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Antibody persistence and booster response following MenACWY-CRM vaccination in children as assessed by two different assay methods

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ABSTRACT

Background: The quadrivalent meningococcal conjugate vaccine MenACWY-CRM has been shown to be immunogenic and well-tolerated in infants and toddlers. We evaluated antibody persistence for up to 4 years after vaccination with MenACWY-CRM in the first years of life and response to a booster dose administered at 60 months of age.

Methods: This was phase 3b, open-label, multicenter extension trial (NCT01148017). We assessed by hSBA and rSBA the persistence of antibody responses to serogroups ACWY in 203 healthy 60-month-olds receiving 4 doses of MenACWY-CRM during infancy (ACWY-4 group), or 2 doses at 12/13 and 15 months or 1 dose at 18 months of age (ACWY-2 group). We administered a MenACWY-CRM dose to 224 primed and 45 naïve 60-month-olds and evaluated safety and antibody response 1 month later.

Results: Antibody persistence measured by both assays was higher in primed than naïve 60-month-olds. The percentages of primed children with hSBA titers ≥8 was low for serogroup A (6–25%) and moderate for serogroups C (27–43%), Y (69–74%) and W (56–69%). For all serogroups, hSBA antibody geometric mean titers (GMTs) tended to be higher in the ACWY-2 than the ACWY-4 group. Post-booster/single dose, ≥96% of primed and ≥73% of naïve children had hSBA titers ≥8 against each serogroup, and hSBA GMTs were higher in primed children. The booster dose was well-tolerated and no safety concern was identified. We further assessed persistence using rSBA across different age groups and detected no overall correlation between rSBA and hSBA titers.

Conclusions: Primary vaccination of infants/toddlers with MenACWY-CRM resulted in moderate antibody persistence against serogroups C, W and Y for up to 4 years after the last priming dose. Regardless of priming schedule, a MenACWY-CRM booster dose at 60 months of age induced a robust immune response against all serogroups and was well-tolerated in all children.

1. Introduction

Invasive meningococcal disease (IMD) is a life-threatening condition, which has a case-fatality rate of 10%, and causes permanent sequelae in 10–20% of survivors [1–4]. The incidence of IMD varies by age group, peaking in infants and in adolescents and young adults [5]. Six *Neisseria meningitidis* serogroups cause most IMD cases, with serogroups B, C and Y predominating in the Americas and Europe, serogroups A and C in Asia, and serogroups B, C, X, and W in Africa [5–8]. More recently, an upsurge of serogroup W-caused IMD has been observed in South America, Europe, Australia and regions of sub-Saharan Africa [9].

The CRM_{197}-conjugate vaccine MenACWY-CRM (*Menveo*, GSK) is licensed for use against meningococcal disease in individuals

Abbreviations: AE, adverse event; ATP, according-to-protocol; CI, confidence interval; CRM, non-toxic cross-reacting mutant of diphtheria toxin isolated from cultures of Corynebacterium diphtheria strain C7 (β197); D, day; fHbp, factor H binding protein; GMT, geometrical mean titers; hSBA, serum bactericidal assay using human complement; IMD, invasive meningococcal disease; MenACWY-CRM, quadrivalent meningococcal conjugate vaccines containing each of meningococcal groups A, C, Y and W capsular polysaccharides; rSBA, serum bactericidal assay using baby rabbit complement; SAE, serious adverse event.

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from 2 months of age in several countries, and from 2 years in Europe [10,11]. In the United States (US), MenACWY-CRM is licensed as a 4-dose infant series (at 2, 4, 6 and 12 months of age), a 2-dose series (administered ≥3 months apart) in 7–23-month-olds, and as a single dose for individuals 2–55 years old, with an additional dose to be administered in 2–5 year-olds at continued risk of disease [11]. The vaccine is immunogenic with an acceptable safety profile [12–15], and a dose administered up to 5 years post-primary vaccination induces a robust booster response in individuals aged >2 years [16]. However, data on the persistence of bactericidal antibodies following infants and toddlers priming with 1 MenACWY-CRM dose are still scarce.

We have previously shown that MenACWY-CRM was highly immunogenic and well tolerated in healthy infants and toddlers when co-administered with routine vaccines [15,17]. Here, we present new data on the persistence of antibody titers in MenACWY-CRM-vaccinated children at ages 40 and 60 months. We also evaluated a potential correlation between human and rabbit complement serum bactericidal assay (hSBA/rSBA) titers, based on serological data generated in the current study and 2 other trials assessing long-term persistence following MenACWY-CRM immunization in infants, children and adolescents (Fig. 1).

2. Methods

2.1. Study design and participants

This was a phase 3b, open-label study (NCT01148017) performed between July 2010 and April 2013 in 19 centers in the US. We evaluated the persistence of antibody titers in 2 groups of healthy 40- and 60-month-olds previously vaccinated with MenACWY-CRM, primed either according to a 3 + 1 schedule at 2, 4, 6, and 12/13 months of age (group ACWY-4) or according to a 2-dose (at 12/13 months and 15 months of age) or single-dose schedule (at 18 months of age) (group ACWY-2) [17]. We enrolled age-matched MenACWY vaccine-naïve children (groups Naïve-40M and Naïve-60M) as controls for immunogenicity analyses (Fig. 2). Text S1 (Supplementary Material) gives the full list of enrolment exclusion criteria. We administered one 0.5 mL dose of MenACWY-CRM to all vaccine-primed and vaccine-naïve children enrolled at age 60 months by intramuscular injection in the left deltoid, as previously described [11]. We also offered a dose to children from the group Naïve-40M.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2019.06.076.

We conducted the study in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. We obtained written informed consent from the study participants or their parents/legally accepted representatives before study enrollment. For each site, an Institutional Review Board reviewed and approved the study protocol, amendments and informed consent forms. The study is registered at http://www.clinicaltrials.gov.

