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REVIEW ARTICLE

A Multi-Component Meningococcal Serogroup B Vaccine (4CMenB): The Clinical Development Program

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Abstract Recently approved in Europe and Australia, the multi-component meningococcal B vaccine, 4CMenB (Bexsero®, Novartis Vaccines and Diagnostics), contains three surface-exposed recombinant proteins (fHbp, NadA, and NHBA) and New Zealand strain outer membrane vesicles (NZ OMV) with PorA 1.4 antigenicity. This comprehensive review of the 4CMenB clinical development program covers pivotal phase I/IIb/III studies in over 7,000 adults, adolescents, and infants. The immunological correlate for clinical protection used was human complement-mediated serum bactericidal activity titers ≥4 or 5 against indicator strains for individual antigens. Based on achievement of protective titers, a four-dose schedule (three primary doses and one booster dose) for infants and a two-dose schedule for adolescents provided the best results. Observed increases in injection site pain/tenderness and fever in infants, and injection site pain, malaise, and headache in adolescents compared with routine vaccines, were mostly mild to moderate; frequencies of rare events (Kawasaki disease, juvenile arthritis) were not significantly different from non-vaccinated individuals. 4CMenB is conservatively estimated to provide 66–91 % coverage against meningococcal serogroup B strains worldwide.

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1 Introduction

The introduction of safe and immunogenic glycoconjugate vaccines against invasive meningococcal disease (IMD) in Europe, Canada, Australia, and Brazil (*Neisseria meningitidis* serogroups ACWY and C) and in the meningitis belt in sub-Saharan Africa (serogroup A) has increased awareness of the need for disease prevention of the remaining prominent cause of IMD: serogroup B (MenB) [1]. Laboratory-confirmed cases of MenB disease resulting in substantial mortality have been documented throughout the world, most notably in countries where surveillance systems are robust and clinical outcomes are well documented [2–4]. Serogroup B is the predominant cause of IMD in most of Europe and Australia, especially where serogroup C vaccination is part of routine recommendations and in New Zealand due to a serogroup B epidemic. Similarly, in Canada, serogroups B, C, and Y represented the main causes of IMD after introduction of serogroup C conjugated vaccines by different provinces from 2001–2009; in 2010, serogroup B was responsible for 64 % of IMD, compared with 19 and 7 % for serogroups Y and C, respectively [4]. In the USA, where vaccination of infants is not practiced but where vaccination of adolescents against serogroups A, C, W, and Y is routinely recommended, serogroups B, C, and Y represent the main causes of IMD in approximately equal proportions [3]. In Latin America, overall, serogroup B has predominated over C, W, and Y, although the situation is highly variable—recent increases in serogroup C have seen it replace serogroup B as the predominant form in Brazil [5], and serogroup W has recently emerged as an important group in Argentina (34 % of all IMD in 2011) and Chile (55 % of IMD in 2012) [6]. The epidemiologic picture in Brazil may continue to change if, as expected, the recently

implemented infant MenC immunization campaign reduces the incidence of serogroup C disease. Serogroup dynamics will most probably continue to change during the future years worldwide.

Prevention of serogroup B-related IMD presents unique challenges. Glycoconjugate vaccines against serogroups A, C, W, and Y exploit the antigenicity of the capsular polysaccharides that characterize the serogroups, but poor immunogenicity of the MenB capsular polysaccharide [7] and antigenic diversity of the MenB surface proteins have significantly complicated vaccine development [8]. To provide broad coverage against the diversity of MenB strains, vaccine candidates require well conserved antigen variants and mixtures of multiple surface proteins, with the capacity to induce bactericidal antibodies against a majority of circulating strains [7].

Strong evidence that a protein-based meningococcal vaccine would provide protective efficacy originated from clinical experience with vaccines derived from meningococcal outer membrane vesicles (OMVs) to control regional outbreaks of specific MenB strains [9–11]. OMVs contain several different molecular moieties, but the porin protein, PorA, is the principle antigenic source of bactericidal antibodies. The limitations of these vaccines are well recognized; effectiveness tends to be limited to strains containing the same PorA protein (serosubtype-specific), especially in young children, limiting use to strain-specific outbreaks [11]. Worldwide, the vast majority of MenB disease is not related to one or even a limited set of disease-causing strains. Thus, advancements toward MenB IMD protection using OMV have been limited, as their effective coverage would not be sufficiently broad to justify development and implementation [1].

