RESEARCH PAPER

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Immunogenicity and safety of a CRM-conjugated meningococcal ACWY vaccine administered concomitantly with routine vaccines starting at 2 months of age

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Background: Infants are at the highest risk for meningococcal disease and a broadly protective and safe vaccine is an unmet need in this youngest population. We evaluated the immunogenicity and safety of a 4-dose infant/toddler regimen of MenACWY-CRM given at 2, 4, 6, and 12 months of age concomitantly with pentavalent diphtheria-tetanus-acellular pertussis-*Hemophilus influenzae* type b-inactivated poliovirus-combination vaccine (DTaP-IPV/Hib), hepatitis B vaccine (HBV), 7- or 13-valent conjugate pneumococcal vaccine (PCV), and measles, mumps, and rubella vaccine (MMR).

Results: Four doses of MenACWY-CRM induced hSBA titers ≥8 in 89%, 95%, 97%, and 96% of participants against serogroups A, C, W-135, and Y, respectively. hSBA titers ≥8 were present in 76–98% of participants after the first 3 doses. A categorical linear analysis incorporating vaccine group and study center showed responses to routine vaccines administered with MenACWY-CRM were non-inferior to routine vaccines alone, except for seroresponse to the pertussis antigen fimbriae. The reactogenicity profile was not affected when MenACWY-CRM was administered concomitantly with routine vaccines.

Conclusion: MenACWY-CRM administered with routine concomitant vaccinations in young infants was well tolerated and induced highly immunogenic responses against each of the serogroups without significant interference with the immune responses to routine infant vaccinations.

Methods: Healthy 2 month old infants were randomized to receive MenACWY-CRM with routine vaccines (n = 258) or routine vaccines alone (n = 271). Immunogenicity was assessed by serum bactericidal assay using human complement (hSBA). Medically attended adverse events (AEs), serious AEs (SAEs) and AEs leading to study withdrawal were collected throughout the study period.

Introduction

Invasive meningococcal disease remains one of the most devastating bacterial infections worldwide and can cause serious disability or death in an otherwise healthy person in a matter of hours.[1,2] Annually, an estimated 500 000 cases of invasive meningococcal disease occur worldwide, causing 50 000 deaths.[3] The vast majority of invasive meningococcal disease (>90%) can be attributed to 1 of 5 immunologically distinct serogroups: A, B, C, W-135, and Y.[4,5] A quadrivalent meningococcal vaccine against serogroups A, C, W-135, and Y offers a broad protection against infection.

The distribution of serogroups varies geographically, with serogroups B and C predominating in Europe, Australia, and New Zealand, serogroups A, C, and W-135 most common in Asia and Africa and serogroups B, C, and Y predominating in the Americas.[6] Infants in their first year of life are at the highest risk for meningococcal disease, with peak incidence occurring in the first 6 mo.[7,8] While serogroup C meningococcal conjugate vaccines have had a profound effect where widely employed,[9,10] a safe and effective vaccine that broadly protects infants from 2 mo of age against meningococcal disease is an unmet need.[11]

MenACWY-CRM (Menveo®, Novartis Vaccines and Diagnostics) is a quadrivalent (A, C, W-135, and Y) meningococcal vaccine conjugated to CRM_{197}, a nontoxic mutant of diphtheria toxin, as the carrier protein.[12] Previous phase 2 and 3 studies demonstrated that MenACWY-CRM elicited highly immunogenic responses and was well-tolerated in adults,[13-15] adolescents,[13,16,17] children,[18,19] and importantly, young infants.[20-23]

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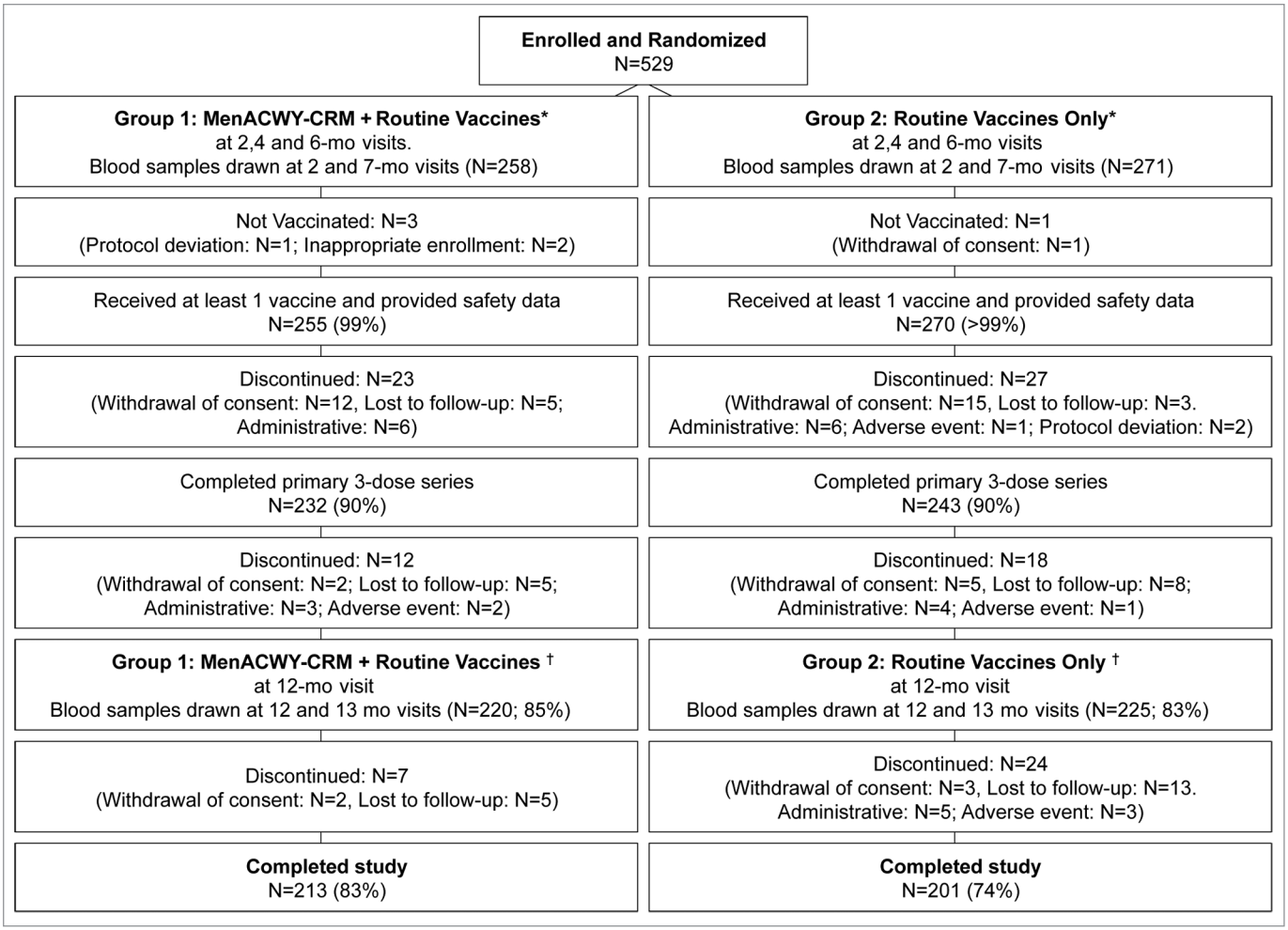