2.2. Study objectives

The primary objective of the study evaluated the persistence of antibody response in groups ACWY-4 and ACWY-2, measured by

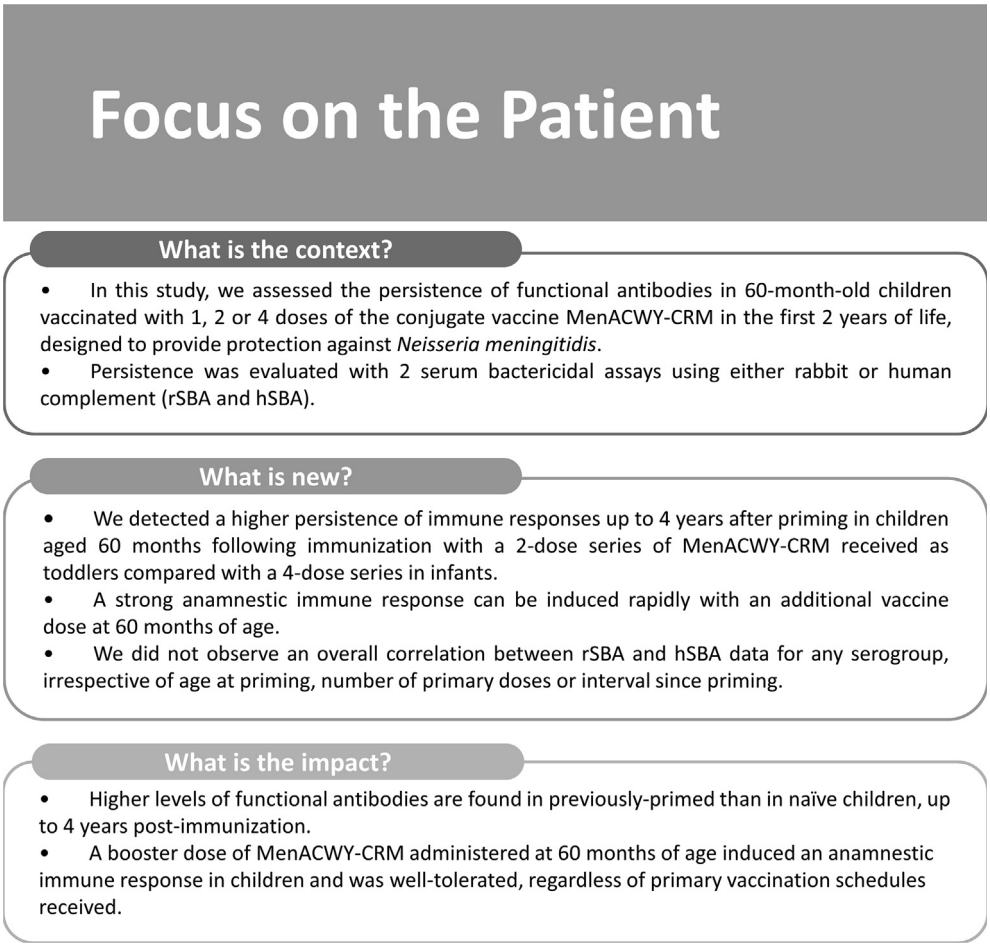

Fig. 1. Focus on the Patient.

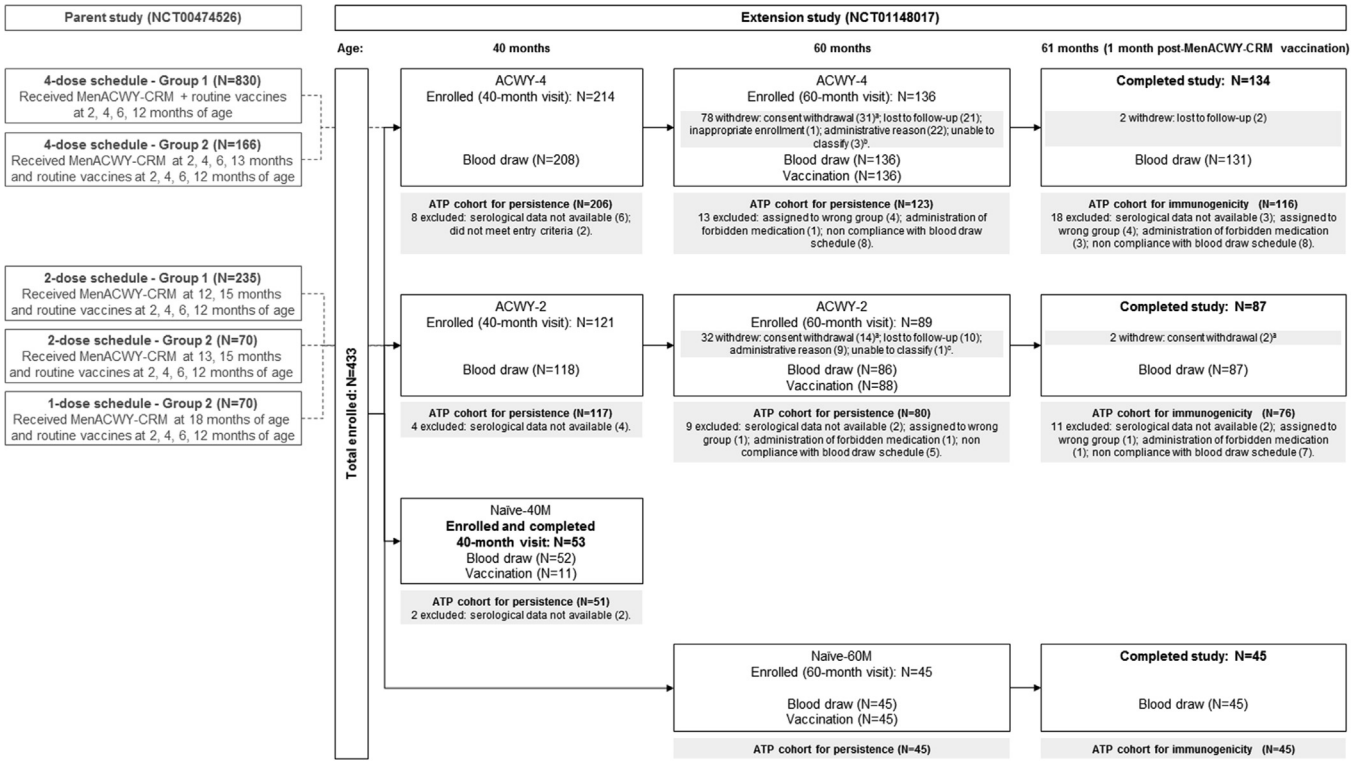

Fig. 2. Participant flow diagram, with study interventions. Footnote: ATP, according-to-protocol; N, number of children in each group; M, month. *Note:* [a]Not due to an adverse event. [b]One participant had latex allergy (erroneously thought to be a criterion for termination), one was ineligible due to latent tuberculosis and autism evaluation, and one developed encephalitis. [c]One participant was withdrawn due to an ongoing social/custody issue.

percentages of children with hSBA titers ≥8 against serogroups A, C, W and Y. Secondary objectives included assessing functional antibody levels in vaccine-naïve children aged 40 and 60 months, and comparing antibody responses 1 month after a single dose of MenACWY-CRM in groups ACWY-4 and ACWY-2 with those in group Naïve-60M. *Post-hoc* exploratory analyses assessed immunogenicity endpoints using rSBA. We assessed the safety and tolerability of the MenACWY-CRM booster/single dose administered at 60 months of age.