To confer broad protection against MenB, a vaccine has to account for high levels of antigenic diversity, variability in types and expression of antigens, and also anticipate the risk of escape mutants [12, 13]. To do so, constituent antigens should be highly conserved, important for virulence and/or survival of *N. meningitidis*, surface-expressed, and able to induce bactericidal antibodies.

In the late 1990s, reverse vaccinology applied a complete analysis of the genetic sequence of a MenB strain to systematically identify numerous surface-exposed proteins that were screened for their ability to induce bactericidal activity against MenB in animal models [14]. The most promising of these candidates were included in investigational vaccine formulations aimed at protecting against a broad spectrum of MenB strains circulating worldwide [13]. Further refinements, including fusion of certain vaccine antigens to stabilize protein conformation, were made in the final formulation [15].

This review focuses on the clinical development program of the Novartis Vaccines and Diagnostics meningococcal vaccine, 4CMenB, now licensed in Europe and Australia as Bexsero® (Fig. 1). The goal was to develop a MenB meningococcal vaccine proven to be safe and immunogenic in all age groups, particularly in infants, who represent the major group at risk of IMD, and to induce long-term immunity across heterologous subtypes to be effective against a majority of circulating strains [16]. 4CMenB contains the four main components, two of which are presented as fusion proteins with two other antigenic proteins identified in the screening process, GNA2091 and GNA1030: *Neisseria* adhesin A (NadA), factor H-binding protein (fHbp) with GNA2091, *Neisseria* heparin-binding antigen (NHBA) with GNA1030, and OMV from the New Zealand outbreak strain NZ98/254 (NZ OMV), which contains the PorA 1.4 protein. The rationale for choosing these components for inclusion in 4CMenB, details of the vaccine development, and the distribution of the vaccine antigens in meningococcal strains have been described in detail elsewhere [15].

We provide updated and consolidated information regarding the 4CMenB clinical program, reviewing results from the early-phase studies, summarizing details of the immunogenicity and safety results from pivotal studies completed in adolescents and infants, and potential coverage of this vaccine based on available global surveillance data. We also address the potential public health impact of this novel vaccine.

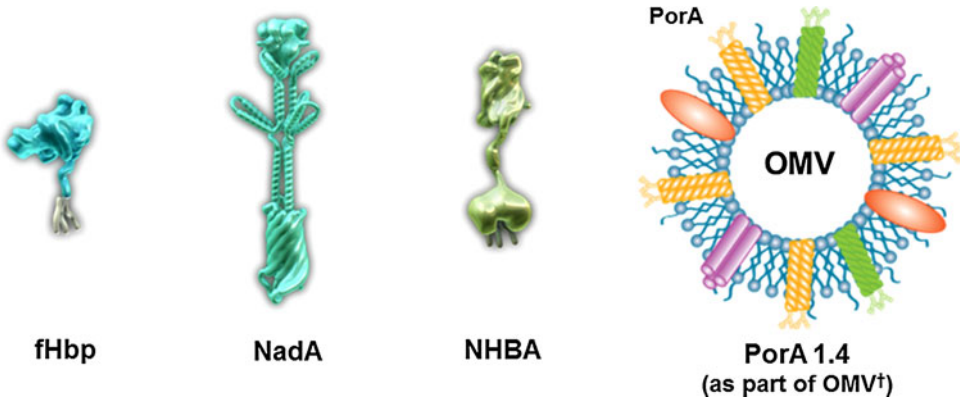

Fig. 1 Representation of the antigenic components of 4CMenB. 4CMenB contains three recombinant antigens, fHbp, NadA, and NHBA, combined with OMV from MenB strain NZ 98/254. *fHbp* factor H-binding protein, *NadA* Neisserial adhesin A, *NHBA* Neisseria heparin-binding antigen, *OMV* outer membrane vesicles, *PorA-OMV NZ* Porin A as part of the New Zealand strain OMV

