Vaccine 36 (2018) 1908–1916

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Immunogenicity and safety of one or two doses of the quadrivalent meningococcal vaccine MenACWY-TT given alone or with the 13-valent pneumococcal conjugate vaccine in toddlers: A phase III, open-label, randomised study

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ARTICLE INFO

Article history:
Received 13 September 2017
Received in revised form 2 February 2018
Accepted 3 February 2018
Available online 2 March 2018

ABSTRACT

Background: We evaluated the immunogenicity and safety of 1 and 2 doses of quadrivalent meningococcal serogroup A, C, W and Y tetanus toxoid-conjugate vaccine (MenACWY-TT) given alone or co-administered with 13-valent pneumococcal conjugate vaccine (PCV13) in toddlers.

Methods: In this phase III, open-label, controlled, multicentre study (NCT01939158), healthy toddlers aged 12–14 months were randomised into 4 groups to receive 1 dose of MenACWY-TT at month (M) 0 (ACWY_1), 2 doses of MenACWY-TT at M0 and M2 (ACWY_2), MenACWY-TT and PCV13 at M0 (Co-ad), or PCV13 at M0 and MenACWY-TT at M2 (PCV13/ACWY). Immune responses were assessed 1 month post-each vaccination. Solicited and unsolicited symptoms were recorded for 4 and 31 days post-each vaccination, respectively; serious adverse events (SAEs) and new onset of chronic illnesses (NOCIs) up to M9 from first vaccination.

Results: 802 toddlers were vaccinated. Post-dose 1 of MenACWY-TT, ≥92.8% of toddlers had rSBA titres ≥1:8, and ≥62.5% had hSBA titres ≥1:4 for each meningococcal serogroup. Post-dose 2 of MenACWY-TT, rSBA titres ≥1:8 were observed in ≥98.0% and hSBA titres ≥1:4 in ≥95.3% of toddlers. Percentages of toddlers with hSBA titres ≥1:4 were higher after 2 doses versus 1 dose of MenACWY-TT for MenW (97.1% versus 62.5–68.9%) and MenY (95.3% versus 64.3–67.6%). Non-inferiority of immune responses to co-administered MenACWY-TT and PCV13 over their separate administration was demonstrated.

Abbreviations: AE, adverse event; ATP, according-to-protocol; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GMT, geometric mean titre; hSBA, human complement serum bactericidal antibody assay; MenACWY-TT, quadrivalent serogroups A, C, W and Y conjugate vaccine using tetanus toxoid as carrier protein; LL, lower limit; M, month; PCV, pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; rSBA, rabbit complement serum bactericidal antibody assay; SAE, serious adverse event; TT, tetanus toxoid.

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https://doi.org/10.1016/j.vaccine.2018.02.013

AEs incidence was comparable among groups. SAEs were reported for 4.9%, 5.1%, 5.5% and 7.5%, and NOCIs for 2.0%, 3.0%, 0.5% and 3.5% of toddlers in the ACWY_1, ACWY_2, Co-ad and PCV13/ACWY groups, respectively; 4 SAEs reported in 3 toddlers were vaccine-related. Two fatal vaccine-unrelated SAEs were reported.

Conclusion: MenACWY-TT was immunogenic when administered as a single dose at 12–14 months of age. A second dose in toddlers increased hSBA responses against MenW and MenY. MenACWY-TT and PCV13 can be co-administered without impairing the immunogenicity or safety profile of either vaccine.

1. Introduction

Invasive meningococcal disease and meningococcal meningitis caused by *Neisseria meningitidis* have their highest incidence in infants, with a second peak in adolescents and young adults [1,2]. The most important disease-causing serogroups are meningococcal serogroups A (MenA), MenB, MenC, MenW and MenY. Their prevalence varies geographically, with MenB, MenC and MenY being more prominent in the Americas and Europe, MenA and MenC in Asia, and MenA, MenC and MenW in Africa [3]. Increases in MenW incidence have been reported recently in the United Kingdom, South America and Australia [4–7].

Meningococcal infections can be prevented through vaccination [2,8]. Three quadrivalent meningococcal conjugate vaccines are currently available, using (i) diphtheria toxoid (*Menactra*, Sanofi Pasteur), (ii) non-toxic diphtheria cross-reacting mutant CRM_{197} (MenACWY-CRM_{197}; *Menveo,* GSK) or (iii) tetanus toxoid (MenACWY-TT; *Nimenrix*, Pfizer) as carrier proteins. MenACWY-TT has been approved for use as a single dose in individuals as of 12 months of age in Europe and Canada [9,10].

Previous studies have shown that a single dose of MenACWY-TT is immunogenic with a clinically acceptable safety profile in infants [11], toddlers and children [12–16], and persistence of the immune response was observed up to 5 years post-vaccination [17–21]. However, data suggest that protection against MenW and MenY in toddlers who received their first dose of MenACWY-TT at 9 months might be improved by administration of a second dose at 12 months of age [12].

This study investigates the short-term (at 1 month post-vaccination) and long-term (at 1, 3, and 5 years post-vaccination) immune responses induced by 1 or 2 doses of MenACWY-TT in toddlers. The study long-term follow-up is ongoing; here, we report the short-term immunogenicity and safety data up to 9 months post-first vaccination. Since the recommended timing of vaccination could coincide with the administration of a booster dose of pneumococcal conjugate vaccine (PCV), according to paediatric immunisation programmes worldwide, we also aimed to determine whether co-administration of MenACWY-TT with the booster dose of the 13-valent PCV (PCV13; Prevnar/Prevenar 13, Pfizer) impacted the immunogenicity or safety of either of the vaccines.

2. Methods

2.1. Study design and participants

This phase III, randomised, open-label, controlled, multicentre study was conducted in Australia, Canada, Czech Republic, Panama, South Africa and Turkey. The study interventions were performed in the vaccination phase (October 2013 to February 2015) lasting up to 3 months from first vaccination. A 6 M extended safety follow-up was completed in August 2015.

Participants were healthy 12–14-month-olds at the time of first vaccination, with documented receipt of the full primary series of PCV13 and diphtheria, tetanus and pertussis-containing vaccines according to local recommendations at least 5 months prior to enrolment.

Toddlers were randomised (1:1:1:1) into 4 groups to receive 1 dose of MenACWY-TT at month [M] 0 (ACWY_1 group), 2 doses of MenACWY-TT at M0 and M2 (ACWY_2 group), 1 dose of MenACWY-TT co-administered with PCV13 at M0 (Co-ad group), or 1 dose of PCV13 at M0 and 1 dose of MenACWY-TT at M2 (PCV13/ACWY group).

Randomisation was performed using a web-based system, with a minimisation algorithm accounting for centre, country and number of PCV13 doses (2 or 3, received before study start) with equal weight. The open-label design was imposed by differences in the vaccines' appearance and vaccination schedules for each group, but the personnel in charge of laboratory testing were blinded to treatment.