Figure 1. Subject disposition flowchart. *Routine vaccines at 2, 4, and 6 mo visits included DTaP-IPV/Hib, HBV, and either PCV-7 or PCV-13. †Routine vaccines at 12 mo visit included MMR and either PCV-7 or PCV-13.

Adding novel vaccines to infant vaccination schedules generally necessitates concomitant administration and requires evidence that vaccines can be given simultaneously without negatively impacting the safety and immunogenicity of either of the vaccines. It was recently demonstrated that MenACWY-CRM induced safe, robust immune responses in infants and toddlers without concern for clinically relevant interference with routine vaccines (pentavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus combination vaccine [DTaP-HBV-IPV; Pediarix®, GlaxoSmithKline], *Hemophilus influenzae* type b-tetanus conjugate vaccine [PRP-T; ActHib®, Sanofi Pasteur], 7-valent pneumococcal conjugate vaccine [PCV7; Prevnar®, Pfizer],[20] and measles, mumps, rubella, and varicella vaccine [MMRV; ProQuad®, Merck and Co]).[21]

Here we present the results of a phase 3 study conducted in healthy infants to assess the immunogenicity and safety of MenACWY-CRM when co-administered with routine vaccines including pentavalent diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Hemophilus influenzae*-type b-combination vaccine (DTaP-IPV/Hib; Pentacel®, Sanofi Pasteur), hepatitis B vaccine (HBV; Engerix-B®, GlaxoSmithKline), and either PCV7 or 13-valent pneumococcal conjugate vaccine (PCV13; Prevnar13®, Wyeth) at 2, 4, and 6 mo of age, and measles, mumps, and rubella vaccine (MMR; M-M-R®II, Merck and Co) and either PCV7 or PCV13 at 12 mo of age. The immunogenicity and safety of DTaP-IPV/Hib, PCV, and HBV, and the safety of MMR when co-administered with MenACWY-CRM are presented.

Results

Enrolment, study flow and demographics

A total of 529 infants were enrolled and randomized, 258 to MenACWY-CRM + Routine and 271 to Routine Only. Of these, 213 in group MenACWY-CRM + Routine and 201 in group Routine Only completed the study (**Fig. 1**). With 200 evaluable subjects per group however, the power of the study for the primary immunogenicity objectives combining the 4 serogroups was still >96%.

Both groups were similar in age, gender, and weight and the majority of participants were Caucasian (**Table 1**).

Immunogenicity

Immunogenicity analyses were performed on the per protocol (PP) set: 411 (78%) participants after the 3-dose infant series (MenACWY-CRM + Routine, n = 202; Routine Only, n = 209), and 352 (67%) after the full 4-dose infant/toddler series (MenACWY-CRM + Routine, n = 172: Routine Only, n = 180). The primary immunogenicity analysis was also conducted on the modified intent to treat (MITT) set: 428 (81%) participants after the infant series and 439 (83%) participants after the toddler dose.

Responses to meningococcal ACWY antigens

The primary immunogenicity objective was sufficiency of the immune response following 4 doses of MenACWY-CRM in terms of the percentage of participants with hSBA titers ≥8. A post-vaccination hSBA titer ≥8 is used as an accepted and conservative correlate of protection against invasive meningococcal disease.[24] In the MenACWY-CRM + Routine group, 1 mo following the primary course, 76%, 94%, 98%, and 94% of infants demonstrated hSBA titers ≥8 against serogroups A, C, W-135, and Y, respectively (**Fig. 2A** [post-infant series at 7 mo of age]). A ≥4-fold increase in hSBA titers against serogroups A, C, W-135, and Y was achieved in 78%, 94%, 93%, and 93% of participants, respectively. At 12 mo of age (before the toddler dose), 7%, 37%, 70%, and 53% of infants demonstrated hSBA titers ≥8 against serogroups A, C, W-135, and Y, respectively (**Fig. 2A**).

