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

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Summary

Background **Effective glycoconjugate vaccines against *Neisseria meningitidis* serogroups A, C, W-135, and Y have been developed, but serogroup B remains a major cause of severe invasive disease in infants and adolescents worldwide. We assessed immunogenicity and tolerability of a four-component vaccine (4CMenB) in adolescents.**

Methods **We did a randomised, observer-blind, placebo-controlled, study at 12 sites in Santiago and Valparaíso, Chile. Adolescents aged 11–17 years received one, two, or three doses of 4CMenB at 1 month, 2 month, or 6 month intervals. Immunogenicity was assessed as serum bactericidal activity using human complement (hSBA) against three reference strains for individual vaccine antigens, and assessed by ELISA against the fourth strain. Local and systemic reactions were recorded 7 days after each vaccination, and adverse events were monitored throughout the study. Participants were initially randomised to five groups (3:3:3:3:1) during the primary phase to receive either one dose, two doses 1 or 2 months apart, or three doses of 4CMenB, or three doses of placebo, with an additional three groups generated for the booster phase. All subjects received at least one dose of 4CMenB. Geometric mean titres, proportions of participants with serum bactericidal antibody titres of 4 or more, and Clopper-Pearson 95% CIs were calculated. The study is registered with ClinicalTrials.gov, number NCT00661713.**

Findings **Overall, 1631 adolescents (mean age 13·8 [SD 1·9] years) received at least one dose of 4CMenB. After two or three doses, 99–100% of recipients had hSBA titres of 4 or more against test strains, compared with 92–97% after one dose (p<0·0145) and 29–50% after placebo. At 6 months 91–100% of participants still had titres of 4 or more for each strain after two or three doses, but only 73–76% after one dose; seroresponse rates reached 99–100% for each strain after second or third doses at 6 months. Local and systemic reaction rates were similar after each 4CMenB injection and did not increase with subsequent doses, but remained higher than placebo. No vaccine-related serious adverse events were reported and no significant safety signals were identified.**

Interpretation **On the basis of immunogenicity responses this study provides evidence for an adolescent 4CMenB vaccine schedule of two doses, 1–6 months apart, to provide protection against meningococcal B infection. The extent of this protection against meningococcus B variants circulating worldwide will be determined by national surveys.**

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Introduction

Epidemic and sporadic *Neisseria meningitidis* disease caused by six major serogroups, A, B, C, W-135, Y, and the recently emerging X, causes substantial morbidity and mortality in otherwise healthy people, with large variations in global epidemiology.[1] Initially, vaccines against serogroups A, C, W135, and Y were developed on the basis of serogroup-specific capsular polysaccharides; vaccines were subsequently improved with the development of polysaccharide-protein conjugates.[2,3] Quadrivalent (A,C,W-135,Y) meningococcal glycoconjugate vaccines for all age groups are now licensed or in late-stage development.[4,5] Following successful implementation of routine childhood vaccination with serogroup C meningococcal conjugate vaccines, serogroup B is now the most serious cause of meningococcal disease in Europe and elsewhere, with a substantial medical burden.[6] In the UK, for example, up to 19% of laboratory-confirmed cases of invasive serogroup B disease between 1999 and 2006 were fatal.[7] In North America, prevalence of serogroups B, C, and Y is almost equal,[1] and serogroup B is the most prevalent in the southern cone of Latin America; over 60% of meningococcal-disease cases in Chile are caused by serogroup B.[8]

The capsular-polysaccharide strategy cannot be applied to the development of a serogroup B vaccine, because serogroup B polysaccharide immunologically resembles the surface of neural-cell adhesion molecules, resulting in poor immunogenicity and the potential for induction of autoimmune antibodies.[9–11] Vaccines successfully developed to combat epidemic outbreaks of serogroup B meningococcal disease used outer membrane vesicles

from the local outbreak strains to induce immune responses.[1,12] Since effectiveness was limited to the clonal outbreak strains, these vaccines do not provide a solution to endemic meningococcal B disease caused by heterologous meningococcal strains worldwide.[12,13]

To develop broadly protective vaccines against serogroup B, whole genome sequencing was used to identify proteins on the surface of many meningococcal strains.[14] In-vitro and in-vivo assessment of targets for bactericidal activity and large-scale production identified three protein components for vaccine development: factor H binding protein, neisserial adhesin A, and neisseria heparin binding antigen.[15,16] Neisseria heparin binding antigen and factor H binding protein components were each presented as fusion proteins with other proteins identified from genome mining.[17] A formulation of these components in an investigational serogroup B meningococcal vaccine with outer membrane vesicles from the New Zealand outbreak strain 98/254 (4CMenB) yielded promising results in feasibility studies in infants and adults.[17–19] We report the results of the first large-scale trial of 4CMenB, in adolescents, the aim of which was to assess the immunogenicity and tolerability of 4CMenB in different schedules.

Methods

Study design and participants

We did a phase 2b/3 randomised, observer-blind, placebo-controlled study at 12 sites in Santiago and Valparaíso, Chile, following good clinical practice and the principles outlined in the Declaration of Helsinki. Ethical committee approval was obtained from Comité de Ética en Investigación en Seres Humanos (Facultad de Medicina, Universidad de Chile, Chile), Comité de Ética de la Investigación del Servicio de Salud (Metropolitano Norte, Santiago, Chile), Comité de Ética Científico Pediátrico, Servicio de Salud (Metropolitano Oriente, Santiago, Chile), and Comité de Evaluación Ético Científico del Servicio de Salud (Valparaíso, San Antonio, Chile). Informed, written consent and assent were obtained from all parents or legal guardians and participants, respectively. An independent data monitoring committee provided guidance for interpretation of safety outcomes.

