ORIGINAL CONTRIBUTION

Immunogenicity and Tolerability of Recombinant Serogroup B Meningococcal Vaccine Administered With or Without Routine Infant Vaccinations According to Different Immunization Schedules
A Randomized Controlled Trial

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MENINGOCOCCAL DISEASE occurs with an incidence of 0.2 to 14 per 100 000 population in industrialized countries.[1] Effective vaccines based on capsular polysaccharides for meningococcal serogroups A, C, W-135, and Y are available,[2] but serogroup B capsular polysaccharide is antigenically similar to human neural cell glycopeptides containing polysialic acid and poorly immunogenic in humans.

For editorial comment see p 614.

Context In the absence of an effective vaccine, serogroup B *Neisseria meningitidis* (MenB) remains a major cause of invasive disease in early childhood in developed countries.

Objective To determine the immunogenicity and reactogenicity of a multicomponent MenB vaccine (4CMenB) and routine infant vaccines when given either concomitantly or separately.

Design, Setting, and Participants Phase 2b, multicenter, open-label, parallel-group, randomized controlled study of 1885 infants enrolled at age 2 months from August 2008 to July 2010 in Europe.

Intervention Participants were randomized 2:2:1:1 to receive (1) 4CMenB at 2, 4, and 6 months with routine vaccines (7-valent pneumococcal and combined diphtheria, tetanus, acellular pertussis, inactivated polio, hepatitis B, *Haemophilus influenzae* type b vaccines); (2) 4CMenB at 2, 4, and 6 months and routine vaccines at 3, 5, and 7 months; (3) 4CMenB with routine vaccines at 2, 3, and 4 months; or (4) routine vaccines alone at 2, 3, and 4 months.

Main Outcome Measures Percentage of participants with human complement serum bactericidal activity (hSBA) titer of 1:5 or greater against 3 MenB strains specific for vaccine antigens (NZ98/254, 44/76-SL, and 5/99).

Results After three 4CMenB vaccinations, 99% or more of infants developed hSBA titers of 1:5 or greater against strains 44/76-SL and 5/99. For NZ98/254, this proportion was 79% (95% CI, 75.2%-82.4%) for vaccination at 2, 4, and 6 months with routine vaccines, 86.1% (95% CI, 82.9%-89.0%) for vaccination at 2, 4, and 6 months without routine vaccines, and 81.7% (95% CI, 76.6%-86.2%) for vaccination at 2, 3, and 4 months with routine vaccines. Responses to routine vaccines given with 4CMenB were noninferior to routine vaccines alone for all antigens, except for the responses to pertactin and serotype 6B pneumococcal polysaccharide. Fever was seen following 26% (158/602) to 41% (247/607) of 4CMenB doses when administered alone, compared with 23% (69/304) to 36% (109/306) after routine vaccines given alone and 51% (306/605) to 61% (380/624) after 4CMenB and routine vaccines administered together.

Conclusion A 4CMenB vaccine is immunogenic against reference strains when administered with routine vaccines at 2, 4, and 6 or at 2, 3, and 4 months of age, producing minimal interference with the response to routine infant vaccinations.

Trial Registration clinicaltrials.gov Identifier: NCT00721396

Serogroup B *Neisseria meningitidis* (MenB) vaccines based on detergent-extracted outer membrane vesicles (OMVs) containing outer membrane proteins, including porin protein A,

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proved effective in clinical trials and controlled a clonal MenB outbreak in New Zealand.[3,4] However, the high strain specificity of these vaccines limits their usefulness, especially in infants and young children.[5,6]

To develop a vaccine with broader protection, novel antigens identified by sequencing the MenB genome—factor-H binding protein variant 1 (fHbp1), Neisserial adhesin A (NadA), and *Neisseria* heparin binding antigen (NHBA)—were combined with OMVs from the New Zealand epidemic strain NZ98/254 in a multicomponent serogroup B meningococcal vaccine (4CMenB).[7] To facilitate manufacture and enhance protein stability, fHbp1 and NHBA were combined with other proteins identified during genome sequencing to create 2 fusion proteins.[8,9] Small studies in infants demonstrated immunogenicity of 4CMenB against reference strains.[8,9]

In this study we assessed the immunogenicity and reactogenicity of 4CMenB in a large cohort of infants when given in 2 different schedules, and concomitantly or separately from routine vaccines.

METHODS
Study Participants and Trial Design

This phase 2b, open-label, parallel-group, randomized controlled trial was conducted between August 2008 and July 2010 in Belgium (6 centers), United Kingdom (4 centers), Czech Republic (4 centers), Germany (25 centers), Italy (5 centers), and Spain (16 centers). Appropriate regulatory authorities in each participating country granted approval for the trial. Participants were healthy, full-term, 2-month-old infants with a birth weight of 2.5 kg or greater, whose parent(s) or legal guardian(s) provided written informed consent. In the United Kingdom, participants were recruited by information letters sent via National Health Service child health computer databases; participants in other study centers were recruited through pediatric hospitals or private practices. Exclusion criteria were any history of meningococcal infection or close contact with

someone experiencing meningococcal disease; prior receipt of meningococcal B or C vaccines or any of the nationally recommended routine infant vaccines; acute or chronic illness; known reactions to vaccine components; known or suspected immune disease or impairment, including the administration of steroids; receipt of antibiotics 6 days prior to enrollment; receipt of blood products; or planned receipt of nonstudy vaccines. Investigator-defined options for race/ethnicity were chosen by the participants' parents for demographic description of the study population.

Intervention

The 4CMenB vaccine consisted of 50 μg each of fHbp1, NadA, and NHBA fusion proteins, 25 μg of detoxified OMV from *N meningitidis* strain NZ98/254, 1.5 of mg aluminum hydroxide, and histidine 10mM in 0.5 mL of water for injection. Participants also received a combined diphtheria, tetanus, acellular pertussis, inactivated polio, hepatitis B, and *Haemophilus influenzae* type b vaccine (DTaP-HBV-IPV/Hib) (Infanrix Hexa; GlaxoSmithKline) and 7-valent pneumococcal glycoconjugate vaccine (PCV7) (Prevnar; Wyeth Pharmaceuticals). These are referred to as routine vaccines, although they do not necessarily reflect the routine vaccines used in each study country.

The study design allowed assessment of 3 primary 4CMenB schedules: ages 2, 4, and 6 months, together with routine infant vaccines (concomitant); 2, 4, and 6 months, with routine vaccines given separately at 3, 5, and 7 months (intercalated); and 2, 3, and 4 months, concomitantly with routine infant vaccines (accelerated). A control group received DTaP-HBV-IPV/Hib and PCV7 only at 2, 3, and 4 months. These immunization time points were chosen to reflect schedules currently used in different countries. All vaccines were administered by intramuscular injection in the anterolateral thigh; 4CMenB and routine vaccines given concomitantly were administered in opposite limbs.