2 Clinical Development Program

For relatively rare diseases such as IMD, with annual disease rates ranging from 0.5 to 5 per 100,000 individuals, efficacy studies are impractical because of the large sample sizes required. Instead, an immunologic correlate of protection using the serum bactericidal assay (SBA) has been accepted as an approach for vaccine licensure [17]. Correlates of protection using SBA with exogenous human complement (hSBA) were obtained approximately 40 years ago in studies performed in US Army recruits that provided data supporting a relationship between susceptibility to serogroup A, B, and C meningococcal disease and absence of bactericidal antibodies [17–19]. These data, combined with the observation of peak disease rates in age groups with the lowest detected hSBA antibody levels (6–24 months of age) support the concept that bactericidal antibodies are sufficient to confer protection against IMD; the level established as surrogate protective correlate is a serum dilution of 1:4 (or titer of 4) in the hSBA assay [17]. These observations have been demonstrated to be accurate in practice and are currently accepted to provide a path to licensure for all disease-causing serogroups, including serogroup B [20].

In the early phase I and II clinical studies of 4CMenB, functional antibody responses were assessed against 7–15 reference strains to estimate the potential efficacy of the protein-based vaccines [21–23]. Strains used in these studies were chosen based on genetic diversity, geographic and temporal distribution, and representation of key pathogenic clonal complexes (Table 1). In later phase III studies, indicator strains were selected, each of which is sensitive to antibodies against only one of the four vaccine antigens on its surface, in order to assess bactericidal killing directed against one particular vaccine antigen [21–23]. As an indicator strain representing bactericidal response directed primarily at the NHBA antigen was only available at the latter stages of the clinical program (strain M10713) [24], serum antibody responses to NHBA were measured by enzyme-linked immunosorbent assay (ELISA) against NHBA as a surrogate for hSBA in the earlier studies [21–23, 25].

SBA titer of 4 was initially used [17]. Subsequent phase III studies used the more conservative limit of $\geq$5, which ensures that within assay variability with a >95 % confidence that the result was at least 4.

3 Early Phase I/II Studies Aimed to Select the Best Vaccine Candidate

The first study of 4CMenB, reported by Toneatto et al. [23], was a randomized, single-center study conducted in Switzerland beginning in February 2006 in healthy adults aged 18–40 years (V72P5) to assess the immunogenicity and tolerability of the three selected recombinant protein antigens in the absence (rMenB) or presence of OMV from the New Zealand strain (NZ OMV, equivalent to the final 4CMenB formulation) or the Norwegian (NW OMV) outbreak strain. Participants were randomized to receive three doses of the respective formulations on a 0-, 1-, and 2-month schedule. Bactericidal responses were observed for participants in all three study arms against a diverse panel of reference strains. At least 75 % of subjects achieved hSBA titers $\geq$4 against 13 of 15, 10 of 15, and 7 of 15 strains after two doses of 4CMenB, rMenB + NW OMV, and rMenB, respectively. Both OMV-containing vaccines elicited immune responses against more meningococcal serogroup B strains than rMenB alone. The 4CMenB formulation also elicited a response to both OMVs tested, including the heterologous NW OMV, and was thus the formulation, along with rMenB, on which further development was focused.

A phase II study beginning in July 2007, reported by Kimura et al. [24], studied the immunogenicity and tolerability of three doses of 4CMenB and a single dose of the quadrivalent glycoconjugate against serogroups A, C, W, and Y (MenACWY-CRM; Menveo®, Novartis Vaccines and Diagnostics) in laboratory workers in Germany and Italy highly exposed to meningococcal isolates. Participants received three doses of 4CMenB on a 0-, 2-, and 6-month schedule followed by a single dose of MenACWY-CRM 1 month after the last 4CMenB (V72P4; NCT00560313). Sera were tested against the fHbp, NadA, and PorA 1.4 indicator strains with 91–100 % achieving hSBA titers $\geq$4 after the second 4CMenB dose, with similar immunogenicity (range 92–100 %) after the third 4CMenB dose. The response to the subsequent MenACWY-CRM was as expected, with 83–100 % achieving hSBA titers $\geq$8 against serogroups A, C, W, and Y. Both vaccines were well tolerated, although reactogenicity was higher with 4CMenB than with MenACWY-CRM, but no serious adverse events associated with either vaccine were observed. This study indicated that a two-dose schedule of 4CMenB is sufficient in adults to provide robust immune responses against the vaccine antigens, although this observation needed to be confirmed in subjects whose occupation does not offer the possibility of priming through pre-exposure to a range of meningococcal strains.