Healthy adolescents of either sex, aged 11–17 years, with no previous history of meningococcal serogroup B vaccination or meningococcal disease, were eligible. In post-menarchal participants a negative pregnancy test was required at study start and before any future injection together with use of contraception throughout. Exclusion criteria were allergy to any vaccine component, household contact with a confirmed case of meningococcal disease within 60 days, any immunisation within 30 days with the exception of influenza vaccination, which was permitted more than 14 days before or after a study injection, receipt of antibiotics within 6 days or blood products or any investigational product within 90 days of enrolment.

4CMenB was supplied as a liquid formulation in prefilled syringes for injection in the deltoid muscle. Each 0·5 mL dose contained 50 µg each of neisserial adhesin A, factor H binding protein, and neisseria heparin binding antigen fusion proteins, and 25 µg of outer membrane vesicles from *N meningitidis* strain NZ98/254, with 1·5 mg $Al(OH)_3$ in 10 mM histidine buffer containing 110–120 mM saline as previously described.[17] Placebo syringes contained 1·5 mg $Al(OH)_3$ in the histidine and saline buffer.

Procedures

The study was done in two phases. In the priming phase all eligible participants received three injections at 1 month intervals. At enrolment, participants were randomised to receive either one dose, two doses 1 month apart, two doses 2 months apart, or three doses of 4CMenB, or three doses of placebo. In the second phase at 6 months, participants previously given one or two doses of 4CMenB in the primary phase were further randomised (1:2) to receive 4CMenB or placebo leading to a total of eight study groups. Participants receiving only placebo during the primary phase received one dose of 4CMenB and those receiving three doses of 4MenCB received placebo at month 6.

Following each study injection participants were observed for 30 min, and provided with 7-day diary cards on which they or their parents or guardians recorded axillary temperature, solicited injection site reactions (pain, induration, erythema, and swelling), and systemic reactions (malaise, myalgia, arthralgia, headache, and nausea), staying home from school, and use of analgesic or antipyretic drugs. Other adverse events were recorded at the following study visit, and medically attended and serious adverse events were reported throughout the study. Induration, erythema, and swelling were measured in millimetres, severity of pain and systemic reactions were assessed using a predefined scale from mild (noticeable) to severe (interference with normal

	4CMenB				Placebo (n=128)
	One dose (n=375)	Two doses 1 month apart (n=375)	Two doses 2 months apart (n=380)	Three doses (n=373)	
Age, years	13·8 (1·9)	13·9 (1·9)	13·7 (1·9)	13·8 (1·9)	13·8 (2·0)
Female	223 (59%)	213 (57%)	211 (56%)	199 (53%)	66 (52%)
Ethnic origin					
Asian	0	0	0	1 (<1%)	0
Hispanic	370 (99%)	375 (100%)	376 (99%)	370 (99%)	128 (100%)
Other	5 (1%)	0	4 (1%)	2 (<1%)	0
Weight, kg	54·76 (11·30)	56·39 (13·17)	56·14 (11·70)	57·76 (13·95)	56·24 (13·18)
Height, cm	157·7 (9·2)	157·6 (9·7)	158·0 (9·4)	158·4 (9·9)	158·4 (9·8)

Data are number (%) or mean (SD).

Table 1: **Demographics of the study population in the primary phase**

activities), and investigators assessed the relation of these events to study injections.

Sera collected at baseline and 1 month after each injection were assessed by analysing serum bactericidal activity using human complement (hSBA) against three meningococcal serogroup B reference strains—strains 44/76, 5/99, and NZ98/254—to establish the individual contributions of the factor H binding protein, neisserial adhesin A, and NZ outer membrane vesicle components.[20] Interpolated hSBA titres were based on the reciprocal of the final serum dilution, which showed 50% or more killing of the colony-forming units after incubation for 60 min, versus the number of units at time zero. The primary immunogenicity endpoint was the percentage of participants with a protective hSBA titre of 4 or more, which has been shown to be the protective level.[21] In the absence of a suitable candidate strain specific for neisseria heparin binding antigen at time of the study, responses to this component were assessed by ELISA.

Randomisation and masking

Participants were randomised to eight groups with a sponsor-supplied computer generated allocation schedule (1:2:1:2:1:2:3:1), but were combined into five groups (3:3:3:3:1) for the primary phase. To maintain study masking, placebo was administered when a 4CMenB dose was not scheduled so that every participant received four injections within 6 months.

Statistical analysis

A planned sample size of 1625 was calculated, which was guided by previous results from a phase 1 study of 4CMenB immunogenicity, and assuming 20% attrition and 85–95% of participants having protective hSBA titres after vaccination. Geometric mean titres, proportions of participants with hSBA titres of 4 or more, and Clopper-Pearson 95% CIs were computed by exponentiating (base 10) the least square means of the log-transformed titres from a two-way ANOVA with factors for vaccine group and study centre. For these calculations, samples with undetectable titres were given a value of two, half the detection limit. Differences between geometric mean titres were calculated by a two-way ANOVA with factors for vaccine group and study centre. The primary criterion for immunogenicity was that the lower limit of the two-sided 95% CI for the percentage of participants with an hSBA titre of 4 or more at 1 month after the first, second, or third 4CMenB vaccination was 85% or higher for strains with an expected response of 95%, and 75% or higher for a strain with an expected response of 85%.

