Immunogenicity and safety of an investigational multicomponent, recombinant, meningococcal serogroup B vaccine (4CMenB) administered concomitantly with routine infant and child vaccinations: results of two randomised trials

Timo Vesikari, Susanna Esposito, Roman Prymula, Ellen Ypma, Igor Kohl, Daniela Toneatto, Peter Dull, Alan Kimura, for the EU Meningococcal B Infant Vaccine Study group

Summary

Background Meningococcal serogroup B disease disproportionately affects infants. We assessed lot-to-lot consistency, safety and immunogenicity, and the effect of concomitant vaccination on responses to routine vaccines of an investigational multicomponent vaccine (4CMenB) in this population.

Methods We did primary and booster phase 3 studies between March 31, 2008, and Aug 16, 2010, in 70 sites in Europe. We used two series of sponsor-supplied, computer-generated randomisation envelopes to allocate healthy 2 month-old infants to receive routine vaccinations (diphtheria-tetanus-acellular pertussis, inactivated poliovirus, hepatitis B plus *Haemophilus influenzae* type b, and seven-valent pneumococcal vaccine) wat 2, 4, and 6 months of age alone, or concomitantly with 4CMenB or serogroup C conjugate vaccine (MenC) in: 1) an open-label, lot-to-lot immunogenicity and safety substudy of three 4CMenB lots compared with routine vaccines alone (1:1:1:1, block size eight); or 2) an observer-blind, lot-to-lot safety substudy of three 4CMenB lots compared with MenC (1:1:1:3, block size six). At 12 months, 4CMenB-primed children from either substudy were randomised (1:1, block size two) to receive 4CMenB booster, with or without measles-mumps-rubella-varicella (MMRV) vaccine. Immunogenicity was assessed by serum bactericidal assay with human complement (hSBA) against serogroup B test strains, and on randomly selected subsets of serum samples for routine vaccines; laboratory personnel were masked to assignment. The first coprimary outcome was lot-to-lot consistency (hSBA geometric mean ratio of all lots between 0·5 and 2·0), and the second was an immune response (hSBA titre ≥5) for each of the three strains. The primary outcome for the booster study was immune response to booster dose. Immunogenicity data for 4CMenB were for the modified intention-to-treat population, including all infants from the open-label substudy who provided serum samples. The safety population included all participants who contributed safety data after at least one dose of study vaccine. These trials are registered with ClinicalTrials.gov, numbers NCT00657709 and NCT00847145.

Findings We enrolled 2627 infants in the open-label phase, 1003 in the observer-blind phase, and 1555 in the booster study. Lot-to-lot consistency was shown for the three 4CMenB lots, with the lowest 95% lower confidence limit being 0·74 and the highest upper limit being 1·33. Of 1181–1184 infants tested 1 month after three 4CMenB doses (all lots pooled), 100% (95% CI 99–100) had hSBA titres of 5 or more against strains selective for factor H binding protein and neisserial adhesin A, and 84% (82–86) for New Zealand outer-membrane vesicle. In a subset (n=100), 84% (75–91) of infants had hSBA titres of 5 or more against neisseria heparin binding antigen. At 12 months of age, waning titres were boosted by a fourth dose, such that 95–100% of children had hSBA titres of 5 or more for all antigens, with or without concomitant MMRV. Immune responses to routine vaccines were much the same with or without concomitant 4CMenB, but concomitant vaccination was associated with increased reactogenicity. 77% (1912 of 2478) of infants had fever of 38·5°C or higher after any 4CMenB dose, compared with 45% (295 of 659) after routine vaccines alone and 47% (228 of 490) with MenC, but only two febrile seizures were deemed probably related to 4CMenB.

Interpretation 4CMenB is immunogenic in infants and children aged 12 months with no clinically relevant interference with routine vaccines, but increases reactogenicity when administered concomitantly with routine vaccines. This breakthrough vaccine offers an innovative solution to the major remaining cause of bacterial meningitis in infant and toddlers.

Funding Novartis Vaccines and Diagnostics.

Lancet 2013; 381: 825–35

Published Online
January 14, 2013
http://dx.doi.org/10.1016/S0140-6736(12)61961-8

See Comment page 785

This publication has been corrected. The corrected version first appeared at thelancet.com on March 8, 2013

University of Tampere Medical School, Tampere, Finland (Prof T Vesikari MD); **Pediatric Clinic 1, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy** (Prof S Esposito MD); **University Hospital Hradec Kralove, Hradec Kralove, Czech Republic** (Prof R Prymula MD); **and Novartis Vaccines and Diagnostics, Cambridge, MA, USA** (E Ypma MSc, I Kohl MD, D Toneatto MD, P Dull MD, A Kimura MD)

Correspondence to:
Prof Timo Vesikari, Vaccine Research Centre, University of Tampere Medical School, 33014 Tampere, Finland
timo.vesikari@uta.fi

Introduction

Meningococcal serogroup B is now the most prominent cause of infant bacterial meningitis and septicaemia in Europe.[1–3] Serogroup B polysaccharide is immunologically similar to that of human neural-cell adhesion molecules and thus is poorly immunogenic, obviating its use in the traditional polysaccharide conjugate-vaccine approach.[1] Serogroup B vaccines have been developed on the basis of

outer membrane vesicles from strains that produce clonal outbreaks in New Zealand, Norway, and Cuba,[1,2,4] but these vaccines provide little protection against heterologous serogroup B strains.[5]

A novel approach to create a broadly protective vaccine against meningococcal serogroup B strains was the identification of antigens by genome mining.[6] Preclinical assessment identified three primary recombinant antigens as candidates for vaccine development: factor H binding protein, neisserial adhesin A, and neisseria heparin binding antigen.[7–9] In preliminary studies, formulations of these three antigens—factor H binding protein–genome-derived neisserial antigen-(GNA)2091 and neisseria heparin binding antigen–GNA1030 as fusion proteins,[6] together with outer membrane vesicle components from the New Zealand outbreak strain NZ98/254—induced antibodies in infants that killed several serogroup B strains in vitro.[10–12]

We report the first large-scale, phase 3 trials of primary doses in infants and a booster dose in children aged 12 months of the investigational formulation, 4CMenB (Novartis Vaccines, Siena, Italy). In these studies we aimed to assess three manufacturing lots to establish lot-to-lot consistency for safety and immunogenicity of 4CMenB vaccine, and to study the effect of concomitant vaccination with 4CMenB on responses to routine vaccines.

Methods

Study design and participants

These multicentre, phase 3, primary and booster studies were done between March 31, 2008, and Aug 16, 2010, in 70 sites in Finland, the Czech Republic, Germany, Austria, and Italy. After obtaining written, informed parental consent, we enrolled healthy 2 month-old infants, excluding infants who had infection within 7 days, or fever within 1 day of enrolment, or those who had received any other vaccine except rotavirus vaccine within 30 days of enrolment. The trials were designed according to existing principles of good clinical practice and the Declaration of Helsinki. We obtained approval from appropriate ethics committees before enrolment.