Participants were randomized to the 4 vaccination groups in a 2:2:1:1 ratio according to a centrally held randomization list (Biostatistics and Clinical Data Management, Novartis) using a fixed block size of 6. The unequal allocation ratio was chosen to efficiently assess primary as well as secondary objectives. This was an open-label study; ie, study personnel and parents both knew which vaccines were being administered.

Immunology

Sera obtained before the first vaccination and 30 (–4/+7) days after the third dose of 4CMenB (or routine vaccines in the control group) were shipped to the Novartis Vaccines Serology Laboratory, Marburg, Germany, for blinded immunology assessments. Human complement serum bactericidal activity (hSBA) was assessed against 3 target strains chosen to determine the immunogenicity of individual vaccine components—strain 44/76-SL for fHBP1, strain 5/99 for NadA, and strain NZ98/254 for OMV. The hSBA was expressed as interpolated titers according to reciprocal serum dilutions yielding 50% or greater killing of the target strain after 60 minutes of incubation compared with growth at time 0. An interpolated titer of 1:5 or greater represented 95% confidence that participants achieving this titer had a protective hSBA titer ($\geq$1:4).[10]

Because no hSBA strain specific for NHBA was available at the time of analysis, antibodies to NHBA were measured by enzyme-linked immunosorbent assay and expressed as geometric mean concentrations (GMCs).

Responses to routine vaccines were determined for participants in the accelerated and control groups only, according to availability of serum, using standard correlates of protection to interpret responses. Geometric mean titers (GMTs) of hSBA, routine vaccine antigen-specific IgG GMCs, and the between-group ratios of GMT and GMC were calculated (with 2-sided 95% CIs) using 2-way analysis of variance with a factor for vaccine group and immunization center.

Safety

Parents recorded local injection site reactions (pain, erythema, swelling, and induration) and systemic reactions (ie, fever [axillary temperature ≥38°C], change in eating habits, sleepiness, unusual crying, vomiting, diarrhea, irritability, rash) for 7 days after each vaccination. Injection site pain was classified by the parents as mild (minimal discomfort when the child's leg was touched), moderate (obvious discomfort when the leg was touched), or severe (pain on movement of the leg). Erythema, induration, and swelling were summarized by maximal severity (1-25 mm, 25-50 mm, and >50 mm, respectively).

Adverse event recording was enhanced by telephone contact in the week after study vaccination. Safety follow-ups were completed 6 months after the last dose of 4CMenB (and at age 10 months in the control group). All serious adverse events reported during the study were recorded. Determination of the relationship between adverse events and the study vaccine was made by a study investigator's judgment based on temporal relationship and biological plausibility criteria.

Statistics

The primary outcome was assessment of percentages of participants with hSBA titers of 1:5 or greater 1 month after immunization with 4CMenB in the concomitant and accelerated groups, vaccine response being sufficient if the lower limit of the 2-sided 95% Clopper-Pearson[11] CI of this percentage was 70% or greater for all 3 reference strains. In addition, hSBA titers were log transformed and their GMTs and 2-sided 95% CIs calculated. The prespecified population for this analysis was the modified intention-to-treat population, including only those who received a study vaccine and provided evaluable serum samples before and after immunization.

The main secondary outcomes were noninferiority of immune responses to 4CMenB and routine vaccines administered together compared with sepa-

rately. The percentage of 4CMenB recipients achieving hSBA titers of 1:5 or greater in the concomitant group, obtained from a categorical linear model, was considered noninferior to that in the intercalated group if the lower limits of the 95% CI for the differences in these percentages (concomitant minus intercalated) was greater than –10%.[12] The same criteria were used to determine noninferiority between the control and accelerated groups for the response to routine vaccines.

Additionally, a post hoc noninferiority criterion for the geometric mean ratios of 4CMenB response (intercalated divided by concomitant) obtained from analysis of variance adjusting for center was defined as the lower limit of the 95% CI being greater than 0.5 for all 3 strains. For pertussis antigens, noninferiority of the accelerated compared with the control group was achieved if the lower limit of the 2-sided 95% CI for the ratio of the antigen-specific IgG GMCs after vaccination was greater than 0.67. All antigen thresholds were chosen based on traditional use and acceptance by regulatory authorities. Responses to antigens administered at the same time were analyzed in a per-protocol population, ie, those who received all designated vaccines on time and provided sera within the scheduled window (23-55 days post vaccination) and who received no prohibited medications (systemic antibiotics or systemic/high-dose inhaled corticosteroids).

Modified intention-to-treat analysis was performed as per-randomized group; the per-protocol population was analyzed as per-immunization course received.

A post hoc analysis was performed that used all available data and included imputed values to replace missing data.[13] Each missing value was replaced with a set of plausible values that represent the uncertainty about the correct values to impute. Variables included in the imputation model were actual vaccine administered, center, age, birth weight, weight, height, sex, race, hSBA titers for strains 44/76-SL, 5/99, and NZ98/254 (at baseline and follow-

ing the third dose), and IgG GMC against NHBA using the adjusted Wald test proposed by Agresti and Coull.[14]

The sample size calculation for the primary outcome was determined using a simulation approach to estimate that 300 participants in the accelerated group would yield 95% power to demonstrate that the lower limit of the 2-sided 95% CI for the true percentage of participants with hSBA titer of 1:5 or greater was 70% or greater. A sample size of 600 in the concomitant and intercalated groups was calculated to provide 87% power to demonstrate noninferiority in response rates for all 3 MenB strains (assuming only 80% provided evaluable sera, response rates to 4CMenB given together with routine immunizations were similar to those in previously published studies,[8,9] and that response rates to 4CMenB given without routine immunizations were 2% higher for each strain).

Individuals who received at least 1 dose of vaccine and provided postbaseline safety data were included in the safety analyses, for which results were reported descriptively with no formal statistical analyses.

Statistical analysis was performed using SAS version 9.1.

RESULTS

A total of 1885 participants were enrolled (United Kingdom, 561; Italy, 371; Germany, 317; Czech Republic, 283; Belgium, 248; Spain, 105), of whom 1810 (96%) completed the study. Of these, 1636 were included in the modified intention-to-treat immunogenicity analysis (**FIGURE 1**) and 1599 in the per-protocol immunogenicity analysis. Median age at enrollment was 68.7 days for all groups, 48.3% to 53.4% of each group were boys, and 93.1% of all participants were white.