At 13 mo of age (1 mo post-toddler dose) 89%, 95%, 97%, and 96% of toddlers who received MenACWY-CRM + Routine vaccines achieved hSBA titers ≥8 against serogroups A, C, W-135, and Y, respectively (**Fig. 2B**). The lower limit (LL) of the 2-sided 95% confidence intervals (LL95%CIs) were 83%, 90%, 93%, and 92% for serogroups A, C, W-135, and Y, respectively, which exceeded the prespecified criteria, demonstrating that the primary endpoint was achieved. As expected, these percentages were very low (1–7%) in participants who received routine vaccines only. The conclusions did not change when these analyses were performed on the MITT population.

Following the 3-dose infant series (at 7 mo), robust increases in geometric mean titers (GMTs) (**Fig. 2C**) were observed in the MenACWY-CRM + Routine group, with 10-, 30-, 27-, and 20-fold increases for serogroups A, C, W-135, and Y, respectively. Immediately prior to the 4th dose at 12 mo, 7%, 37%, 70%, and 53% of toddlers who received MenACWY-CRM + Routine vaccines still demonstrated hSBA titers ≥8 against serogroups A, C, W-135, and Y, respectively (**Fig. 2A**), and GMTs were 2.5, 6.0, 15.0, and 8.4 for the respective serogroups.

One month following the toddler dose, 89%, 92%, 95%, and 96% of participants showed a ≥4-fold increase in pre-toddler dose titers against serogroups A, C, W-135, and Y, respectively. Robust increases in GMTs were also observed in this group with 21-, 23-, 14-, and 22-fold increases against serogroups A, C, W-135, and Y, respectively (**Fig. 2C**).

Responses to co-administered antigens

Following the 3-dose infant series (**Fig. 3A**), the LL95%CIs for geometric mean concentration (GMC) ratios for pertussis antigens were all >0.67 and the LL95%CIs for GMC ratios for all other antigens were >0.5 (non-inferiority criterion met).

Table 1. Study population demographics

	MenACWY-CRM + Routine (N = 258)	Routine Only (N = 271)
Age, days (Mean ± SD)	64.7 ± 6.5	65.4 ± 7.4
Male, n (%)	133 (52)	141 (52)
Female, n (%)	125 (48)	130 (48)
Weight, kg (Mean ± SD)	5.4 ± 0.7*	5.4 ± 0.7
Ethnicity		
Asian, n (%)	3 (1)	1 (<1)
Black, n (%)	20 (8)	29 (11)
Caucasian, n (%)	168 (65)	177 (65)
Hispanic, n (%)	44 (17)	45 (17)
Other, n (%)	23 (9)	19 (7)
Met entry criteria, n (%)	254 (98)	266 (98)

SD, standard deviation; *N = 257

Non-inferiority criteria for the difference in seroresponse rates were met for all of the antigens except pertussis antigens pertussis toxin (PT) (LL95%CI: –12.1%) and fimbriae (FIM) (LL95%CI: –10.6%) (**Fig. 3A**), and pneumococcal serotypes 6B (LL95%CI: –10.3%) and 23F (LL95%CI: –11.4%), only marginally exceeding the non-inferiority threshold (**Fig. 3B**). Post-hoc analyses incorporating group and study center were performed for these 4 antibody responses. After adjustment for center differences, responses to PT, 6B, and 23F met non-inferiority criteria (LL95%CI: –3.9, –1.8, and –2.3, respectively). However, the CI for the response to FIM remained just outside the non-inferiority boundary (group difference – 2%, [95%CI: –10.2, 5.9]).

Following the 4th dose of PCV at 12 mo of age, the LL95%CIs for group differences in seroresponse rates were > –10% for all pneumococcal antigens, and the LL95%CIs for GMC ratios for all antigens were >0.5 (non–inferiority criteria met) (**Fig. 3C**).

Although all participants received a 4-dose series of concomitant PCV, individual participants received 0–4 doses of PCV13 due to the transition from PCV7 to PCV13 that occurred during the study. **Table 2** shows the ratio of pneumococcal conjugate polysaccharide (PnC) concentrations (MenACWY-CRM + Routine/Routine Only) for each serotype common to both vaccines, per number of PCV13 doses received. The ratios of PnC concentrations were consistently >1 in participants who received 4 doses of PCV13, but no conclusions can be drawn due to the fact that very few participants received 4 doses of PCV13.

Reactogenicity and Safety

Of 529 enrolled participants, 525 (99%) were exposed to ≥1 study vaccination and contributed to the safety analyses. Adverse event (AE) rates were similar between groups, with low rates of "possibly vaccine-related" events (**Table 3**). Noting that there was