	Primary series			Booster	
	Routine plus 4CMenB (N=2481)	Routine only (N=659)	Routine plus MenC (N=490)	4CMenB plus MMRV (N=766)	4CMenB only (N=789)
Sex					
Boys	1262 (51%)	341 (52%)	256 (52%)	401 (52%)	378 (48%)
Girls	1219 (49%)	318 (48%)	234 (48%)	365 (48%)	411 (52%)
Ethnic group					
Asian	17 (1%)	1 (<1%)	22 (4%)	..	..
Black	4 (<1%)	0	4 (1%)	..	..
White	2432 (98%)	654 (99%)	449 (92%)	..	..
Hispanic	16 (1%)	0	14 (3%)	..	..
Other	12 (<1%)	4 (1%)	1 (<1%)	..	..
Age (days or months)*	73·7 (9·5)	74·7 (9·3)	70·6 (9·7)	12·3 (0·5)	12·3 (0·5)
Weight (kg)	5·72 (0·75)	5·74 (0·73)	5·52 (0·72)	10·01 (1·14)	10·02 (1·22)

Data are n (%), or mean (SD). MenC=meningococcal serogroup C conjugate vaccine. MMRV=measles, mumps, rubella, and varicella vaccine. *Age in days at first dose of primary series, and in months for booster.

Table 1: **Baseline characteristics for the enrolled primary and booster populations**

Procedures

The primary phase and booster phase were separate studies with several parts. In the primary phase, all infants received routine vaccines (diphtheria-tetanus-acellular pertussis, inactivated poliovirus, and hepatitis B plus *Haemophilus influenzae* type b [DTaP-IPV-HBV/Hib; Infanrix Hexa, GlaxoSmithKline] and seven-valent pneumococcal vaccine (PCV7; Prevenar, Pfizer) at ages 2, 4, and 6 months. This phase was done in two parts: an immunogenicity substudy and a safety substudy.

For the immunogenicity study, designed to assess the effect of the 4CMenB vaccine on immune responses to routine vaccines, we enrolled infants in Finland and the Czech Republic and randomly assigned them to four groups to receive routine vaccines alone, or routine vaccines concomitantly with one of three lots of 4CMenB. Blood samples were collected from these participants. For an observer-blind assessment of safety we enrolled a cohort of infants in Austria, Germany, Italy, and Finland and randomly assigned them to receive routine vaccines either concomitantly with one of the three lots of 4CMenB, or a meningococcal serogroup C conjugate vaccine (MenC, Menjugate; Novartis Vaccines and Diagnostics). Blood was not drawn from this cohort of participants.

Parents of all infants who completed the primary 4CMenB series in either the immunogenicity or safety parts of the study were invited to enrol their child in the booster phase of the study at 12 months of age. Participants were randomised to receive 4CMenB booster dose, either concomitantly with measles-mumps-rubella-varicella vaccine (MMRV; Priorix-Tetra, GlaxoSmithKline) or alone, when MMRV was given 1 month later. Blood samples were collected from these participants.

4CMenB vaccine was supplied in prefilled syringes, each 0·5 mL dose containing 50 μg (each) of factor H binding protein–GNA2091, neisserial adhesin A, and neisseria heparin binding antigen–GNA1030, 25 μg of outer membrane vesicles from NZ98/254, and 1·5 mg of aluminium hydroxide. Other vaccines were commercial preparations, prepared and used according to instructions. On day 1, vaccines were administered as intramuscular injections in the anterolateral area of the thigh, 4CMenB or MenC in the right leg and routine vaccines in the left leg when given concomitantly. When given alone, routine vaccines were administered in different legs. In the booster study, MMRV was administered subcutaneously in the opposite limb to 4CMenB, either deltoid muscle or anterior thigh when deltoid mass was insufficient.

We observed vaccinees for 30 min after vaccination for immediate reactions. Parents recorded solicited injection

site (injection site tenderness, erythema, swelling, induration) and systemic reactions (change in eating habits, sleepiness, vomiting, diarrhoea, irritability, unusual crying) and other data (rectal temperature, rash, or antipyretic medication), and any adverse events on diary cards for 7 days after vaccination. In 12 month-old children, either rectal or axillary temperature (at parental discretion) was monitored daily for 7 days after 4CMenB or for 28 days after MMRV vaccination. Medically attended and serious adverse events were to be reported throughout the study. Investigators assessed spontaneously reported adverse events for severity and relation to study vaccines.

We collected serum samples from the immunogenicity cohort at baseline, 1 month after the third dose, and immediately before and 1 month after 4CMenB booster and 2 months after MMRV. Immunogenicity assessments were done at the Novartis Clinical Serology Laboratory (Marburg, Germany), with blinding maintained.

The primary endpoints for the primary series were lot-to-lot consistency, and consistency of post-immune geometric

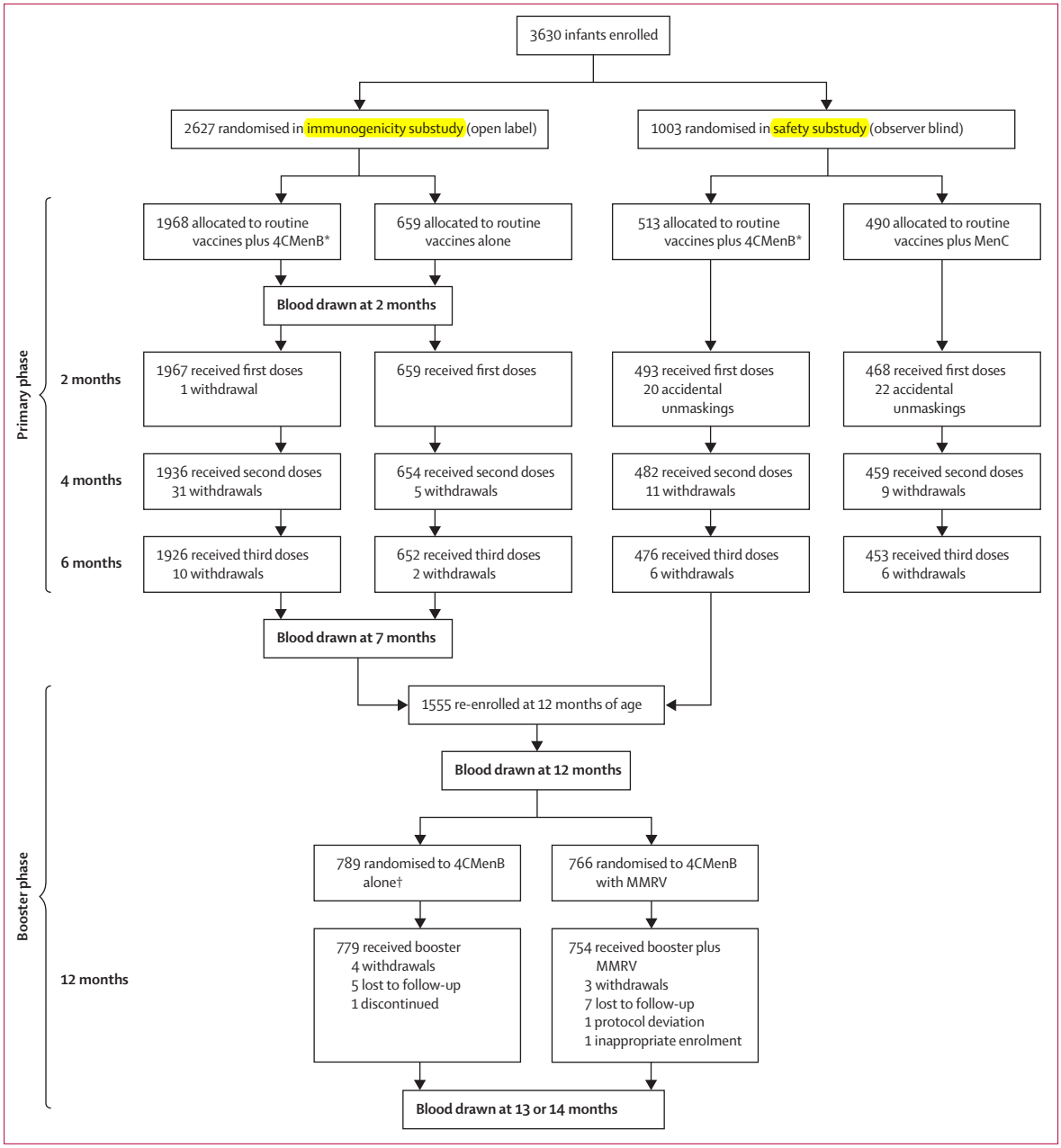

Figure 1: **Trial profile**
MenC=meningococcal serogroup C vaccine. MMRV=measles, mumps, rubella, and varicella vaccine. *Originally randomised to three equal groups for 4CMenB lot-to-lot analyses. †These children received MMRV one month after 4CMenB booster.