Immunogenicity

Primary Outcome. After immunization with 4CMenB and routine vaccines together at either 2, 4, and 6 or 2, 3, and 4-months, 99% or more of participants had hSBA titers of 1:5 or greater for strains 44/76-SL and 5/99 in the modified intention-to-treat analy-

sis (FIGURE 2 and TABLE 1). For strain NZ98/254, percentages were 79.0% (417/528 [95% CI, 75.2% to 82.4%]) and 81.7% (219/268 [95% CI, 76.6% to 86.2%]) following concomitant or accelerated schedules. Lower limits of the 95% CIs were greater than 70% for all 3 tested reference strains, thereby meet-

ing the predefined criteria of a sufficient immune response. Results from multiple imputation analysis were similar (Table 1).

Secondary Outcomes. The difference in the percentage of participants with hSBA titers of 1:5 or greater in the concomitant group minus the

intercalated group met the prespecified noninferiority criterion for 44/76-SL and 5/99 but not NZ98/254 (−7.1% [95% CI, −11.7% to −2.6%]) (Table 1). Although the lower limit of the 95% CI for the geometric mean concomitant to intercalated hSBA ratios were all above 0.5, thus meeting

Figure 1. Flowchart of the Modified Intention-to-Treat (MITT) Population
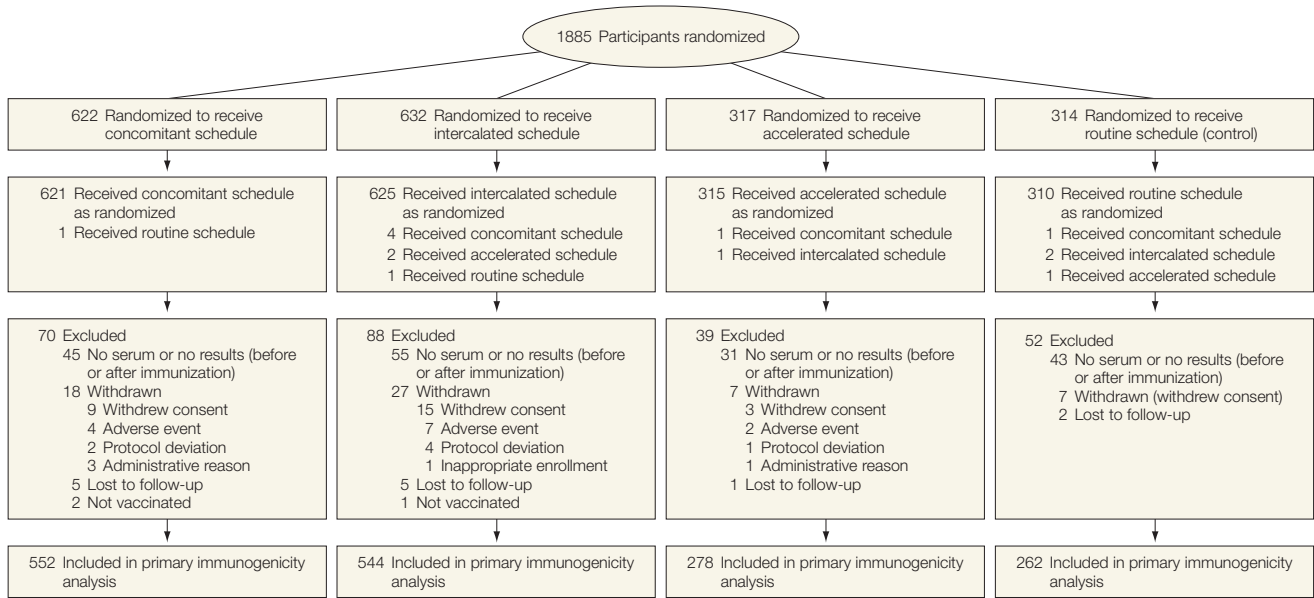
See "Methods" for definitions of groups. The number of infants approached, assessed for eligibility, and primarily excluded is unknown. Six-month follow-up in the safety population was completed by 597 participants in the concomitant group, 599 in the intercalated group, 309 in the accelerated group, and 305 in the routine (control) group. This chart does not show the flow of participants for the per-protocol population (used for calculations of *Neisseria* heparin binding antigen enzyme-linked immunosorbent assay data and response to concomitant vaccines).

Figure 2. Reverse Cumulative Distribution of hSBA Titers at 1 Month After the Third 4CMenB Vaccination, by Meningococcal Strain (MITT Population)
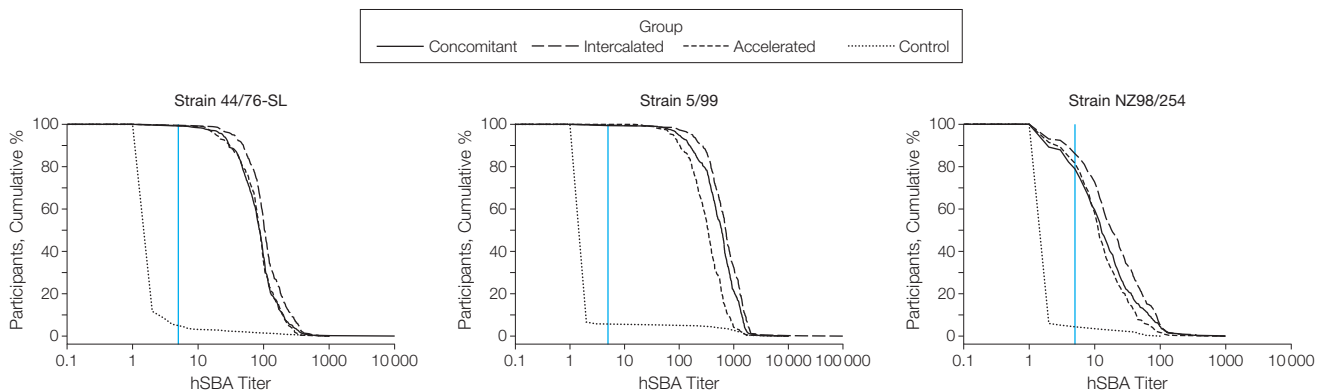
See "Methods" for definitions of groups. Numbers of participants in each group are as reported in Figure 1. Blue vertical lines indicate reference for hSBA titers of 1:5 or greater. 4CMenB indicates multicomponent serogroup B meningococcal vaccine; hSBA, human complement serum bactericidal activity; MITT, modified intention-to-treat.

the prespecified noninferiority criterion, it is notable that the upper limit of the 95% CI for these ratios were all less than 1 (**Table 2**).

