Safety and immunogenicity of a CRM or TT conjugated meningococcal vaccine in healthy toddlers

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ABSTRACT

Background: MenACWY-CRM (Menveo®; GlaxoSmithKline) and MenACWY-TT (Nimenrix®; Pfizer) are two meningococcal vaccines licensed in the European Union for use in both children and adults. While both vaccines target meningococcal serogroups A, C, W and Y, immunogenicity and reactogenicity of these quadrivalent meningococcal conjugate vaccines may differ due to differences in formulation processes and chemical structure. Yet data on the comparability of these two vaccines are limited.
Methods: The reactogenicity and immunogenicity of one dose of either MenACWY-CRM or MenACWY-TT were evaluated in healthy toddlers aged 12–15 months. Immunogenicity was assessed using serum bactericidal antibody assays (SBA) with human (hSBA) and rabbit (rSBA) complement.
Results: A total of 202 children aged 12–15 months were enrolled to receive one dose of MenACWY-CRM or MenACWY-TT. Similar numbers of subjects reported solicited reactions within 7 days following either vaccination. Tenderness at the injection site was the most common local reaction. Systemic reactions reported were similar for both vaccines and mostly mild to moderate in severity: irritability, sleepiness and change in eating habits were most commonly reported. Immunogenicity at 1 month post-vaccination was generally comparable for both vaccines across serogroups. At 6 months post-vaccination antibody persistence against serogroups C, W, and Y was substantial for both vaccines, as measured by both assay methodologies. For serogroup A, hSBA titers declined in both groups, while rSBA titers remained high.
Conclusion: Despite differences in composition, the MenACWY-CRM and MenACWY-TT vaccines have comparable reactogenicity and immunogenicity profiles. Immediate immune responses and short-term antibody persistence were largely similar between groups. Both vaccines were well-tolerated and no safety concerns were identified.

1. Introduction

Neisseria meningitidis is a leading cause of bacterial meningitis and sepsis worldwide, particularly in young children under 5 years of age. Invasive disease caused by this bacterium is often severe, develops rapidly and is associated with high rates of morbidity and mortality, even with early antibiotic treatment [1].

To date, meningococcal serogroups A, B, C, W, and Y have been identified as responsible for the majority of invasive meningococcal disease (IMD) cases worldwide, and several quadrivalent conjugate meningococcal vaccines offering protection against serogroups A, C, W, and Y are widely available. In the European Union (EU), there are two licensed quadrivalent conjugate meningococcal vaccines: MenACWY-CRM (Menveo®; GlaxoSmithKline) and MenACWY-TT (Nimenrix®; Pfizer). MenACWY-CRM is an oligosaccharide diphtheria CRM_{197} conjugate vaccine containing bacterial capsular oligosaccharides for serogroups A, C, W, and Y conjugated to a protein carrier cross reactive material 197 (CRM_{197}). Oligosaccharides are prepared from purified polysaccharides by hydrolysis,

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sizing and reductive amination, and then covalently linked to the CRM_{197} [2]. MenACWY-TT is a tetravalent meningococcal polysaccharide conjugated vaccine consisting of *N. meningitidis* capsular polysaccharides A, C, W, and Y each coupled to tetanus toxoid (TT) as the carrier protein. The serogroup A and C polysaccharides are conjugated with an adipic dihydrazide (AH) spacer and indirectly conjugated to the TT, whereas the W and Y polysaccharides are conjugated directly to the TT. MenACWY-CRM was licensed in 2010 and is currently approved for individuals over 2 years of age in the EU and from 2 months to 55 years of age in the USA. MenACWY-TT was licensed in 2012 and is currently approved in the EU for individuals over 12 months of age.

Both MenACWY-CRM and MenACWY-TT protect against IMD caused by serogroups A, C, W, and Y. However, due to differences in polysaccharide chain length, conjugation chemistry for protein−polysaccharide coupling, and carrier protein, it is possible that the reactogenicity and immunogenicity of these vaccines differ. To date, only a single study has directly compared these vaccines when administered as a booster dose in adolescents previously vaccinated with meningococcal C conjugate vaccines [3]. Both vaccines induced bactericidal antibodies to levels associated with protection in comparable proportions of subjects. Both vaccines were also well-tolerated and no safety concerns were identified. However, a similar evaluation of the two vaccines has not been performed in young children or after primary vaccination. Indirect comparisons across studies are inappropriate due to differences in serological assays used in different laboratories and in methods used to assess reactogenicity.

A serum bactericidal assay using human complement (hSBA) is commonly used to measure vaccine immunogenicity against *N. meningitidis*. It is based on the observation that an hSBA titer ≥ 4 correlates with protection against IMD, as described by Goldschneider and colleagues [2,3]. During the development of MenACWY-CRM, a more conservative hSBA titer level (≥ 8) was selected in order to avoid overestimation of protection [4]. However, despite the acceptance of hSBA data as the basis for vaccine licensure, this assay is difficult to standardize across laboratories due to the nature of the reagents. For example, different sources of human complement may yield different results, as can the selection and management of test strains. Ultimately, while substantial efforts have been made to standardize the hSBA assay and its components [5,6], results from different laboratories cannot be readily compared.