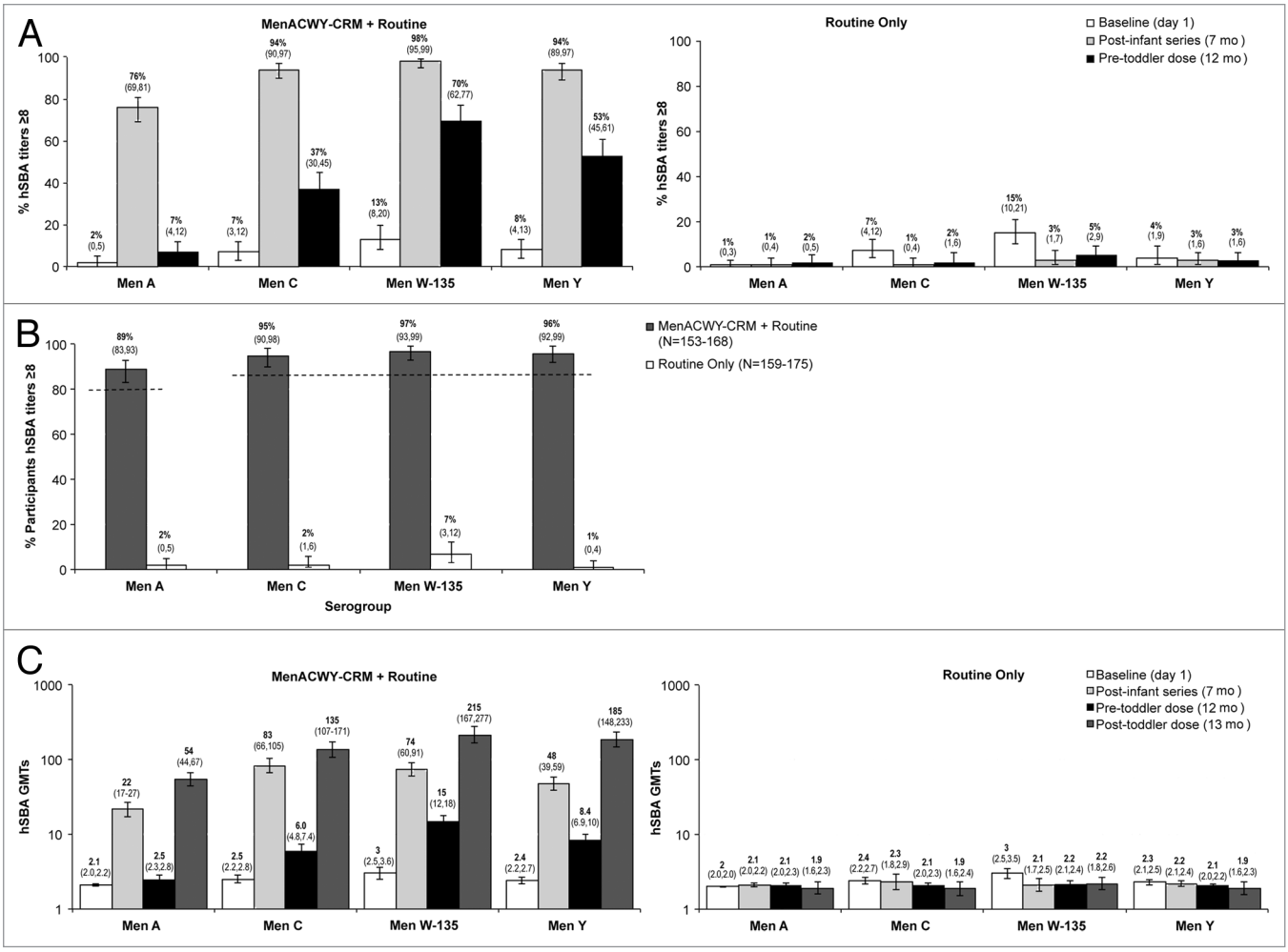

Figure 2. (**A**) Percentage of participants (95% CI) in MenACWY-CRM + Routine (left) and Routine Only (right) groups with hSBA titers ≥8 at baseline, 1-mo following the 3-dose infant series (7 mo) and prior to the toddler dose, at 12 mo, per serogroup. (**B**) Immunogenicity results after 4 doses of MenACWY-CRM. Percentage of participants (95% CI) with hSBA titers ≥8 at 1-mo after the 4th vaccination (13 mo) in MenACWY-CRM + Routine and Routine Only groups, per serogroup.* Dashed lines illustrate criterion for response sufficiency, i.e., LL95%CI ≥85% for serogroups C, W-135, and Y and ≥80% for serogroup A after 4 doses of MenACWY-CRM. (**C**) GMTs (95% CI) in MenACWY-CRM + Routine (left) and Routine only (right) groups before vaccination, 1-mo after the 3-dose infant series (7 mo), prior to toddler dose (12 mo), and 1-mo following toddler dose (13 mo), per serogroup.

no placebo control group, the most commonly reported AE by preferred term was upper respiratory infection (56% MenACWY-CRM + Routine, 57% Routine Only), followed by otitis media (39% both groups), and conjunctivitis (23% MenACWY-CRM + Routine, 19% Routine Only). Overall, serious AEs (SAEs) were reported in 21 participants (8%) receiving MenACWY-CRM + Routine vaccines and in 20 participants (7%) receiving Routine Only. No SAEs were considered vaccine-related. There were few study withdrawals due to AEs (1% MenACWY-CRM + Routine, 2% Routine Only group) and none were vaccine-related. There were no deaths.

Discussion

Young children are at highest risk for meningococcal disease, especially in the first year of life.[7,25,26] The development of a sufficiently immunogenic vaccine to broadly protect infants from meningococcal disease has proven challenging. Recent studies in this vulnerable population have demonstrated promising results for MenACWY-CRM as indicated by robust immune responses against serogroups A, C, W-135, and Y and a well-tolerated safety profile.[20-23] To incorporate new vaccines into routine childhood immunization schedules, evidence is required that vaccines can be given concomitantly without compromising their immune responses and reactogenicity and safety profiles.