The geometric mean ratios (concomitant divided by accelerated) of hSBA titers were similar for strains 44/76-SL and NZ98/254 (confidence intervals crossing 1) but higher for strain 5/99 (1.61 [95% CI, 1.41 to 1.84]). IgG GMCs against NHBA 1 month after the third 4CMenB vaccination were 4342 (95% CI, 4067-4635) when 4CMenB was administered separately from routine vaccines, compared with 3211 (95% CI, 2949-3495) to 3332 (95% CI, 3120-3558) when administered together (**Table 3**).

The percentages of participants achieving the threshold of response for DTaP-HBV-IPV/Hib components were noninferior in the accelerated group compared with the control group (lower limits of 95% CI of the differences in these values being greater than −10%), with the exception of pertactin (difference, −4% [95% CI, −11% to 3%]). Noninferiority was demonstrated for 6 of the 7 serotypes in the pneumococcal vaccine (serotypes 4, 9V, 14, 18C, 19F, 23F) but not for serotype 6B (difference, −5% [95% CI, −14% to 3%]), because the lower limit of the difference in the percentage of participants with antibody response greater or equal to the prespecified cutoff level was −14% (**Table 4**).

Reactogenicity and Safety

The numbers of participants experiencing adverse reactions after immunization are shown in the eTable available at http://www.jama.com. Throughout the study, fewer than 1% of all participants experienced severe erythema, swelling, or induration at either of the vaccination sites. However, 12% to 16% in the concomitant and accelerated groups, respectively, experienced severe local pain after a dose of 4CMenB, compared with 1% to 3% after doses of DTaP-HBV-IPV/Hib or PCV7 in the control group.

Fever ($\geq$38.0°C) after any vaccination was described in 80% (501/624) in the concomitant group and 76% (243/318) in the accelerated group, compared with 51% (160/311) in control group and 71% (447/627) in the intercalated group. For the intercalated group there were twice as many immunization days, and therefore more opportunity for fever to be experienced. Rates of fever per 4CMenB dose are shown in the eTable.

Table 1. Results of the Number of Infants Achieving Bactericidal Titers of 1:5 or Greater and the Percentages at Baseline and 1 Month After the Third Vaccination (MITT Population and Multiple Imputation) and Vaccine Group Differences[a]

| | % (95% CI)[b] | | | | Vaccine Group Differences | |
Strain	Concomitant	Intercalated	Accelerated	Control	Concomitant Minus Intercalated	Concomitant Minus Accelerated
Strain 44/76-SL						
Baseline, No./total[c]	47/525	38/531	16/272	16/248		
MITT[d]	9.0 (6.7 to 11.7)	7.2 (5.1 to 10.0)	5.9 (3.4 to 9.4)	6.5 (3.7 to 10.3)	1.8 (−1.5 to 5.1)	3.1 (−0.6 to 6.8)
Multiple imputation[e]	8.7 (6.8 to 11.2)	6.80 (5.1 to 9.2)	6.8 (4.2 to 9.8)	6.5 (4.1 to 9.7)	1.9 (−1.2 to 4.9)	2.3 (−1.4 to 5.9)
Post third dose, No./total[c]	521/525	528/531	270/272	11/248		
MITT[d]	99.2 (98.1 to 99.8)	99.4 (98.4 to 99.9)	99.3 (97.4 to 99.9)	4.4 (2.2 to 7.8)	−0.2 (−1.2 to 0.8)	−0.03 (−1.3 to 1.2)
Multiple imputation[e]	98.9 (96.6 to 99.1)	98.8 (96.4 to 99.1)	98.4 (94.3 to 98.7)	10.5 (8.2 to 16.0)	0.1 (−1.4 to 1.6)	0.5 (−1.5 to 2.4)
Strain 5/99						
Baseline, No./total[c]	28/520	34/517	12/266	15/226		
MITT[d]	5.4 (3.6 to 7.7)	6.6 (4.6 to 9.1)	4.5 (2.3 to 7.7)	6.6 (3.7 to 10.7)	−1.2 (−4.1 to 1.7)	0.9 (−2.3 to 4.0)
Multiple imputation[e]	5.5 (4.0 to 7.7)	6.7 (4.9 to 8.9)	4.9 (3.0 to 7.8)	6.0 (3.7 to 9.2)	−1.1 (−3.9 to 1.7)	0.7 (−2.4 to 3.8)
Post third dose, No./total[c]	517/520	513/517	266/266	12/226		
MITT[d]	99.4 (98.3 to 99.9)	99.2 (98.0 to 99.8)	100 (98.6 to 100)	5.3 (2.8 to 9.1)	0.2 (−0.8 to 1.2)	−0.6 (−1.2 to 0.01)
Multiple imputation[e]	99.1 (96.8 to 99.3)	98.7 (96.2 to 98.9)	98.6 (94.6 to 98.9)	11.6 (9.0 to 17.2)	0.5 (−1.0 to 2.0)	0.4 (−1.6 to 2.3)
Strain NZ98/254						
Baseline, No./total[c]	18/528	5/526	6/268	2/250		
MITT[d]	3.4 (2.0 to 5.3)	1.0 (0.3 to 2.2)	2.2 (0.8 to 4.8)	0.8 (0.1 to 2.9)	2.5 (0.7 to 4.2)	1.2 (−1.2 to 3.5)
Multiple imputation[e]	3.2 (1.9 to 5.0)	1.0 (0.4 to 2.3)	2.0 (0.8 to 4.3)	0.8 (0.1 to 2.8)	2.2 (0.5 to 3.8)	1.2 (−1.0 to 3.4)
Post third dose, No./total[c]	417/528	453/526	219/268	11/250		
MITT[d]	79.0 (75.2 to 82.4)	86.1 (82.9 to 89.0)	81.7 (76.6 to 86.2)	4.4 (2.2 to 7.7)	−7.1 (−11.7 to −2.6)	−2.7 (−8.5 to 3.05)
Multiple imputation[e]	79.0 (74.8 to 81.5)	86.1 (82.0 to 88.1)	79.1 (73.5 to 82.9)	9.7 (7.2 to 14.7)	−7.0 (−11.6 to −2.44)	−0.76 (−6.5 to 5.0)

Abbreviation: MITT, modified intention-to-treat.
[a] See "Methods" for definition of groups.
[b] Two-sided 95% CI calculated using Clopper-Pearson method.
[c] Total numbers may differ from MITT population in Figure 1 because human complement serum bactericidal activities failed for individual strains.
[d] Unadjusted for center effect.
[e] n=610 for the concomitant group; n=613 for the intercalated group; n=306 for the accelerated group; and n=309 for the routine group. See "Methods" for variables included in imputation model.