To estimate the power to meet the primary endpoint criteria for true percentages ranging from 85–95%, 5000 simulations were done. On the basis of these simulations, 300 evaluable participants per group would result in 99% power. Therefore, allowing for attrition, a planned sample size of about 1625 participants would be needed to attain a total of 1300 evaluable participants (300 per vaccine group and 100 placebo recipients) and 99% power. Safety analyses were descriptive only, with no predefined statistical criteria. Ad-hoc analyses for differences between 4CMenB and placebo recipients were done using χ^2 test.

The study is registered with ClinicalTrials.gov, number NCT00661713.

Role of the funding source

The study sponsor and investigators designed and developed the study. The sponsor of the study had no role in data collection and data interpretation, but did provide support for writing of the report. The principle investigator and the corresponding author had full access to all the data in the study.

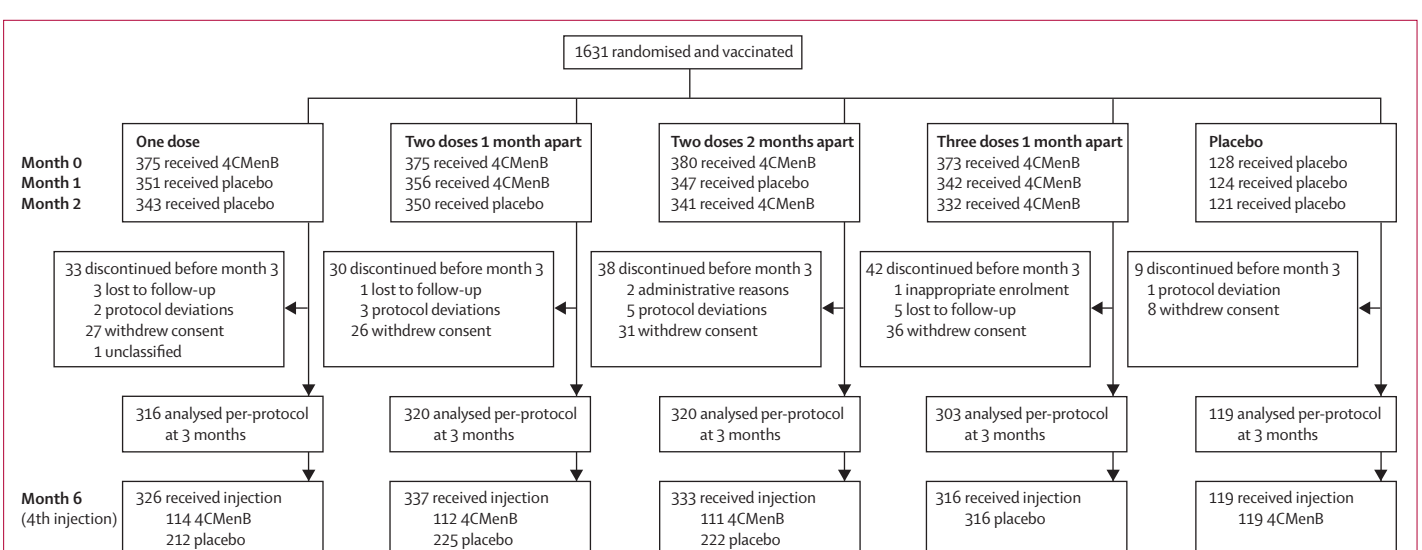

Figure 1: **Study profile**

Results

Between June, 2008, and December, 2010, 1631 adolescents were enrolled, of which 1378 (84%) completed assessments after the primary doses (table 1, figure 1). At 6 months 1431 (88%) adolescents continued their participation, of whom 456 received a dose of 4CMenB and 975 received a placebo injection. 367 (80%) of those who received a dose of 4CMenB and 794 (81%) of those who received a placebo injection had sera analysed at 7 months. Drop-outs occurred at similar rates in all groups throughout the study, with withdrawal of consent the most common reason. Only one participant withdrew due to an adverse event (juvenile arthritis, described below).