Results from the present study demonstrate that 4 doses of MenACWY-CRM given at 2, 4, 6, and 12 mo of age with concomitant pentavalent DTaP-IPV/Hib, hepatitis B, 7- or 13-valent PCV and MMR vaccines induced robust immune responses against all 4 serogroups, as evidenced by 89%, 95%, 97%, and 96% of participants with hSBA titers ≥8 against serogroups A, C, W-135, and Y, respectively. GMTs increased 14–23-fold from pre- to post-toddler dose and 89–96% of participants showed a ≥4-fold increase. Importantly, robust immune responses were also observed

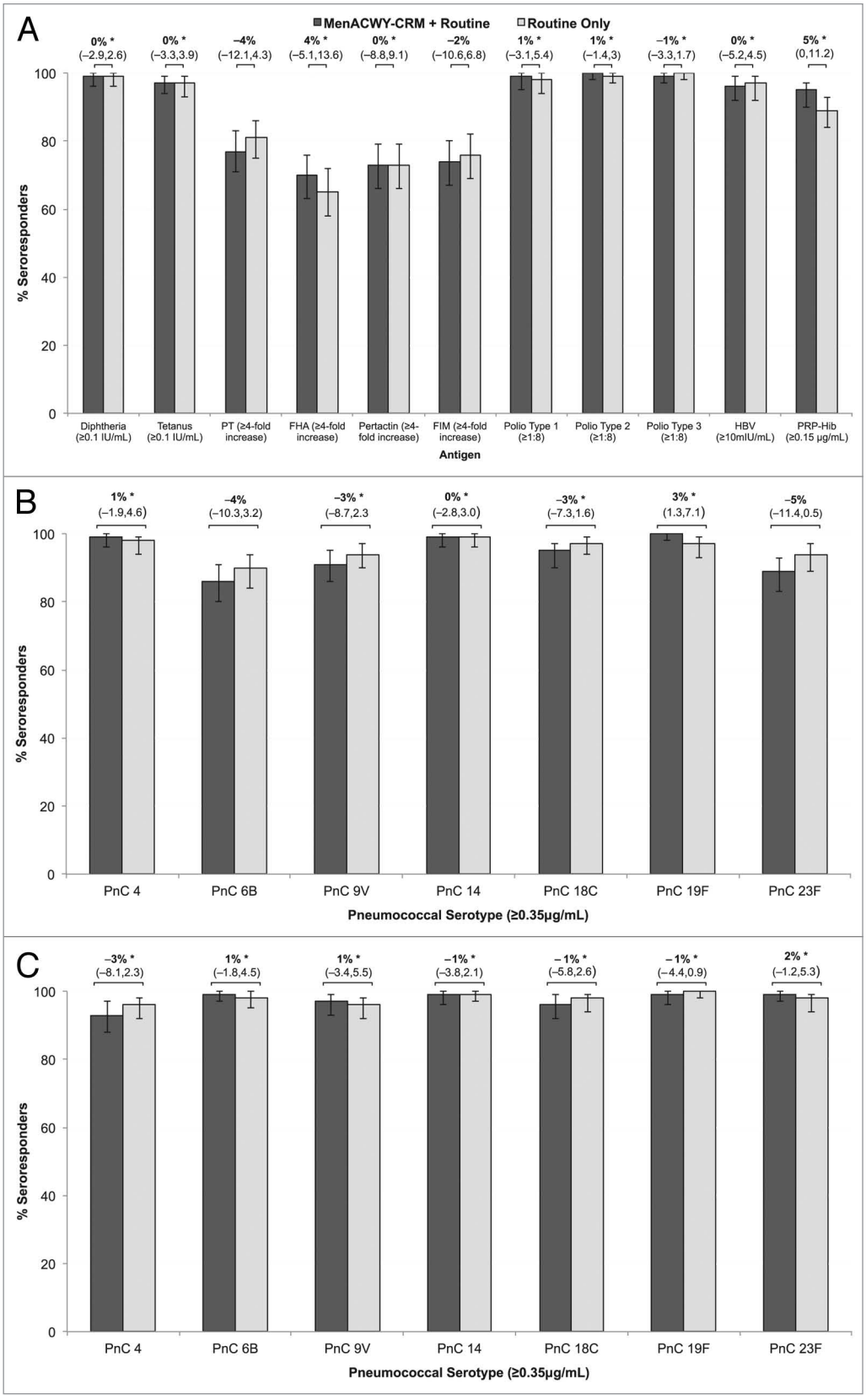

Figure 3. For figure legend, see page 285.

Figure 3 (See opposite page). Seroresponse in participants with and without MenACWY-CRM after routine vaccinations at 2, 4, 6, and 12 mo. The difference in percentage of seroresponders (95% CI) is shown. (**A**) Percentage of participants with seroresponse to concomitant vaccinations DTaP-IPV/Hib 1-mo after the 3-dose infant series, at 7 mo. (**B**) Percentage of participants with seroresponse to pneumococcal conjugate 1-mo after the 3-dose infant series, at 7 mo. (**C**) Percentage of participants with seroresponse to pneumococcal conjugate 1-mo after the 4th vaccine dose, at 13 mo. *Non-inferiority criteria met for the difference in percentage of seroresponders, i.e., LL95%CI ≥−5% for poliovirus and −10% for all other antigens.

after the 3-dose infant series of MenACWY-CRM + Routine with 76%, 94%, 98%, and 94% of infants achieving hSBA titers ≥8 against serogroups A, C, W-135, and Y, respectively.