2.3. Immunogenicity assessments

We collected blood samples (10 mL) at 40 and 60 months of age, and at 1 month post-vaccination (Fig. 2).

We evaluated antibody levels using hSBA (GSK Clinical Laboratory Sciences, Marburg, Germany) or rSBA (Public Health England, United Kingdom).

We assessed immune responses to MenACWY-CRM as the percentage of individuals with reciprocal hSBA/rSBA titers ≥8 against each serogroup and geometric mean titers (GMTs), as previously described [18–21]. We also evaluated the percentage of individuals with titers against each serogroup ≥4 for hSBA and ≥128 for rSBA.

2.4. Safety assessments

Investigators recorded solicited local and systemic adverse events (AEs) occurring within 30 min post-vaccination for all children receiving a MenACWY-CRM dose at 60 months of age. Parents/legally accepted representatives recorded any solicited AE occurring within 7 days post-vaccination on diary cards. Investigators also recorded unsolicited AEs from day (D) 1 to D7 and medically-attended AEs from D8 to D28 post-vaccination and followed all events until resolved. We collected serious AEs (SAEs) throughout the study.

For all participants enrolled at 40 months of age, investigators collected SAEs at least possibly related to study procedures within 24 h from the blood draw. For children who received a MenACWY-CRM dose at 40 months of age, investigators also collected SAEs and medically attended AEs (graded by severity and relatedness to vaccination) reported within 28 days post-vaccination.

2.5. Statistical analyses

We planned to enroll 200 children in each of the ACWY-4 and ACWY-2 groups. Assuming a drop-out rate of 20%, this would result in a sample size of 160 participants in the according-to-protocol (ATP) cohorts, including children with evaluable serum samples at each timepoint and no major protocol deviations.

In the ATP cohort, we performed hSBA analyses for persistence at 40 and 60 months of age and for immunogenicity at 1 month post-vaccination. We randomly selected subsets for rSBA assessments from groups ACWY-4 (N = 50), ACWY-2 (N = 75), Naïve-40M (N = 50) and Naïve-60M (N = 45).

We included all exposed children with solicited/unsolicited safety data at each timepoint in the safety analyses.

In addition, we conducted exploratory analyses assessing the persistence of antibody levels up to 5 years following initial vaccination with MenACWY-CRM. In order to include different age groups, the same analyses were conducted for 2 other studies which we previously reported the design and hSBA results in detail. Briefly, they assessed antibody persistence in healthy individuals aged 2–10 years [12] and 11–18-years [22] at first vaccination with MenACWY-CRM according to a single- or 2-dose (in the 2–10 years group only) schedule, as compared with MenACWY-naïve aged-matched participants. We performed *ad hoc* analyses

for rSBA assessments in the subsets of previously vaccinated participants (i.e., not including naïve children). We assessed correlation between rSBA and hSBA titers against each serogroup by calculating the Pearson correlation coefficient and its p-value, overall and by timepoint. The null hypothesis was that the true correlation coefficient [ρ] is equal to 0 (indicating no correlation).

We performed all analyses using SAS version 9.3.

3. Results

3.1. Demographics

We enrolled 388 children 40 months of age: 214 in group ACWY-4, 121 in group ACWY-2 and 53 naïve children. The ATP cohorts for persistence included 210 in group ACWY-4, 119 in group ACWY-2 and 51 children in group Naïve-40M (Fig. 2).

Of these subjects, 270 children continued in the study at the second assessment timepoint at 60 months of age (136 in group ACWY-4, 89 in group ACWY-2 and 45 naïve participants), of whom 269 received a MenACWY-CRM dose. The ATP cohorts for persistence at this timepoint included 203 primed (123 and 80 in groups ACWY-4 and ACWY-2, respectively) and 45 naïve children (Fig. 2).

Overall, the groups were relatively balanced in terms of baseline characteristics, although the naïve groups at both timepoints included more female participants compared with the primed groups and there was a slight imbalance in terms of ethnic origin (Table 1).

3.2. Immunogenicity

At 40 months of age, the percentages of children with hSBA titers ≥8 and antibody hSBA GMTs were higher in groups ACWY-2 and ACWY-4 than in group Naïve-40M for serogroups C, W and Y (Table 2). For serogroup A, these values were similar between the ACWY-4 and Naïve-40M groups (as shown by overlapping 95% CIs), and lower than in group ACWY-2. We observed a trend for higher antibody persistence against all serogroups in group ACWY-2 compared with ACWY-4 (Table 2).

By 60 months of age, the percentages of primed children with hSBA titers ≥8 were lower, but remained higher than in naïve children for serogroups W and Y (with non-overlapping 95% CIs) (Table 2). Among primed children, the percentages with hSBA titers ≥8 were low for serogroup A (6–25%) and moderate for serogroups C (27–43%), Y (69–74%) and W (56–69%). Antibody persistence tended to be higher in group ACWY-2 than in ACWY-4 for each serogroup (Table 2).

At 1 month post-booster/single-dose vaccination, ≥96% of primed and ≥73% of naïve children had hSBA titers ≥8 against each serogroup. Antibody GMTs against all serogroups were higher in primed children than in group Naïve-60M. Antibody GMTs against serogroups A and C were higher in group ACWY-2 than in ACWY-4 (Table 2).

Similar trends were observed for percentages of children with hSBA titers ≥4 at all timepoints (Table 2).

At 40 months of age, the percentages with rSBA titers ≥8 and rSBA GMTs were higher in primed than in age-matched naïve children for all serogroups except serogroup C. At 60 months of age, the percentages of children with rSBA titers ≥8 and rSBA GMTs were higher in the ACWY-2 group for all serogroups except serogroup C than in the ACWY-4 and Naïve groups. For serogroup A, at both pre-vaccination timepoints, rSBA GMTs were higher in the previously primed children than in the naïve group (Table 3). Post-booster/single dose vaccination, ≥93% of children achieved rSBA titers ≥8 against each serogroup (Table 3). We observed similar trends for rSBA titers ≥128 (data not shown).

3.3. Safety

Overall, 14–42% of primed 60-month-olds reported solicited local reactions, compared with 20–49% in the naïve cohort. Most reactions were mild to moderate, with severe reactions occurring in <7% of primed children and <14% in naïve participants. Pain was the most frequent solicited local reaction, reported in 40–42% of children in groups ACWY-4 and ACWY-2, and 49% of children in group Naïve-60M (Table 4), with no reports of severe pain across groups. Erythema was the most frequently reported severe reaction. The incidence of solicited general reactions was comparable between groups, with irritability being the most frequently reported in 17–19% of children in groups ACWY-4 and ACWY-2, and 16% in group Naïve-60M. Less than 3% of primed and 6% of naïve children reported rash or fever, and none of the AEs were severe (Table 4).