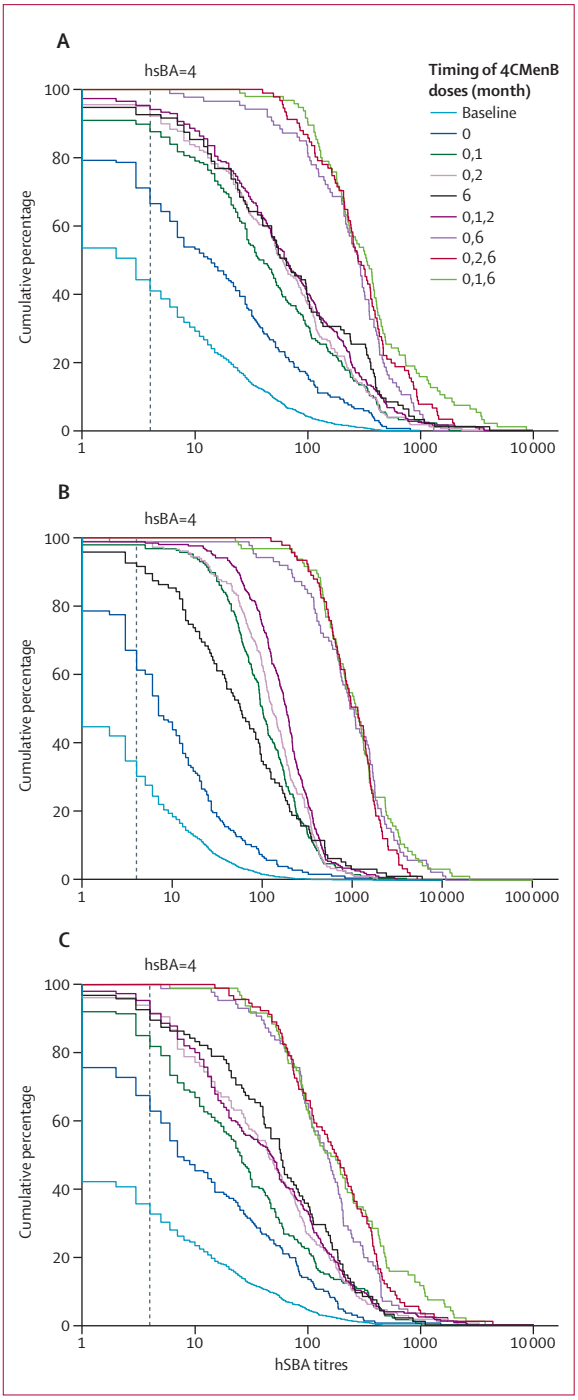

Figure 2: **Reverse cumulative distribution curves for serum bactericidal activity using human complement against the three test strains for all participants at baseline, and for the individual study groups at 7 months after the different vaccination regimens**
Strain 44/76-SL (factor H binding protein; A). Strain 5/99 (neisserial adhesin A; B). Strain NZ98/254 (NZ outer membrane vesicle; C).

See Online for webappendix

The per-protocol population at each visit included participants with no major protocol deviations who provided sera for immune testing. At baseline, 643 (44%) of 1471 participants had hSBA titres of 4 or more to the test strains for factor H binding protein, 500 (34%) of 1471 for neisserial adhesin A, and 516 (35%) of 1470 for NZ outer membrane vesicles. Geometric mean titre ranges at baseline were 3·3–4·2 (factor H binding protein), 2·3–3·2 (neisserial adhesin A), and 2·8–3·4 (NZ outer membrane vesicles). These values remained consistent throughout the study in participants who only received placebo. After one dose of 4CMenB 1347 (93%) of 1449 of participants had hSBA titres of 4 or more for strains specific to factor H binding protein, 1396 (96%) of 1448 to neisserial adhesin A, and 1349 (93%) of 1448 to NZ outer membrane vesicles (webappendix pp 1–3). Geometric mean titre ranges after one dose of 4CMenB were 44–60 (factor H binding protein), 53–81 (neisserial adhesin A), and 42–49 (NZ outer membrane vesicles; webappendix pp 4–6). After a second dose, 1041 (>99%) of 1042 participants had hSBA titres of 4 or more against the indicator test strain factor H binding protein, 1040 (>99%) of 1044 against neisserial adhesin A, and 1041 (>99%) of 1043 against NZ outer membrane vesicles ($p<0·0001$ compared with one dose for all antigens), irrespective of the time interval between doses. After two or three doses, 99–100% of recipients had hSBA titres of 4 or more against the test strains, compared with 92–97% of those who only received one dose ($p<0·0145$). Geometric mean titres increased 1 month after the final dose when the interval between vaccine doses was increased from 1 month to 2 months or 6 months (figure 2), especially for neisserial adhesin A and NZ outer membrane vesicles, but as noted, the proportion of participants with hSBA titres of 4 or more were already 99% or higher. A third dose had a small incremental effect on geometric mean titres, especially when given at 6 months, but did not increase the proportion of participants achieving protective titres: 488 (>99%) of 489 achieving hSBA titres of 4 or more against factor H binding protein ($p=0·584$), 489 (100%) against neisserial adhesin A ($p=0·171$), and 485 (99%) of 488 against NZ outer membrane vesicles ($p=0·176$).

Waning of all antibody titres was evident 2 months after the first dose, with geometric mean titres continuing to decline after 6 months in participants who did not receive a second dose, but protective titres

were maintained in 69–81% of participants. The booster response to a second or third dose of 4CMenB at 6 months re-established hSBA titres of 4 or more in 99–100% of vaccinated participants.

When participants were assessed on the basis of their baseline serological status (ie, hSBA titre <4 or ≥4), differences occurred in proportions with protective titres 1 month after the first dose (table 2) with subsequent decreases occurring in these values up to 6 months. After administration of a second dose, proportions with protective titres were similar, irrespective of baseline status, with high levels of protection maintained up to 6 months. Similar differences in geometric mean titres were seen (data not shown).