Over the 8- to 10-month study, 166 serious adverse events were reported in 158 participants: 63 (10%) in the concomitant group, 57 (9%) in the intercalated group, 19 (6%) in the accelerated group, and 19 (6%) in the control group.

Of these, 20 (9 concomitant, 7 intercalated, 3 accelerated, and 1 control) were thought to be possibly related to 4CMenB or routine vaccinations.

Four possibly related serious adverse events were seizures, 1 each following

routine vaccination in the intercalated and accelerated groups, and 2 following vaccination with 4CMenB in the intercalated group, 1 of the latter being a febrile seizure 2 days after the second 4CMenB dose. Two hypotonic hypore-

Table 2. Geometric Mean Titers and Ratios (MITT Population), by Vaccination Group[a]

	GMT (95% CI)				GMR (95% CI)	
Strain	Concomitant	Intercalated	Accelerated	Control	Concomitant to Intercalated	Concomitant to Accelerated
Strain 44/76-SL[b]	n = 525	n = 531	n = 272	n = 248		
Baseline						
MITT[c]	1.51 (1.41-1.61)	1.38 (1.29-1.48)	1.34 (1.23-1.46)	1.27 (1.18-1.37)	1.09 (1.00-1.18)	1.12 (1.01-1.24)
Multiple imputation[d]	1.54 (1.44-1.65)	1.40 (1.32-1.50)	1.39 (1.28-1.51)	1.29 (1.20-1.38)	1.10 (1.01-1.19)	1.11 (1.01-1.22)
Post third dose						
MITT[c]	83 (77-90)	110 (102-119)	82 (75-91)	1.26 (1.13-1.39)	0.75 (0.69-0.83)	1.01 (0.90-1.49)
Multiple imputation[d]	85 (76-92)	106 (97-116)	80 (72-90)	1.71 (1.45-2.01)	0.80 (0.72-0.88)	1.05 (0.93-1.19)
Strain 5/99[b]	n = 520	n = 517	n = 266	n = 226		
Baseline						
MITT[c]	1.29 (1.20-1.38)	1.28 (1.19-1.37)	1.20 (1.10-1.31)	1.24 (1.14-1.35)	1.01 (0.93-1.10)	1.07 (0.97-1.19)
Multiple imputation[d]	1.28 (2.00-1.37)	1.29 (1.21-1.38)	1.21 (1.11-1.32)	1.24 (1.15-1.33)	1.00 (0.92-1.08)	1.06 (0.97-1.17)
Post third dose						
MITT[c]	520 (475-570)	669 (611-731)	323 (287-363)	1.43 (1.18-1.74)	0.78 (0.70-0.87)	1.61 (1.41-1.84)
Multiple imputation[d]	533 (475-598)	636 (572-709)	315 (274-361)	1.77 (1.66-2.73)	0.84 (0.74-0.95)	1.69 (1.44-1.99)
Strain NZ98/254[b]	n = 528	n = 526	n = 268	n = 250		
Baseline						
MITT[c]	1.14 (1.09-1.19)	1.08 (1.04-1.13)	1.07 (1.01-1.13)	1.06 (1.03-1.10)	1.05 (0.90-1.11)	1.06 (1.00-1.14)
Multiple imputation[d]	1.13 (1.08-1.17)	1.08 (1.04-1.13)	1.06 (1.01-1.12)	1.06 (1.02-1.10)	1.04 (0.99-1.10)	1.06 (1.00-1.12)
Post third dose						
MITT[c]	12 (10-13)	17 (15-19)	11 (9-12)	1.16 (1.06-1.25)	0.68 (0.59-0.80)	1.10 (0.91-1.32)
Multiple imputation[d]	12 (10-13)	16 (14-19)	10 (9-12)	1.40 (1.25-1.57)	0.71 (0.61-0.83)	1.15 (0.95-1.38)

Abbreviations: GMR, geometric mean ratio; GMT, geometric mean titer; MITT, modified intention-to-treat.
[a] See "Methods" for definitions of groups.
[b] Total numbers may differ from MITT population in Figure 1 because human complement serum bactericidal activities failed for individual strains.
[c] Adjusted for center effect.
[d] n=610 for the concomitant group; n=613 for the intercalated group; n=306 for the accelerated group; and n=309 for the routine group. See "Methods" for variables included in imputation model.

Table 3. Geometric Mean Bactericidal Antibody Concentrations Measured by ELISA Against Vaccine Antigen NHBA (per-Protocol Population and Multiple Imputation Analysis)[a]

	ELISA Geometric Mean Antibody Concentration							
	Concomitant		Intercalated		Accelerated		Control	
Group	U/mL (95% CI)	Sample Size, No.	U/mL (95% CI)	Sample Size, No.	U/mL (95% CI)	Sample Size, No.	U/mL (95% CI)	Sample Size, No.
Baseline								
Per-protocol population[b]	23 (21-24)	569	23 (22-24)	559	22 (21-24)	293	22 (21-23)	287
Multiple imputation[c]	23 (22-24)	615	23 (22-24)	615	23 (21-24)	307	22 (21-23)	307
Post third dose								
Per-protocol population[b]	3332 (3120-3558)	545	4342 (4067-4635)	557	3211 (2949-3495)	281	21 (20-21)	269
Multiple imputation[c]	3261 (2965-3587)	615	4039 (3697-4414)	609	3049 (2732-3402)	307	30 (26-36)	307
Ratio of post third dose to baseline								
Per-protocol population[b]	150 (136-165)	531	191 (174-210)	539	145 (128-164)	275	0.94 (0.89-0.99)	257
Multiple imputation[c]	143 (128-161)	615	175 (157-196)	609	135 (118-156)	307	1.39 (1.17-1.66)	307

Abbreviations: ELISA, enzyme-linked immunosorbent assay; NHBA, *Neisseria* heparin binding antigen.
[a] See "Methods" for definitions of groups.
[b] Adjusted for center effect.
[c] n=610 for the concomitant group; n=613 for the intercalated group; n=306 for the accelerated group; and n=309 for the routine group. See "Methods" for variables included in imputation model.

sponsive episodes were reported, 1 within 12 hours of concomitant 4CMenB and routine vaccines and 1 within 6 hours after routine vaccines in the intercalated group. Two cases of Kawasaki disease were reported, 1 of which was considered possibly related to the study vaccine by an independent expert panel. Six children were observed in the hospital because they had experienced fever within 2 days after vaccination with 4CMenB. The remaining 5 possibly

related serious adverse events were aseptic meningitis, retinal dystrophy (believed to be congenital), transient synovitis of the right hip, a transient hearing loss noted by a parent, and transient apnea after concomitant vaccination.