Post-vaccination at age 60 months, parents reported any unsolicited AEs in 7% of children in ACWY-4, 8% in ACWY-2, and 12% in Naïve-60M groups. In 4% of children in ACWY-4, 6% in ACWY-2, and 6% in Naïve-60M groups, investigators considered the AEs as related to vaccination, with most being injection-site reactions.

We collected medically-attended AEs for 8% of children in ACWY-4, 13% in ACWY-2 and 10% in Naïve-60M groups; none were related to vaccination (Table 4).

Among children in all 3 groups, there were no SAEs considered at least possibly related to study procedures following the blood

Table 1
Demographic characteristics of participants, at enrollment.

	Enrolled at 40 months visit			Enrolled at 60 months visit		
	ACWY-4 N = 214	ACWY-2 N = 121	Naïve-40M N = 53	ACWY-4 N = 136	ACWY-2 N = 89	Naïve-60M N = 45
Age (±SD), months	39.6 ± 3.4	39.5 ± 2.4	38.7 ± 1.8	59.7 ± 2.2	59.6 ± 2.4	60.0 ± 1.7
Female, n (%)	97 (45%)	55 (45%)	29 (55%)	64 (47%)	42 (47%)	26 (58%)
Ethnic origin, n (%)						
Asian	15 (7%)	8 (7%)	3 (6%)	12 (9%)	6 (7%)	7 (16%)
Black	25 (12%)	8 (7%)	2 (4%)	14 (10%)	6 (7%)	5 (11%)
Caucasian	129 (60%)	82 (68%)	28 (53%)	80 (59%)	60 (67%)	20 (44%)
Hispanic	33 (15%)	16 (13%)	12 (23%)	22 (16%)	11 (12%)	7 (16%)
Other	12 (6%)	7 (6%)	8 (15%)	8 (6%)	6 (7%)	6 (13%)
Weight (±SD), kg	16.00 ± 2.35	16.01 ± 2.12	14.83 ± 2.04	–	–	20.06 ± 3.08
Height (±SD), cm	98.52 ± 5.76[a]	98.82 ± 4.91[a]	96.65 ± 4.10	–	–	110.71 ± 4.84
Months since vaccination (±SD)[b]	–	–	–	47.1 ± 2.3	43.1 ± 3.4	–

N, number of children in each group; n (%), number (percentage) of children in each category; SD, standard deviation.
Note:
[a] Data for 213 children in the ACWY-4 group and 120 children in the ACWY-2 group.
[b] For children receiving a booster dose of MenACWY-CRM at 60 months of age.

Table 2
Percentages of children with hSBA titers ≥8 and ≥4 and hSBA GMTs before and after vaccination with MenACWY-CRM (according-to protocol cohorts for persistence [40 and 60 months of age]/immunogenicity [1 month post-vaccination] at each timepoint).

	N; % of children with hSBA titers ≥8 (95% CI)			% of children with hSBA titers ≥4 (95% CI)			GMT (95% CI)		
	ACWY-4	ACWY-2	Naïve[a]	ACWY-4	ACWY-2	Naïve[a]	ACWY-4	ACWY-2	Naïve[a]
Serogroup A									
40 M of age[b]	206; 10 (6–15)	117; 35 (26–44)	51; 2 (0.05–10)	13 (9–18)	40 (31–50)	2 (0.05–10)	2.54 (2.23–2.9)	4.81 (4.06–5.69)	2.02 (1.57–2.61)
60 M of age[b]	123; 6 (2–11)	80; 25 (16–36)	45; 2 (0.056–12)	9 (5–15)	33 (22–44)	2 (0.056–12)	2.27 (1.95–2.63)	3.74 (3.12–4.48)	2.14 (1.68–2.74)
1 M post-vac at 60 M of age	116; 97 (91–99)	76; 97 (91–100)	45; 87 (73–95)	97 (91–99)	97 (91–100)	89 (76–96)	159 (120–210)	330 (237–459)	47 (30–73)
Serogroup C									
40 M of age[b]	206; 34 (28–41)	116; 51 (41–60)	51; 12 (4–24)	43 (36–50)	61 (52–70)	16 (7–29)	6.14 (4.98–7.59)	9.24 (7.05–12)	2.52 (1.68–3.78)
60 M of age[b]	123; 27 (19–36)	80; 43 (32–54)	45; 22 (11–37)	46 (37–55)	60 (48–71)	33 (20–49)	5.17 (3.96–6.75)	9.26 (6.71–13)	3.87 (2.5–5.98)
1 M post-vac at 60 M of age	115; 96 (90–99)	74; 99 (93–100)	45; 84 (71–94)	97 (93–99)	100 (95–100)	87 (73–95)	195 (142–267)	569 (392–826)	44 (27–71)
Serogroup W									
40 M of age[b]	204; 76 (70–82)	115; 83 (74–89)	51; 47 (33–62)	81 (75–86)	88 (80–93)	49 (35–63)	26 (21–32)	29 (22–39)	8.25 (5.47–12)
60 M of age[b]	121; 69 (60–77)	78; 74 (63–84)	45; 40 (26–56)	74 (66–82)	83 (73–91)	42 (28–58)	17 (13–22)	20 (14–28)	6.63 (4.25–10)
1 M post-vac at 60 M of age	104; 100 (97–100)	70; 100 (95–100)	44; 89 (75–96)	100 (97–100)	100 (95–100)	91 (78–97)	1950 (1408–2701)	1645 (1124–2407)	37 (23–61)
Serogroup Y									
40 M of age[b]	205; 67 (60–73)	116; 71 (62–79)	51; 22 (11–35)	76 (69–81)	80 (72–87)	24 (13–37)	16 (13–20)	18 (14–24)	3.69 (2.44–5.57)
60 M of age[b]	122; 56 (46–65)	80; 69 (57–79)	44; 25 (13–40)	65 (56–73)	74 (63–83)	25 (13–40)	11 (8.1–14)	14 (9.94–19)	4.1 (2.61–6.45)
1 M post-vac at 60 M of age	111; 100 (97–100)	74; 100 (95–100)	44; 73 (57–85)	100 (97–100)	100 (95–100)	80 (65–90)	1089 (812–1461)	922 (659–1290)	18 (11–28)

CI, confidence interval; GMT, geometric mean titer; hSBA, serum bactericidal assay using human complement; N, number of children in each group with available results; M, month(s); post-vac, post-vaccination. GMTs and 95% CIs were computed by exponentiation of the least square means of the log10-transformed titers and their 95% CIs obtained from a 2-way analysis of variance with factors accounting for vaccine group and center.