These findings are in line with recently published pivotal phase 3 infant data,[20] in which another pentavalent DTaP-HBV-IPV was co-administered. In that study, hSBA titers ≥8 were present in 94–100% of participants after the full 4-dose series and in 67–97% after the 3-dose infant series. Antibody persistence was demonstrated before the 4th dose in 12–69% of participants, and GMTs increased 4.5–38-fold from before to after dose 4.

The study by Klein et al. also demonstrated significantly higher GMTs for all serogroups after a 4th dose of MenACWY-CRM at 12 mo, compared with a first dose given at 12 mo (range of ratios 4.5–38). These findings support previous results demonstrating that meningococcal conjugate vaccines induce immunological memory, bringing about an elevated immune response to subsequent vaccination.[22,27] By contrast, only modest immunogenicity after a 3-dose infant series was observed with a quadrivalent meningococcal vaccine conjugated to diphtheria toxoid.[28] In that study, immunogenicity was tested by SBA using rabbit complement (rSBA), which results in higher titers compared with when human complement is used. One month after the 3-dose series, rSBA titers ≥8 were present in 54–92% of children, depending on the serogroup and the dose of polysaccharide contained in the vaccine.[28] The role of carrier proteins may be of relevance in this context, as previous studies have demonstrated differential immunogenicity in Hib vaccines using diphtheria toxoid or CRM_{197} carrier proteins.[29,30]

The percentages of subjects with hSBA titers ≥8 were lower against serogroup A than the other serogroups, an observation that has been reported in other studies.[20,23,31,32] Measures of immunogenicity by rSBA after MenACWY-CRM have yielded much higher titers with prolonged persistence of antibodies.[33] However, the clinical relevance of these differences between serogroup responses are yet to be determined.

As has been observed with other glycoconjugate vaccines,[34-37] as well as previous MenACWY-CRM studies,[20,23,31] there was evidence of waning antibodies after infant vaccination by 12 mo of age. In the appropriate epidemiologic setting, these data support the importance of the dose in the second year of life, which this study and others have demonstrated to elicit an anamnestic response.[20,23,31]

Immune responses to routine infant vaccinations (pneumococcal, IPV, HBV, Hib, DTaP) were generally not affected when administered concomitantly with MenACWY-CRM. Non-inferiority of the immune response to all routine vaccine antigens was demonstrated after the 3-dose infant series and after the toddler dose at 12 mo, except for slight differences in the post-infant seroresponse rates for pertussis antigens pertactin (PRN) and FIM, and pneumococcal serotypes 6B and 23F, although the non-inferiority criterion for the GMC ratio was achieved. Klein et al. recently demonstrated that responses to routine vaccines with MenACWY-CRM were non-inferior to routine vaccines alone, except for the seroresponse rate for pneumococcal serotype 6B (after the 3-dose infant series but not after dose 4) and PRN after dose 3. After adjustment for center differences in our study, only FIM continued to exceed the non-inferiority CI boundary for seroresponse rate. Non-inferiority was achieved for this antigen in terms of GMC ratio, and the difference in percentage of seroresponders between MenACWY-CRM + Routine and Routine Only groups was only 2% (**Fig. 3A**); however, the clinical significance of this is unclear.

This study has some limitations, including a high withdrawal rate. In total 115 subjects (22% of enrolled population) withdrew from the study early, mainly due to withdrawal of informed consent not related to AEs (8%), lost to follow-up (7%), or

Table 2. Ratio of concentrations of antibodies against PnC antigens (MenACWY-CRM + Routine/Routine Only) by PnC serotype and number of PCV13 doses

Number of doses per PCV vaccine	0 PCV13, 4 PCV7	1 PCV13, 3 PCV7	2 PCV13, 2 PCV7	3 PCV13, 1 PCV7	4 PCV13, 0 PCV7
Number of participants[1]	66 (34/32)	63 (31/32)	90 (48/42)	102 (41/61)	10 (7/3)
Serotype[2]					
4	0.75 (0.51–1.11)	1.19 (0.71–2.0)	1.11 (0.78–1.58)	0.85 (0.58–1.26)	4.12 (0.8–21)
6b	0.60 (0.36–1)	0.71 (0.42–1.18)	0.78 (0.51–1.18)	0.82 (0.56–1.18)	2.41 (0.58–10)
9v	0.64 (0.43–0.97)	0.94 (0.6–1.46)	0.98 (0.7–1.38)	0.84 (0.57–1.24)	3.19 (1.43–7.09)
14	0.75 (0.46–1.23)	1.26 (0.7–2.27)	1.25 (0.88–1.77)	0.9 (0.61–1.32)	3.29 (0.37–29)
18C	0.67 (0.47–0.96)	1.06 (0.68–1.68)	1.19 (0.82–1.71)	1.03 (0.72–1.48)	2.08 (1.04–4.17)
19F	0.63 (0.43–0.9)	0.94 (0.54–1.63)	0.94 (0.67–1.3)	1.02 (0.72–1.46)	1.42 (0.23–8.73)
23F	0.79 (0.53–1.19)	0.95 (0.54–1.67)	0.93 (0.63–1.37)	0.73 (0.48–1.12)	1.33 (0.2–8.67)

Note: [1]Numbers of participants are reported as total N ($N_{MenACWY\text{-}CRM+Routine}$ / $N_{Routine\ Only}$). [2]GMC ratios were computed as $GMC_{MenACWY\text{-}CRM+Routine}$ / $GMC_{Routine\ Only}$ and are reported as GMC ratio (CI).