mean titres across the three 4CMenB lots in a serum bactericidal assay using human complement (hSBA) against three serogroup B reference strains.[4,11–14] These strains were selected because they are each specifically killed by bactericidal antibodies raised to one of the major vaccine components (44/76-SL for factor H binding protein; 5/99 for neisserial adhesin A; and NZ98/254 for PorA P1.4, the major outer-membrane protein antigen).[8] The primary endpoint for the booster study was sufficient immune response to the booster dose.

Because only small volumes of serum can be obtained from infants we had to randomise the selection of serum samples to be able to do all the required immunogenicity analyses, while ensuring that such subsets were representative of the whole study population. Subsets of 400 infants from each of the three 4CMenB lots were randomly selected to ensure 350 assessable samples for testing for the three antigens listed, together with 120 randomly selected samples from the routine vaccines only group. For testing of routine vaccine antigens we used two randomly selected groups of 270 samples (consisting of 90 participants from each of the three 4CMenB lots), one for the polio antigens and one for the other routine vaccines. Similarly, we took two randomly selected groups of 270 samples from the routine vaccines only group. The study randomisation analyst selected serum samples and supplied four lists of participant numbers (one for hSBA and three for the routine vaccines, without vaccine group information) to laboratory technicians for preparation of serum samples for testing, so that testing personnel remained blinded to study groups.

While the study was underway, strain M10713 was identified as a suitable reference strain for measurement of bactericidal responses to the neisseria heparin binding antigen and we did a post-hoc hSBA with this strain on two subsets (n=100 each) of serum samples from 4CMenB vaccinees, randomly selected from those with sufficient serum samples remaining from before and after vaccination in primary and booster phases.

	4CMenB plus routine vaccines		Routine vaccines	
	n	GMT (95% CI)	n	GMT (95% CI)
44/76-SL (factor H binding protein)				
Baseline	1195	1·2 (1·1–1·2)	122	1·1 (1·0–1·2)
1 month after dose 3	1181	91 (88–95)	120	1·2 (1·1–1·4)
GMR†	1129	79 (75–84)	114	1·1 (1·0–1·3)
5/99 (neisserial adhesin A)				
Baseline	1193	1·2 (1·1–1·2)	123	1·2 (1·1–1·3)
1 month after dose 3	1184	634 (606–664)	119	1·1 (1·0–1·3)
GMR†	1129	537 (505–571)	114	0·9 (0·8–1·1)
NZ98/254 (outer membrane vesicle)				
Baseline	1199	1·1 (1·0–1·1)	123	1·0 (1·0–1·0)
1 month after dose 3	1183	14 (13–15)	124	1·1 (1·0–1·2)
GMR†	1132	14 (13–15)	117	1·1 (1·0–1·2)
M10713 (neisseria heparin binding antigen)				
Baseline	100	3·2 (2·5–4·0)	..	..
1 month after dose 3	100	16 (13–21)	..	..
GMR†	100	5·2 (3·8–7·2)	..	..

GMT=geometric mean titre. GMR=GM ratio. *All infants from the open-label substudy who provided serum samples for immune testing. †GMR of titres after vaccination to baseline.

Table 2: **Immunogenicity of 4CMenB components before and after the three dose primary series (modified intention-to-treat analysis*)**

Randomisation and masking

For all parts and phases of the studies, randomisation was with sponsor-supplied, computer-generated randomisation envelopes. For the immunogenicity study, participants were randomised to the four groups in a 1:1:1:1 ratio, with a block size of eight, stratified according to centre. This substudy was open label because the 4CMenB vaccine was evidently an additional injection, but immunogenicity assessors were masked to assignment. For the observer-blind assessment of safety, participants were randomised to four groups in a 1:1:1:3 ratio, with a block size of six, stratified according to centre. Opening of randomisation envelopes and vaccine administrations were done by appropriately qualified study personnel separate from investigators and parents, who remained masked to assignment throughout this phase of the study. For the booster study, participants were randomised in a 1:1 ratio with a block size of two, stratified according to centre. This study was open label, but immunogenicity assessors were masked to assignment.

Statistical analysis

Immunogenicity data for 4CMenB were based on the modified intention-to-treat population, including all infants from the open-label substudy who provided serum samples for immune testing. The safety population included all participants who received at least one dose of study vaccine and contributed safety data after vaccination.

Interpolated hSBA titres were based on the reciprocal of the final serum dilution giving more than 50% killing at 60 min, compared with the number of colony-forming units at time 0. We computed geometric mean titres and 95% CIs by exponentiating (base 10) the least square means of the log-transformed titres from an ANOVA with factors for vaccine group and centre. Titres below the limit of detection were set at half the limit of detection. Percentages of participants with an hSBA titre of 5 or more, representing 95% confidence of having the accepted correlate of protection against meningococcal disease (titre ≥4),[15] a four-fold increase in titre from baseline, and geometric mean ratios were assessed. We assessed immune responses to routine vaccines with commonly accepted seroresponse measures.

The planned sample size for lot-to-lot comparison (350 assessable participants per 4CMenB lot) was based

on earlier study data.[4] The present study had 98% power to assess the first coprimary objective of lot-to-lot consistency, more than 99% power to show the second coprimary objective of a sufficient immune response for each of the three strains (4CMenB lots combined), and 94% power to show sufficient immune response for a booster dose given with or without MMRV. Lot-to-lot consistency of response after the primary series was defined as met when the two-sided 95% CI of the ratio of hSBA geometric mean titres of all three pairs of lots for each of the three strains fell between predefined values (0·5–2·0). A sufficient immune response was defined as being achieved when the lower limit of the two-sided 95% CI for the percentage of participants with hSBA titres of 5 or more was at least 70% for the three reference strains after the primary series and at least 75% after the booster. For responses to routine vaccines, the predefined non-inferiority criterion was that the lower limit of the 95% CI around the between-group difference (4CMenB minus routine) in seroresponders would be 10% or less. There were no prespecified safety analyses.

These trials are registered with ClinicalTrials.gov, numbers NCT00657709 and NCT00847145.

Role of the funding source

The investigators and representatives of the study sponsor (as stated in author affiliations) designed and did the study. Investigators collected data, which was collated and analysed by the Biostatistics and Clinical Data Management department of the study sponsor. Study authors—investigators and sponsor clinical staff—interpreted the data for study report and manuscript preparation. All authors contributed to manuscript development, the corresponding author having the final responsibility for the content and decision to submit for publication.