Since no suitable candidate strain was available to assess bactericidal responses against neisseria heparin binding antigen at the time of testing, samples of each study group were tested at each timepoint by ELISA for antibodies to this antigen (webappendix p 7). Neisseria heparin binding antigen ELISA concentrations showed the same pattern of responses as hSBA titres against the other antigens, a low concentration of antibodies at baseline (geometric mean concentration range 32–48), which was stable in the placebo group until 6 months, but substantially increased 1 month after a dose of 4CMenB (194–521). In the absence of further doses, titres waned over 6 months, but were boosted by an order of magnitude by a second dose 1 month, 2 months, or 6 months later. By comparison, a third dose only elicited a small increase in this response.

The safety population included all participants. Two deaths during the study were due to causes unrelated to vaccination. The only serious adverse event that led to study discontinuation was a vasovagal reaction and convulsion immediately after a first dose of 4CMenB, in a participant with a paternal history of epilepsy. This event was judged by the investigator to be related to the vaccination procedure, and not to the investigational product.

	1 dose (0)		Two doses (0, 1)		Two doses (0, 2)		Three doses (0, 1, 2)	
	Titres <4	Titres ≥4	Titres <4	Titres ≥4	Titres <4	Titres ≥4	Titres <4	Titres ≥4
Strain 44/76-SL (factor H binding protein)								
Month 0	0/183 (0%, 0–2)	152/152 (100%, 98–100)	0/210 (0%, 0–2)	134/134 (100%, 97–100)	0/193 (0%, 0–2)	149/149 (100%, 98–100)	0/179 (0%, 0–2)	155/155 (100%, 98–100)
Month 1	159/183 (87%, 81–91)*	150/152 (99%, 95–100)*	188/210 (90%, 85–93)*	133/134 (99%, 96–100)*	166/193 (86%, 80–91)*	147/149 (99%, 95–100)*	161/178 (90%, 85–94)*	155/155 (100%, 98–100)*
Month 2	147/175 (84%, 78–89)	144/146 (99%, 95–100)	202/202 (100%, 98–100)*	128/128 (100%, 97–100)*	150/183 (82%, 76–87)	138/141 (98%, 94–100)	165/166 (99%, 97–100)*	141/141 (100%, 97–100)*
Month 3	132/172 (77%, 70–83)	140/143 (98%, 94–100)	192/194 (99%, 96–100)	126/126 (100%, 97–100)	179/179 (100%, 98–100)*	140/140 (100%, 97–100)*	163/163 (100%, 98–100)*	139/140 (99%, 96–100)*
Month 6	93/163 (57%, 49–65)	119/125 (95%, 90–98)	164/186 (88%, 83–92)	112/112 (100%, 97–100)	167/175 (95%, 91–98)	125/125 (100%, 97–100)	147/155 (95%, 90–98)	123/123 (100%, 97–100)
Strain 5/99 (neisserial adhesin A)								
Month 0	0/211 (0%, 0–2)	124/124 (100%, 97–100)	0/240 (0%, 0–2)	104/104 (100%, 97–100)	0/223 (0%, 0–2)	119/119 (100%, 97–100)	0/215 (0%, 0–2)	119/119 (100%, 97–100)
Month 1	201/210 (96%, 92–98)*	122/124 (98%, 94–100)*	229/240 (95%, 92–98)*	104/104 (100%, 97–100)*	213/223 (96%, 92–98)*	117/119 (98%, 94–100)*	203/214 (95%, 91–97)*	119/119 (100%, 97–100)*
Month 2	185/201 (92%, 87–95)	118/120 (98%, 94–100)	230/230 (100%, 98–100)*	100/100 (100%, 96–100)*	190/212 (90%, 85–93)	112/112 (100%, 97–100)	195/196 (99%, 97–100)*	112/112 (100%, 97–100)*
Month 3	169/198 (85%, 80–90)	115/118 (97%, 93–99)	221/222 (100%, 98–100)	98/98 (100%, 96–100)	209/211 (99%, 97–100)*	109/109 (100%, 97–100)*	193/193 (100%, 98–100)*	110/110 (100%, 97–100)*
Month 6	125/188 (66%, 59–73)	93/100 (93%, 86–97)	205/209 (98%, 95–99)	89/89 (100%, 96–100)	199/201 (99%, 96–100)	99/99 (100%, 96–100)	181/182 (99%, 97–100)	96/96 (100%, 96–100)
Strain NZ98/254 (NZ outer membrane vesicles)								
Month 0	0/212 (0%, 0–2)	123/124 (100%, 97–100)	0/227 (0%, 0–2)	117/117 (100%, 97–100)	0/219 (0%, 0–2)	123/123 (100%, 97–100)	0/224 (0%, 0–2)	109/109 (100%, 97–100)
Month 1	186/211 (88%, 83–92)*	123/123 (100%, 97–100)*	206/227 (91%, 86–94)*	117/117 (100%, 97–100)*	191/219 (87%, 82–91)*	123/123 (100%, 97–100)*	205/223 (92%, 88–95)*	109/109 (100%, 97–100)*
Month 2	146/201 (73%, 66–79)	119/119 (100%, 97–100)	218/219 (100%, 97–100)*	111/111 (100%, 97–100)*	156/211 (74%, 67–80)	111/112 (99%, 95–100)	206/207 (100%, 97–100)*	100/100 (100%, 96–100)*
Month 3	128/198 (65%, 58–71)	117/117 (100%, 97–100)	204/212 (96%, 93–98)	107/108 (99%, 95–100)	208/208 (100%, 98–100)*	111/111 (100%, 97–100)*	201/203 (99%, 96–100)*	97/98 (99%, 94–100)*
Month 6	114/190 (60%, 53–67)	96/97 (99%, 94–100)	175/203 (86%, 81–91)	95/95 (100%, 96–100)	186/197 (94%, 90–97)	102/102 (100%, 96–100)	183/191 (96%, 92–98)	86/86 (100%, 96–100)

Data are number (%, 95% CI). *Assessments done 1 month after a dose of 4CMenB.