Note:

[a] Data for group Naïve-40M at 40 months of age, and group Naïve-60M at 60 months of age and 1-month post-vaccination timepoints.

[b] Antibody persistence was evaluated at 40 months (22–28 months post-primary vaccination) and 60 months (3.5–4 years post-primary vaccination).

draw at 40 months of age. Among vaccinated 40-month-olds (Naïve-40M group), we recorded 5 medically-attended AEs in 3/11 (27%) children: acute otitis media, upper respiratory tract infection, viral pharyngitis, arthralgia and ingrown nail; none were related to vaccination. Only 1 AE (arthralgia) was moderate in severity, while the other 4 were mild.

We recorded no SAEs and no deaths throughout the study.

3.4. Correlation between hSBA and rSBA results for antibody persistence

The subsets of children with rSBA assessments in each of the 3 studies included in the exploratory analyses had comparable demographic characteristics at each timepoint (Table S1).

Antibody persistence at 5 years post-vaccination with MenACWY-CRM as assessed by hSBA in children aged 2–10 years [23] and adolescents aged 11–18 years [23] at first vaccination has been previously reported; rSBA results are presented in Table S2. The percentages of individuals with rSBA titers ≥8 and antibody GMTs were higher in groups previously vaccinated with MenACWY-CRM than in the corresponding naïve cohorts, for all age categories and serogroups (including A), except for serogroup C for children 7–10 years of age receiving either 1 or 2 doses of MenACWY-CRM 5 years earlier (Table S2).

At 40 and 60 months of age across all groups, the percentages with hSBA titers ≥4 were lower than for rSBA titers ≥8 for serogroup A, tended to be higher for serogroups C and W and tended to be lower for serogroup Y, (Tables 2 and 3). Although a few combinations of serogroup, age, timepoint of vaccination and number of doses had a statistically significant correlation coefficient between rSBA and hSBA titers, we could not identify an overall correlation between the 2 assays (Table S3).

4. Discussion

This is the first study to assess long-term persistence of functional antibodies after a multi-dose regimen of MenACWY-CRM in infants and toddlers, using both rSBA and hSBA. We demonstrated that, at 4 years post-immunization, antibody levels measured by both assays were overall higher in children vaccinated with MenACWY-CRM during the first 2 years of life than in naïve children of similar age, although some exceptions were noted for hSBA results for serogroup A.

We also found that for all meningococcal serogroups, antibody levels in 60-month-old children were higher following immunization as toddlers with a 1- or 2-dose series compared with a 4-dose series during infancy, although similar percentages of children had protective titers regardless of the age at primary vaccination. This finding might be explained by the difference in maturity of the immune system between toddlers and infants [16]. Our observation is further supported by trials in 2–18-year-olds which showed less pronounced waning of antibody titers over time in individuals receiving vaccination in adolescence [23–25] than at younger ages [16,26]. Nevertheless, as IMD incidence is the highest in infancy and peaks again in adolescence [5], these age groups remain the main target for vaccination against meningococcal disease.

The hSBA and rSBA antibody titers in our study were comparable to those in prior trials assessing the persistence of MenACWY-CRM [16,25,27,28] or MenACWY-TT [29,30] after vaccination of infants and toddlers, as well as other age groups. Consistent with these studies, at 4 years post-last primary dose, we observed that the percentages of children with hSBA titers ≥4 were considerably lower than those with rSBA titers ≥8 for serogroup A and that antibody hSBA GMTs were lower than those measured by rSBA for all serogroups. However, the percentages of children with hSBA titers

Table 3
Percentages of children with rSBA titers ≥8 and rSBA GMTs before and after vaccination with MenACWY-CRM (according-to-protocol subsets for persistence [40 and 60 months of age]/immunogenicity [1 month post-vaccination] at each timepoint).

	N; % of children with rSBA titers ≥8 (95% CI)			GMT (95% CI)		
	ACWY-4	ACWY-2	Naïve[a]	ACWY-4	ACWY-2	Naïve[a]
Serogroup A						
40 M of age[b]	49; 80 (66–90)	70; 91 (82–97)	50; 46 (32–61)	279 (127–613)	1087 (632–1869)	44 (17–114)
60 M of age[b]	49; 96 (86–100)	74; 95 (87–99)	44; 80 (65–90)	968 (565–1659)	1408 (903–2195)	219 (97–494)
1 M post-vac at 60 M of age	45; 100 (92–100)	71; 100 (95–100)	43; 100 (92–100)	15,644 (11005–22239)	15,006 (11341–19856)	11,308 (7891–16206)
Serogroup C						
40 M of age[b]	48; 21 (10–35)	70; 29 (18–41)	50; 4 (0–14)	4.00 (2.74–5.84)	6.5 (4.11–10)	2.27 (1.90–2.70)
60 M of age[b]	48; 27 (15–42)	74; 30 (20–41)	44; 20 (10–35)	5.19 (3.13–8.59)	7.28 (4.42–12)	4.19 (2.57–6.85)
1 M post-vac at 60 M of age	44; 95 (85–99)	71; 99 (92–100)	43; 93 (81–99)	747 (433–1290)	1752 (1140–2692)	611 (352–1062)
Serogroup W						
40 M of age[b]	49; 37 (23–52)	70; 60 (48–72)	50; 2 (0.051–11)	8.83 (4.87–16)	59 (29–118)	2.27 (1.76–2.91)
60 M of age[b]	49; 31 (18–45)	74; 47 (36–59)	44; 14 (5–27)	10 (4.59–22)	27 (14–53)	4.91 (2.33–10)
1 M post-vac at 60 M of age	45; 96 (85–99)	71; 99 (92–100)	43; 95 (84–99)	4290 (2430–7572)	8272 (5262–13004)	3543 (1981–6336)
Serogroup Y						
40 M of age[b]	49; 67 (52–80)	68; 65 (52–76)	50; 22 (12–36)	51 (24–110)	86 (42–177)	6.32 (3.4–12)
60 M of age[b]	49; 71 (57–83)	74; 80 (69–88)	44; 45 (30–61)	77 (36–166)	115 (65–205)	21 (8.8–48)
1 M post-vac at 60 M of age	45; 98 (88–100)	71; 100 (95–100)	43; 98 (88–100)	3734 (2465–5657)	4343 (3121–6045)	2015 (1318–3082)

CI, confidence interval; GMT, geometric mean titer; N, number of children in each group with available results; rSBA, serum bactericidal assay using rabbit complement; M, month(s); post-vac, post-vaccination. GMTs and 95% CIs were computed by exponentiation of the least square means of the log10-transformed titers and their 95% CIs obtained from a 2-way analysis of variance with factors accounting for vaccine group and center.
Note:
[a] Data for group Naïve-40M at 40 months of age, and group Naïve-60M at 60 months of age and 1-month post-vaccination timepoints.
[b] Antibody persistence was evaluated at 40 months (22–28 months post-primary vaccination) and 60 months (3.5–4 years post-primary vaccination).