Two phase II studies were conducted in parallel in UK infants to evaluate inclusion of OMV, in different vaccination schedules, and concomitant administration with routine infant vaccines. Both studies included groups who received 4CMenB or rMenB.

The first study, reported by Findlow et al. [21], was an open-label, randomized, controlled trial beginning in

Table 1 Characteristics of MenB strains used to assess immunogenicity of 4CMenB

Strain	Phenotype	Similarity of antigens to 4CMenB		
		Homologous	Heterologous	Lack of antigen
NZ98/254	B:4:P1.7-2,4	PorA, NHBA	fHbp	NadA
44/76-SL	B:15:P1.7,16	fHbp	NHBA, PorA	NadA
5/99	B:2b:P1.5,2	–	fHbp, NadA, PorA, NHBA	–
M10713	B:NT:P1.17,16-3		fHbp, NHBA, PorA	NadA
M01 240101	B:NT:P1.19,15	–	fHbp, NHBA, PorA	NadA
M00 242922	B:4:P1.7-2,4	NHBA, PorA	fHbp	NadA
M01 240364	B:2aP1.5,2	–	fHbp, PorA, NHBA, NadA	–
M01 240355	B:1:P1.22,14	–	fHbp, PorA, NadA, NHBA	–
1000	B:NT:P1.5-1,10-4	–	fHbp, NHBA, PorA	NadA
2996	B:2bP1.5,2	NadA	fHbp, PorA, NHBA	–
95N477	B:2a:P1.2	–	fHbp, NHBA, PorA	NadA
CU385	B:4:P1.15	fHbp	NadA, NHBA, PorA	–
M01 240013	B:NT:P1.22,9	–	fHbp, NHBA, PorA	NadA
M01390	B:15:P1.7-2,4	NHBA, PorA	fHbp,	NadA
M03812	B:NT:P1.5-1,10-4	–	fHbp, NHBA, PorA	NadA
M04105	B:4,7:P1.7,4	NHBA, PorA	fHbp,	NadA
M04458	B:NT:P1.18-1,3	NadA	fHbp, NHBA, PorA	
M06190	B:2a:P1.5,2	–	fHbp, NHBA, PorA	NadA[a]
MC58	B:15:P1.17,16-2	fHbp	NadA, NHBA, PorA	–

fHbp factor H-binding protein, *NadA* Neisserial adhesin A, *NHBA* Neisseria heparin-binding antigen, *OMV* outer membrane vesicles, *PorA-OMV NZ* Porin A as part of the New Zealand strain OMV

[a] *nadA* gene in M6190 is present but interrupted by an insertion sequence element and is therefore, functionally NadA-negative

The four indicator strains used in most studies are indicated by italics

September 2006 in 147 healthy 2-month-old infants (V72P6; NCT00381615). Two groups of infants received either rMenB or 4CMenB at 2, 4, 6, and 12 months of age, while a third group of MenB vaccine-naïve toddlers received one dose of rMenB or 4CMenB at 12 months. In the cohort who received 4CMenB, 74–100 % achieved hSBA titers ≥4 against the fHbp, NadA, and PorA 1.4 indicator strains after the second dose and 85–95 % after the third dose. Responses in the rMenB cohort, with the exception of NadA, were lower both in terms of proportions achieving ≥4 and as geometric mean titers (GMT). Sera were also tested against a broader panel of MenB heterologous reference strains, which reinforced the immunologic benefit of including the OMV component. Percentages achieving hSBA titers ≥4 against these heterologous strains were lower than the indicator strains, but killing was evident across a broad panel of strains as early as 5 months of age. At 13 months of age, following a booster dose at 12 months, a majority (57–100 %) of infants receiving four doses of 4CMenB achieved hSBA titers ≥4 against six of seven strains, while the rMenB-only group elicited responses in over 20 % of subjects against three of seven strains (44/76-SL, 100 %; 5/99, 97 %; M01 240364, 70 %). Comparisons of titers after third and fourth doses, and in toddlers given their first dose of 4CMenB at 12 months of age, indicated that the increases in titers after the fourth dose of 4CMenB were anamnestic responses, suggesting this was a true booster response. At 5 months of age, following the second dose at 4 months, although percentages achieving hSBA titers ≥4 against reference strains were lower, killing was already evident across a broad panel of strains.