Table 2: **Numbers of participants with a serum bactericidal activity using human complement (hSBA) titre of 4 or more against the three strains according to baseline hSBA titres of less than 4 or 4 or more**

Most adverse events were injection-site disorders reported within 7 days of study injection. Overall, reaction rates after 4CMenB vaccination were similar after each injection: 352 (93%) of 380 participants in the two doses 2 months apart group and 356 (95%) of 375 in the three doses 1 month apart group reported a reaction after the first dose, with slight decreases in rates to 306 (90%) of 341 in the two doses 2 months apart group and 312 (91%) of 342 in the three doses 1 month apart group after the second dose and 289 (87%) of 332 after the third dose. A similar decrease in reactogenicity was reported in participants who received three placebo injections (118 [92%] of 128 had adverse events at month 0, 104 [84%] of 124 at month 1, and 94 [78%] of 121 at month 2). For overall rates of solicited local and systemic reactions for any injection, 3330 doses of 4CMenB were associated with higher rates than with the 2739 placebo injections; most reactions were described as mild to moderate in severity (figure 3), resolving within a few days of each vaccination.

The most common local reaction was pain, reported after 2863 [86%] of 3330 of 4CMenB injections versus 1648 [60%] of 2739 after placebo injections, with 563 [17%] of 3330 cases described as severe after 4CMenB injections versus 105 [4%] of 2739 after placebo injections (p<0·0001). When severe pain occurred, the duration was short with onset on the day of vaccination and 535 [95%] of 563 cases in recipients of 4CMenB and 103 [98%] of 105 in recipients of placebo resolving within 3 days of vaccination.

Most common systemic reactions were malaise (1703 [51%] of 3330 4CMenB and 809 [30%] of 2739 placebo, p<0·0001) and headache (1412 [42%] of 3330 4CMenB and 741 [27%] of 2739 placebo, p<0·0001). Fever (≥38°C) was reported after 123 (4%) of 3329 4CMenB doses compared with 44 (2%) of 2738 after placebo injections (p<0·0001); only 20 (1%) of 2738 participants given 4CMenB and eight (<1%) of 2738 given placebo had a fever of 39°C or above (p=0·0689). Medical attention for fever (four [<1%] of 1480 4CMenB and two [<1%] of 1290 placebo) and use of antipyretics (61 [4%] of 1461 4CMenB and 22 [2%] of 689 placebo, p<0·0002) reflected these rates.

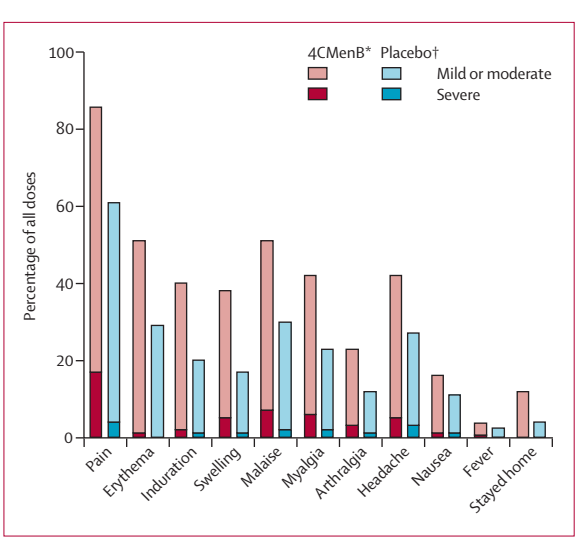

Figure 3: **Cumulative reactions for all doses of 4CMenB and placebo injections**
*Number of doses 3330. †Number of doses 2739.

Unsolicited adverse events were reported by 641 (43%) of 1503 recipients of 4CMenB and 57 (45%) of 128 recipients of placebo (p=0·679). Events judged by the investigator as possibly and probably related to study injection were reported by 240 (16%) of 1503 recipients of 4CMenB and 15 (12%) of 128 recipients of placebo (p=0·204). Two cases of juvenile arthritis, individually assessed as possibly and probably related to 4CMenB vaccination, were reported 170 days and 198 days, respectively, after the third of three doses of 4CMenB in the 0, 1, 2 months group. Although asymptomatic at study entry, the participant who reported juvenile arthritis 170 days after the third dose had symptoms of ankle pain and tendinitis before study entry. Other reported serious adverse events, all judged to be unrelated to study injection by the investigator, included four cases of appendicitis, and individual cases of shigella infection, drug-related toxic effects, pneumococcal meningitis, urticaria, and asthmatic crisis. Each of these events was reported 23–95 days after the latest study injection, and resolved within 3 days except for the case of meningitis, which lasted 8 days.