≥4 compared with those with rSBA titers ≥8 were higher for serogroups C and W in the current study, regardless of the number of MenACWY-CRM doses received. We also observed a more rapid decline of hSBA, but not rSBA, antibody levels against serogroup A compared with the other serogroups, over 4 years after priming. The more pronounced difference between assays for serogroup A is not readily explained but might suggest that serogroup A bacteria are more dependent on factor H for survival. *N. meningitidis* strains express the factor H binding protein, which is able to selectively bind the key negative regulator of the alternative complement pathway (human factor H), and enables the pathogen to evade alternative complement-mediated killing by the host innate

Table 4
Incidence of solicited and unsolicited adverse events in children vaccinated at 60 months (solicited and unsolicited safety sets).

Solicited AEs	ACWY-4, n (%)		ACWY-2, n (%)		Naïve-60M, n (%)	
	Any	Severe	Any	Severe	Any	Severe
	N = 129		N = 83		N = 49	
Any	80 (62%)	10 (8%)	53 (64%)	10 (12%)	37 (76%)	8 (16%)
Any local AE	67 (52%)	9 (7%)	44 (53%)	9 (11%)	29 (59%)	7 (14%)
Erythema	26 (20%)	8 (7%)	20 (24%)	9 (11%)	13 (27%)	7 (14%)
Induration	18 (14%)	4 (3%)	14 (17%)	5 (6%)	10 (20%)	1 (2%)
Pain	54 (42%)	0 (0%)	33 (40%)	0 (0%)	24 (49%)	0 (0%)
Any systemic AE	45 (35%)	1 (<1%)	26 (31%)	1 (1%)	23 (47%)	1 (2%)
Change in eating habits	14 (11%)	0 (0%)	5 (6%)	0 (0%)	4 (8%)	0 (0%)
Sleepiness	20 (16%)	0 (0%)	9 (11%)	0 (0%)	7 (14%)	0 (0%)
Irritability	24 (19%)	0 (0%)	14 (17%)[a]	0 (0%)	8 (16%)	0 (0%)
Vomiting	4 (3%)	0 (0%)	0 (0%)	0 (0%)	3 (6%)	0 (0%)
Diarrhea	7 (5%)	0 (0%)	1 (1%)	0 (0%)	1 (2%)	0 (0%)
Arthralgia	2 (2%)	0 (0%)	4 (5%)	0 (0%)	0 (0%)	0 (0%)
Headache	6 (5%)	0 (0%)	6 (7%)	1 (1%)	2 (4%)	0 (0%)
Rash	4 (3%)	0 (0%)	2 (2%)	0 (0%)	3 (6%)	0 (0%)
Fever[a]	4 (3%)	0 (0%)	2 (2%)	0 (0%)	2 (4%)	0 (0%)
Unsolicited AEs	Any	Possibly related	Any	Possibly related	Any	Possibly related
	N = 132[c]		N = 87		N = 50	
D1–D7 post-vaccination	9 (7%)	5 (4%)	7 (8%)	5 (6%)	6 (12%)	3 (6%)
D8–D28 post-vaccination[b]	11 (8%)	0 (0%)	11 (13%)	0 (0%)	5 (10%)	0 (0%)
Serious AEs	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Deaths	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

AE, adverse event; D, day; N, number of children in each group; n (%), number (percentage) of children in each category. Severe reactions were defined as "diameter >50 mm" for erythema and induration, "body temperature ≥40 °C" for fever, and "preventing normal daily activity" for all other symptoms. Serious AEs were defined as any event that resulted in death, was life-threatening, required or prolonged hospitalization, or jeopardized the child.
Note:
[a] Data reported for N = 83 for irritability, and N = 126 (group ACWY-4), 81 (group ACWY-2) and 49 (group Naïve-60M) for fever.
[b] Unsolicited AEs collected from D8 through study end were medically-attended AEs.
[c] One child from group ACWY-4 was lost to follow-up on D3. Therefore safety data for D8 to study termination was not available.

immune system and to survive in human serum and blood [31]. Factor H is not present in baby rabbit complement, which impacts the complement-mediated killing of *N. meningitidis* and leads to higher titers [32].

We demonstrated that a booster dose of MenACWY-CRM at 60 months of age induced a robust anamnestic response. Although we observed higher rSBA GMTs than hSBA GMTs for all age groups and serogroups, the percentages of children with hSBA titers ≥4 and rSBA titers ≥8 were comparable. We did not evaluate how rapidly the anamnestic response could occur at this age, however a previous study found that 98–100% of adolescents receiving a booster dose 5 years after primary vaccination with MenACWY-CRM had hSBA titers ≥8 by 7 days post-vaccination [27].

Among 60-month-olds, a dose of MenACWY-CRM was well tolerated in both primed and naïve children. We observed no differences between the incidence of AEs in primed children, regardless of having previously received a 1-, 2- or 4-dose primary vaccination series. In the current study, the safety profile was similar to that reported for 2–5-year-olds receiving 1 dose of MenACWY-CRM [12].

When assessing the evolution of hSBA and rSBA titers over time in the same individual, we could not establish any correlation between the assays for any of the serogroups regardless of age at priming, number of primary doses or interval since priming. Similar results were seen in a previous study in adolescents who received a single dose of either MenACWY-CRM or a quadrivalent ACWY polysaccharide vaccine [33].

An hSBA titer ≥4 is considered the accepted correlate of protection against serogroup C [19], a value which has been extrapolated to all other meningococcal serogroups, while rSBA titers ≥8 correlate with serogroup C vaccine efficacy estimates from post-licensure surveillance studies [18]. Consistent with this, another study showed that the majority of unvaccinated individuals with rSBA titers ≥8 also had hSBA titers ≥4 and that rSBA titers ≤8 indicated susceptibility to IMD [34]. Thus, even in the absence of a clear correlation between the two assays, there is evidence that both hSBA and rSBA can be used to monitor response to vaccination and persistence of immune response in individuals of all ages.