One 0.5 mL-dose of MenACWY-TT contained 5 μg of each MenA, MenC, MenW and MenY polysaccharide conjugated to TT (total TT content ~44 μg). PCV13 composition was previously described [22]. At each vaccination, a 0.5 mL dose was administered intramuscularly in the left (MenACWY-TT) or right (PCV13) anterolateral thigh or deltoid.

The study was conducted in agreement with the Declaration of Helsinki and the principles of Good Clinical Practice. Written informed consent was obtained from parents/legally acceptable representatives prior to enrolment. The study protocol, amendments and informed consent forms were approved by independent ethics committees at each site. The study is registered at www.clinicaltrials.gov (NCT01939158) and a protocol summary is available at https://www.gsk-clinicalstudyregister.com (116892).

2.2. Objectives

The immunogenicity of 1 or 2 doses of MenACWY-TT was compared at 1 month following last vaccination, in terms of serum bactericidal activity using rabbit complement (rSBA) titres. Evaluation of the persistence of the immune response at years 1, 3 and 5 is still ongoing (exploratory primary objective). The same comparisons were performed in terms of serum bactericidal activity using human complement (hSBA) titres.

The non-inferiority of co-administration of MenACWY-TT and PCV13 versus administration of either MenACWY-TT or PCV13 alone was evaluated at 1 month following last vaccination with MenACWY-TT and PCV13, respectively (confirmatory primary objectives).

Other secondary objectives evaluated the immunogenicity, reactogenicity and safety of the study vaccines in all groups.

2.3. Assessments

Blood samples (~5 mL) were collected from toddlers at pre-vaccination, and 1 month post-each vaccination. Immune responses to MenACWY-TT were evaluated using rSBA and hSBA assays [23] and to PCV13 antigens by both 22F-inhibition

enzyme-linked immunosorbent assay [24] and opsonophagocytic activity assay [25], as detailed in Table S1. Thresholds of rSBA titres ≥1:8 and hSBA titres ≥1:4 have been previously associated with seroprotection against MenC [23,26,27], and were applied to all 4 serogroups in this study [26,27].

Parents recorded solicited local and general symptoms during a 4-day (days 0–3) and unsolicited adverse events (AEs) during a 31-day (days 0–30) post-vaccination period, using diary cards. The occurrence of new onset of chronic illnesses and serious AEs (SAEs) were reported during the entire extended safety follow-up. Vaccine-related SAEs will continue to be recorded throughout the study.

2.4. Statistical analyses

Eight hundred toddlers were planned to be enrolled (200 per group) to achieve a sample size of 160 evaluable children in each group. The power to assess the confirmatory objectives, calculated based on rSBA response rates previously observed for MenACWY-TT in toddlers [14], was ≥99%.

Immunogenicity analyses were performed on the according-to-protocol cohorts at each timepoint, which included all evaluable vaccinated toddlers for whom immunogenicity results were available. Immune responses for some vaccine antigens were evaluated in subsets of participants (Table S1).

The confirmatory objectives were assessed hierarchically: the first objective had to be met to demonstrate the second one. Co-administration of MenACWY-TT and PCV13 was considered non-inferior to administration of MenACWY-TT alone if the lower limit (LL) of the 2-sided 95% confidence interval (CI) for the difference between the Co-ad group and the ACWY_pooled group (pooled ACWY_1 and ACWY_2 data) in the percentage of toddlers with rSBA titres ≥1:8 at M1 was ≥−10% for each meningococcal serogroup. Co-administration of PCV13 and MenACWY-TT was considered non-inferior to administration of PCV13 alone if the LL of the 95% CI for the geometric mean concentration (GMC) ratio between the Co-ad group and the PCV13 group was ≥0.5 for each vaccine serotype.

Immune responses in the ACWY_2 group (at M3) and the ACWY_1 group (at M1) were compared in exploratory analyses (Text S1). Details on computing GMTs/GMCs and other derived data are provided in Text S1.

Safety analyses were conducted on the total vaccinated cohort, including toddlers who received ≥1 study vaccine dose.

All analyses were performed using the Statistical Analysis System software (SAS Institute Inc., Cary, United States).

3. Results

3.1. Demographics

A total of 804 toddlers were enrolled and 802 (167 in Australia, 137 in Canada, 206 in the Czech Republic, 29 in Panama, 204 in South Africa and 59 in Turkey) were included in the total vaccinated cohort (Fig. 1). The groups were well-balanced in terms of age, gender and geographic distribution (Table 1).

3.2. Immunogenicity

3.2.1. Immunogenicity of 1 or 2 doses of MenACWY-TT

One month post-dose 1, percentages of toddlers with rSBA titres ≥1:8 in the ACWY_1 and ACWY_2 groups were ≥96.8% for MenA, ≥95.0% for MenC, ≥94.9% for MenW and ≥92.8% for MenY. In both groups, rSBA GMTs increased ≥271.3-, ≥87.9-, ≥461.4-, and ≥179.5-fold from pre-vaccination levels for MenA, MenC, MenW and MenY respectively. One month post-dose 2, 98.0%, 98.7%, 100% and 99.3% of toddlers in the ACWY_2 group had rSBA titres ≥1:8 and rSBA GMTs increased 250.3-, 152.2-, 803.0-, and 218.0-fold from pre-vaccination levels for MenA, MenC, MenW and MenY, respectively (Table 2).

One month post-dose 1, percentages of toddlers with hSBA titres ≥1:4 in the ACWY_1 and ACWY_2 groups were ≥96.0% for MenA, ≥95.7% for MenC, ≥62.5% for MenW and ≥64.3% for MenY, with GMTs increasing ≥49.2-, ≥69.0-, ≥12.5- and ≥12.8-fold from pre-vaccination levels, respectively. Post-dose 2, percentages of toddlers with hSBA titres ≥1:4 in the ACWY_2 group were 97.0%, 100%, 97.1% and 95.3%, and hSBA GMTs increased 65.6-, 876.7-, 378.4- and 205.2-fold from pre-vaccination levels, for MenA, MenC, MenW and MenY, respectively (Table 3).

In exploratory analyses, higher values were observed in the ACWY_2 group (at M3) than in the ACWY_1 group (at M1) for percentages of participants with rSBA/hSBA titres above the pre-specified cut-offs for MenW and MenY, and for GMTs for MenC, MenW (both rSBA and hSBA) and MenY (hSBA) (Table S2).

Percentages of toddlers with rSBA titres ≥1:128 and hSBA titres ≥1:8 for each serogroup are presented in Table S3.