The bactericidal assay using complement derived from baby rabbit serum (rSBA) is a widely accepted alternative to the hSBA, in which an rSBA titer of ≥ 128 is approximately equivalent to an hSBA titer of ≥ 4 [7]. However, while rSBA titers of ≥ 128 reliably predict protection, more recent data have suggested that this threshold may be unnecessarily stringent and that an rSBA titer of ≥ 8 is sufficient [8,9].

In the present study, we directly assessed the tolerability and immunogenicity of a single dose of MenACWY-CRM or MenACWY-TT in 12–15 month old toddlers using uniform safety assessments and serological assays (hSBA and rSBA).

2. Methods

This Phase II, randomized, controlled, observer-blind, multicenter study was conducted in four centers in Italy between November 2013 and October 2014. The primary objective was to assess the reactogenicity of single doses of MenACWY-CRM or Men ACWY-TT vaccines in healthy toddlers (NCT01994629). The study was designed and conducted in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, applicable local regulations, and the Declaration of Helsinki.

2.1. Subjects

Children aged 12–15 months in good health, whose parent(s)/legal guardian(s) had provided written informed consent, and who were expected to be available for all study visits were included. Exclusion criteria included: serious, acute, or chronic illness; a history of allergy to any vaccine component; previous or suspected disease caused by *N. meningitidis*; previous immunization with any meningococcal vaccine; planned or actual receipt of any vaccines within 14 days prior to or 30 days after administration of the study vaccine (with the exception of the influenza vaccine).

Subjects were enrolled and randomized according to a 1:1 ratio into either the ACWY-CRM or the ACWY-TT group, each to receive one dose of study vaccine on Day 1 (see Fig. 1 for study design). Randomization was performed using a centralized randomization system (interactive response technology [IRT]). Blood samples were collected from all toddlers on Days 1 (prevaccination), 29 (1 month) and 180 (6 months).

2.2. Vaccines

The MenACWY-CRM vaccine was reconstituted immediately prior to injection by adding a vial of the liquid MenCWY component to a vial of the lyophilized MenA component. Each reconstituted dose of MenACWY-CRM contained 10 μg MenA and 5 μg each of MenC, MenW, and MenY. The MenACWY-TT vaccine was reconstituted by adding the entire contents of the prefilled syringe of solvent to the vial containing the powdered components. Each reconstituted dose contained 5 μg each of MenA, MenC, MenW, and MenY. Both vaccines were administered as a 0.5 mL dose by intramuscular injection into the anterolateral right thigh.

2.3. Safety assessments

Reactogenicity was measured as percentages of subjects reporting at least one severe solicited adverse event (AE) within 7 days post-vaccination. Solicited AEs included tenderness, erythema, induration, irritability, sleepiness, change in eating habits, vomiting, diarrhea, and fever (body temperature $\geq 38\,°C$). Solicited AEs were collected using diary cards.

Secondary safety assessments included solicited local and systemic AEs, of any severity, within 7 days post-vaccination, any unsolicited AEs through Day 29, and any medically attended AEs, serious AEs (SAEs) or AEs leading to premature withdrawal during the entire study.

2.4. Immunogenicity assessments

The immunogenicity objectives were to assess the immunogenicity of one dose of either MenACWY-CRM or MenACWY-TT on Day 29, and to assess the persistence of immune responses on Day 180. Immune responses against serogroups A, C, W, and Y were measured by serum bactericidal assay (SBA) as described previously [10] using human and rabbit complement (hSBA and rSBA). Results are expressed as: (1) percentages of subjects with hSBA/rSBA titers ≥ 8; (2) percentages of subjects with rSBA titers ≥ 128; (3) percentages of subjects with hSBA seroresponse (an increase in hSBA titers from <4 pre-vaccination to ≥ 8 post-vaccination, or an at least 4-fold increase in hSBA titers for subjects with pre-vaccination titers ≥ 4); (4) percentages of subjects with a 4-fold increase in rSBA titers; (5) hSBA/rSBA geometric mean titers (GMTs); and (6) geometric mean ratios (GMRs; post-vaccination/baseline).hSBA testing was performed at Clinical Laboratory Sciences, Novartis Vaccines and Diagnostics (now GlaxoSmithKline), Marburg. The qualification procedures for human complement were performed according to the laboratory standard
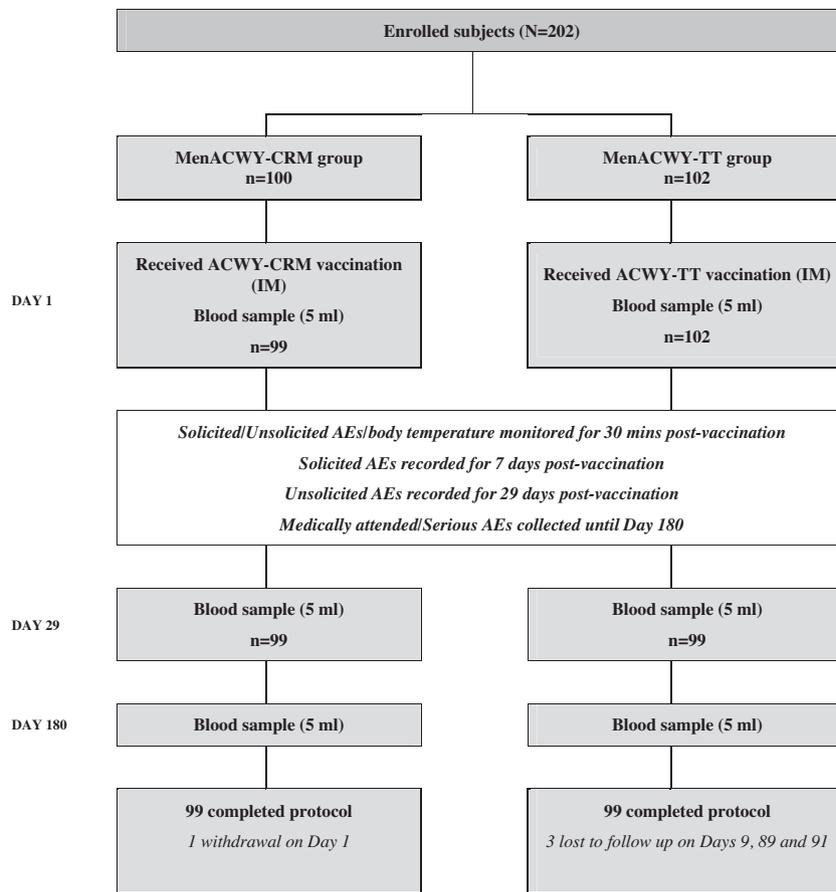
Fig. 1. Study design.