COMMENT

This study of more than 1800 infants shows that when administered together with routine vaccines to healthy infants, a primary immunization course

of 4CMenB is immunogenic against 3 reference strains expressing 1 of 3 vaccine antigens. Furthermore, 4CMenB was immunogenic when administered in a 2-, 3-, and 4-month schedule, an important finding given the high rates of MenB disease in the first 6 months of life.[15,16] These results suggest that there can be some flexibility in the incorporation of 4CMenB into the various immunization schedules used in different countries.

Table 4. Immunogenicity of Routine Vaccines (DTaP-HBV-IPVHib and 7-Valent Pneumococcal) Given Concomitantly With or Without 4CMenB; Total Number and Percentage of Infants With Seroresponse Greater Than or Equal to a Prespecified Level and Geometric Mean Antibody Concentrations (per-Protocol Population)[a]

Vaccine Antigen	Threshold of Response	No. of Participants With Positive Threshold/Total No. Tested (% Participants Achieving Threshold of Response) [95% CI]			Geometric Mean Antibody Concentration/Titers (95% CI)		
		Accelerated Group	Control Group	Difference in Response, %	Accelerated Group	Control Group	Geometric Mean Ratio
Diphtheria	IgG ≥0.10 IU/mL	238/238 (100) [98 to 100]	209/210 (100) [97 to 100]	0 (–1 to 3)	1.43 (1.30 to 1.57)	1.69 (1.53 to 1.87)	0.85 (0.75 to 0.95)
Tetanus	IgG ≥0.10 IU/mL	238/238 (100) [98 to 100]	210/210 (100) [98 to 100]	0 (–2 to 2)	1.91 (1.71 to 2.13)	2.00 (1.78 to 2.25)	0.95 (0.83 to 1.09)
Pertussis	≥4-fold increase in IgG						
FHA		182/238 (76) [71 to 82]	156/210 (74) [68 to 80]	2 (–6 to 10)	71 (65 to 79)	71 (64 to 79)	1.01 (0.89 to 1.14)
Pertactin		195/238 (82) [76 to 87]	181/210 (86) [81 to 91]	–4 (–11 to 3)	143 (125 to 164)	185 (160 to 214)	0.77 (0.65 to 0.91)
Pertussis toxoid		204/238 (86) [81 to 90]	186/210 (89) [83 to 93]	–3 (–9 to 4)	35 (31 to 39)	37 (33 to 41)	0.94 (0.82 to 1.08)
Polio	Titers ≥1: 8						
Type 1		174/175 (99) [97 to 100]	162/164 (99) [96 to 100]	1 (–2 to 4)	103 (83 to 128)	151 (120 to 189)	0.68 (0.53 to 0.89)
Type 2		162/174 (93) [88 to 96]	155/164 (95) [90 to 97]	–1 (–7 to 4)	62 (48 to 80)	89 (69 to 115)	0.70 (0.52 to 0.94)
Type 3		175/175 (100) [98 to 100]	159/164 (97) [93 to 99]	3 (1 to 7)	257 (201 to 329)	366 (284 to 472)	0.70 (0.52 to 0.94)
PRP-Hib	IgG ≥0.15 µg/mL	233/236 (99) [96 to 100]	204/209 (98) [95 to 99]	1 (–2 to 4)	2.39 (2.01 to 2.85)	2.51 (2.10 to 3.01)	0.95 (0.77 to 1.18)
Hepatitis type B virus	IgG ≥10 mIU/mL	199/206 (97) [93 to 99]	189/194 (97) [94 to 99]	–1 (–5 to 3)	243 (193 to 305)	319 (252 to 405)	0.76 (0.58 to 1.01)
Pneumococcal serotype	IgG ≥0.35 µg/mL						
PnC 4		215/228 (94) [90 to 97]	192/203 (95) [91 to 97]	0 (–5 to 4)	1.97 (1.67 to 2.32)	2.08 (1.74 to 2.48)	0.95 (0.77 to 1.17)
PnC 6B		162/228 (71) [65 to 77]	155/203 (76) [70 to 82]	–5 (–14 to 3)	0.87 (0.68 to 1.09)	1.16 (0.91 to 1.50)	0.74 (0.55 to 1.00)
PnC 9V		224/228 (98) [96 to 100]	198/203 (98) [9 to 99]	1 (–2 to 4)	4.31 (3.71 to 5.00)	3.61 (3.08 to 4.24)	1.19 (0.99 to 1.44)
PnC 14		223/228 (98) [95 to 100]	192/203 (95) [91 to 97]	3 (0 to 8)	5.06 (4.28 to 6.00)	4.12 (3.44 to 4.93)	1.23 (0.99 to 1.53)
PnC 18C		223/228 (98) [95 to 100]	195/203 (96) [92 to 98]	2 (–2 to 6)	4.61 (3.91 to 5.42)	3.67 (3.09 to 4.37)	1.25 (1.02 to 1.54)
PnC 19F		224/228 (98) [96 to 100]	198/203 (98) [94 to 99]	1 (–2 to 4)	4.58 (3.89 to 5.39)	4.48 (3.77 to 5.34)	1.02 (0.83 to 1.26)
PnC 23F		212/228 (93) [89 to 96]	195/203 (96) [92 to 98]	–3 (–7 to 2)	2.38 (1.98 to 2.87)	2.68 (2.19 to 3.26)	0.89 (0.70 to 1.13)

Abbreviations: DTaP-HBV-IPV/Hib, combined diphtheria, tetanus, acellular pertussis, inactivated polio, hepatitis B, *Haemophilus influenzae* type b; FHA, filamentous hemagglutinin; 4CMenB, multicomponent serogroup B meningococcal vaccine; PRP-Hib, polyribosylribitol phosphate *Haemophilus influenzae* type b polysaccharide.
[a]See "Methods" for definitions of groups.