The persistence of these immune responses was examined in a small follow-up study (NCT01027351) at 40 months of age following vaccination of infants and toddlers at 2, 4, 6, and 12 months [25]. At 40 months of age, 41–76 % of the 17 children tested still maintained protective bactericidal antibody titers (titer ≥4) to each of the four vaccine components following 4CMenB (fHbp, 65 %; NadA, 76 %; NZ OMV, 41 %; NHBA, 67 %). When a booster dose of 4CMenB was administered, there was an anamnestic response to achieve protective titers against the four reference strains in 89–100 % of subjects 1 month later, showing that memory had been induced. Similarly high responses were observed against a panel of other strains, with 94–100 % achieving titers ≥4 against three strains, and 50 % against a fourth.

The second study, described by Snape et al. [22], was performed in 60 older infants who were vaccinated with two doses of either 4CMenB or rMenB beginning at 6–8 months of age with a third dose at 12 months of age (V72P9, NCT00433914), and their responses assessed against the same panel of seven strains as the V72P6 trial. Reflecting the results in younger infants, after two doses of 4CMenB most infants (67–100 %) achieved an hSBA ≥4 against six of the seven test strains, but this was only true for three of seven strains after rMenB (95–100 %). Persistence of the immune responses in this study were also investigated at 40 months of age [26], and were consistent with the previous study [25]; 36 % had titers ≥4 against fHbp, 100 % against NadA, 14 % against NZ OMV, and 79 % against NHBA after 4CMenB vaccination 2 years earlier. Persistence was lower in those vaccinated only with the experimental rMenB formulation. All subjects responded anamnestically to a booster dose of 4CMenB, and also demonstrated responses against four other non-reference strains as in the previous study.

In both of these infant phase II studies, the two vaccine formulations were generally well tolerated, with infrequent severe local or systemic reactogenicity, although with a modest increase observed in reactogenicity in the 4CMenB group [21, 22]. When present, fever was of short duration and no medically attended fever events occurred.

This phase I study in adults (V72P5) and early phase II studies in infants and toddlers (V72P6, V72P9) were the first indication that 4CMenB had the potential to confer a more robust, and/or broader range of protection against MenB strains compared with OMV-only or rMenB-only vaccines; including OMV enhanced the immune response over rMenB alone both in magnitude of the hSBA response and with a broader coverage of different strains, with protection evident across a wide range of ages, including infants. These data resulted in the 4CMenB formulation advancing into larger phase IIb/III trials.

4 Pivotal Phase IIb/III Studies

4.1 Immunogenicity—Infants

Further evaluation in infants was carried out in two large European studies (Table 2). The first (V72P12; NCT00721396) was a phase IIb, open-label, parallel-group, randomized, controlled trial conducted in Belgium, the UK, the Czech Republic, Germany, Italy, and Spain in 1,885 subjects, beginning in August 2008. This study investigated different schedules for three doses of 4CMenB in infants—at 2, 3, and 4 months, or 2, 4, and 6 months concomitantly with routine infant vaccines (DTaP-IPV-HBV/Hib [combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio, and *Haemophilus influenzae* type b vaccine] and PCV7 [7-valent pneumococcal glycoconjugate]) or in a schedule of 2, 4, and 6 months, with intercalated routines at 3, 5, and 7 months. As reported by Gossger et al. [27], immunogenicity against the fHbp, NadA, and NZ OMV test strains produced by vaccination at 2, 4, and 6 months was not substantially different from vaccination at 2, 3, and 4 months. The dosing schedule of 4CMenB (three doses given at 1- or 2-month intervals) and the addition of concomitant infant vaccines did not appear to affect the proportion of subjects who achieved these protective hSBA responses. At least 99 % of infants had hSBA titers of ≥5 against fHbp and NadA 1 month after the primary series completion, irrespective of the schedule. For NZ OMV, the proportion ranged from 79 to 86 % across the treatment groups. Furthermore, responses to routine vaccines were unaffected by concomitant 4CMenB administration, with only those to the pertussis pertactin component and pneumococcal serotype 6B not achieving non-inferiority due to small decreases in response of improbable clinical significance.