3.2.2. Co-administration of MenACWY-TT and PCV13

One month post-dose 1, the LLs of the 95% CIs for the differences between the Co-ad and pooled ACWY_1 and ACWY_2 groups in percentages of toddlers with post-vaccination rSBA titres ≥1:8 ranged between −9.16 and −3.59 for all meningococcal serogroups (Table S4). Therefore, non-inferiority of the immune response to MenACWY-TT when co-administered with PCV13 versus MenACWY-TT given alone was demonstrated.

For each PCV13 serotype, the LLs of the 95% CIs for the GMC ratios between the Co-ad and the PCV13 group ranged between 0.87 and 1.03 (Table S5). Thus, immune response to PCV13 when co-administered with MenACWY-TT was non-inferior to PCV13 administered alone.

One month post-vaccination with MenACWY-TT, percentages of toddlers with rSBA titres ≥1:8 ranged from 90.0% to 96.1% in the Co-ad group and from 95.3% to 97.1% in the PCV13/ACWY group (Table 2). Exploratory comparisons showed comparable rSBA GMTs for MenA and MenC between the Co-ad and PCV13/ACWY groups, whereas higher MenW and MenY GMTs were observed in the PCV13/ACWY group than in the Co-ad group (data not shown).

One month post-vaccination with PCV13, for each vaccine serotype, percentages of toddlers with antibody concentrations ≥0.35 μg/ml ranged between 79.0% and 100%, and antibody GMCs were comparable among the Co-ad and PCV13/ACWY groups (Table 4, Table S5). For each PCV13 serotype, except serotype 3, ≥99.4% and ≥98.3% of toddlers had antibody concentrations ≥0.15 and ≥0.26 μg/ml, respectively (Table S6). Percentages of toddlers with opsonophagocytic activity assay titres ≥1:8 ranged from 90.7% to 100% in both groups (Table 4).

3.3. Safety

Following MenACWY-TT administration, the most common local symptom was redness, reported in 34.2%, 37.4% and 37.1% of toddlers in the ACWY_1, ACWY_2 and Co-ad groups, respectively, post-dose 1, and 33.9% of toddlers in group ACWY_2, post-dose 2. Redness was also the most frequent symptom following PCV13 vaccination, reported in 44.4% of toddlers in the Co-ad group and 45.9% in the PCV13/ACWY group. Grade 3 symptoms were reported in ≤5.1% of toddlers (Fig. 2A).

The most frequent solicited general symptom was irritability/-fussiness, reported in 44.7%, 47.7%, 54.3% and 43.4% of toddlers in the ACWY_1, ACWY_2, Co-ad and PCV13 groups, respectively, post-dose 1, and 40.2% and 35.1% of toddlers in the ACWY_2 and

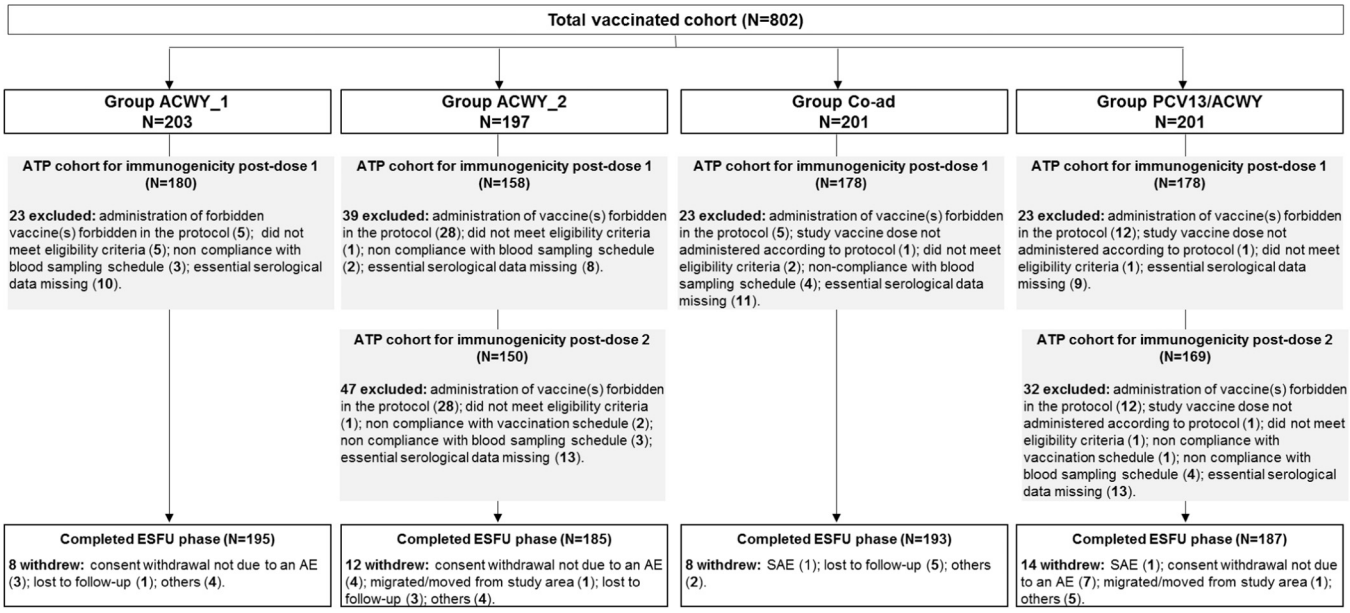

Fig. 1. Flow of participants. ACWY_1, participants who received 1 dose of MenACWY-TT at Month 0; ACWY_2, participants who received 2 doses of MenACWY-TT at Month 0 and Month 2; Co-ad, participants who received 1 dose of MenACWY-TT and 1 dose of PCV13 at Month 0; PCV13/ACWY, participants who received 1 dose of PCV13 at Month 0 and 1 dose of MenACWY-TT at Month 2; N, number of children in each group; ATP, according-to-protocol; AE, adverse event; ESFU, extended safety follow-up; SAE, serious adverse event.

Table 1
Demographic characteristics of toddlers (total vaccinated cohort).

	ACWY_1 N = 203	ACWY_2 N = 197	Co-ad N = 201	PCV13/ACWY N = 201
Mean age at first vaccination (SD), months	12.8 (0.9)	12.8 (0.9)	12.8 (0.9)	12.7 (0.9)
Female, n (%)	94 (46.3)	85 (43.1)	98 (48.8)	98 (48.8)
Geographic ancestry, n%				
White-Caucasian/European Heritage	135 (66.5)	127 (64.5)	135 (67.2)	133 (66.2)
African Heritage/African American	33 (16.3)	33 (16.8)	31 (15.4)	30 (14.9)
Other	34 (16.7)	34 (17.3)	29 (14.4)	33 (16.4)

ACWY_1, participants who received 1 dose of MenACWY-TT at Month 0; ACWY_2, participants who received 2 doses of MenACWY-TT at Month 0 and Month 2; Co-ad, participants who received one dose of MenACWY-TT and one dose of PCV13 at Month 0; PCV13/ACWY, participants who received one dose of PCV13 at Month 0 and one dose of MenACWY-TT at Month 2; N, number of participants with available results; SD, standard deviation; n (%), number (percentage) of participants in a given category.