Results

We enrolled 3630 infants in the primary series (2627 infants in Finland and the Czech Republic, immunogenicity substudy; 1003 infants in Austria, Germany, Italy, and Finland, safety subset) and 1555 children aged 12 months in the booster study (table 1; figure 1). Baseline characteristics were much the same across the primary and booster study groups (table 1). Immunogenicity data for 4CMenB are based on the modified intention-to-treat population, of 1282 infants from the open-label substudy who provided serum samples for immune testing.

For the immunogenicity substudy, lot-to-lot consistency was shown for the three 4CMenB lots, with the lowest 95% lower confidence limit in comparison between lots being 0·74 and the highest upper limit being 1·33, so within the predefined limits of 0·5–2·0 (appendix). As outlined in the protocol, we present pooled data for all three lots for the second coprimary objective. At baseline, geometric mean titres were low (table 2) such that the proportions of infants who had hSBA titres of 5 or more against the indicator strains were 3·0% (39 of 1317) for factor H binding protein, 4·3% (57 of 1316) for neisserial adhesin A, and 1·2% (16 of 1322) for NZ outer membrane vesicle. We recorded much the same proportions at 7 months of age in the 122 infants tested from the routine vaccines only group, with 2–3% having a titre of 5 or more against any of the antigens (data

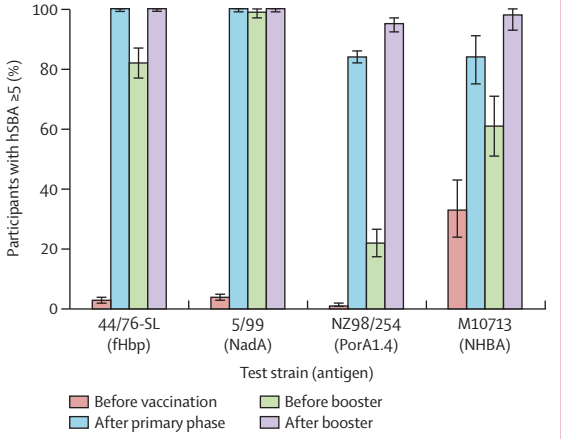

Figure 2: **Participants with hSBA titres of 5 or more against each of the test strains selective for the four vaccine antigens, before and after three primary doses of 4CMenB, and before and after a booster vaccination**
Modified intention-to-treat analyses, tables 2 and 3 show number of participants in each group. Primary 4CMenB doses were coadministered with routine vaccines. Booster 4CMenB was administered alone or with measles, mumps, rubella, and varicella vaccine. PorA1.4 I the major component of New Zealand strain outer-membrane vesicle. fHbp=factor H binding protein. NadA=neisserial adhesin A. NHBA=neisseria heparin binding antigen.

See Online for appendix

	4CMenB with MMRV		4CMenB alone	
	n	GMT (95% CI)	n	GMT (95% CI)
44/76-SL (factor H binding protein)				
Before booster	213	11·0 (9·3–12·0)	224	10·0 (9·1–12·0)
1 month after booster	212	139 (124–156)	222	119 (105–132)
GMR†	209	13 (12–14)	218	11 (10–13)
5/99 (neisserial adhesin A)				
Before booster	212	82 (72–93)	222	80 (70–91)
1 month after booster	211	1500 (1338–1681)	222	1403 (1255–1568)
GMR†	207	18 (16–20)	216	18 (16–20)
NZ98/254 (outer membrane vesicle)				
Before booster	213	2·1 (1·8–2·4)	224	2·3 (2·0–2·6)
1 month after booster	213	39 (33–46)	223	31 (27–37)
GMR†	210	19 (16–22)	219	14 (12–17)
M10713 (neisseria heparin binding antigen)				
Before booster	..	..	100	8·0 (6·1–10·0)
1 month after booster	..	..	100	42 (35–50)
GMR†	..	..	100	5·3 (4·1–6·8)

MMRV=measles, mumps, rubella, varicella. GMT=geometric mean titre. GMR=GM ratio. *All infants from the open-label substudy who provided serum samples for immune testing. †GMR of titres after vaccination to baseline.

Table 3: **Immunogenicity of 4CMenB components before and after the booster dose at 12 months of age (modified intention-to-treat analysis*)**

not shown), suggesting that these low titres did not evolve during the primary series. When tested 1 month after their third 4CMenB dose (n=1149–1152), 100% of infants had an hSBA titre of 5 or more against factor H binding protein and neisserial adhesin A indicator strains and 84% (992 of 1183) of infants had an hSBA titre of 5 or more against the NZ outer-membrane vesicle indicator strain (figure 2). The lower limits of the two-sided 95% CI of these percentages were all more than 70%, the lowest being 82% for NZ outer membrane vesicle, thus meeting the second coprimary objective. In the subset of 100 infants tested post hoc for neisseria heparin binding antigen responses with the M10713 strain, the proportion with hSBA titre of 5 or more increased from 33% (33 of 100) at baseline to 84% (84 of 100) after three doses of 4CMenB (figure 2).

For the booster 4CMenB vaccination substudy, although antibodies waned such that geometric mean titres were lower in the immunogenicity subset at 12 months than 1 month after vaccination (tables 2, 3) these values remained higher than the prevaccination baseline, and the proportion of children with hSBA titres of 5 or more were 81% (355 of 437) against factor H binding protein, 99% (429 of 434) against neisserial adhesin A, 22% (96 of 437) against NZ outer membrane vesicle, and 61% (61 of 100) against neisseria heparin binding antigen. In 4CMenB vaccine-naive children at 12 months of age, the proportions of children with hSBA titres of 5 were 4·2% (11 of 261) against factor H binding protein, 1·5% (11 of 260) against neisserial adhesin A, 0·8% (2 of 262) against NZ outer membrane vesicle, and 18% (16 of 87) against neisseria heparin binding antigen (data not shown). We recorded much the same responses to a fourth dose of 4CMenB when it was administered either concomitantly with MMRV (n=211); or alone, with MMRV given 1 month later (n=215; table 3). We recorded a booster response against all test strains, resulting in hSBA titres of 5 or more against the four antigens in 95–100% of recipients (figure 2).

We examined the immunogenicity of routine vaccines in subsets of infants (n=245 with 4CMenB, n=252 with routine vaccines alone). Responses to all constituent antigens reached accepted thresholds (tables 4, 5) with no evidence of interference on the basis of a predefined non-inferiority analysis, except for poliovirus type 2 responses. The difference in proportions with a poliovirus type 2 titre of 8 or more between the groups who received routine vaccines with 4CMenB (88%; 95% CI 84 to 92) and routine vaccines only (94%; 90 to 97) was –5% (–11 to –1).

	4CMenB plus routine vaccines				Routine vaccines only				Difference between groups after third vaccination (95% CI)†
	Baseline		1 month after third vaccination		Baseline		1 month after third vaccination		
	n	Response (95% CI)	n	Response (95% CI)	n	Response (95% CI)	n	Response (95% CI)	
Diphtheria	241	38% (32 to 44)	239	100% (98 to 100)	246	38% (32 to 45)	244	100% (98 to 100)	0% (–1 to 2)
Tetanus	241	93% (88 to 96)	239	100% (98 to 100)	246	95% (91 to 97)	244	100% (98 to 100)	0% (–2 to 2)
PRP (Hib)	..	54% (47 to 60)	239	99% (97 to 100)	246	55% (49 to 62)	244	100% (98 to 100)	–1% (–3 to 1)
HBV	241	22% (17 to 28)	241	98% (95 to 99)	248	15% (10 to 20)	248	100% (99 to 100)	–2% (–5 to –1)
Polio 1	245	75% (69 to 80)	243	95% (92 to 98)	246	72% (66 to 77)	248	97% (94 to 99)	–1% (–5 to 2)
Polio 2	245	68% (62 to 74)	243	88% (84 to 92)	246	69% (63 to 74)	248	94% (90 to 97)	–5% (–11 to –1)
Polio 3	245	48% (41 to 54)	243	97% (94 to 99)	246	49% (42 to 55)	248	98% (95 to 99)	–1% (–4 to 2)

DTaP-HBV-IPV/Hib=diphtheria-tetanus-acellular pertussis, inactivated poliovirus, hepatitis B plus *Haemophilus influenzae* type b. PRP=polyribosylribitol phosphate.
*Responses assessed as percentages of participants achieving: ELISA antibodies ≥0·1 IU/mL for diphtheria and tetanus; ELISA antibodies ≥0·15 μg/mL against Hib-PRP; ELISA antibodies ≥10 mIU/mL against hepatitis B surface antigen; neutralisation test titres ≥8 against polio type 1, 2, and 3 antigens. †4CMenB with routine vaccines minus routine vaccines only.