The second large infant study (V72P13; NCT00657709) was a phase III randomized controlled trial conducted in Finland, the Czech Republic, Austria, Germany, and Italy, involving 3,630 infants, beginning in March 2008 [28]. A primary aim of this study was the regulatory requirement of a lot-to-lot comparison of three consecutive manufacturing lots of 4CMenB, as well as comparison of routine infant vaccine responses when administered with or without concomitant 4CMenB. Safety was also a primary objective, studied in separate open-label and observer-blind cohorts. Importantly, this study included the full recommended 4CMenB schedule of three doses in the first year of life and a booster dose at 12 months of age. The investigators, Vesikari et al. [28], reported that 84–100 % of infants had hSBA titers ≥5 against the vaccine antigens (fHbp, NadA, NHBA, and NZ OMV) after the three-dose primary series at 7 months of age (Fig. 2a). Titers declined before the booster dose, especially for the PorA 1.4 of NZ OMV, but there was a robust booster response when 4CMenB was given concomitantly with the measles-mumps-rubella-varicella vaccine at 12 months of age, resulting in hSBA titers ≥5 against the four antigens in 95–100 % of recipients (Fig. 2a). A similar pattern consistent with a booster response was seen for the GMTs for each vaccine antigen (Fig. 2b). Concomitant antigen testing was also performed and, in contrast to the other smaller phase III study, non-inferiority for pertactin and pneumococcal serotype 6B was achieved.

Table 2 Vaccination schedules for pivotal trials

Reference, *ClinicalTrials.gov no.* Phase	Age at first vaccination	Participants, n (countries)	Vaccination schedule (all vaccinations)	Vaccination schedule summary (4CMenB groups only)
Vesikari et al. [28], (V72P13 / V72P13E1), *NCT00657709, NCT00847145*, phase III	2 months	3,630 infants in primary series; 1,555 toddlers in booster study (Finland, Czech Republic, Germany, Austria, Italy)	At 2, 4, and 6 months of age: 4CMen B (3 lots) + routine or routine alone (1:1:1:1 open-label immunogenicity subset); 4CMenB (3 lots) + routine or MenC (Menjugate) + routine (1:1:1:3 observer-blind) At age 12 months: subset of those who completed the primary 4CMenB series received 4CMenB booster ± MMRV (if no MMRV given with 4CMenB at 12 months, MMRV was given at 13 months)	3 doses of 4CMenB at 2-month intervals (primary) 1 dose 4CMenB booster at age 12 months
Gossger et al. [27], (V72P12), *NCT00721396*, phase IIb	2 months	1,885 infants (Belgium, UK, Czech Republic, Germany, Italy, Spain)	4CMenB at 2, 4, and 6 months with routine vaccines (concomitant); 4CMenB at 2, 4, and 6 months with routine vaccines at 3, 5, and 7 months (intercalated); 4CMenB at 2, 3, and 4 months with routine vaccines (accelerated)	3 doses of 4CMenB at 2-month intervals with routine vaccines (concomitant) 3 doses of 4CMenB at 2-month intervals with routine vaccines following months (intercalated) 3 doses of 4CMenB at 1-month intervals with routine vaccines (accelerated)
Santolaya et al. [29], (V72P10), *NCT00661713*, phase IIb/III	11–17 years	1,631 adolescents (Chile)	1, 2, or 3 doses of 4CMenB 1–6 months apart; All subjects received 3 injections, 4CMenB or placebo	1 dose of 4CMenB at study month 0 or 6 2 doses of 4CMenB at 0, 1 month 2 doses of 4CMenB at 0, 2 months 2 doses of 4CMenB at 0, 6 months 3 doses of 4CMenB at 0, 1, 2 months 3 doses of 4CMenB at 0, 1, 6 months 3 doses of 4CMenB at 0, 2, 6 months

