/12 (33%)	25/204 (12%)	24/194 (12%)
Severe¹	1/99 (1%)	0/10	0/12	1/204 (<1%)	1/194 (1%)
Arthralgia – any	28/99 (28%)	1/10 (10%)	6/12 (50%)	45/203 (22%)	40/192 (21%)
Severe¹	10/99 (10%)	0/10	1/12 (8%)	6/203 (3%)	2/192 (1%)
Rash	13/99 (13%)	0/10	0/12	15/201 (7%)	10/192 (5%)

	Follow-on cohort			Vaccine-naïve cohort – Group 4	
	Group 1	Group 2	Group 3	Dose 1	Dose 2
Fever					
≥38.0°C⁶	16/99 (16%)	4/10 (40%)	5/12 (42%)	20/204 (10%)	16/189 (8%)
≥39.0°C	0/99	0/10	2/12 (17%)	3/204 (1%)	3/189 (2%)
≥40.0°C	0/99	0/10	0/12	2/204 (1%)	0/189
Fever management					
Treatment given	18/99 (18%)	4/10 (40%)	5/12 (42%)	22/204 (11%)	24/193 (12%)
Preventive therapy given	5/99 (5%)	2/10 (20%)	2/12 (17%)	17/204 (8%)	23/192 (12%)
Medical attention sought	2/99 (2%)	1/10 (10%)	0/12	2/204 (1%)	4/192 (2%)

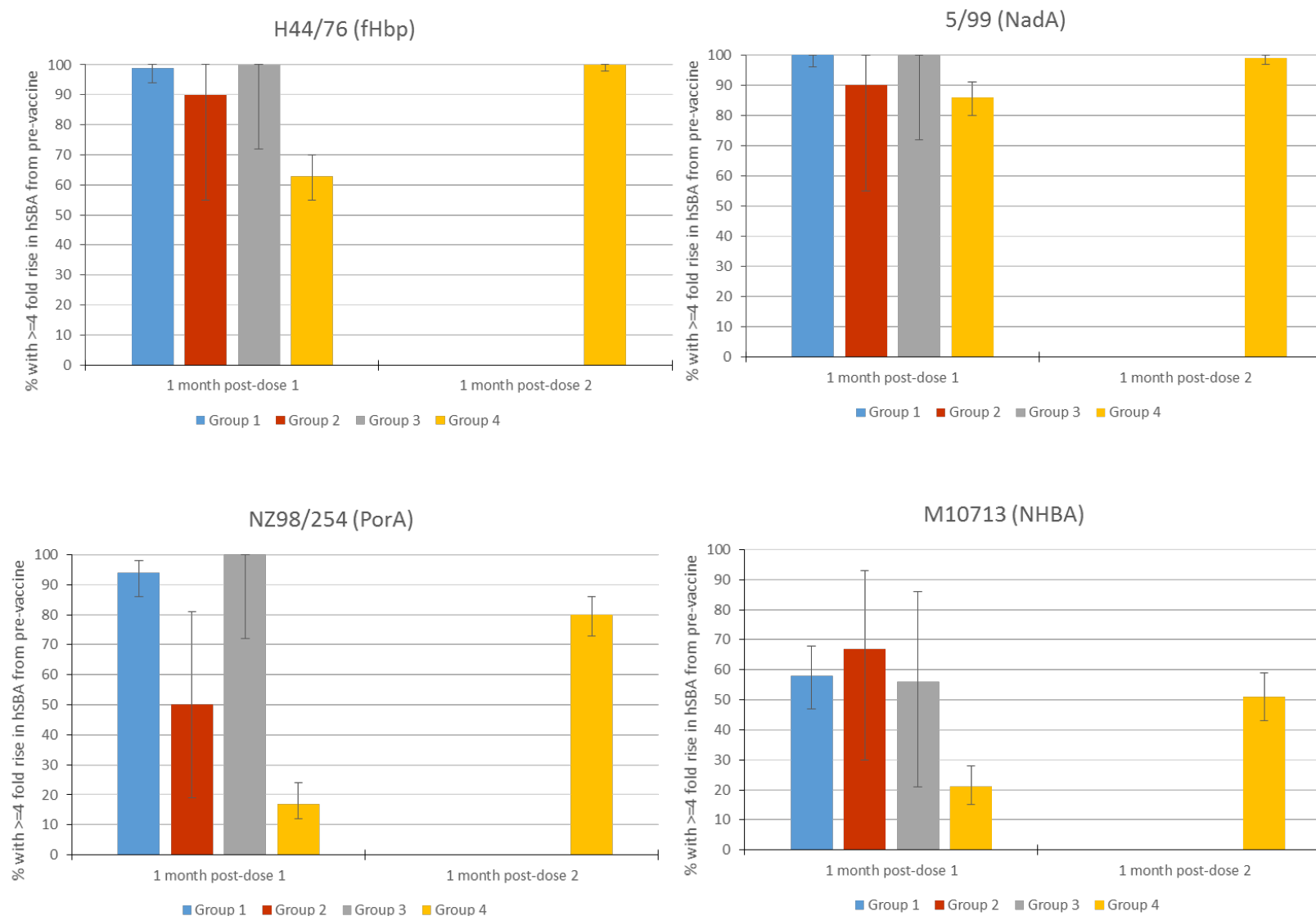
¹unable to perform daily activity; ²severe erythema, swelling or induration defined as ≥50mm; ³no meals all day; ⁴requires intravenous hydration; ⁵6 or more watery stools per day or requires intravenous hydration; ⁶axillary temperature; ⁷Two additional systemic non-severe adverse events occurred within 30 minutes after vaccination – 1 episode of sleepiness and 1 episode of irritability.