Table 4: **Responses* to DTaP-HBV-IPV/Hib antigens, and differences between groups receiving routine vaccines with or without 4CMenB**

	Seroconversion*			Four-fold increase		
	4CMenB plus routine vaccines (N=238)	Routine vaccines alone (N=243)	Difference between groups	4CMenB plus routine vaccines (N=238)	Routine vaccines alone (N=243)	Difference between groups†
Pertussis toxin	98% (95 to 99)	100% (9 to 100)	–2% (–5 to –1)	92% (88 to 95)	95% (91 to 97)	–2% (–7 to 2)
Filamentous haemagglutinin	95% (91 to 97)	99% (96 to 100)	–4% (–8 to –1)	84% (79 to 88)	87% (82 to 91)	–3% (-9 to 4)
Pertactin	94% (90 to 96)	98% (95 to 99)	–4% (–8 to –1)	79% (73 to 84)	88% (84 to 92)	–9% (–16 to –3)

*Pertussis seroconversion is defined as a four-fold increase for each pertussis antigen or in individuals who were initially seropositive, persistence of the post-vaccination antibody concentration to at least the same antibody concentration as before vaccination, to take into account the decay of maternal antibodies.†4CMenB with routine vaccines minus routine vaccines only.

Table 5: **Pertussis antigens 1 month after third vaccination**

	Primary series								Booster	
	4CMenB plus routine vaccines			Routine vaccines only		Routine vaccines plus MenC			4CMenB plus MMRV† (N=765)	4CMenB (N=789)
	4CMenB (N=2477)	DTaP (N=2478)	PCV7 (N=2478)	DTaP (N=659)	PCV7 (N=659)	DTaP (N=490)	PCV7 (N=490)	MenC (N=490)		
Tenderness	2147 (87%)	1986 (80%)	1961 (79%)	388 (59%)	352 (53%)	331 (68%)	303 (62%)	266 (54%)	546 (71%)	563 (71%)
Severe*	723 (29%)	607 (24%)	596 (24)	51 (8%)	38 (6%)	47 (10%)	40 (8%)	23 (5%)	104 (14%)	115 (15%)
Erythema	2049 (83%)	1900 (77%)	1724 (70%)	469 (71%)	408 (62%)	363 (74%)	307 (63%)	261 (53%)	504 (66%)	539 (68%)
Induration	1908 (77%)	1674 (68%)	1360 (55%)	424 (64%)	321 (49%)	368 (75%)	271 (55%)	227 (46%)	388 (51%)	424 (54%)
Swelling	1174 (47%)	919 (37%)	794 (32%)	222 (34%)	176 (27%)	146 (30%)	99 (20%)	84 (17%)	284 (37%)	287 (36%)

Data are n (%). DTaP=diphtheria-tetanus-acellular pertussis, inactivated poliovirus, hepatitis B plus *Haemophilus influenzae* type b vaccine. PCV7=seven-valent pneumococcal vaccine. MenC=meningococcal serogroup C conjugate vaccine. MMRV=measles, mumps, rubella, varicella. *Cried when limb moved. †Reactions at 4CMenB site only because MMRV administered subcutaneously.

Table 6: **Participants with any local and systemic reactions within 7 days of any dose of the respective vaccines**

Because reactogenicity profiles in open-label and blinded study cohorts were much the same, and did not change with subsequent doses, we pooled the safety data and report data for all doses combined (tables 6, 7). Injection-site reactions peaked on day 1, with a steep decrease in occurrence noted on day 2. The most frequent reaction was tenderness, reported in 87% of 4CMenB recipients; 29% of cases were described as severe, defined as crying when the limb was moved (table 6). When administered concomitantly with 4CMenB and PCV7, DTaP-HBV-IPV/Hib vaccine elicited much the same occurrence of local tenderness—any (80%) and severe (24%). Without 4CMenB occurrence of any tenderness was lower: 59% with PCV7 only, and 68% with both PCV7 and MenC (table 6). Although erythema and induration, and to a lesser extent swelling, were reported frequently (table 6), fewer than 1% of these reactions were reported as severe (data not shown). Booster doses of 4CMenB in children aged 12 months elicited lower occurrence of all injection-site reactions, including any (71% with and without MMRV) and severe tenderness (15% with MMRV, 14% without MMRV; table 6).

We identified no major differences in safety reporting between the open-label subset and the smaller blinded cohort (appendix) except for the proportions of participants with medically attended fever. In the open-label substudy, medical attention for fever after any vaccination was sought for 1·4% (28 of 1966) of infants in the 4CMenB plus routine vaccines group and 1·8% (12 of 659) of infants in the routine vaccines only group. In the observer-blind substudy these proportions were 5·3% (26 of 493) for the 4CMenB group and 2·8% (13 of 470) for the routine vaccines plus MenC group.

Pooled data for both subsets are presented for other adverse events and reactions (table 7; figure 3). The most notable systemic reaction in both infants and children aged 12 months was fever, usually occurring on the same day as vaccination (figure 3). Temperatures in infants were highest 6 h after vaccination, decreased on day 2, and were normal by day 3 (figure 3A). Rectal temperature of 38·5°C or more was reported in 65·3% (1612 of 2468) of infant 4CMenB recipients within 6 h of vaccination, compared with 32·2% (212 of 658) receiving routine vaccines alone, or 33·7% (165 of 489) with routine vaccines and MenC. Fever of 40·0°C or more was reported in 1·2% (29 of 2468), 0%, and 0·2% (one of 489) of these groups, respectively. Antipyretic use was frequent in all groups (table 7), but rates of medical attention for fever were low (2–3% of doses), and medical procedures were infrequent (1·0% [25 of 2468] with 4CMenB, 1·1% [seven of 658] with routine vaccines alone, 1·0% [five of 489] after routine vaccines plus MenC).