operation procedures. A single lot of human complement was used for testing of all clinical samples against each vaccine serogroup. rSBA testing was performed at Public Health England (PHE, UK).

2.5. Statistical methods

For the primary endpoint, two-sided 95% confidence intervals (CIs) for the difference in percentages of subjects with severe solicited AEs between the groups (ACWY-CRM and ACWY-TT) were computed using the methodology of Miettinen and Nurminen [11]. All other reactogenicity, immunogenicity and safety analyses were descriptive; two-sided 95% CIs were calculated using the Clopper-Pearson method. Safety analyses included all subjects who received at least one dose of vaccine and provided any safety data. Since <10% of subjects were terminated from the study or reported protocol deviations, the immunogenicity analysis was based on all subjects who received a study vaccine and provided an evaluable serum sample at Day 180 (Full Analysis Set).

With 100 enrolled subjects per arm, the study has power of 80% to detect a 11% increase in proportion of subjects with severe solicited AEs between the ACWY-CRM and the ACWY-TT group, assuming the rate of severe reactions in a reference group of 5% and alpha of 0.05.

3. Results

3.1. Demographics

A total of 202 subjects were enrolled in this study, of which 201 subjects (99 in the ACWY-CRM group and 102 in the

ACWY-TT group) received the study vaccine (see Fig. 1). Demographic and baseline characteristics were balanced between the groups (Table 1). The mean age of the subjects was 12.7 months and the majority were Caucasian (95%). Nearly all subjects (99% in the ACWY-CRM group and 97.1% in the ACWY-TT group) completed the study. Four subjects withdrew prematurely; of these, one subject (ACWY-CRM group) withdrew consent and three subjects (ACWY-TT group) were lost to follow-up.

3.2. Safety

Of the 201 vaccinated subjects, 200 (99 in the ACWY-CRM group and 101 in the ACWY-TT group) were included in safety evaluations; one subject was excluded due to lack of post-vaccination safety data.

Table 1
Demographics and baseline characteristics of subjects.

	MenACWY-CRM $N = 100$	MenACWY-TT $N = 102$	Total $N = 202$
Age in months(mean ± SD)	12.8 ± 1.0	12.7 ± 0.9	12.7 ± 0.9
Sex, n (%)			
Female	48 (48)	46 (45.1)	94 (46.5)
Male	52 (52)	56 (54.9)	108 (53.3)
Ethnic origin, n (%)			
Asian	0	1 (1)	1 (0.5)
Black or African American	0	2 (2)	2 (1)
Caucasian	96 (96)	96 (94.1)	192 (95)
Other	4 (4)	3 (2.9)	7 (3.5)
Weight in Kg (mean ± SD)	10 ± 1.1	10 ± 1.3	10 ± 1.2
Height in cm (mean ± SD)	77 ± 3.2	76.5 ± 3.6	76.8 ± 3.4

3.2.1. Solicited reactions

Two hundred subjects were evaluated for solicited AEs reported within 7 days post-vaccination. The frequencies of severe solicited AEs reported were comparable between groups: four (4%) subjects in the ACWY-CRM group and two (2%) subjects in the ACWY-TT group, an intergroup difference of 2% (95% CI -3.4%, 8.2%). Similar numbers of subjects reported at least one solicited AE within 7 days in both the ACWY-CRM (67.7%) and ACWY-TT (63.4%) groups (Table 2). A greater number of subjects in the ACWY-CRM group (34.3%) reported local solicited AEs than in the ACWY-TT group (28.7%). Tenderness at the injection site was the most common local solicited AE, reported by 29.9% of subjects in ACWY-CRM group and 26% of subjects in the ACWY-TT group. The incidence of erythema (ACWY-CRM: 4.1%; ACWY-TT: 2%) or induration (ACWY-CRM: 6.2%; ACWY-TT: 4%) was similar in both groups. There was only one severe local reaction (induration) reported by one subject in the ACWY-CRM group.