Table 3. Adverse events reported in ≥5% of participants in the MenACWY-CRM + Routine and Routine Only groups

N (%)	MenACWY-CRM + Routine	Routine Only
Between 2 and 4 months		
Any AE	99 (39%)	105 (39%)
AEs reported in ≥5% of participants		
Otitis Media	12 (5%)	16 (6%)
Upper respiratory tract infection	46 (18%)	37 (14%)
Between 4 and 6 months		
Any AE	118 (49%)	114 (45%)
AEs reported in ≥5% of participants		
Otitis Media	21 (17%)	17 (7%)
Upper respiratory tract infection	31 (13%)	39 (15%)
Between 6 and 12 months		
Any AE	187 (78%)	197 (79%)
AEs reported in ≥5% of participants		
Conjunctivitis	25 (10%)	17 (7%)
Diarrhea	18 (8%)	15 (6%)
Teething	11 (5%)	-
Vomiting	-	14 (6%)
Pyrexia	24 (10%)	25 (10%)
Bronchiolitis	-	12 (5%)
Candidiasis	11 (5%)	-
Gastroenteritis	22 (9%)	15 (6%)
Otitis Media	68 (28%)	63 (25%)
Otitis Media Acute	14 (6%)	19 (8%)
Pharyngitis	14 (6%)	14 (6%)
Upper respiratory tract infection	86 (36%)	94 (38%)
Viral infection	20 (8%)	25 (10%)
Cough	16 (7%)	13 (5%)
Within 28 Days After 12-Month		
Any AE	80 (36%)	78 (35%)
AEs reported in ≥5% of participants		
Otitis Media	21 (10%)	21 (9%)
Upper respiratory tract infection	28 (13%)	19 (8%)
Between 13 and 18 months (End of study)		
Any AE	137 (64%)	137 (67%)
AEs reported in ≥5% of participants		
Conjunctivitis	21 (10%)	15 (7%)
Pyrexia	14 (7%)	-
Otitis Media	54 (25%)	53 (26%)
Otitis Media Acute	17 (8%)	-
Rhinitis	10 (5%)	-
Upper respiratory tract infection	53 (25%)	52 (25%)
Viral infection	15 (7%)	21 (10%)
Dermatitis Diaper	-	10 (5%)

administrative reasons (5%). The higher than expected drop-out rate decreases the power to conclude on non-inferiority for routine childhood vaccines (especially for pneumococcal antigens at 1 mo after the third vaccination). Furthermore, the study was conducted during the transition from PCV7 to PCV13, and therefore the subjects may have received a mix of Prevnar 7® and Prevnar 13®. As a result, only data for the 7 serogroups that are common in both vaccines (4, 6B, 9V, 14, 18C, 19F, and 23F) were evaluated.

Overall, all vaccines were well tolerated and the safety profile was not affected when MenACWY-CRM was administered concomitantly with routine vaccines. Altogether, the current findings support recently published data that MenACWY-CRM is highly immunogenic and well tolerated in healthy infants without major concern for clinical immunological interference with routine infant vaccination.[20,21]

Conclusions

The full 4-dose series of MenACWY-CRM administered with routine concomitant pentavalent DTaP-IPV/Hib, HBV, and PCV vaccines at 2, 4, and 6 mo and with PCV and MMR vaccines at 12 mo of age was well tolerated and induced highly immunogenic responses against all 4 serogroups without clinically relevant interference with immune responses to routine vaccinations.

Methods

Study design

This phase 3, randomized, open-label, controlled, multi-center study was conducted at 42 sites in the United States, 3 sites in Australia, and 1 site in Canada from November 2009–November 2011 (Clinicaltrials.gov identifier: NCT01000311) and was designed according to Good Clinical Practice and the 1975 Declaration of Helsinki. Ethics review committees of participating centers approved the protocol and amendments, and written informed consent was obtained from parents/legal guardians prior to enrolment.

Participants

A total of 529 healthy 2 mo old infants were randomized 1:1 to receive 4 doses of MenACWY-CRM co-administered with routine vaccines (MenACWY-CRM + Routine; n = 258) at 2, 4, 6, and 12 mo of age or routine vaccines alone (Routine Only; n = 271) (**Fig. 1**). Randomization was performed using site-specific envelopes, whereby each site was assigned a set of randomization blocks containing the 1:1 ratio with a block size of 4.

Eligible participants were healthy infants aged 2 mo (55–89 d, inclusive) at the time of enrolment. Exclusion criteria included exposure to or vaccination against: *N. meningitidis*; *C. diphtheriae*; *C. tetani*; *B. pertussis*;

poliovirus; Hib; pneumococcus; or receipt of the second dose of HBV. Other exclusion criteria were history or likelihood of anaphylaxis or adverse reactions to vaccine components; history or ongoing (chronic) illness likely to interfere with results; impairment/alteration of the immune system; significant acute or chronic infection within 7 d, or fever ≥38 °C within 3 d of enrolment; receipt of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation; and previous receipt or intent to receive any investigational agents or vaccines prior to study completion. Also, family or household members of study staff and participants whose parents/legal representatives were planning to leave the area during the study were excluded.

Vaccines

MenACWY-CRM (lot numbers X79P45I1 and 091601) was prepared by extemporaneous mixing of the lyophilized MenA component with the liquid MenCWY component before intramuscular injection. Each vaccine contained 10 µg of meningococcal serogroup A and 5 µg each of capsular polysaccharide of serogroups C, W-135, and Y conjugated to CRM_{197} in a 0.5 mL dose.[17] The 4-dose infant/toddler MenACWY-CRM regimen consisted of a 3-dose infant series (at 2, 4, and 6 mo) followed by a toddler dose at 12 mo.