After a booster dose of 4CMenB alone, 31·6% (248 of 783) of children had a temperature (rectal or axillary) of 38·0°C or more within 6 h. The proportion at 6 h was similar (30·6% [233 of 761]) when 4CMenB was given concomitantly with MMRV, but this group then displayed the characteristic increase in temperature associated with MMRV vaccine,[16] peaking around day 9 when 25·3% (190 of 727) had temperature of 38·0°C or more

	Primary series			Booster	
	4CMenB plus routine vaccines (N=2478)	Routine vaccines only (N=659)	Routine vaccines plus MenC (N=490)	4CMenB plus MMRV (N=765)	4CMenB (N=789)
Change in eating habits	1787 (72%)	329 (50%)	257 (52%)	312 (41%)	318 (40%)
Sleepiness	2159 (87%)	476 (72%)	353 (72%)	362 (47%)	355 (45%)
Vomiting	662 (27%)	104 (16%)	116 (24%)	54 (7%)	36 (5%)
Diarrhoea	1086 (44%)	218 (33%)	164 (33%)	188 (25%)	160 (20%)
Irritability	2296 (93%)	544 (83%)	370 (76%)	560 (73%)	540 (68%)
Unusual crying	2109 (85%)	424 (64%)	352 (72%)	327 (43%)	294 (37%)
Rash	318 (13%)	77 (12%)	43 (9%)	57 (7%)	56 (7%)
Medically attended fever	57 (2%)	12 (2%)	16 (3%)	8 (1%)	13 (2%)
Antipyretic use	2302 (93%)	471 (71%)	325 (66%)	436 (57%)	406 (51%)

Data are n (%). MenC=meningococcal serogroup C conjugate vaccine. MMRV=measles, mumps, rubella, varicella vaccine.

Table 7: **Participants with any systemic reactions within 7 days of any dose of the respective vaccines**

(figure 3B). Fever of 40·0°C or more was rare in children aged 12 months—two cases occurring within 6 h with 4CMenB plus MMRV, no cases in the 4 days after 4CMenB alone, and six cases during the MMRV peak around day 9.

Seizures temporally associated with 4CMenB vaccination were rare in infants. Two cases reported as febrile seizures, and assessed as probably related to 4CMenB, occurred within 24 h of the second vaccinations with 4CMenB and routine vaccines; one case was a complex febrile seizure in a child with underlying neurological and renal pathologies and developmental delay, with no previous history of seizures. After withdrawal from the study, this child had another apparent febrile seizure 5 months later. Four other reported febrile seizures, occurring 2, 6, 8, and 25 weeks after the third vaccinations with 4CMenB plus routine vaccines, were deemed by investigators to be associated with underlying infections. Two additional seizures—one case reported as leg convulsions and another of jerking movements of the right arm—occurred on the same day as the first vaccinations with 4CMenB and routine vaccines. These events occurred in the presence of fever, were deemed mild or moderate in severity and possibly related to 4CMenB, and resolved spontaneously. Nine febrile seizures reported in the booster phase occurred between 9 days and 6 months after receipt of 4CMenB vaccine, which are beyond the duration of fever associated with 4CMenB vaccination. The case occurring 9 days after receiving 4CMenB with MMRV was assessed as possibly related to MMRV, because 9 days is a plausible time interval for fever associated with live viral vaccines.[16]

Four suspected cases of Kawasaki disease were reported during the primary phase, which were assessed

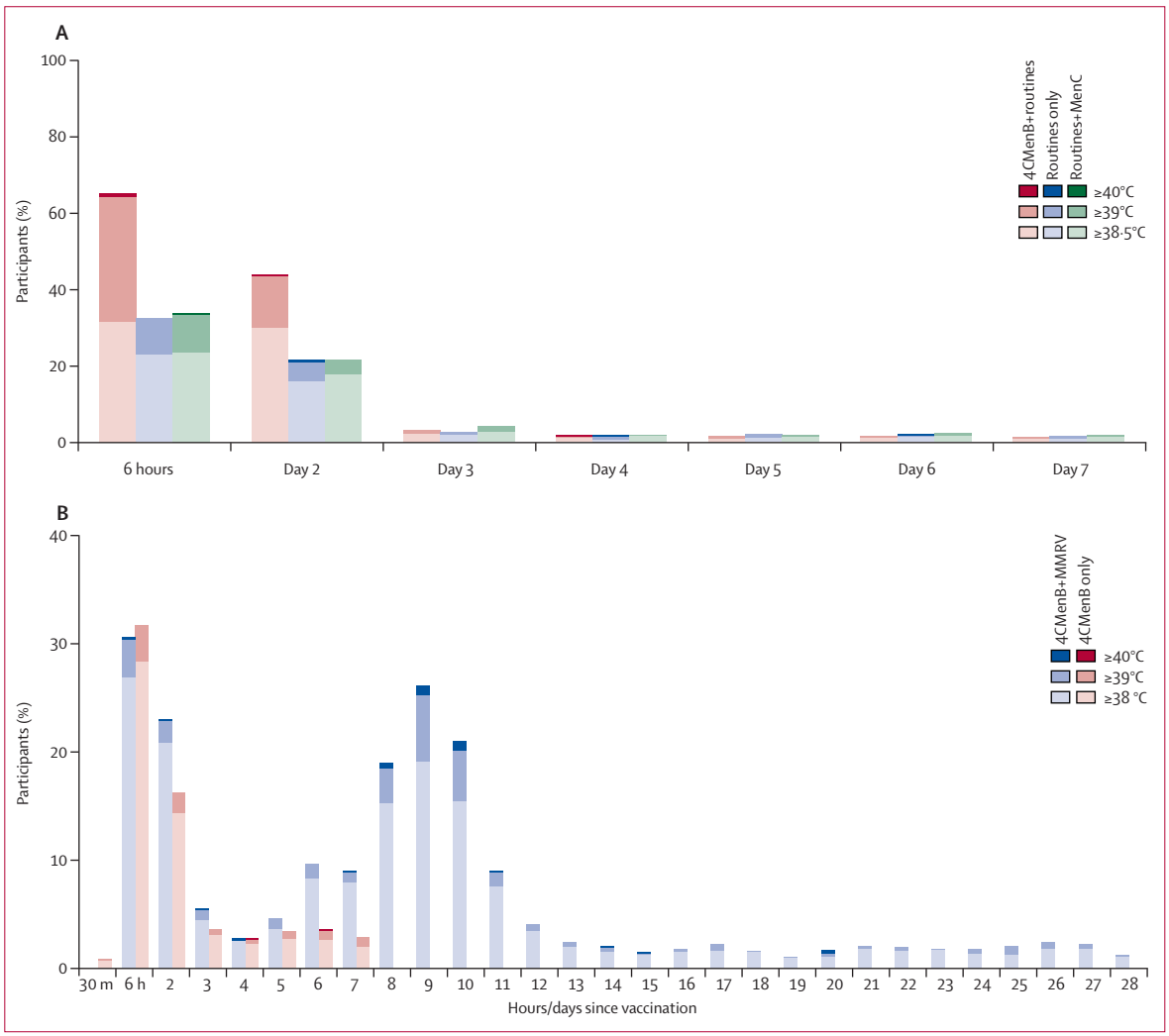

Figure 3: **Temperatures in infants (A) for 7 days after vaccination with 4CMenB, routine, and MenC vaccines, and in children aged 12 months (B) monitored for 7 days after administration of 4CMenB alone, or for 28 days after 4CMenB plus MMRV vaccine**

Infant temperatures were rectal, temperatures in children aged 12 months were either rectal or axillary. MenC=meningococcal serogroup C. MMRV=measles, mumps, rubella, and varicella.

by an independent expert panel as three confirmed cases and one unconfirmed case of Kawasaki disease, on the basis of the classic clinical criteria (appendix).[17] Two confirmed cases and the unconfirmed case occurred among the 2480 infants given 4CMenB and routine vaccines, 3, 7, and 14 weeks after vaccination, respectively; the other confirmed case was at 23 weeks in one of the 1149 infants who received MenC and routine vaccines. No cases of Kawasaki disease were reported after the 4CMenB booster.