Immunogenicity of 4CMenB

When administered alone in the intercalated group rather than with routine vaccines, 4CMenB elicited higher hSBA GMTs for all strains. Similarly, administration of 4CMenB in a 2-, 4-, and 6-month schedule, rather than an accelerated 2-, 3-, and 4-month schedule, resulted in higher hSBA GMTs for the 5/99 strain. However, immunization schedules need to balance the advantages of optimal immunogenicity and the practical need to minimize immunization visits and ensure early immunization. The clinical significance of any reduction in hSBA GMTs is uncertain, given that the proportion of participants with an hSBA titer of 1:5 or greater was at least 79% for all strains in all 4CMenB recipients. Lower hSBA GMTs may lead to shorter persistence of bactericidal antibodies following primary immunization, as observed with the serogroup C meningococcal vaccines[17-19] and with the New Zealand MenB vaccine (MeNZB).[8,20] A follow-up study of the same participants assessing antibody persistence and the response to a booster dose of 4CMenB has been undertaken[21] and will further inform the relative benefits of the different immunization schedules.

4CMenB immunogenicity in this study is consistent with previous small trials that found 4CMenB to be more broadly immunogenic when given with OMVs,[8,9] possibly because of a synergistic or enhancing effect of the OMV. Furthermore, the OMV component of 4CMenB was used as a vaccine to control a clonal outbreak of MenB disease in New Zealand, with an apparent effectiveness of 85% in children aged 6 months to 5 years[22] after inducing very similar hSBA titers against strain NZ98/254.[23] This provides confidence that the levels of functional antibody induced by this vaccine could indeed induce protection against strains expressing the vaccine antigens.

Immunogenicity of Routine Vaccines

Concomitant 4CMenB impaired the immunogenicity of only 2 routine vaccine antigens: pertactin and pneumococcal serotype 6B. This is unlikely to be of clinical significance, because acellular pertussis vaccines that lack pertactin are known to be effective, and evidence suggests that pneumococcal disease resulting from the 6B serotype is lower in countries that have implemented vaccination with the PCV7 vaccine, although the putative response threshold of IgG level of 0.35 µg/mL or greater was achieved only by a relatively low percentage of the immunized population, possibly resulting from both direct protection and herd immunity.[24]

Breadth of Protection

Three strains expressing key vaccine antigens were used for hSBA analysis, demonstrating "proof of principle" immunogenicity of these antigens but allowing only limited conclusions regarding cross-protection against other naturally occurring strains. Also, data from assays using strains genetically engineered to express a range of fHbp1 subvariants[25] and from early phase 2 studies[8,9] suggest that genotypic analysis of panels of invasive strains is not sufficient to predict the likely breadth of coverage of 4CMenB immunization against invasive meningococcal disease in infants.

Further information regarding the breadth of protection could be achieved by testing against a more extended panel of strains, as in previous 4CMenB infant studies,[8,9] but the number of strains that can be tested by hSBA is limited by the volume of sera available from infants, the limited supply of suitable human complement, and the unsuitability of many strains for hSBA.[26] This, combined with the evolutionary and geographical variation in the target MenB antigens, makes selection of strain panels difficult.[27] The meningococcal antigen testing system[28] using an antigen-specific enzyme-linked immunosorbent assay (evaluating both immunological cross-reactivity and antigen expression) has an accuracy of 86% in predicting the hSBA response and might be suitable to assess the propor-tion of MenB strains likely to be covered by the vaccine on a regional basis. Recently presented data suggest that pooled sera from children immunized with the 4CMenB vaccine would be bactericidal against 78% (95% CI, 66% to 91%) of invasive strains in Europe[29] and against 76% (95% CI, 59% to 87%) of such strains in Australia.[30] However, the ability of these laboratory assays to accurately predict the effects of a MenB vaccine—possibly also on non-MenB pathogens expressing vaccine antigens—is unknown and can only be evaluated properly following implementation in a program with close surveillance.

Reactogenicity

Outer membrane vesicle vaccines (eg, MeNZB) have been administered to more than 3 million children across all age groups and are considered well tolerated and safe.[23] In trials of MeNZB to control clonal outbreaks in New Zealand, fever was the most pronounced adverse reaction,[31] with no additional reactogenicity burden with concomitant administration of MenB and routine vaccines. In contrast, in this study 4CMenB appeared to be less reactogenic when administered separately rather than together with routine vaccines (although the study made no formal statistical comparisons of reactogenicity rates between groups). The majority of children in this study became febrile after receiving their first and second dose of concomitant 4CMenB and routine vaccines, although only 1 child in our study had a febrile convulsion (2 days following 4CMenB administration). There is therefore a need to compare the risk profile of a relatively common, but transient, postimmunization fever with the benefit of reducing the risk of an uncommon, but potentially fatal, meningococcal infection. This must be carefully communicated to parents in the event of routine implementation if the possibility of increased systemic reactogenicity is to be an accepted component in the control of this serious disease. Rates and

magnitude of fever in this study are comparable with those seen with other pediatric vaccines, notably after the second dose of ASO3$_B$-adjuvanted split virion H1N1 influenza vaccine used in children aged 6 months to 5 years[32] and whole-cell pertussis vaccine in 1 study,[33] and were actually lower than those observed in another study of the pertussis vaccine.[34]

Limitations

The open-label design could potentially bias reporting of adverse events if parents were more alert to subjective reactions such as irritability if their child received 4CMenB rather than routine vaccines. This would be less likely to influence objective measures such as temperature and extent of swelling or erythema. The 3 strains selected for hSBA analysis do not specifically evaluate the immunogenicity of NHBA, because the only strain expressing NHBA also contained porin protein A homologous to the OMV component. Concentrations of IgG specific to NHBA increased following administration of 4CMenB; however, this was also observed following administration of a vaccine containing the recombinant proteins without OMVs in early phase 2 studies. This increase in IgG GMCs was not associated with an increase in hSBA titers against NZ98/254,[8,9] raising doubts about the bactericidal qualities of these antibodies. A naturally occurring strain allowing the assessment of NHBA-induced bactericidal activity has recently been identified[35] and will provide important additional information regarding the immunogenicity of this antigen.

CONCLUSIONS

In conclusion, 4CMenB was immunogenic, generally well tolerated, and showed minimal interference with routine vaccines in the first year of life. The flexibility in schedule allows it to be incorporated into a range of country-specific immunization schedules and for primary immunization to be completed in early infancy. If licensed, the decisions regarding vaccine introduction will require detailed assessment of potential vaccine coverage at a regional level and monitoring after implementation to determine the accuracy of such predictions. Nevertheless, this vaccine could potentially provide improved protection for infants against meningococcal disease beyond the protection provided by currently licensed vaccines.

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REFERENCES

1. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine.* 2009; 27(suppl 2):B51-B63.
2. Menactra (Meningococcal [Groups A, C, Y and W-135] Polysaccharide Diphtheria Toxoid Conju-

gate Vaccine) for Intramuscular Injection [package insert]. Swiftwater, PA: Sanofi Pasteur; 2011. US Food and Drug Administration Web site. http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM131170.pdf. Accessed November 23, 2011.