Systemic solicited AEs were reported by similar numbers of subjects in both groups (56.6% and 56.4% in the ACWY-CRM and ACWY-TT groups, respectively). Irritability, sleepiness, and change in eating habits were most commonly reported solicited systemic AEs (Table 2). A total of three subjects in the ACWY-CRM group reported eight severe systemic AEs, while two subjects in the ACWY-TT group reported four severe systemic AEs. Fever (temperature $\geq 38\,°C$) was reported by 14.1% of subjects in the ACWY-CRM group and by 13% of subjects in the ACWY-TT group. There were no instances of severe fever ($\geq 40\,°C$). Most reported solicited AEs were mild to moderate and resolved by Day 7.

Table 2
Number of subjects (%) with solicited and unsolicited adverse events post- vaccination with MenACWY-TT and MenACWY-CRM.

	MenACWY-CRM (N = 99)	MenACWY-TT (N = 101)
Any Solicited AEs (Days 1−7)	67 (67.7%)	64 (63.4%)
Any Severe AEs	4 (4%)	2 (2%)
Any Local Solicited AEs	34 (34.3%)	29 (28.7%)
Tenderness		
Any	29 (29.9%)	26 (26%)
Severe	0	0
Erythema		
Any	4 (4.1%)	2 (2%)
>50 mm	0	0
Induration		
Any	6 (6.2%)	4 (4%)
>50 mm	1 (1%)	0
Any Systemic Solicited AEs	56 (56.6%)	57 (56.4%)
Change in eating habits		
Any	22 (22.9%)	26 (26%)
Severe	3 (3.1%)	1 (1%)
Sleepiness		
Any	23 (24%)	26 (26.5%)
Severe	2 (2.1%)	0
Irritability		
Any	30 (31.3%)	39 (39.4%)
Severe	3 (3.1%)	1 (1%)
Vomiting		
Any	6 (6.3%)	9 (9.1%)
Severe	0	1 (1%)
Diarrhea		
Any	17 (17.7%)	18 (18.2%)
Severe	0	1 (1%)
Fever ($\geq 38\,°C$)		
Any	14 (14.1%)	13 (13%)
Severe ($\geq 40\,°C$)	0	0
Any Unsolicited AEs	73 (73.7%)	71 (70.3%)
Possibly/probably related AEs	12 (12.1%)	14 (13.9%)
Serious AEs (Through Day 180)	8 (8.1%)	3 (3%)
Possibly/probably related SAEs	0	0
Medically attended AEs	73 (73.7%)	68 (67.3%)
Death	0	0

3.2.2. Unsolicited AEs

Unsolicited AEs were reported by 73.7% of subjects in the ACWY-CRM group and by 70.3% of subjects in the ACWY-TT group (Table 2). Percentages of possibly related unsolicited AEs and medically attended AEs were similar between groups. Eleven SAEs (eight in the ACWY-CRM group and three in the ACWY-TT group) were reported: five infectious illnesses (all between Days 14 and 170 post-vaccination), two injuries (Days 22 and 65), two febrile convulsions (Day 2 [concurrent with infectious illness] and Day 38), one respiratory disorder (Day 109) and one skin rash (Day 48); none were considered related to the study vaccines (Table 2). Overall, the MenACWY-CRM and MenACWY-TT vaccines had similar reactogenicity profiles in healthy toddlers.

3.3. Immunogenicity

At baseline, only 0–6% of subjects had hSBA titers ≥ 8 against serotypes A, C, W, or Y (Fig. 2). 1 month post-vaccination (Day 29), hSBA responses to both MenACWY-CRM and MenACWY-TT were comparable, albeit serogroup specific. Namely, the majority of subjects in the ACWY-CRM and ACWY-TT groups had hSBA titers ≥ 8 against serogroup A (90% and 88%, respectively) and C (96% and 86%, respectively), while the proportions of subjects with hSBA titers ≥ 8 against serogroups W and Y were lower (62–72% and 41–56%, respectively).

This trend was also seen across other immunogenicity endpoints. At Day 29, hSBA GMTs against serogroups A and C were ~9- to 18-fold higher than baseline across vaccine groups, but only ~3- to ~6-fold higher against serogroups W and Y (Table 3). Likewise, greater proportions of subjects achieved seroresponses against serogroups A (87–88%) and C (84–95%) than against serogroups W (54–73%) and Y (39–54%).

Immune responses, as measured by rSBA, were generally higher than those measured by hSBA.

In both vaccine groups, rSBA titers ≥ 8 and ≥ 128 on Day 29 were observed in all (100%) subjects against serogroup A, and in the majority of subjects against serogroups C, W and Y (89–97% had rSBA titers ≥ 8 and 65–90% had rSBA titers ≥ 128; Fig. 3). Serogroup-specific increases in rSBA GMTs on Day 29 (Table 3) were likewise similar between the two vaccine groups for serogroups C, W, and Y, with increases in rSBA GMTs against serogroup C being lower (76- to 79-fold increase over baseline) than those against serogroups Y (240- to 285-fold) and W (380- to 482-fold). For serogroup A, rSBA GMTs increased more in the ACWY-CRM group (854-fold higher than baseline) than in the ACWY-TT group (228-fold higher than baseline). A 4-fold increase in rSBA titers over baseline was also observed for all serogroups in most subjects (65–100%) across both vaccine groups.