PCV13 groups, respectively, post-dose 2. General symptoms had comparable incidences among the 4 groups, and grade 3 symptoms were reported in ≤6.6% of toddlers (Fig. 2B).

Following first vaccination, ≥1 unsolicited AE was reported in 42.3–46.2% and ≥1 grade 3 AE in 7.4–8.0% of participants in the 4 groups. For 5.4–7.6% of participants across all groups, the AEs were considered causally related to vaccination, with diarrhoea, vomiting, pyrexia, rash and bruise at injection site being the most common. Following second vaccination, ≥1 unsolicited AE was reported in 34.5% and 30.3% and ≥1 grade 3 unsolicited AE in 7.1% and 5.5% of toddlers in the ACWY_2 and PCV13/ACWY groups, respectively. Vaccine-related AEs were reported in 4.6% and 2.5% of participants in the ACWY_2 and PCV13/ACWY groups, respectively, with diarrhoea and vomiting being the most common.

Up to M9, 10 (4.9%) toddlers in the ACWY_1 group, 10 (5.1%) in the ACWY_2 group, 11 (5.5%) in the Co-ad group, and 15 (7.5%) in the PCV13/ACWY group experienced ≥1 SAE. Four SAEs were considered related to vaccination: leukopenia and neutropenia (2 days post-dose 1) and pneumonia (169 days post-dose 2) for 2 toddlers in the ACWY_2 group, and pneumonia (45 days post-vaccination) for a toddler in the Co-ad group; all were resolved by M9. Two fatal SAEs were reported: 1 toddler in the Co-ad group died due to asphyxiation (121 days post-vaccination) and another in the PCV13/ACWY group due to aspiration of food (26 days post-vaccination with PCV13). Neither death was considered to be causally related to vaccination.

Up to M9, new onset of chronic illnesses were reported in 4 (2.0%), 6 (3.0%), 1 (0.5%) and 7 (3.5%) toddlers in the ACWY_1, ACWY_2, Co-ad and PCV13/ACWY groups, respectively. Eczema and asthma were the most commonly reported symptoms.

4. Discussion

This study demonstrated that 1 or 2 doses of MenACWY-TT were immunogenic in toddlers. After 1 dose, ≥92.8% of toddlers had rSBA titres ≥1:8 for each meningococcal serogroup. rSBA GMTs and, to a lesser extent, hSBA GMTs increased markedly compared with pre-vaccination levels. A second dose administered after 2 months elicited comparable rSBA and hSBA immune responses, with the vast majority of toddlers having titres indicative of protection and substantial increases in GMTs from pre-vaccination levels. Co-administration of MenACWY-TT and PCV13 in toddlers did not impact the immunogenicity or safety profile of either vaccine.

After 1 dose of MenACWY-TT, immune responses as measured by rSBA were higher than those assessed by hSBA, in line with previous observations in toddlers [12,21]. A recent study comparing

Table 2
Percentage of toddlers with rSBA titres ≥1:8 and rSBA geometric mean titres against the 4 meningococcal serogroups (adapted ATP cohort for immunogenicity).

	ACWY_1			ACWY_2			Co-ad			PCV13/ACWY		
	N	% (95% CI)	GMT (95% CI)	N	% (95% CI)	GMT (95% CI)	N	% (95% CI)	GMT (95% CI)	N	% (95% CI)	GMT (95% CI)
MenA												
M0	175	2.9 (0.9–6.5)	4.3 (4.0–4.6)	153	4.6 (1.9–9.2)	4.7 (4.1–5.4)	169	2.4 (0.6–5.9)	4.2 (4.0–4.5)	171	2.9 (1.0–6.7)	4.3 (4.0 –4.6)
M1	180	97.8 (94.4–99.4)	1437.0 (1118.3–1846.6)	158	96.8 (92.8–99.0)	1275.2 (970.5–1675.4)	178	94.9 (90.6–97.7)	1146.4 (867.9–1514.3)	–	–	–
M3	–	–	–	150	98.0 (94.3–99.6)	1176.3 (921.8–1501.0)	–	–	–	169	96.4 (92.4–98.7)	1957.7 (1513.4–2532.3)
MenC												
M0	175	2.9 (0.9–6.5)	4.4 (4.0–4.8)	153	0.7 (0.0–3.6)	4.2 (3.8–4.5)	170	1.2 (0.1–4.2)	4.1 (3.9–4.4)	171	0.0 (0.0–2.1)	4.0 (4.0–4.0)
M1	179	95.0 (90.7–97.7)	452.3 (345.6–591.9)	157	95.5 (91.0–98.2)	369.3 (280.9–485.5)	176	96.0 (92.0–98.4)	337.3 (263.8–431.1)	–	–	–
M3	–	–	–	150	98.7 (95.3–99.8)	639.1 (521.8–782.9)	–	–	–	169	95.3 (90.9–97.9)	376.4 (284.7–497.6)
MenW												
M0	175	2.3 (0.6–5.7)	4.5 (4.0–5.1)	153	2.6 (0.7–6.6)	4.4 (4.0–4.8)	169	1.2 (0.1–4.2)	4.2 (3.9–4.5)	171	1.2 (0.1–4.2)	4.2 (3.9–4.6)
M1	180	95.0 (90.7–97.7)	2120.2 (1601.0–2807.8)	158	94.9 (90.3–97.8)	2030.1 (1510.7–2728.2)	177	93.2 (88.5–96.4)	1550.9 (1137.4–2114.7)	–	–	–
M3	–	–	–	150	100 (97.6–100)	3533.0 (2914.5–4282.7)	–	–	–	169	96.4 (92.4–98.7)	3490.5 (2643.3–4609.3)
MenY												
M0	175	9.7 (5.8–15.1)	6.0 (5.0–7.3)	153	5.2 (2.3–10.0)	5.2 (4.3–6.1)	169	5.9 (2.9–10.6)	5.1 (4.4–6.0)	171	7.0 (3.7–11.9)	5.2 (4.5–6.1)
M1	180	92.8 (88.0–96.1)	951.8 (705.0–1284.9)	157	93.6 (88.6–96.9)	933.3 (692.3–1258.3)	177	89.8 (84.4–93.9)	778.5 (566.2–1070.4)	–	–	–
M3	–	–	–	150	99.3 (96.3–100)	1133.6 (944.5–1360.5)	–	–	–	169	97.0 (93.2–99.0)	1481.2 (1158.4–1893.9)

rSBA, rabbit complement serum bactericidal antibody assay; ACWY_1, toddlers who received 1 dose of MenACWY-TT at month 0; ACWY_2, toddlers who received 2 doses of MenACWY-TT at month 0 and month 2; Co-ad, toddlers who received 1 dose of MenACWY-TT and 1 dose of PCV13 at month 0; PCV13/ACWY, toddlers who received 1 dose of PCV13 at month 0 and 1 dose of MenACWY-TT at month 2; ATP, according-to-protocol; N, number of toddlers with available results; CI, confidence interval; %, percentage of toddlers with rSBA titres above the specified cut-off; M, month; GMT, geometric mean titre.
Note: Analyses were carried out on the ATP cohort for immunogenicity post-dose 1 for M0 and M1, and the ATP cohort for immunogenicity post-dose 2 for the M3 timepoint.