MMRV measles, mumps, rubella vaccine

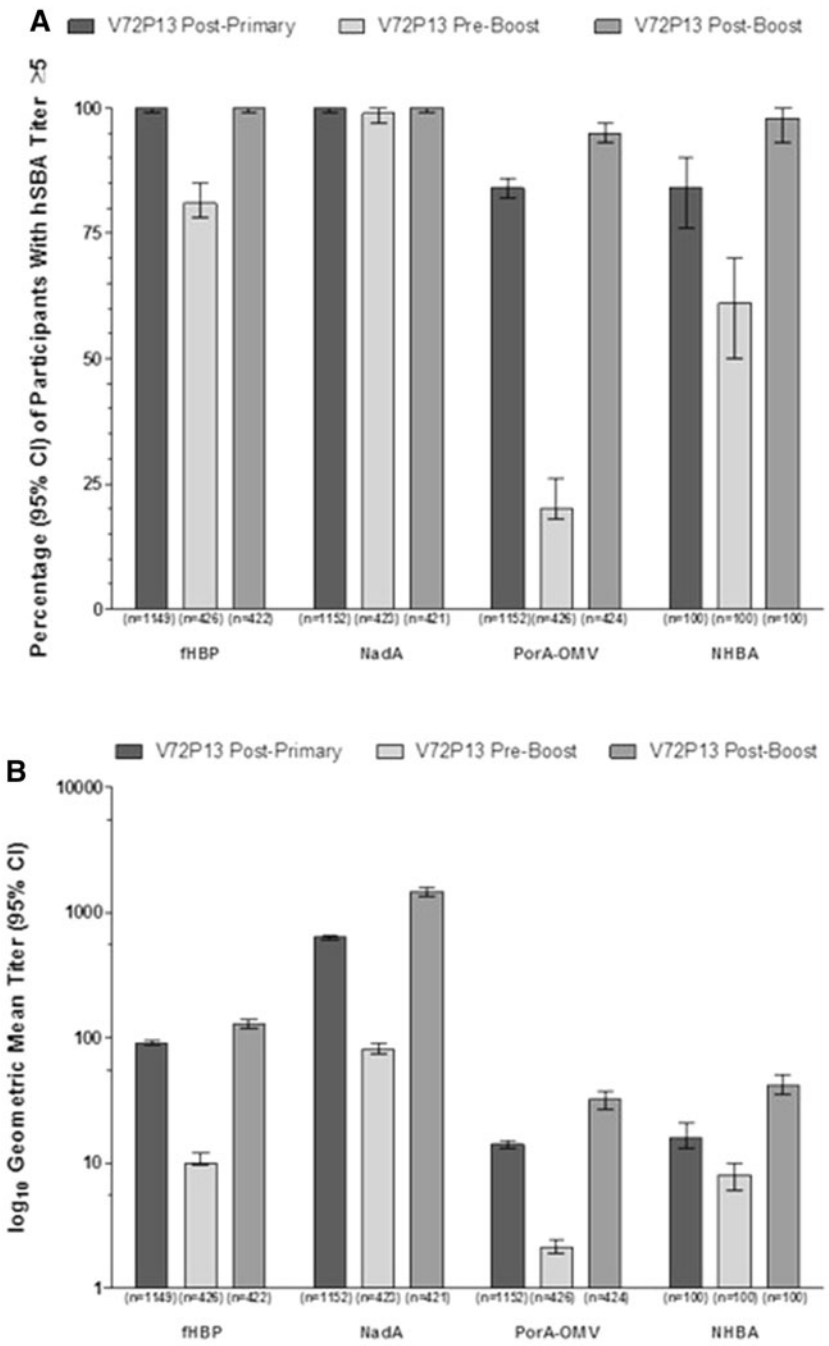

Fig. 2 Immunogenicity in response to four doses of 4CMenB in infants. **a** Percentages of participants with hSBA titer ≥5. **b** Geometric mean titers. *Error bars* represent 95 % confidence intervals. Target antigens are indicated on the *x* axis. Immunogenicity was assessed 1 month after receipt of the third of three doses at 2, 4, and 6 months of age co-administered with routine vaccines, at 12 months of age (pre-boost) before administration of the fourth (booster) dose co-administered with the measles-mumps-rubella vaccine, and 1 month (post-boost) after the booster vaccination. *fHbp* factor H-binding protein, *hSBA* human serum bactericidal assay, *NadA* Neisseria adhesin A, *NHBA* Neisseria heparin-binding antigen, *PorA-OMV NZ* porin A as part of the New Zealand strain outer membrane vesicles

In studies V72P12 and V72P13, the use of the licensed rotavirus vaccines (Rotarix™ and Rotateq™) was permitted but not required according to the study protocol. Although not prospectively studied, over 300 study participants throughout the two studies received at least one dose of rotavirus vaccine concomitantly with 4CMenB and routine vaccines. Given the differences in routes of administration (oral vs. intramuscular), and vaccine classification (live-attenuated vs. inactivated), it was not unexpected that, after three doses of 4CMenB in 200–202 of these subjects, there were no clinically relevant effects on the responses to fHbp, NadA, or NZ OMV; 99–100 % of all subjects achieved hSBA titers ≥5 against fHbp and NadA, irrespective of receipt of rotavirus vaccine, while 76–84 % of rotavirus recipients achieved this level against NZ OMV compared with 83–84 % of those who had no history of rotavirus vaccination.