Routine vaccines included DTaP-IPV/Hib, HBV, and PCV at 2, 4, and 6 mo of age and MMR and PCV at 12 mo (**Fig. 1**). During the study, there was a transition from PCV7 to PCV13, and infants received the latter in accordance with national recommendations. Vaccines were administered into the anterolateral region of either thigh, except the MMR vaccine, which was administered subcutaneously to the deltoid region of the arm.

Immunogenicity

Blood samples (2–5 mL) were obtained for immunogenicity analyses before and 1-mo after the 3-dose infant vaccinations (at 2 and 7 mo), and before and 1-mo after the 12-mo toddler dose (at 12 and 13 mo). For hSBA and HBV, sera were analyzed using validated methods at the Novartis laboratory (Clinical Serology, Novartis Vaccines). For all other antigens, sera were analyzed at two external contract labs; one lab analyzed antigens against DTaP-IPV/Hib and the other analyzed antigens against PCV. The laboratory staff who performed the serology assays were blinded to study group allocation and visit associated with the samples.

Immunogenicity was assessed by serum bactericidal assay using human complement (hSBA), expressed as hSBA GMTs, percentage of participants with hSBA titers ≥8, and with ≥4-fold increase in prevaccination titers. Immune responses to routine vaccines were assessed by standard ELISA or neutralization test methods (poliovirus). Seroprotection was defined as antibody concentration ≥0.1 IU/mL for diphtheria and tetanus, 1:8 dilution for poliovirus types 1, 2, and 3, 0.15 µg/mL for PRP/Hib, 10 mIU/mL for hepatitis B, and 0.35 µg/mL for pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. For pertussis antigens PT, filamentous hemagglutinin, PRN and FIM types 2 and 3, response was defined as antibody concentrations ≥4 times the LL of quantification for initially seronegative participants, or ≥4-fold increase in pre-vaccination antibody concentration for initially seropositive participants.

Safety

Participants were observed for ≥15 min after receipt of each vaccination to monitor for immediate reactions and parents/guardians were provided with a worksheet to record AEs until the next study visit. Reports of medically attended AEs, SAEs, and AEs leading to study withdrawal were collected throughout the study (up to 18 mo of age). AEs were classified by the investigator as not vaccine-related, possibly related, or probably related.

Statistical analyses

Analyses were performed using SAS software version 8.2 or higher (SAS Institute, Cary).

A sample of 260 participants per group was estimated to provide adequate power to assess the primary and key secondary endpoints. The primary immunogenicity objective was sufficiency of the immune response following 4 doses of MenACWY-CRM in terms of the percentage of participants with hSBA titers ≥8 at 1-mo post-4th dose, for each serogroup. The criterion for response sufficiency was LL95%CI ≥85% for serogroups C, W-135, and Y and ≥80% for serogroup A, as previously agreed with the Center for Biologics Evaluation and Research (CBER). With 200 evaluable subjects per study group, the power of the study to demonstrate the sufficiency of immune response for each serogroup was >99% and >96% for the 4 serogroups combined (the primary immunogenicity objective). The key secondary objective was non-inferiority of routine vaccinations (PCV, IPV, HBV, Hib, and DTaP) when administered concomitantly compared with administration without MenACWY-CRM. Non-inferiority criteria were the LL95%CI of the GMC ratio of MenACWY-CRM + Routine/Routine Only >0.67 for pertussis antigens and >0.50 for all other antigens. For non-inferiority, the LL95%CI for the difference in seroresponse rates ([MenACWY-CRM + Routine] – [Routine Only]) would be > –5% for poliovirus and > –10% for all other antigens. A pooled analysis of responses to the 7 shared serotypes of any combination of PCV7 and PCV13 was performed.

Log10-transformed antibody titers were analyzed using an analysis of variance model that included group and center effects for each serogroup or antigen, and GMTs/GMCs, their ratios, and corresponding LL95%CI were calculated. Titers below the limit of detection were set at half that limit. To further explore non-inferiority of seroresponses, a categorical linear model analysis that incorporated group and center in the model was performed.

Safety data were summarized as the number and percentage of participants reporting an event, per group. Immunogenicity analyses were run on the PP set: participants who received all relevant vaccine doses correctly, provided serum samples at appropriate time points, and had no major protocol violations. Primary immunogenicity analyses were also conducted on the MITT set: participants who received a study vaccination and provided ≥1 evaluable serum sample after baseline. Safety was analyzed for all participants exposed to study vaccines who provided safety data.

Disclosure of Potential Conflicts of Interest

T.M.N. received travel support from GSK, CSL, Novartis, Sanofi-Pasteur, and Wyeth (Pfizer) for participation in

investigator meetings or for the presentation of scientific data at research meetings, and honoraria for participation in a GSK independent data monitoring and safety board for an unrelated vaccine. His institution has received project-specific research funding from the same organizations to conduct clinical trials. He is a member of the WHO SAGE (Scientific Advisory Group of Experts on immunization), and chairs the Australian Government's Australian Technical Advisory Group on Immunisation (ATAGI). M.D.N. has received travel grants from Pfizer (previously Wyeth Australia) to present independent research at international meetings, and currently and previously has been the principal investigator for clinical trials sponsored by Abbott, Baxter, CSL, GSK, MedImmune, Merck, Novartis, Sanofi-Pasteur, Wyeth, and Pfizer. L.B., M.H., T.O., and P.M.D. are employees of Novartis Vaccines and Diagnostics.

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