Discussion

In adolescents given 4CMenB vaccine, protective hSBA titres against three meningococcal serogroup B strains developed in over 90% of participants after one dose, increasing to 99–100% when two doses were administered at 1 month, 2 month, or 6 month intervals (panel). A third dose of 4CMenB provided no additional immunological benefit. Reactogenicity did not increase with subsequent doses of 4CMenB, rather it declined slightly, which together with the low drop-out rates show overall good tolerance. The preferred schedule seems to be two doses of 4CMenB, with the second dose administered between 1 month and 6 months after the first, allowing for flexibility in the use of the vaccine, such as in school-based programmes or for travellers.

Immunogenicity in this study was assessed by means of hSBA against three meningococcal serogroup B strains chosen to assess the individual contributions of the three vaccine components. These test strains are either mismatched to the vaccine antigens, as for the major outer membrane protein antigen PorA, or lack either genotypic or phenotypic expression of the vaccine component antigen, and so provide a means to assess the potential for the individual factor H binding protein, neisserial adhesin A, and outer membrane vesicle components to provide protection through bactericidal antibodies.[20] Studies are underway to identify a suitable meningococcal hSBA test strain for the fourth component, neisseria heparin binding antigen. In our study ELISA showed that this antigen induced formation of antibodies

in most participants, with a pattern of responses similar to that of hSBA against the three other antigens. Preliminary data for hSBA against a strain indicative of neisseria heparin binding antigen responses are consistent with the ELISA results (data not shown).

Our study was not designed to provide data on vaccine coverage of meningococcal B strains circulating worldwide, which will vary with presence and expression of the individual components in strains circulating in different regions. The vaccine antigens in 4CMenB induced immune responses in adults against 85% of an investigational panel of 124 serogroup B strains.[22] Studies are in progress to estimate age-specific, country-specific, and region-specific coverage by the represented strains, and first data suggest coverage in children of between 73–87% of recently circulating strains in five European countries.[23] Furthermore, as the vaccine antigens are not necessarily linked to specific serogroups, individual meningococcal surface proteins, such as factor H binding protein, could provide vaccine coverage for other serogroups, including A, C, W-135, Y, and even serogroup X, for which no vaccine currently exists.[24,25] Further clinical data to support such an exciting prospect are needed.[26]

At baseline, a high proportion of participants in each vaccine group had pre-existing hSBA titres against meningococcal test strains. This high baseline level could be due to nasopharyngeal carriage, which also suggests a high level of environmental exposure to meningococcal strains in Santiago and Valparaiso, where recent data show a predominance of serogroup B (61%) over serogroups C (22%) and W-135 (17%).[8] Importantly, protective hSBA titres were developed in 99–100% of vaccinated adolescents after two or three doses of 4CMenB, irrespective of their baseline status, although persistent differences in antibody concentrations occurred, but the clinical significance of these differences is unknown.

Tolerability outcomes were acceptable to the study participants, as shown by the comparative reactogenicity profiles of 4CMenB and placebo and the low rate of withdrawals. Reactogenicity was higher in recipients of 4CMenB vaccines than in recipients of placebo, with most local and systemic reactions mild or moderate in severity. The reaction most commonly reported as severe was pain at the injection site after injection with either 4CMenB or placebo and was more common in recipients of 4CMenB, but it was a transient local response. Importantly, rates of fever (≥38°C) were low and reports of fever 39°C or higher were rare. No evidence of increasing rates of reactions with subsequent doses of 4CMenB was identified. The overall reactogenicity profile of 4CMenB was generally consistent with clinical experience of NZ98/254 outer membrane vesicle vaccines, which effectively limited epidemic disease in New Zealand with an acceptable tolerability and overall safety profile in general use.[27]

Panel: Research in context

Systematic review

The immunodominant antigen of outer membrane vesicles, PorA, is strain specific and so is unsuitable as the basis of a broadly effective meningococcal B vaccine. The new recombinant multivalent meningococcal serogroup B vaccine (4CMenB) has multiple recombinant protein antigens together with outer membrane vesicles. Previous phase 2 studies have shown these recombinant proteins elicit robust immune responses, which are enhanced by inclusion of outer membrane vesicles in adults[19] and in infants when administered at 6 months and 12 months of age,[17] or at 2 months, 4 months, 6 months, and 12 months of age.[18] The primary target populations for meningococcal immunisation are infants and adolescents, and this study was done as the pivotal study in the adolescent population, as part of the licensure process for 4CMenB.

Interpretation

This pivotal study shows that two doses of the novel 4MenCB vaccine separated by 1, 2, or 6 months provide a potentially protective immune response in almost 100% of adolescents irrespective of previous antibody status. Actual levels of protection will depend on geographical variation of strains.

As in feasibility trials of the 4CMenB vaccine in adults[19] and infants,[17,18] we reported substantial immune effects against three antigen-specific meningococcal serogroup B strains and a generally favourable tolerability profile for 4CMenB vaccination in adolescents in Chile. On the basis of correlates of hSBA titres with clinical efficacy,[21] and the linkage provided by expression assays on circulating invasive strains of disease,[22] the results of our study suggest that two doses of 4CMenB given to healthy adolescents can impart substantial protection against meningococcal serogroup B disease. Further study is needed to provide information about the immunogenicity and tolerability of 4CMenB in various age groups, including infants, who bear the largest disease burden worldwide.[2]

Contributors

MES, MLO'R, DT, HW, RC, and PMD conceived and designed the study. MES, MLO'R, MTV, VP, RV, and AM undertook the study, managed by DT, GG, and PMD. Results were analysed by HW, interpreted by MES, MLO'R, PMD, and RC. All authors reviewed and revised the first draft report, written by MLO'R and MES with assistance from KV, and approved the final manuscript for submission.