MenACWY-TT and MenACWY-CRM in 12–15-month-olds also found more prominent rSBA than hSBA responses following administration of either vaccine [28]. Although hSBA titres are believed to underestimate complement-mediated killing of some *N. meningitidis* strains [29], both assays are currently used to evaluate meningococcal vaccines for licensure purposes.

Higher hSBA responses to MenA and MenC than to MenY and MenW have been previously reported following vaccination with MenACWY-TT in toddlers [12,21] and infants [11] or MenACWY-CRM in toddlers [30,31]. This difference is not fully understood, but it was no longer observed in our study after administration of the second MenACWY-TT dose, similarly with previous reports [12].

Following the second MenACWY-TT dose, ≥98.0% of toddlers had rSBA titres ≥1:8 and ≥95.3% hSBA titres ≥1:4. Moreover, robust increases in GMTs from pre-vaccination levels were noted, in line with previous observations in toddlers [12]. Exploratory analyses showed that for MenW and MenY, percentages of toddlers with rSBA and hSBA titres indicative of protection and hSBA GMTs were higher following 2 doses compared to 1 dose of MenACWY-TT. This suggests that a 2-dose regimen may substantially improve short-term immune responses to these serogroups. Higher percentages of toddlers with MenC, MenW and MenY hSBA titres ≥1:4 and higher hSBA GMTs were previously reported following vaccination with 2 doses versus 1 dose of MenACWY-TT [12]. In the persistence study, differences in terms of percentages of children with MenW hSBA titres ≥1:4 were also significant at 5 years, but not at 3 years post-vaccination, with no significant differences observed for the other serogroups [17]. Our study is still ongoing to evaluate the persistence of responses to 1 or 2 doses of MenACWY-TT beyond 1 year post-vaccination.

Our study demonstrated the non-inferiority of the immune responses to MenACWY-TT and PCV13 when co-administered versus their separate administration in toddlers. This is in line with previous studies showing that MenACWY-TT can be co-administered with other routine paediatric vaccines [14,16] or with 10-valent PCV (PHiD-CV) [32] in the second year of life. While lower responses to serotype 18C were previously observed when MenACWY-TT was co-administered with PHiD-CV compared with PHiD-CV alone [32], this was not observed with PCV13. A potential explanation for this difference could be the reduced polysaccharide responses induced by multivalent conjugate vaccines using TT as carrier protein compared with CRM_{197} since the capsular polysaccharide of serotype 18 is conjugated to TT in PHiD-CV and CRM_{197} in PCV13 [33]. However, an enhancement of the immune response, especially for MenC and MenY, was observed for co-administration of MenACWY-TT with other TT-conjugate vaccines, potentially attributed to the higher quantity of TT [14,34].

The incidence of AEs observed in our study following vaccination with MenACWY-TT was similar to that previously reported in toddlers [21]. The overall incidence of AEs following co-administration of MenACWY-TT with PCV13 was comparable to that following separate administration, as observed in this and previous studies [10,35]. Therefore, the safety profiles of either vaccine was not impacted by co-administration. The incidence of local AEs was generally lower following MenACWY-TT compared to PCV13 vaccination, despite the higher content of carrier protein in MenACWY-TT. No increase in the incidence of AEs was observed after the administration of a second MenACWY-TT dose, which supports the tolerability of a 2-dose schedule in toddlers.

The main strength of this study is that it allows the comparison of 1- and 2-dose MenACWY-TT regimens in toddlers. Non-inferiority endpoints were assessed with sufficient power. Meningococcal antibody persistence will continue to be evaluated at subsequent timepoints. Study limitations include the fact that the 2-dose schedule of MenACWY-TT co-administered with PCV13 was not assessed. In addition, the open-label design might

Table 3
Percentage of toddlers with hSBA titres ≥1:4, and hSBA geometric mean titres against the 4 meningococcal serogroups (adapted ATP cohort for immunogenicity).

	ACWY_1			ACWY_2		
	N	% (95% CI)	GMT (95% CI)	N	% (95% CI)	GMT (95% CI)
MenA						
M0	78	7.7 (2.9–16.0)	2.4 (2.1–2.8)	62	9.7 (3.6–19.9)	2.6 (2.1–3.2)
M1	74	95.9 (88.6–99.2)	118.0 (86.8–160.5)	66	97.0 (89.5–99.6)	132.9 (98.1–180.1)
M3	–	–		66	97.0 (89.5–99.6)	170.5 (126.2–230.2)
MenC						
M0	82	3.7 (0.8–10.3)	2.2 (2.0–2.4)	66	0.0 (0.0–5.4)	2.0 (2.0–2.0)
M1	78	98.7 (93.1–100)	151.9 (104.8–220.4)	70	97.1 (90.1–99.7)	160.8 (109.8–235.5)
M3	–			69	100 (94.8–100)	1753.3 (1278.7–2404.2)
MenW						
M0	73	1.4 (0.0–7.4)	2.2 (1.8–2.6)	62	0.0 (0.0–5.8)	2.0 (2.0–2.0)
M1	72	62.5 (50.3–73.6)	27.5 (16.1–46.8)	61	68.9 (55.7–80.1)	26.2 (16.0–43.0)
M3	–	–		70	97.1 (90.1–99.7)	756.8 (550.1–1041.3)
MenY						
M0	65	6.2 (1.7–15.0)	2.5 (2.0–3.1)	60	8.3 (2.8–18.4)	2.5 (2.1–3.0)
M1	71	67.6 (55.5–78.2)	41.2 (23.7–71.5)	56	64.3 (50.4–76.6)	31.9 (17.6–57.9)
M3	–	–		64	95.3 (86.9–99.0)	513.0 (339.4–775.4)

hSBA, human complement serum bactericidal antibody assay; ACWY_1, toddlers who received 1 dose of MenACWY-TT at month 0; ACWY_2, toddlers who received 2 doses of MenACWY-TT at month 0 and month 2; Co-ad, toddlers who received 1 dose of MenACWY-TT and 1 dose of PCV13 at month 0; PCV13/ACWY, toddlers who received 1 dose of PCV13 at month 0 and 1 dose of MenACWY-TT at month 2; ATP, according-to-protocol; N, number of toddlers with available results; %, percentage of toddlers with hSBA titres above the specified cut-off; CI, confidence interval; GMT, geometric mean titre; M, month.
Note: Analysis were carried out on the ATP cohort for immunogenicity post-dose 1 for M0 and M1, and the ATP cohort for immunogenicity post-dose 2 for the M3 timepoint.

have biased the reporting of AE towards increasing frequencies in the Co-ad group. Finally, the results of exploratory analyses presented in this manuscript should be interpreted cautiously as they were performed without accounting for multiplicity.