The decline in hSBA titers over time is known to be age- and serogroup-dependent [16]. Generally, immune responses against serogroups C, W and Y tend to be similar whether measured by hSBA or rSBA, although rSBA GMTs run several-fold higher than hSBA GMTs. Against serogroup A, however, hSBA and rSBA responses wane differently, with hSBA titers usually dropping rapidly post-vaccination, while rSBA titers remain high [16]. Data from a mass-vaccination campaign with a monovalent serogroup A conjugate vaccine in African countries also showed this divergence between waning hSBA and relatively-constant rSBA antibody levels over 1-year post-immunization [35]. Nevertheless, there were no cases of serogroup A disease, and nasopharyngeal carriage remained low in both vaccinated and unvaccinated populations 2 years after campaign implementation, despite low hSBA levels [36–38], raising the question of whether rSBA titers could also be used to evaluate the persistence of the immune response.

The immunogenicity results obtained in our study complement those obtained in other persistence trials [16], providing evidence that hSBA antibodies persist for up to 4 years post-MenACWY-CRM vaccination in all age categories for which it is indicated. However, this study was limited by the open design and the absence of a control group. In the primed groups, we enrolled only children who had previously been vaccinated in the parent study whose parents/legally accepted representatives were willing to participate in the extension trial; therefore, randomization was not possible and this led to a slight imbalance between groups in the participants' baseline characteristics. While our immunogenicity results are in agreement with those of other persistence trials [16], we did not perform any formal statistical analyses. Further, our comparisons of hSBA with rSBA assessments for antibody persistence were limited by the relatively small number of participants who had both rSBA and hSBA titers assessed, as well as the different number of previously administered doses within the same age category. Finally, in addition to the difference in source complement, the two assays were performed in different laboratories with each following its own protocol and strain selection procedure.

5. Conclusions

Vaccination of infants and toddlers with MenACWY-CRM resulted in moderate antibody persistence against serogroups C, W and Y at 60 months of age. While hSBA antibody levels against serogroup A declined over time, high rSBA GMTs were maintained for up to 4 years post-primary vaccination. A booster dose of MenACWY-CRM in 60-month-olds elicited a strong anamnestic response 1 month after vaccination and did not raise any safety concerns. Regardless of prior vaccination status, a dose of MenACWY-CRM at 60 months of age was immunogenic and well-tolerated in all children.

Trademark statement

Menveo is a trademark owned by the GSK group of companies.

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Declaration of Competing Interest

NK received a grant from Novartis (now part GSK groups of companies) during the conduct of this study; SBa and PK are employees of the GSK groups of companies. IS was an employee of Novartis (now part of GSK groups of companies) during the time of the study. BE has no conflict of interest to report. SBl received a research grant from Novartis (now part GSK groups of companies).

Authors' contribution

SBl and IS were involved in the design of the study, SBl, NK, BE and IS performed the enrollment and were involved in the collection of the data. All authors were involved in the analysis of the data and in the development of this manuscript.

References

[1] World Health Organization. Meningococcal meningitis. Available from: http://www.who.int/mediacentre/factsheets/fs141/en/ [accessed 2019 January 19].

[2] Halperin SA, Bettinger JA, Greenwood B, Harrison LH, Jelfs J, Ladhani SN, et al. The changing and dynamic epidemiology of meningococcal disease. Vaccine 2012;30(Suppl 2):B26–36. https://doi.org/10.1016/j.vaccine.2011.12.032.

[3] Pace D, Pollard AJ. Meningococcal disease: clinical presentation and sequelae. Vaccine 2012;30(Suppl 2):B3–9. https://doi.org/10.1016/j.vaccine.2011.12.062.

[4] Sabatini C, Bosis S, Semino M, Senatore L, Principi N, Esposito S. Clinical presentation of meningococcal disease in childhood. J Prev Med Hyg 2012;53:116–9. https://doi.org/10.1186/s12879-014-0663-4.

[5] Jafri RZ, Ali A, Messonnier NE, Tevi-Benissan C, Durrheim D, Eskola J, et al. Global epidemiology of invasive meningococcal disease. Popul Health Metr 2013;11:17. https://doi.org/10.1186/1478-7954-11-17.

[6] Martinon-Torres F. Deciphering the burden of meningococcal disease: conventional and under-recognized elements. J Adolesc Health 2016;59:S12–20. https://doi.org/10.1016/j.jadohealth.2016.03.041.

[7] Pelton SI. The global evolution of meningococcal epidemiology following the introduction of meningococcal vaccines. J Adolesc Health 2016;59:S3–S11. https://doi.org/10.1016/j.jadohealth.2016.04.012.

[8] World Health Organization. Invasive meningococcal disease-Serogroup distribution; 2018. Available from: https://www.who.int/emergencies/diseases/meningitis/serogroup-distribution-2018.pdf?ua=1 [accessed 2019 May 14].

[9] Booy R, Gentile A, Nissen M, Whelan J, Abitbol V. Recent changes in the epidemiology of *Neisseria meningitidis* serogroup W across the world, current vaccination policy choices and possible future strategies. Hum Vaccin Immunother 2019;15:470–80. https://doi.org/10.1080/21645515.2018.1532248.

[10] European Medicines Agency. Menveo: EPAR – Product information. Available from http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001095/WC500090147.pdf [accessed 2019 May 14].

[11] U.S. Food and Drug Administration. Package insert – Menveo: highlights of prescribing information. Available from: http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM201349.pdf [accessed 2019 May 14].

[12] Halperin SA, Gupta A, Jeanfreau R, Klein NP, Reisinger K, Walter E, et al. Comparison of the safety and immunogenicity of an investigational and a licensed quadrivalent meningococcal conjugate vaccine in children 2–10 years of age. Vaccine 2010;28:7865–72. https://doi.org/10.1016/j.vaccine.2010.09.092.

[13] Keshavan P, Pellegrini M, Vadivelu-Pechai K, Nissen M. An update of clinical experience with the quadrivalent meningococcal ACWY-CRM conjugate vaccine. Expert Rev Vaccin 2018;17:865–80. https://doi.org/10.1080/14760584.2018.1521280.

[14] Reisinger KS, Baxter R, Block SL, Shah J, Bedell L, Dull PM. Quadrivalent meningococcal vaccination of adults: phase III comparison of an investigational conjugate vaccine, MenACWY-CRM, with the licensed vaccine, Menactra. Clin Vaccine Immunol 2009;16:1810–5. https://doi.org/10.1128/cvi.00207-09.

[15] Tregnaghi M, Lopez P, Stamboulian D, Grana G, Odrljin T, Bedell L, et al. Immunogenicity and safety of a quadrivalent meningococcal polysaccharide CRM conjugate vaccine in infants and toddlers. Int J Infect Dis 2014;26:22–30. https://doi.org/10.1016/j.ijid.2014.03.1390.

[16] Baxter R, Keshavan P, Welsch JA, Han L, Smolenov I. Persistence of the immune response after MenACWY-CRM vaccination and response to a booster dose, in adolescents, children and infants. Hum Vaccin Immunother 2016;12:1300–10. https://doi.org/10.1080/21645515.2015.1136040.