Most other adverse events were common childhood illnesses or events consistent with solicited reactions. In the primary series, serious adverse events were reported in 210 of 2480 (8%) infants who received at least one dose of 4CMenB with the routine vaccines, 51 of 658 (8%) who received one or more doses of the routine vaccines only, and 28 of 490 (6%) who received one or more doses of MenC with routine vaccines. In 15 children, 17 serious adverse events (one with routine vaccines alone, 16 with routine vaccines and 4CMenB) were deemed vaccine-related by investigators. All events, except for a case of blindness associated with microcephaly in a 4CMenB recipient, resolved by final follow-up. After booster, two serious adverse events were deemed possibly related to vaccination with 4CMenB and MMRV, the febrile convulsion reported previously, and pyrexia 2 days after vaccination.

Discussion

In infants given three doses of the 4CMenB vaccine, all participants developed protective hSBA titres against strains selective for factor H binding protein and neisserial adhesin A, and 84% developed protective hSBA titres against strains selective for NZ outer membrane vesicle. In a subset, 84% of participants had protective hSBA titres against neisseria heparin binding antigen. The three 4CMenB lots used gave consistent results. Immune responses to routine vaccines were much the same with or without concomitant 4CMenB, but concomitant vaccination was associated with increased reactogenicity. At 12 months of age, waning titres were boosted by a fourth dose of 4CMenB, and 95–100% of participants developed protective hSBA titres for all antigens, irrespective of whether 4CMenB was administered with or without MMRV.

The formulation of several protective protein antigens, identified by genome mining, into an investigational vaccine, 4CMenB, is a breakthrough in the effort to develop a broadly protective vaccine to combat meningococcal serogroup B disease (panel). Use of several independent surface antigens could provide synergistic killing, improve strain coverage, and insure against mutations of individual target proteins. Previous studies[4,11] assessed 4CMenB responses against strain panels that were chosen to provide initial information about the immunogenicity of these components. Data from these studies showed some enhancement of the response to recombinant components by the inclusion of New Zealand outer membrane vesicle components in addition to the response directed at the PorA P1.4 component.[4,11]

We have assessed responses to meningococcal serogroup B indicator strains displaying individual vaccine antigens—factor H binding protein, neisserial adhesin A, neisseria heparin binding antigen, or PorA—while lacking genotypic or phenotypic expression of variant antigens that elicit cross-reactive antibodies.[8] We identified robust immune responses against all four strains in infants after the primary series and in children aged 12 months after a booster dose. After infant priming, hSBA titres of 5 or more, representing 95% confidence of the accepted correlate of protection against meningococcal disease (titre ≥4),[15] were achieved in 100% of 4CMenB recipients against two of three recombinant vaccine components, and in 84% against the NZ outer membrane vesicle components. The latter response is consistent with the reported efficacy of the monocomponent MeNZB outer-membrane vesicle vaccine.[20] We noted no clinically meaningful immunological interference for coadministered routine (DTaP-IPV-HBV/Hib, PCV7, MMRV) vaccines. These responses are consistent with a previously reported phase 2b study[12] of 4CMenB administered as three doses with routine vaccines at 2, 4, and 6 months of age; or 2, 3, and 4 months of age; or as separate vaccinations between routine vaccinations at ages 3, 5, and

Panel: Research in context

Systematic review

The traditional polysaccharide-protein conjugate vaccine approach used for other meningococcal serogroups is not possible for serogroup B because it is immunologically similar to the polysaccharide of human neural cell molecules. Vaccines developed to control local serogroup B outbreaks with outer membrane vesicles containing the immunodominant antigen PorA are only suitable for homologous strains and do not provide broad protection.[5] Other meningococcal proteins have been shown to elicit protective immune responses, and a vaccine containing two variants of factor H binding protein was immunogenic in children aged 18–36 months in a phase 1 study.[18] A novel investigational vaccine, rMenB, was developed with recombinant protein technology to generate several antigens to provide a broader immune response. Phase 2 studies showed that the presence of outer membrane vesicles from the New Zealand outbreak strain enhances the immune response to the recombinant proteins,[11] and that the resulting vaccine formulation, 4CMenB, could be administered concomitantly with routine infant vaccines.[12] In adolescents, who are one important target for MenB vaccination, two doses of 4CMenB 1–6 months apart were sufficient to induce immune responses against all constituent antigens in almost all participants.[19] We present the results of a pivotal study of 4CMenB in another important target population, infants from 2 months of age, when administered concomitantly with their routine infant vaccinations. We did not do a search of the scientific literature before undertaking this study, because no precedent exists for this vaccine.

Interpretation

This large phase 3 study confirms the feasibility of administering a three dose primary schedule of 4CMenB to infants, concomitantly with routine vaccines, which elicited protective immune responses to all antigen components with an acceptable safety profile. We identified an anamnestic response to a fourth dose administered at 12 months of age.

7 months; and studies of investigational formulations of the recombinant proteins with and without the NZ outer-membrane vesicle component.[4,11]

A meningococcal antigen typing system (MATS) with a sandwich ELISA has been developed to predict the likelihood that strains will be killed in the hSBA. MATS analysis of a panel of strains can be done in weeks, rather than the months or years of work that would be required to test each strain individually and provides vaccine coverage estimates from large panels of bacterial strains.[21,22] Reference laboratories worldwide are currently screening strain collections to estimate potential local vaccine coverage of serogroup B disease isolates. We could not assess vaccine efficacy, which must be inferred regionally from forthcoming meningococcal antigen typing system coverage data and the serum protective responses we have shown. However, a preliminary report[23] of meningococcal antigen typing system analyses of 1011 serogroup B clinical isolates from six European countries (England and Wales, France, Germany, Italy, Norway) shows coverage of 77·5% (95% CI 66·3–91·9).

Importantly, factor H binding protein, neisserial adhesin A, and neisseria heparin binding antigen proteins, and PorA in the NZ outer-membrane vesicle, are common to other meningococcal serogroups and might therefore protect against non-serogroup B strains, in a similar approach to that currently being explored for pneumococcal vaccines.[24] For example, meningococcal disease outbreaks in Africa due to serogroup X,[25] for which no vaccine is available, were caused by a strain containing the same factor H binding protein component as 4CMenB.[26]

We noted an additive effect on reactogenicity of the routine vaccines in 4CMenB recipients, who had more frequent and more pronounced solicited reactions and fevers than did recipients of routine vaccines given alone or with MenC. However, the occurrence of febrile seizures and the proportion of infants receiving antipyretics were much the same as those reported from other combination vaccine studies.[27–30] In children aged 12 months, we noted a rapid onset of fever in about 30% of children, but no cases of high fever. These reactions might partly be attributable to the NZ outer membrane vesicle component, because high reactogenicity was also recorded with the MeNZB vaccine.[20] Therefore, additional information from ongoing studies in which 4CMenB is administered on its own, and studies to investigate prophylactic use of antipyretics or formulations with different quantities of the NZ outer membrane vesicle component will be helpful.

We recorded two confirmed and one unconfirmed case of Kawasaki disease in 4CMenB recipients. Although causality to 4CMenB and routine vaccinations is unclear, we could not rule out a possible vaccine association for the case at 3 weeks—the only one with cardiac findings—and that at 7 weeks after vaccination, although this case was deemed less likely to be related to vaccination. The small numbers identified here preclude definitive conclusions, but their detection shows the need for continued surveillance in future trials and after licensure to determine whether occurrence is within background rates for the infant population, as these rates continue to increase with improving diagnostic techniques and increasing awareness of this disorder.[31,32] Data from this and other studies suggest that reactogenicity decreases with age, from infants through toddlers to adolescents.[19]

In summary, we identified strong immune responses against four independent meningococcal serogroup B protein antigens, which were predicted to provide broad coverage against a representative panel of European disease isolates. Although 4CMenB contributed to a noticeable increase in the overall reactogenicity profile when administered concomitantly with the routine vaccinations given at 2, 4, and 6 months to infants in Europe, infants bear the largest meningococcal disease burden worldwide,[2] and the promising immunogenicity of 4CMenB needs to be balanced against this reactogenicity.