5. Conclusions

Vaccination with either 1 or 2 doses of MenACWY-TT in toddlers elicited robust short-term immune responses to all serogroups, with substantial increases in GMTs from pre-vaccination levels after each vaccination. Further follow-up will allow to determine if the higher responses for MenW and MenY observed after the second dose result in improved antibody persistence.

MenACWY-TT and PCV13 can be co-administered without impairing the immunogenicity or safety profile of either vaccine. The implementation of co-administered MenACWY-TT and PCV13 vaccinations in paediatric immunisation programmes could be warranted, as the reduced number of visits might lead to higher compliance and an improved uptake and coverage for both vaccines.

Funding

GlaxoSmithKline Biologicals SA was the funding source and was involved in all stages of the study conduct and analysis as well as the development of the manuscript and its approval for submission. GlaxoSmithKline Biologicals SA also took in charge all costs associated to the development and publishing of the present manuscript.

Authors' contribution

All authors had full access to the data and had final responsibility to submit for publication. Drafts were developed by a professional publication writer according to the recommendations, documentation, and outline provided by the lead author. TN, SH, PR, HM, MH, DK and MvdW were involved in the design of the study; CLC, TN, SH, KA, KP, PR, HM, MH, MC collected the data; CLC, TN, SH, KA, PR, HM, MH, MC carried out the study, ZK, MC and DK contributed with materials/analyses, CLC, TN, SJ, KP, PR, HM, MH, DK, and MvdW were involved in analyses and interpretation of the data.

Conflict of interest

CLC reports grants from GSK during the conduct of the study and grants outside the submitted work, and is an investigator on vaccine trials sponsored by industry including GSK, Sanofi, Novartis, Novavax, MedImmune and Pfizer during the conduct of the study. PR reports grants from GSK during the conduct of the study and participated in scientific advisory board for GSK for novel pneumococcal vaccine with no personal honorarium. TN reports

Table 4
Percentage of toddlers with ELISA antibody concentrations ≥0.35 μg/ml and antibody geometric mean concentrations, and OPA titres ≥1:8 and OPA geometric mean titres against the PCV13 serotypes, at 1 month post-vaccination with PCV13 (ATP cohort for immunogenicity).

		ELISA assessments			OPA assessments		
	Group	N	% (95% CI)	GMC (95% CI)	N	% (95% CI)	GMT (95% CI)
1	Co-Ad	162	99.4 (96.6–100)	2.94 (2.56–3.36)	81	93.8 (86.2–98.0)	116.1 (83.8–160.7)
	PCV13/ACWY	171	98.2 (95.0–99.6)	2.62 (2.27–3.01)	86	90.7 (82.5–95.9)	106.1 (77.0–146.2)
3	Co-Ad	151	83.4 (76.5–89.0)	0.80 (0.69–0.91)	76	98.7 (92.9–100)	137.6 (106.4–178.0)
	PCV13/ACWY	162	79.0 (71.9–85.0)	0.71 (0.62–0.81)	80	100 (95.5–100)	122.0 (100.6–147.9)
4	Co-Ad	163	96.9 (93.0–99.0)	2.46 (2.14–2.83)	77	100 (95.3–100)	2195 (1660–2902)
	PCV13/ACWY	172	97.1 (93.3–99.0)	1.96 (1.69–2.26)	80	100 (95.5–100)	2210 (1735–2815)
5	Co-Ad	163	98.8 (95.6–99.9)	2.09 (1.84–2.37)	81	100 (95.5–100)	452.3 (360.4–567.5)
	PCV13/ACWY	172	94.8 (90.3–97.6)	1.67 (1.46–1.91)	87	100 (95.8–100)	404.6 (319.1–512.9)
6A	Co-Ad	163	99.4 (96.6–100)	8.59 (7.50–9.85)	81	100 (95.5–100)	10177 (7785–13305)
	PCV13/ACWY	171	100 (97.9–100)	7.28 (6.33–8.37)	88	100 (95.9–100)	7354 (5707–9476)
6B	Co-Ad	161	99.4 (96.6–100)	7.36 (6.33–8.55)	75	100 (95.2–100)	4952 (3734–6567)
	PCV13/ACWY	171	99.4 (96.8–100)	6.68 (5.70–7.83)	78	100 (95.4–100)	3881 (3056–4930.)
7F	Co-Ad	163	99.4 (96.6–100)	5.14 (4.49–5.88)	81	100 (95.5–100)	8957 (7306–10982)
	PCV13/ACWY	172	100 (97.9–100)	4.81 (4.23–5.47)	87	100 (95.8–100)	8526 (6926–10497)
9 V	Co-Ad	163	99.4 (96.6–100)	2.03 (1.78–2.30)	81	100 (95.5–100)	3375.5 (2539.6–4486.6)
	PCV13/ACWY	172	97.7 (94.2–99.4)	1.79 (1.59–2.03)	88	100 (95.9–100)	2332 (1766–3080)
14	Co-Ad	161	100 (97.7–100)	13.10 (11.38–15.07)	73	100 (95.1–100)	3444 (2453–4834)
	PCV13/ACWY	172	99.4 (96.8–100)	11.94 (10.43–13.66)	78	100 (95.4–100)	3158 (2445–4078)
18C	Co-Ad	163	98.8 (95.6–99.9)	2.53 (2.19–2.92)	81	100 (95.5–100)	3881 (2947–5111)
	PCV13/ACWY	172	99.4 (96.8–100)	2.13 (1.84–2.47)	88	100 (95.9–100)	3290 (2514–4306)
19A	Co-Ad	162	100 (97.7–100)	9.60 (8.41–10.97)	81	100 (95.5–100)	3362 (2640–4283)
	PCV13/ACWY	170	100 (97.9–100)	8.62 (7.53–9.87)	88	100 (95.9–100)	2494 (1987–3132)
19F	Co-Ad	162	100 (97.7–100)	8.40 (7.33–9.62)	81	98.8 (93.3–100)	1795 (1306–2469)
	PCV13/ACWY	172	100 (97.9–100)	7.98 (6.96–9.15)	88	100 (95.9–100)	1648 (1288–2108)
23F	Co-Ad	161	99.4 (96.6–100)	5.33 (4.57–6.21)	77	98.7 (93.0–100)	7756 (5451–11035)
	PCV13/ACWY	169	99.4 (96.7–100)	4.47 (3.83–5.23)	80	98.8 (93.2–100)	5677 (4255–7575)