3.4. Antibody persistence

By Day 180, the proportion of subjects with hSBA titers ≥ 8 against serogroup A declined in both groups but to a greater degree in the ACWY-TT group (ACWY-TT: 30% had hSBA titers ≥ 8; ACWY-CRM: 65% had hSBA titers ≥ 8).

Against serogroup C, the proportion of subjects with hSBA titers ≥ 8 remained constant, with a trend toward increased antibody persistence in the ACWY-TT group. Unexpectedly, the proportion of subjects with hSBA titers ≥ 8 against serogroups W and Y on Day 180 increased above those on Day 29 with titers in the ACWY-TT group being higher than in the ACWY-CRM group (Fig. 2). Similar patterns were observed for hSBA GMTs on Day 180 (6 months; Table 3).

In contrast to the decrease in hSBA titers against serogroup A on Day 180, the proportion of subjects with rSBA titers ≥ 8 or ≥ 128 against serogroup A on Day 180 remained relatively constant
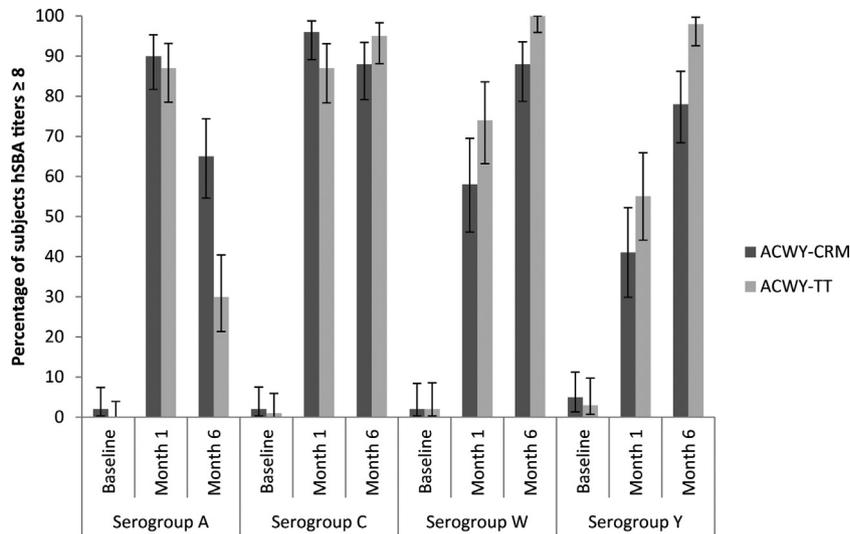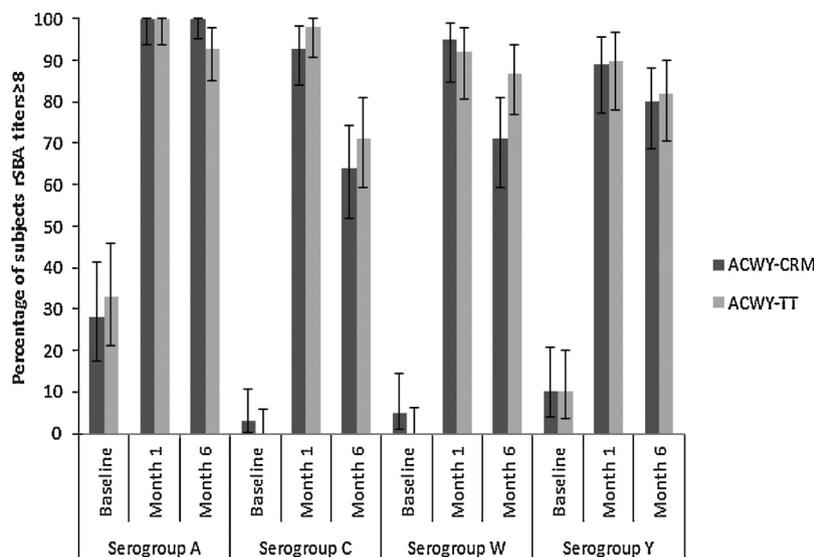
Fig. 2. Percentages of subjects (95% CI) with hSBA titers ≥ 8, against serogroups A, C, W and Y at baseline, 1 month and 6 months post-vaccination with MenACWY-TT or MenACWY-CRM.

Table 3
hSBA and rSBA GMTs and GMRs (95% CI) against *N. meningitidis* serogroups A, C, W and Y at baseline, 1 month and 6 months post-vaccination with MenACWY-TT or MenACWY-CRM.