Collaborators

The V72P10 Meningococcal B Adolescent Vaccine Study group included the following researchers who were involved in enrolment, vaccination, and follow-up of participants: C Arriagada, I Avendaño, M Bastías, C Bravo, C Bustamante, G Bustos, X Cerda, M Espinoza, C Fuentes, T Grez, M T Henriques, C Ibañez, G Izquierdo, J Krauss, M Manriquez, E Muñoz, M A O'Ryan-Soriano, C Osorio, M Rabello, M Reyes, M Salvatierra, S Schiaccaluga, D Silva, D Simian, P Ulloa, A Veliz, K Vera, A Vergara, R Villena, S Vorphal.

Conflicts of interest

DT, GG, HW, RC, and PMD are full-time employees of Novartis Vaccines and Diagnostics. All other authors received funding from Novartis Vaccines and Diagnostics for undertaking the study.

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References

1 Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine* 2009; **27** (suppl 3)**:** B51–63.

2 Tan LK, Carlone GM, Borrow R. Advances in the development of vaccines against *Neisseria meningitidis*. *N Engl J Med* 2010; **362:** 1511–20.

3 Stephens DS. Conquering the meningococcus. *FEMS Microbiol Rev* 2007; **31:** 3–14.

4 Jackson LA, Baxter R, Reisinger K, et al. Phase III comparison of an investigational quadrivalent meningococcal conjugate vaccine with the licensed meningococcal ACWY conjugate vaccine in adolescents. *Clin Infect Dis* 2009; **49:** e1–10.

5 Baxter R, Baine Y, Ensor K, Bianco V, Friedland LR, Miller JM. Immunogenicity and safety of an investigational quadrivalent meningococcal ACWY tetanus toxoid conjugate vaccine in healthy adolescents and young adults 10 to 25 years of age. *Pediatr Infect Dis J* 2011; **30:** e41–48.

6 Racloz VN, Luiz SL. The elusive meningococcal meningitis serogroup: a systematic review of serogroup B epidemiology. *BMC Infect Dis* 2010; **10:** 175.

7 European Union Invasive Bacterial Infections Surveillance Network. Invasive *Neisseria meningitidis* in Europe 2006. http://www.hpa-bioinformatics.org.uk/euibis/documents/2006_meningo.pdf (accessed Dec 9, 2011).

8 Ministerio de Salud, Gobierno de Chile. http://epi.minsal.cl/epi/html/bolets/reportes/Meningitis/meningitis.pdf (accessed Dec 9, 2011).

9 Wyle FA, Artenstein MS, Brandt BL, et al. Immunologic response of man to group B meninogococcal polysaccharide vaccines. *J Infect Dis* 1972; **126:** 514–21.

10 Finne J, Bitter-Suermann D, Goridis C, Finne U. An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. *J Immunol* 1987; **138:** 4402–07.

11 Nedelec J, Boucraut J, Garnier JM, Bernard D, Rougon G. Evidence for autoimmune antibodies directed against embryonic neural cell adhesion molecules (N-CAM) in patients with group B meningitis. *J Neuroimmunol* 1990; **29:** 49–56.

12 Sadarangani M, Pollard AJ. Serogroup B meningococcal vaccines—an unfinished story. *Lancet Infect Dis* 2010; **10:** 112–24.

13 Granoff DM. Review of meningococcal group B vaccines. *Clin Infect Dis* 2010; **50** (suppl 2)**:** S54–65.

14 Pizza M, Scarlato V, Masignami V, et al. Identification of vaccine candidates against ssrogroup B meningococcus by whole-genome sequencing. *Science* 2000; **287:** 1816–20.

15 Giuliani MM, Adu-Bobie J, Comanducci M, et al. A universal vaccine for serogroup B meningococcus. *PNAS* 2006; **103:** 10834–39.

16 Serruto D, Spadafina T, Ciuccui L, et al. *Neisseria meningitidis* GNA2132, a heparin-binding protein that induces protective immunity in humans. *PNAS* 2010; **107:** 3770–75.

17 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.

18 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.

19 Kimura A, Toneatto D, Kleinschmidt A, Wang H, Dull P. Immunogenicity and safety of a multicomponent meningococcal serogroup B vaccine and a quadrivalent meningococcal CRM_{197} conjugate vaccine against serogroups A, C, W-135, and Y in adults who are at increased risk for occupational exposure to meningococcal isolates. *Clin Vaccine Immunol* 2011; **18:** 483–86.

20 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.

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

22 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.

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 conference, Ljubljana, Slovenia; May 18–20, 2011. Abstract O 08.

24 Beernick 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.

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 Jiang HQ, Hoiseth SK, Harris SL, et al. Broad vaccine coverage predicted for a bivalent recombinant factor H binding protein based vaccine to prevent serogroup B meningococcal disease. *Vaccine* 2010; **28:** 6086–93.

27 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. *Hum Vacc* 2007; **3:** 196–204.