ACWY_1, toddlers who received 1 dose of MenACWY-TT at month 0; ACWY_2, toddlers who received 2 doses of MenACWY-TT at month 0 and month 2; Co-ad, toddlers who received 1 dose of MenACWY-TT and 1 dose of PCV13 at month 0; PCV13/ACWY, toddlers who received 1 dose of PCV13 at month 0 and 1 dose of MenACWY-TT at month 2; ELISA, enzyme-linked immunosorbent assay; OPA, opsonophagocytic activity; ATP, according-to-protocol; N, number of toddlers with available results; %, percentage of toddlers with antibody concentrations/titres above the specified threshold; GMC, geometric mean concentration; CI, confidence interval; GMT, geometric mean titre.

grants from GSK during the conduct of the study. KP's institution, Murdoch Childrens Research Institute (MCRI), has received research grants from GSK, Novartis, Pfizer, Novavax, and MedImmune during the conduct of the study. HM is an investigator on clinical vaccine studies sponsored by industry including GSK, Pfizer and Novavax. Her institution has received funding for investigator led research from GSK, Pfizer and Seqirus. PR is an investigator on vaccine trials sponsored by industry including GSK, Novartis and Pfizer. PR's institution receives funding for investigator led studies from industry (including Pfizer and GSK). PR has been a member of scientific vaccine advisory boards for industry (Pfizer, GSK, Sanofi) but has not received any personal payments from industry. DK and MH were employees of GSK at the time of the study. MvdW is an employee of the GSK group of companies. MvdW declared stock ownership in the GSK group of companies. The other authors have no conflict of interest to disclose.

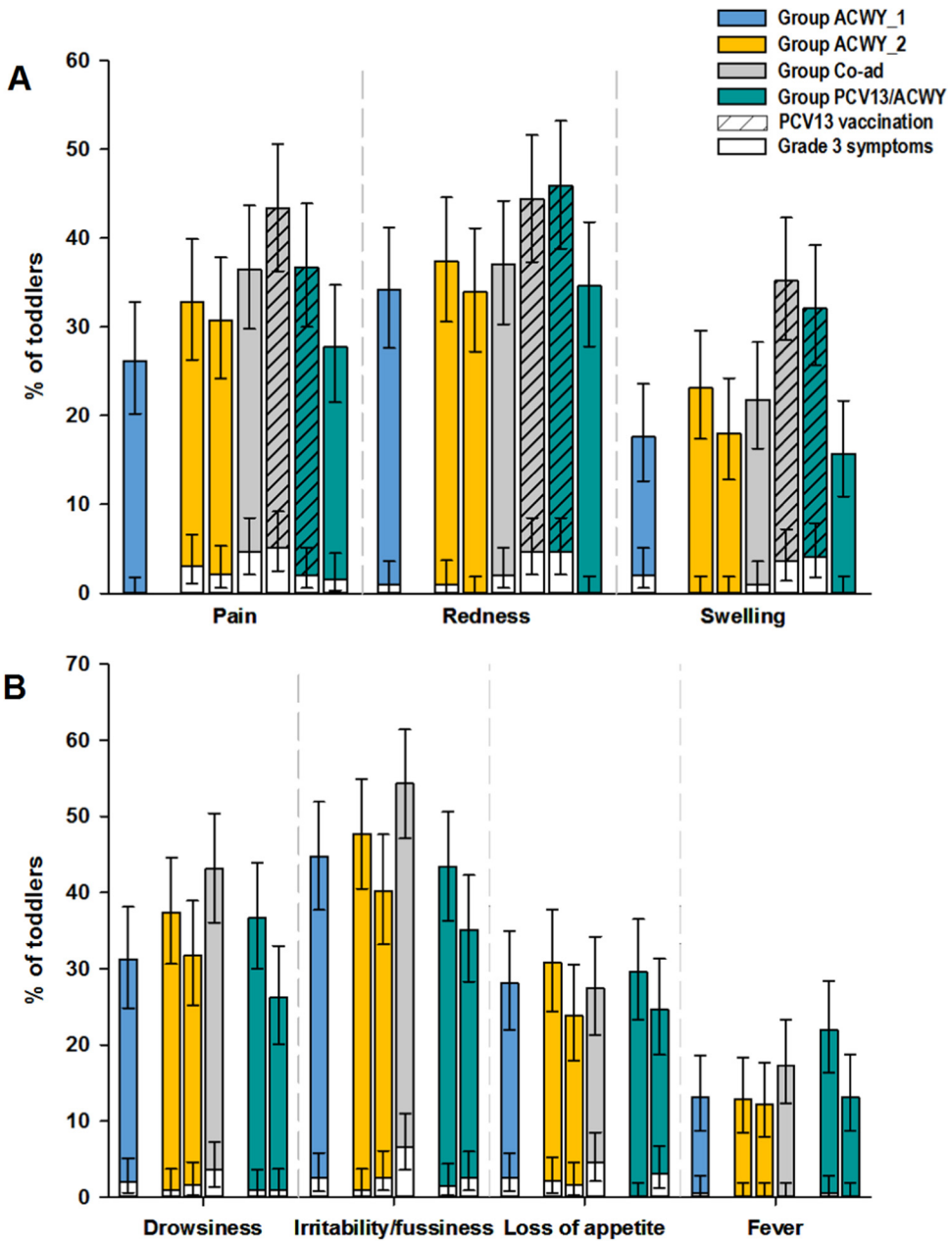

Fig. 2. Percentage of toddlers with reported solicited local (A) and general (B) symptoms (total vaccinated cohort). ACWY_1, toddlers who received 1 dose of MenACWY-TT at month 0; ACWY_2, toddlers who received 2 doses of MenACWY-TT at month 0 and month 2; Co-ad, toddlers who received 1 dose of MenACWY-TT and 1 dose of PCV13 at month 0; PCV13/ACWY, toddlers who received 1 dose of PCV13 at month 0 and 1 dose of MenACWY-TT at month 2. Note: Grade 3 symptoms were defined as "cries when limb is moved/spontaneously painful" for pain, "surface >30 mm" for redness and swelling, "not eating at all" for loss of appetite, "rectal temperature >40 °C" for fever, and "interfering with normal activity" for all other symptoms. Error bars represent 95% confidence intervals.

Trademark statement

Menactra is a trademark of Sanofi Pasteur. *Menveo* is a trademark of the GSK group of companies. *Nimenrix* is a trademark of the GSK group of companies, licensed to Pfizer. *Prevenar/Prevenar 13* is a trademark of Pfizer.