Serogroup			hSBA		rSBA	
			MenACWY-CRM	MenACWY-TT	MenACWY-CRM	MenACWY-TT
A	GMT	Baseline	2.2 (2.0–2.4) (N = 95)	2.0 (1.9–2.2) (N = 92)	8.6 (3.9–19) (N = 60)	15 (6.8–35) (N = 61)
		1 Month	41 (31–55) (N = 89)	30 (22–39) (N = 93)	5275 (3742–7437) (N = 57)	2884 (2061–4035) (N = 56)
		6 Months	13 (9.5–18) (N = 97)	4.7 (3.4–6.4) (N = 96)	2815 (1741–4550) (N = 74)	950 (592–1523) (N = 75)
	GMR	1 Month/Baseline	18 (13–25) (N = 87)	14 (11–19) (N = 90)	854 (364–2004) (N = 50)	228 (92–567) (N = 46)
		6 Months/Baseline	5.9 (4.3–8.2) (N = 95)	2.3 (1.6–3.1) (N = 92)	296 (123–715) (N = 60)	59 (24–146) (N = 61)
C	GMT	Baseline	2.4 (2.2–2.6) (N = 94)	2.27 (2.1–2.5) (N = 92)	2.5 (2.0–3.1) (N = 64)	2.1 (1.7–2.6) (N = 63)
		1 Month	30 (23–38) (N = 91)	22 (17–28) (N = 92)	202 (130–314) (N = 61)	160 (104–248) (N = 57)
		6 Months	24 (18–30) (N = 96)	41 (32–53) (N = 95)	20 (12–33) (N = 77)	22 (13–36) (N = 76)
	GMR	1 Month/Baseline	12 (9.6–16) (N = 89)	9.4 (7.3–12) (N = 90)	79 (46–133) (N = 54)	76 (43–133) (N = 48)
		6 Months/Baseline	9.9 (7.6–13) (N = 94)	18 (13–23) (N = 92)	6.9 (3.9–12) (N = 64)	8.7 (4.9–16) (N = 63)
W	GMT	Baseline	2.4 (2.1–2.7) (N = 83)	2.3 (2.0–2.6) (N = 81)	2.6 (2.0–3.2) (N = 58)	2.0 (1.6–2.6) (N = 58)
		1 Month	8.6 (6.2–12) (N = 74)	15 (10–20) (N = 78)	1478 (768–2846) (N = 55)	974 (509–1864) (N = 50)
		6 Months	21 (17–27) (N = 88)	56 (45–70) (N = 89)	91 (46–182) (N = 73)	142 (72–282) (N = 70)
	GMR	1 Month/Baseline	3.5 (2.4–5.0) (N = 71)	6.5 (4.5–9.5) (N = 71)	482 (213–1093) (N = 48)	380 (157–916) (N = 42)
		6 Months/Baseline	8.6 (6.72–11) (N = 83)	24 (19–31) (N = 81)	24 (11–54) (N = 58)	54 (24–122) (N = 58)
Y	GMT	Baseline	2.3 (2.1–2.6) (N = 88)	2.3 (2.0–2.5) (N = 87)	3.0 (1.9–5.0) (N = 61)	2.9 (1.7–4.8) (N = 59)
		1 Month	5.8 (4.2–8.0) (N = 81)	8.1 (5.9–11) (N = 87)	762 (367–1583) (N = 54)	566 (273–1173) (N = 50)
		6 Months	16 (12–21) (N = 92)	26 (20–34) (N = 95)	248 (124–495) (N = 74)	140 (70–278) (N = 71)
	GMR	1 Month/Baseline	2.7 (1.9–3.8) (N = 78)	3.8 (2.7–5.3) (N = 81)	240 (103–559) (N = 48)	285 (114–714) (N = 42)
		6 Months/Baseline	6.8 (5.1–9.0) (N = 88)	11 (8.7–15) (N = 87)	88 (39–196) (N = 61)	55 (24–125) (N = 59)

Fig. 3. Percentages of subjects (95% CI) with rSBA titers ≥ 8, against serogroups A, C, W and Y at baseline, 1 month and 6 months post-vaccination with MenACWY-TT or MenACWY-CRM.

(93–100% and 91–100% had rSBA titers ≥ 8 and ≥ 128, respectively). For serogroups C, W, and Y, rSBA titers decreased between Days 29 and 180 in both groups. Despite the decreases in rSBA titers by Day 180, rSBA titers remained substantial in both vaccine groups (64–71%, 71–87%, 80–82% subjects had rSBA ≥ 8 for serogroups C, W, and Y, respectively, and 25–33%, and 62–66%, 62–73% subjects had rSBA ≥ 128). These serogroup-specific patterns were also observed for rSBA GMTs, which declined for all serogroups to a comparable extent in both vaccine groups (Table 3).

4. Discussion

This Phase 2 study verified that a single dose of either the MenACWY-CRM or MenACWY-TT vaccine elicit comparable reactogenicity and immunogenicity responses in healthy toddlers.

In an earlier report, Ishola and colleagues [3] compared booster doses of MenACWY-CRM and MenACWY-TT in adolescents previously vaccinated with MenC vaccines. In their study, overall reactogenicity to both vaccines was similar, and severe solicited reactions were "generally rare", although details regarding solicited or unsolicited AEs following the vaccinations were not provided. In the present study, severe solicited AEs were likewise reported by only a few subjects (ACWY-CRM: 4/99; ACWY-TT: 2/100), and local and systemic solicited AEs were comparable between the groups. Moreover, the incidence of local and systemic solicited AEs is consistent with previous studies of MenACWY-CRM [12–14] and MenACWY-TT [15–18] in toddlers. However, in some studies, the reported incidence of erythema following MenACWY-TT or MenACWY-CRM vaccination is higher than reported here [13–19].

Serum bactericidal assay with human complement was selected as the primary assay for this study since hSBA titer ≥4 is a well-accepted surrogate of protection against meningococcal disease [4,20]. However, hSBA results are highly dependent on the human complement used for the assay. In the present study, rigorous complement qualification procedures were routinely implemented to guarantee comparability of the results over time within the laboratory, and serum from a single qualified donor was used as a source of complement for testing all the clinical samples for each vaccine serogroup.