Contributors

TV, SE, EY, IK, DT, PD, and AK conceived and designed the study. TV, SE, and RP did the study, managed by IK, DT, PD, and AK. The study analysis was done by EY, and interpreted by TV, SE, RP, IK, DT, PD, and AK. TV, SE, PD, and AK contributed to a draft cowritten with KV, which was reviewed and revised by all authors, who also approved the final version.

Conflicts of interest

EY, IK, DT, PD, and AK are full-time employees of Novartis Vaccines and Diagnostics. Other authors received funding for doing the study from Novartis Vaccines and Diagnostics.

Acknowledgments

We thank all the parents and guardians of all participants and the many investigators and study staff for their individual contributions to this study. Keith Veitch (Novartis Vaccines and Diagnostics, Amsterdam, the Netherlands) provided editorial guidance and writing and graphical support.

References

1 Tan LK, Carlone GM, Borrow R. Advances in the development of vaccines against *Neisseria meningitidis*. *N Engl J Med* 2010; **362:** 1511–20.
2 Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine* 2009; **27** (suppl 3)**:** B51–63.
3 Sadarangani M, Pollard AJ. Serogroup B meningococcal vaccines—an unfinished story. *Lancet Infect Dis* 2010; **10:** 112–24.
4 Snape MD, Dawson T, Oster P, et al. Immunogenicity of two investigational serogroup B meningococcal vaccines in the first year of life. A randomized comparative trial. *Pediatr Infect Dis J* 2010; **29:** e71–79.
5 Holst J, Martin D, Arnold R, et al. Properties and clinical performance of vaccines containing outer membrane vesicles from *Neisseria meningitidis*. *Vaccine* 2009; **27** (suppl 2)**:** B3–12.
6 Tappero JW, Lagos R, Ballesteros AM, et al. Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines: a randomized controlled trial in Chile. *JAMA* 1999; **281:** 1520–27.
7 Giuliani MM, Adu-Bobie J, Comanducci M, et al. A universal vaccine for serogroup B meningococcus. *PNAS* 2006; **103:** 10834–39.
8 Giuliani MM, Biolchi A, Serruto D, et al. Measuring antigen-specific bactericidal responses to a multicomponent vaccine against serogroup B meningococcus. *Vaccine* 2010; **28:** 5023–30.
9 Serruto D, Spadafina T, Ciucchi L, et al. *Neisseria meningitidis* GNA2132, a heparin-binding protein that induces protective immunity in humans. *PNAS* 2010; **107:** 3770–75.

10 Bambini S, Muzzi A, Olcen P, Rappuoli R, Pizza M, Comanducci M. Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus. *Vaccine* 2009; **27:** 2794–803.

11 Findlow J, Borrow R, Snape MD, et al. Multicentre, open-label, randomised phase II controlled study of an investigational recombinant serogroup B vaccine with and without outer membrane vesicles, administered in infancy. *Clin Infect Dis* 2010; **51:** 1127–37.

12 Gossger N, Snape MD, Yu L-M, et al. Immunogenicity and tolerability of recombinant meningococcal serogroup B vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. *JAMA* 2012; **307:** 575–82.

13 Borrow R, Carlone GM, Rosenstein N, 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:** 5093–107.

14 Santos GF, Giuliani M, Santini L, et al. Investigation on the effect of immune selection on resistance to bactericidal antibodies to group B meningococci in vitro. *Clin Vaccine Immunol* 2009; **16:** 1693–95.

15 Frasch CE, Borrow R, Donnelly J. Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease. *Vaccine* 2009; **27** (suppl 2)**:** B112–16.

16 Knuf M, Habermehl P, Zepp F, et al. Immunogenicity and safety of two doses of tetravalent measles-mumps-rubella-varicella vaccine in healthy children. *Pediatr Infect Dis J* 2006; **25:** 12–18.

17 Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki Disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Pediatrics* 2004; **114:** 1708–33.

18 Marshall HS, Richmond PC, Nissen MD, et al. Safety and immunogenicity of a meningococcal B bivalent rLP2086 vaccine in healthy toddlers aged 18 to 36 months: a phase 1 randomized controlled clinical trial. *Pediatr Infect Dis J* 2012; **31:** 1061–68.

19 Santolaya ME, O'Ryan ML, Valenzuela MT, et al. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. *Lancet* 2012; **379:** 617–24.

20 Oster P, O'Hallahan J, Aaberge I, Tilman S, Ypma E, Martin D. Immunogenicity and safety of a strain-specific MenB OMV vaccine delivered to under 5-year olds in New Zealand. *Vaccine* 2007; **25:** 3075–79.

21 Donnelly J, Medini D, Boccadifuoco G, et al. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *PNAS* 2010; **107:** 19490–95.

22 Lucidarme J, Newbold LS, Findlow J, et al. Molecular targets in meningococci: efficient routine characterization and optimal outbreak investigation in conjunction with routine surveillance of the meningococcal group B vaccine candidate, fHbp. *Clin Vaccine Immunol* 2011; **18:** 194–202.

23 Donnelly J, Medini D, Giuliani M, et al. Estimating the potential strain coverage in Europe of a multicomponent vaccine targeting serogroup B meningococci. 11th EMGM meeting; Ljubljana, Slovenia; May 18–20, 2011. Abstract O 08.

24 Dinleyici EC. Current status of pneumococcal vaccines: lessons to be learned and new insights. *Exp Rev Vaccines* 2010; **9:** 1017–22.

25 Boisier P, Nicolas P, Djibo S, et al. Meningococcal meningitis: unprecedented incidence of serogroup X-related cases in 2006 in Niger. *Clin Infect Dis* 2007; **44:** 657–63.

26 Beerninck PT, Caugent DA, Welsch JA, Koeberling O, Granoff DM. Meningococcal factor H-binding protein variants expressed by epidemic capsular group A, W-135, and X strains from Africa. *J Infect Dis* 2009; **199:** 1360–68.

27 Marcy SM, Kohl KS, Dagan R, et al. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. *Vaccine* 2004; **22:** 551–56.

28 McNicholas A, Galloway Y, Stehr-Green P, et al. Post-marketing safety monitoring of a new group B meningococcal vaccine in New Zealand, 2004–2006. *Human Vaccines* 2007; **3:** 196–204.

29 Van de Berg BJ, Yerushamy J. Studies on convulsive disorders in young children. *Pediatr Res* 1969; **3:** 298–304.

30 Verity CM, Bulter NR, Golding J. Febrile convulsion in a national cohort followed up from birth. I—Prevalence and recurrence in the first five years of life. *BMJ* 1985; **290:** 1307–10.

31 Fischer TK, Holman RC, Yorita KL, Belay ED, Melbye M, Koch A. Kawasaki syndrome in Denmark. *Pediatr Infect Dis J* 2007; **26:** 411–15.

32 Heuclin T, Dubos F, Hue V, et al. Increased detection rate of Kawasaki Disease using new diagnostic algorithm, including the early use of echocardiography. *J Pediatr* 2009; **155:** 695–99.