3. Jackson C, Lennon DR, Sotutu VT, et al. Phase II meningococcal B vesicle vaccine trial in New Zealand infants. *Arch Dis Child.* 2009;94(10):745-751.

4. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis. Lancet.* 2007;369(9580):2196-2210.

5. 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(16):1520-1527.

6. Wong SH, Lennon DR, Jackson CM, et al. Immunogenicity and tolerability in infants of a New Zealand epidemic strain meningococcal B outer membrane vesicle vaccine. *Pediatr Infect Dis J.* 2009; 28(5):385-390.

7. Giuliani MM, Adu-Bobie J, Comanducci M, et al. A universal vaccine for serogroup B meningococcus. *Proc Natl Acad Sci U S A.* 2006;103(29):10834-10839.

8. Findlow J, Borrow R, Snape MD, et al. Multicenter, open-label, randomized phase II controlled trial of an investigational recombinant Meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy. *Clin Infect Dis.* 2010; 51(10):1127-1137.

9. 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 (11):e71-e79.

10. Frasch CE, Borrow R, Donnelly J. Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease. *Vaccine.* 2009; 27(suppl 2):B112-B116.

11. Clopper CP, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika.* 1934;26(4):404-413.

12. Miettinen O, Nurminen M. Comparative analysis of two rates. *Stat Med.* 1985;4(2):213-226.

13. *Statistical Analysis With Missing Data.* Rubin DB, ed. New York, NY: John Wiley & Sons; 1987.

14. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. *Am Stat.* 1998;52(52):119-126.

15. Ramsay ME. Current epidemiology of meningococcal disease in the UK and Europe, including issues for surveillance relating to a MenB vaccine. Presented at: Meningitis Research Foundation Conference 2011; November 8-9, 2011; London, United Kingdom.

16. Harrison LH. Epidemiological profile of meningococcal disease in the United States. *Clin Infect Dis.* 2010;50(suppl 2):S37-S44.

17. Campbell H, Borrow R, Salisbury D, Miller E. Meningococcal C conjugate vaccine: the experience in England and Wales. *Vaccine.* 2009;27(suppl 2):B20-B29.

18. Snape MD, Kelly DF, Green B, Moxon ER, Borrow R, Pollard AJ. Lack of serum bactericidal activity in preschool children two years after a single dose of serogroup C meningococcal polysaccharide-protein conjugate vaccine. *Pediatr Infect Dis J.* 2005;24(2): 128-131.

19. Perrett KP, Winter AP, Kibwana E, et al. Antibody persistence after serogroup C meningococcal conjugate immunization of United Kingdom primaryschool children in 1999-2000 and response to a booster: a phase 4 clinical trial. *Clin Infect Dis.* 2010; 50(12):1601-1610.

20. Jackson C, Lennon D, Wong S, et al. Antibody persistence following MeNZB vaccination of adults and children and response to a fourth dose in toddlers. *Arch Dis Child.* 2011;96(8):744-751.

21. Novartis. Safety, tolerability and immunogenicity of meningococcal B recombinant vaccine administered as booster dose at 12, 18 or 24 months of age in toddlers (12-24 months) primed with a three-dose immunization series as infants in Study V72P12 [NCT00944034]. ClinicalTrials.gov Web site. http://clinicaltrials.gov/ct2/show/NCT00944034. 2009. Accessed October 1, 2009.

22. Galloway Y, Stehr-Green P, McNicholas A, O'Hallahan J. Use of an observational cohort study to estimate the effectiveness of the New Zealand group B meningococcal vaccine in children aged under 5 years. *Int J Epidemiol.* 2009;38(2):413-418.

23. 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-B12.

24. Whitney CG, Pilishvili T, Farley MM, et al. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. *Lancet.* 2006;368(9546):1495-1502.

25. Brunelli B, Del Tordello E, Palumbo E, et al. Influence of sequence variability on bactericidal activity sera induced by Factor H binding protein variant 1.1. *Vaccine.* 2011;29(5):1072-1081.

26. Borrow R, Aaberge IS, Santos GF, et al. Interlaboratory standardization of the measurement of serum bactericidal activity by using human complement against meningococcal serogroup b, strain 44/76-SL, before and after vaccination with the Norwegian MenBvac outer membrane vesicle vaccine. *Clin Diagn Lab Immunol.* 2005;12(8):970-976.

27. Vogel U. Molecular epidemiology of meningococci: application of DNA sequence typing. *Int J Med Microbiol.* 2010;300(7):415-420.

28. 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. *Proc Natl Acad Sci U S A.* 2010; 107(45):19490-19495.

29. Donnelly J, Medini D, Giuliani M, et al. Estimating the potential strain coverage in Europe of a multicomponent vaccine targeting serogroup B meningococci. Presented at: Meningitis Research Foundation Conference 2011; November 8-9, 2011; London, United Kingdom.

30. Smith HNM, Sloots T, Tozer S, et al. Estimating the potential strain coverage in Australia of a multicomponent vaccine targeting serogroup B meningococci. Presented at: 7th World Congress of the World Society for Pediatric Infectious Diseases (WSPID); November 16-19, 2011; Melbourne, Australia.

31. Nøkleby H, Aavitsland P, O'Hallahan J, Feiring B, Tilman S, Oster P. Safety review: two outer membrane vesicle (OMV) vaccines against systemic *Neisseria meningitidis* serogroup B disease. *Vaccine.* 2007; 25(16):3080-3084.

32. Waddington CS, Walker WT, Oeser C, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. *BMJ.* 2010;340:c2649.

33. Kitchin N, Southern J, Morris R, et al. A randomised controlled study of the reactogenicity of an acellular pertussis-containing pentavalent infant vaccine compared to a quadrivalent whole cell pertussis-containing vaccine and oral poliomyelitis vaccine, when given concurrently with meningococcal group C conjugate vaccine to healthy UK infants at 2, 3 and 4 months of age. *Vaccine.* 2006;24(18): 3964-3970.

34. Gustafsson L, Hallander HO, Olin P, Reizenstein E, Storsaeter J. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. *N Engl J Med.* 1996;334 (6):349-355.

35. Biolchi A, Kleinschmidt A, Boccadifuoco G, et al. Evaluation of the contribution of protein antigen NHBA to bactericidal antibody responses in sera from human vaccinees enrolled in clinical trials. Presented at: 17th International Pathogenic Neisseria Conference; September 11-16, 2010; Banff, Alberta, Canada.