[17] Klein NP, Reisinger KS, Johnston W, Odrljin T, Gill CJ, Bedell L, et al. Safety and immunogenicity of a novel quadrivalent meningococcal CRM-conjugate vaccine given concomitantly with routine vaccinations in infants. Pediatr Infect Dis J 2012;31:64–71. https://doi.org/10.1097/INF.0b013e31823dce5c.

[18] Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. Clin Diagn Lab Immunol 2003;10:780–6. https://doi.org/10.1128/CDLI.10.5.780-786.2003.

[19] Goldschneider I, Gotschlich EC. Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129:1307–26. https://doi.org/10.1084/jem.129.6.1307.

[20] Goldschneider I, Gotschlich EC. Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. J Exp Med 1969;129:1327–48. https://doi.org/10.1084/jem.129.6.1327.

[21] McIntosh ED, Broker M, Wassil J, Welsch JA, Borrow R. Serum bactericidal antibody assays – the role of complement in infection and immunity. Vaccine 2015;33:4414–21. https://doi.org/10.1016/j.vaccine.2015.07.019.

[22] Jackson LA, Baxter R, Reisinger K, Karsten A, Shah J, Bedell L, et al. Phase III comparison of an investigational quadrivalent meningococcal conjugate vaccine with the licensed meningococcal ACWY conjugate vaccine in adolescents. Clin Infect Dis 2009;49:e1–e10. https://doi.org/10.1086/599117.

[23] Baxter R, Reisinger K, Block SL, Izu A, Odrljin T, Dull P. Antibody persistence and booster response of a quadrivalent meningococcal conjugate vaccine in adolescents. J Pediatr 2014;164. https://doi.org/10.1016/j.jpeds.2014.02.025. 1409–15 e4.

[24] Baxter R, Reisinger K, Block SL, Percell S, Odrljin T, Dull PM, et al. Antibody persistence after primary and booster doses of a quadrivalent meningococcal conjugate vaccine in adolescents. Pediatr Infect Dis J 2014;33:1169–76. https://doi.org/10.1097/inf.0000000000000438.

[25] Block SL, Christensen S, Verma B, Xie F, Keshavan P, Dull PM, et al. Antibody persistence 5 years after vaccination at 2 to 10 years of age with quadrivalent MenACWY-CRM conjugate vaccine, and responses to a booster vaccination. Vaccine 2015;33:2175–82. https://doi.org/10.1016/j.vaccine.2015.02.049.

[26] Gill CJ, Baxter R, Anemona A, Ciavarro G, Dull P. Persistence of immune responses after a single dose of Novartis meningococcal serogroup A, C, W-135 and Y CRM-197 conjugate vaccine (*Menveo*®) or *Menactra*® among healthy adolescents. Hum Vaccin 2010;6:881–7. https://doi.org/10.4161/hv.6.11.12849.

[27] Jacobson RM, Jackson LA, Reisinger K, Izu A, Odrljin T, Dull PM. Antibody persistence and response to a booster dose of a quadrivalent conjugate vaccine for meningococcal disease in adolescents. Pediatr Infect Dis J 2013;32:e170–7. https://doi.org/10.1097/INF.0b013e318279ac38.

[28] Khatami A, Snape MD, Davis E, Layton H, John T, Yu LM, et al. Persistence of the immune response at 5 years of age following infant immunisation with investigational quadrivalent MenACWY conjugate vaccine formulations. Vaccine 2012;30:2831–8. https://doi.org/10.1016/j.vaccine.2012.02.046.

[29] Borja-Tabora C, Montalban C, Memish ZA, Van der Wielen M, Bianco V, Boutriau D, et al. Immune response, antibody persistence, and safety of a single dose of the quadrivalent meningococcal serogroups A, C, W-135, and Y tetanus toxoid conjugate vaccine in adolescents and adults: results of an open, randomised, controlled study. BMC Infect Dis 2013;13:116. https://doi.org/10.1186/1471-2334-13-116.

[30] Vesikari T, Forsten A, Boutriau D, Bianco V, Van der Wielen M, Miller JM. Randomized trial to assess the immunogenicity, safety and antibody persistence up to three years after a single dose of a tetravalent meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine in toddlers. Hum Vaccin Immunother 2012;8:1892–903. https://doi.org/10.4161/hv.22166.

[31] Granoff DM, Welsch JA, Ram S. Binding of complement factor H (fH) to *Neisseria meningitidis* is specific for human fH and inhibits complement activation by rat and rabbit sera. Infect Immun 2009;77:764–9. https://doi.org/10.1128/iai.01191-08.

[32] Seib KL, Scarselli M, Comanducci M, Toneatto D, Masignani V. *Neisseria meningitidis* factor H-binding protein fHbp: a key virulence factor and vaccine antigen. Expert Rev Vaccin 2015;14:841–59. https://doi.org/10.1586/14760584.2015.1016915.

[33] Gill CJ, Ram S, Welsch JA, Detora L, Anemona A. Correlation between serum bactericidal activity against *Neisseria meningitidis* serogroups A, C, W-135 and Y measured using human versus rabbit serum as the complement source. Vaccine 2011;30:29–34. https://doi.org/10.1016/j.vaccine.2011.10.068.

[34] Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: reevaluation of correlates of protection. Infect Immun 2001;69:1568–73. https://doi.org/10.1128/iai.69.3.1568-1573.2001.

[35] Price GA, Hollander AM, Plikaytis BD, Mocca BT, Carlone G, Findlow H, et al. Human complement bactericidal responses to a group A meningococcal conjugate vaccine in Africans and comparison to responses measured by 2 other group A immunoassays. Clin Infect Dis 2015;61(Suppl 5):S554–62. https://doi.org/10.1093/cid/civ504.

[36] MenAfriCar Consortium. The diversity of meningococcal carriage across the African Meningitis Belt and the impact of vaccination with a group A meningococcal conjugate vaccine. J Infect Dis 2015;212:1298–307. https://doi.org/10.1093/infdis/jiv211.

[37] Kristiansen PA, Ba AK, Ouedraogo AS, Sanou I, Ouedraogo R, Sangare L, et al. Persistent low carriage of serogroup A *Neisseria meningitidis* two years after mass vaccination with the meningococcal conjugate vaccine, MenAfriVac. BMC Infect Dis 2014;14:663. https://doi.org/10.1186/s12879-014-0663-4.

[38] Kristiansen PA, Diomande F, Ba AK, Sanou I, Ouedraogo AS, Ouedraogo R, et al. Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity. Clin Infect Dis 2013;56:354–63. https://doi.org/10.1093/cid/cis892.