Supplementary Information

Supplementary Table 1. List of study sites

Country	Study Site
United Kingdom	Oxford Vaccine Group, University of Oxford Vaccine Institute, St George's, University of London Bristol Children's Vaccine Centre, University of Bristol South West Medicine, Cornwall
Italy	Fondazione IRCCS Policlinico Mangiagalli e Regina Elena Ospedale Maggiore della Carita A.O.U. Meyer Dip Scienze per la Salute della Donna e Bambino Azienda Osp Padova Dip AIS per la salute Donna e Bambino
Spain	Centro Superior de Investigacion en Salud Publica Hospital Universitario Dr Peset de Valencia Servicio de Ped Hospital Clinico Universitario de Santiago Hospital Xeral Cies
Czech Republic	Ordinace praktickeho lekare - Csukasova Ordinace praktickeho lekare – Tyce Ordinace praktickeho lekare – Eimerova Samostatna ordinace praktickeho lekare - Drazan Ordinace praktickeho lekare - Hrunka Samostatna ordinace praktickeho lekare - Slavik Samostatna ordinace praktickeho lekare - Karlova Ordinace praktickeho lekare - Dvorakova Ordinace praktickeho lekare - Machytka Ordinace praktickeho lekare - Machytkova Ordinace praktickeho lekare - Horakova Ordinace praktickeho lekare - Brandova Ordinace praktickeho lekare - Hanzl Ordinace praktickeho lekare - Semerakova Ordinace praktickeho lekare - Machackova Ordinace praktickeho lekare - Kavalkova Ordinace praktickeho lekare - Striteska Samostatna ordinace praktickeho lekare - Verdanova Samostatna ordinace praktickeho lekare - Zizkova

Supplementary Figure 1. Proportion of participants with ≥ 4 -fold rise in hSBA titre from pre-vaccine time-point after one and/or two doses of 4CMenB



Proportion of participants with ≥ 4 -fold rise in hSBA titre from pre-vaccine to 1 month post-dose 1 (all groups) and 1 month post-dose 2 (Group 4 only). Bars represent overall percentage of subjects achieving ≥ 4 -fold rise in hSBA titre, with error bars representing 95% confidence intervals. Data shown separately for the four indicator strains H44/76 (fHbp response), 5/99 (NadA response), NZ98/254 (PorA response) and M10713 (NHBA response). hSBA, human serum bactericidal antibody; 4CMenB, capsular group B meningococcal vaccine.