Acknowledgements

The authors would like to thank the parents and their children who participated in this trial and acknowledge the assistance of all the investigators, study nurses, clinicians, laboratory personnel and other staff members in conducting the study. The authors would also like to thank Claire Verbelen and Petronela M. Petrar (XPE Pharma & Science c/o GSK) for medical writing support and Regis Azizieh (XPE Pharma & Science c/o GSK) for manuscript coordination and editorial support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.02.013.

References

[1] Martinon-Torres F. Deciphering the burden of meningococcal disease: conventional and under-recognized elements. J Adolesc Health 2016;59:S12–20.
[2] McIntyre PB, O'Brien KL, Greenwood B, van de Beek D. Effect of vaccines on bacterial meningitis worldwide. Lancet 2012;380:1703–11.

[3] 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.

[4] Campbell H, Parikh SR, Borrow R, Kaczmarski E, Ramsay ME, Ladhani SN, et al. Presentation with gastrointestinal symptoms and high case fatality associated with group W meningococcal disease (MenW) in teenagers, England, July 2015 to January 2016. Euro Surveill July 2015;2016:21.

[5] Lucidarme J, Hill DM, Bratcher HB, Gray SJ, du Plessis M, Tsang RS, et al. Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. J Infect 2015;71:544–52.

[6] Mustapha MM, Marsh JW, Harrison LH. Global epidemiology of capsular group W meningococcal disease (1970–2015): Multifocal emergence and persistence of hypervirulent sequence type (ST)-11 clonal complex. Vaccine 2016;34:1515–23.

[7] Carville KS, Stevens K, Sohail A, Franklin LJ, Bond KA, Brahmi A, et al. Increase in meningococcal serogroup W disease, Victoria, Australia, 2013–2015. Emerg Infect Dis 2016;22:1785–7.

[8] Khatami A, Pollard AJ. The epidemiology of meningococcal disease and the impact of vaccines. Expert Rev Vaccines 2010;9:285–98.

[9] Croxtall JD, Dhillon S. Meningococcal quadrivalent (serogroups A, C, W135 and Y) tetanus toxoid conjugate vaccine *(Nimenrix)*. Drugs 2012;72:2407–30.

[10] Assaf-Casals A, Dbaibo G. Meningococcal quadrivalent tetanus toxoid conjugate vaccine (MenACWY-TT, *Nimenrix*): A review of its immunogenicity, safety, co-administration, and antibody persistence. Hum Vaccin Immunother 2016;12:1825–37.

[11] Merino Arribas JM, Carmona Martinez A, Horn M, Perez Porcuna XM, Otero Reigada MD, Mares Bermudez J, et al. Safety and immunogenicity of the quadrivalent meningococcal serogroups A, C, W and Y tetanus toxoid conjugate vaccine coadministered with routine childhood vaccines in European infants: an open, randomized trial. Pediatr Infect Dis J 2017;36: e98–e107.

[12] 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. Pediatr Infect Dis J 2013;32:760–7.

[13] 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;28:744–53.

[14] 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;29:4264–73.

[15] Memish ZA, Dbaibo G, Montellano M, Verghese VP, Jain H, Dubey AP, et al. Immunogenicity of a single dose of tetravalent meningococcal serogroups A, C, W-135, and Y conjugate vaccine administered to 2- to 10-year-olds is noninferior to a licensed-ACWY polysaccharide vaccine with an acceptable safety profile. Pediatr Infect Dis J 2011;30:e56–62.

[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;29:4274–84.

[17] Klein NP, Baine Y, Kolhe D, Baccarini CI, Miller JM, Van der Wielen M. Five-year antibody persistence and booster response after 1 or 2 doses of meningococcal A, C, W and Y tetanus toxoid conjugate vaccine in healthy children. Pediatr Infect Dis J 2016;35:662–72.

[18] Knuf M, Baine Y, Bianco V, Boutriau D, Miller JM. Antibody persistence and immune memory 15 months after priming with an investigational tetravalent meningococcal tetanus toxoid conjugate vaccine (MenACWY-TT) in toddlers and young children. Hum Vaccin Immunother 2012;8:866–72.

[19] Vesikari T, Forsten A, Bianco V, Van der Wielen M, Miller JM. Immunogenicity, safety and antibody persistence of a booster dose of quadrivalent meningococcal ACWY-tetanus toxoid conjugate vaccine compared with monovalent meningococcal serogroup C vaccine administered four years after primary vaccination using the same vaccines. Pediatr Infect Dis J 2015;34:e298–307.

[20] Vesikari T, Forsten A, Bianco V, Van der Wielen M, Miller JM. Antibody persistence up to 5 years after vaccination of toddlers and children between 12 months and 10 years of age with a quadrivalent meningococcal ACWY-tetanus toxoid conjugate vaccine. Hum Vaccin Immunother 2016;12:132–9.

[21] 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.

[22] European Medicines Agency. Prevenar 13. Summary of product characteristics. Available from: <http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001104/WC500057247.pdf> [accessed 2017 February 7].

[23] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129:1307–26.

[24] Concepcion NF, Frasch CE. Pneumococcal type 22F polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. Clin Diagn Lab Immunol 2001;8:266–72.

[25] Romero-Steiner S, Libutti D, Pais LB, Dykes J, Anderson P, Whitin JC, et al. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. Clin Diagn Lab Immunol 1997;4:415–22.

[26] 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.

[27] Borrow R, Balmer P, Miller E. Meningococcal surrogates of protection-serum bactericidal antibody activity. Vaccine 2005;23:2222–7.

[28] Bona G, Castiglia P, Zoppi G, de Martino M, Tasciotti A, D'Agostino D, et al. Safety and immunogenicity of a CRM or TT conjugated meningococcal vaccine in healthy toddlers. Vaccine 2016;34:3363–70.

[29] 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 Vaccines 2015;14:841–59.

[30] 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 2010;29:259–67.

[31] 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.

[32] Ruiz-Palacios GM, Huang LM, Lin TY, Hernandez L, Guerrero ML, Villalobos AL, et al. Immunogenicity and safety of a booster dose of the 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine coadministered with the tetravalent meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine in toddlers: a randomized trial. Pediatr Infect Dis J 2013;32:62–71.

[33] Dagan R, Poolman J, Siegrist CA. Glycoconjugate vaccines and immune interference: a review. Vaccine 2010;28:5513–23.

[34] Leonardi M, Latiolais T, Sarpong K, Simon M, Twiggs J, Lei P, et al. Quadrivalent meningococcal (MenACWY-TT) conjugate vaccine or a fourth dose of *H. influenzae-N. meningitidis* C/Y conjugate vaccine (HibMenCY-TT) is immunogenic in toddlers who previously received three doses of HibMenCY-TT in infancy. Vaccine 2015;33:933–41.

[35] Plosker GL. 13-valent pneumococcal conjugate vaccine: a review of its use in infants, children, and adolescents. Paediatr Drugs 2013;15:403–23.