One month post-vaccination (Day 29), a robust immune response (indicated by the percentage of subjects with hSBA titers ≥ 8 and hSBA GMTs) was seen in both vaccine groups against all serogroups, particularly A and C. Previous reports of immunogenicity one month after a single dose of MenACWY-CRM [14,21] or MenACWY-TT [15,22] in toddlers have also demonstrated higher hSBA responses to serogroups A and C than for serogroups W and Y. In general, while overall immunogenicity against MenACWY-CRM and MenACWY-TT in the present and previous studies is comparable, there is substantial variability across studies in terms of hSBA immune response to each serogroup [14,22,23]. Differences in immune responses in the current and previous studies could reflect variations in study design, assay methodologies and human complement qualification procedures used by different laboratories as well as different regional demographics or IMD epidemiology.

With the exception of serogroup A, short-term persistence of immune responses to all serogroups at 6 months post-vaccination with MenACWY-CRM or MenACWY-TT was substantial. hSBA titers to serogroup A, however, declined in both vaccine groups, to a greater extent in the ACWY-TT group. Consistent with the present study, Baxter et al. [20] recently reported that hSBA titers to serogroup A 1 year post single vaccination in adolescents with MenACWY-TT were markedly lower than to serogroups C, W, and Y.

Interestingly, the percentages of subjects with hSBA titers ≥8 against serogroups W and Y were higher at 6 months than at 1 month post-vaccination. A similar increase in seropositivity rates against these two serogroups after a single dose vaccination in toddlers was observed in previous studies with MenACWY-TT [15] and MenACWY-CRM [NCT01345721]. This could reflect natural exposure to circulating serogroups W and Y (or cross-reacting pathogens) in this age group, or could indicate that the kinetics of hSBA immune response after a single vaccination with conjugate meningococcal vaccines is serogroup-specific. Note that no increase in hSBA titers was observed at approximately 6 months after a two-dose vaccination with MenACWY-CRM in toddlers (NCT01345721).

Serum bactericidal assay with rabbit complement was used as a secondary assay in this study.

Standardized rabbit complement is commercially available for use in rSBA assays and is known to be associated with less inter-laboratory variability when compared with the hSBA assay. However, although a strong correlation between rSBA and hSBA assays has been consistently demonstrated for serogroup C, it has

not for serogroups A, W, and Y [24]. Moreover, samples lacking detectable hSBA activity were frequently positive by rSBA [24,25].

Both study vaccines induced robust rSBA responses at 1 month after a single dose, with a vast majority of subjects achieving rSBA titer ≥ 8. A moderate and comparable decline in rSBA titers against serogroups C, W, and Y was observed at 6 months after vaccinaton in both vaccine groups. This pattern of antibody decline was similar to that previously described for MenACWY-TT [15] and MenACWY-CRM [26] vaccines.

The proportion of subjects with rSBA titers ≥ 8 against serogroup A remained constant within 6 months after vaccination. This observation is in line with recent studies of MenAfriVac vaccine, which demonstrated sustained antibody persistence for serogroup A (up to 5 years post-vaccination) using the rSBA assay [27,28]. The persistence of rSBA titers in MenAfriVac studies corresponds, in turn, with sustained reduction in invasive meningococcal disease and reduction of carriage in countries with high incidence of serogroup A disease [20,29,30]. This may indicate that rSBA might be more appropriate for monitoring antibody persistence and associated protection against IMD caused by serogroup A. Expanded immunologic and epidemiologic surveillance is needed to improve the interpretation of differences between the immunoassays [31].

Overall, taking hSBA and rSBA results together, there were no appreciable differences in the immune responses between the MenACWY-CRM and MenACWY-TT groups 1 month after a single vaccination in toddlers, and no consistent difference in the persistence of antibody titers at 6 months. However, there are several limitations to consider: Firstly, the investigation was not sufficiently powered to perform statistical between-group comparisons. Secondly, this study included only four study sites from a single country; results may not be representative of other regions with different meningococcal disease epidemiology.

In conclusion, the reactogenicity and safety profiles of the MenACWY-CRM and MenACWY-TT vaccines were similar in healthy 12–15 month-old toddlers, with no safety concerns identified. Furthermore, single doses of MenACWY-CRM or MenACWY-TT-induced robust immune responses with limited differences between groups at 1 month (immunogenicity), and 6 months (antibody persistence) post-vaccination.

Conflicts of interest statement

Acknowledgements

References

[1] Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. Vaccine 2009;27(Suppl. 2):B51–63.
[2] Broker M, Dull PM, Rappuoli R, Costantino P. Chemistry of a new investigational quadrivalent meningococcal conjugate vaccine that is immunogenic at all ages. Vaccine 2009;27(41):5574–80.
[3] Ishola DA, Andrews N, Waight P, Yung CF, Southern J, Bai X, et al. Randomized trial to compare the immunogenicity and safety of a CRM or TT conjugated quadrivalent meningococcal vaccine in teenagers who received a CRM or TT conjugated serogroup C vaccine at preschool age. Pediatr infect Dis J 2015;34(8):865–74.
[4] Frasch CE, Borrow R, Donnelly J. Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease. Vaccine 2009;27(Suppl. 2):B112–6.
[5] Borrow R, Carlone GM, Rosenstein N, Blake M, Feavers I, Martin D, et al. Neisseria meningitidis group B correlates of protection and assay standardization–international meeting report Emory University: Atlanta, Georgia, United States, 16–17 March 2005. Vaccine 2006;24(24):5093–107.
[6] Findlow H, Plikaytis BD, Aase A, Bash MC, Chadha H, Elie C, et al. Investigation of different group A immunoassays following one dose of meningococcal group A conjugate vaccine or A/C polysaccharide vaccine in adults. Clin Vaccine Immunol 2009;16(7):969–77.
[7] 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(3):1568–73.
[8] Miller E, Salisbury D, Ramsay M. Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. Vaccine 2001;20(Suppl. 1):S58–67.
[9] 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(5):780–6.
[10] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129(6):1307–26.
[11] Miettinen O, Nurminen M. Comparative analysis of two rates. Stat Med 1985;4(2):213–26.
[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(50):7865–72.
[13] Klein NP, Reisinger KS, Johnston W, Odrljin T, Gill JC, 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(1):64–71.
[14] Halperin SA, Diaz-Mitoma F, Dull P, Anemona A, Ceddia F. Safety and immunogenicity of an investigational quadrivalent meningococcal conjugate vaccine after one or two doses given to infants and toddlers. Eur J Clin Microbiol Infect Dis Official Publ Eur Soc Clin Microbiol 2010;29(3):259–67.
[15] 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 Vaccines Immunother 2012;8(12):1892–903.
[16] Vesikari T, Karvonen A, Bianco V, Van der Wielen M, Miller J. Tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine is well tolerated and immunogenic when co-administered with measles-mumps-rubella-varicella vaccine during the second year of life: an open, randomized controlled trial. Vaccine 2011;29(25):4274–84.
[17] Knuf M, Pantazi-Chatzikonstantinou A, Pfletschinger U, Tichmann-Schumann I, Maurer H, Maurer L, et al. An investigational tetravalent meningococcal serogroups A: C, W-135 and Y-tetanus toxoid conjugate vaccine co-administered with Infanrix hexa is immunogenic, with an acceptable safety profile in 12-23-month-old children. Vaccine 2011;29(25):4264–73.
[18] Knuf M, Kieninger-Baum D, Habermehl P, Muttonen P, Maurer H, Vink P, et al. A dose-range study assessing immunogenicity and safety of one dose of a new candidate meningococcal serogroups A: C, W-135, Y tetanus toxoid conjugate (MenACWY-TT) vaccine administered in the second year of life and in young children. Vaccine 2010;28(3):744–53.
[19] Tregnaghi M, Lopez P, Stamboulian D, Graña 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 DisOfficial Publ Int Soc Infect Dis 2014;26C:22–30.
[20] Baxter R, Baine Y, Kolhe D, Baccarini CI, Miller JM, Van der Wielen M. Five-year antibody persistence and booster response to a single dose of meningococcal A, C, W and Y tetanus toxoid conjugate vaccine in adolescents and young adults: an open, randomized trial. Pediat Infect Dis J 2015;34(11):1236–43.
[21] Tregnaghi M, Lopez P, Stamboulian D, Graña 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.
[22] Klein NP, Baine Y, Bianco V, Lestrate PR, Naz A, Blatter M, et al. One or two doses of quadrivalent meningococcal serogroups A: C, W-135 and Y tetanus toxoid

conjugate vaccine is immunogenic in 9- to 12-month-old children. Pediat Infect Dis J 2013;32(7):760–7.

[23] Summary, of, Product, Characteristics. Nimenrix. Available at: https://www.medicines.org.uk/emc/medicine/26514.

[24] 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(1):29–34.

[25] McIntosh ED, Bröker M, Wassil J, Welsch JA, Borrow R. Serum bactericidal antibody assays–The role of complement in infection and immunity. Vaccine 2015;33(36):4414–21.

[26] 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 Vaccines Immunother 2016 (epub ahead of print).

[27] Diallo A, Sow SO, Idoko OT, Hirve S, Findlow H, Preziosi MP, et al. Antibody persistence at 1 and 4 years following a single dose of MenAfriVac or quadrivalent polysaccharide vaccine in healthy subjects aged 2–29 years. Clin Infect Dis Official Publ Infect DisSoc Am 2015;61(Suppl. 5):S521–30.

[28] Tapia MD, Findlow H, Idoko OT, Preziosi MP, Kulkarni PS, Enwere GC, et al. Antibody persistence 1–5 years following vaccination with MenAfriVac in African children vaccinated at 12–23 months of age. Clin Infect Dis Off Publ Infect Dis Soc Am 2015;61(Suppl. 5):S514–20.

[29] Lingani C, Bergeron-Caron C, Stuart JM, Fernandez K, Djingarey MH, Ronveaux O, et al. Meningococcal meningitis surveillance in the African Meningitis Belt, 2004–2013. Clin Infect Dis 2015;61(Suppl. 5):S410–5.

[30] Daugla DM, Gami JP, Gamougam K, Naibei N, Mbainadji L, Narbé M, et al. Effect of a serogroup A meningococcal conjugate vaccine (PsA-TT) on serogroup A meningococcal meningitis and carriage in Chad: a community study. Lancet 2014;383(9911):40–7.

[31] 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.