4.2 Immunogenicity—Adolescents

In parallel with the phase IIb/III infant studies, a large study was performed in adolescents, who represent a

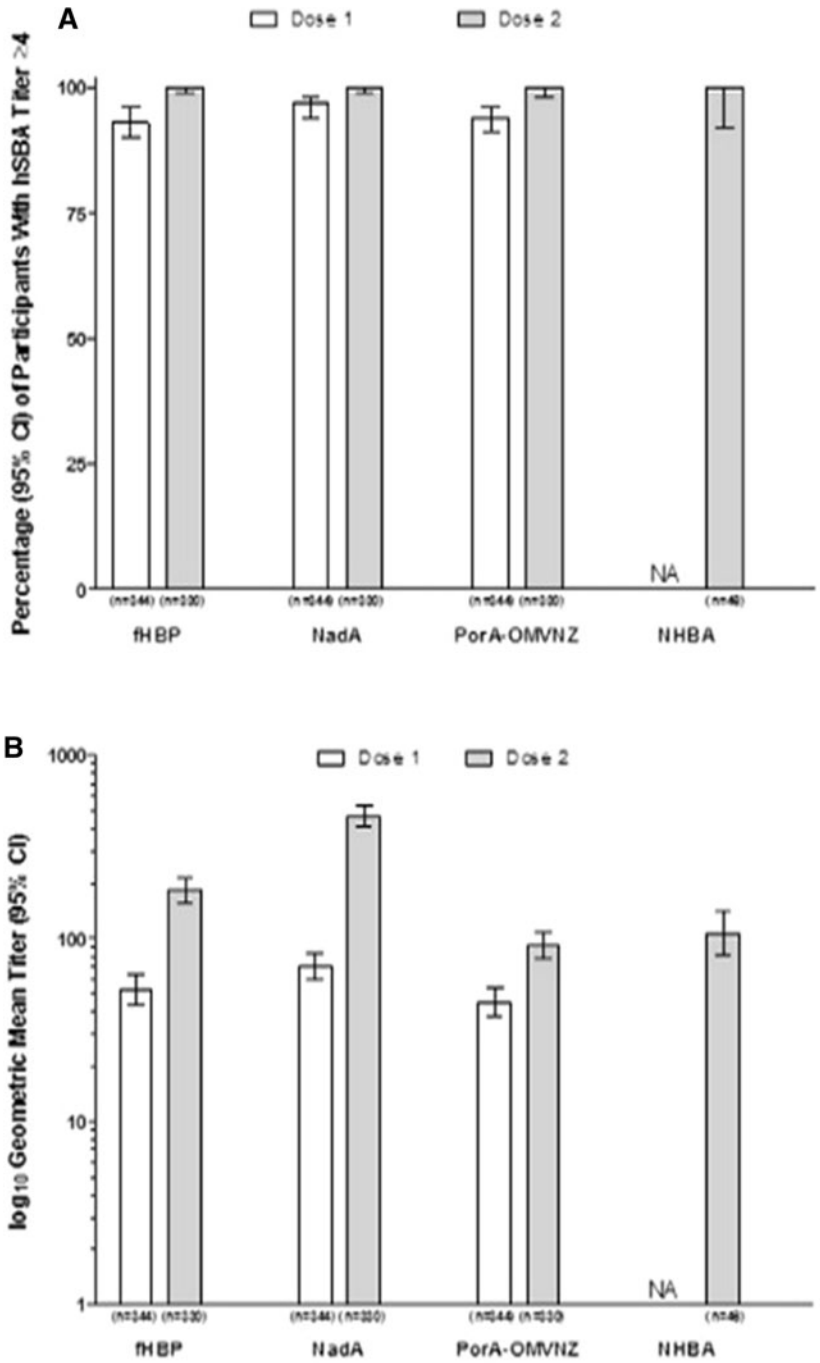

Fig. 3 Immunogenicity in response to two doses of 4CMenB given 1 month apart in adolescents. **a** Percentage of participants with hSBA titer ≥4 at 1 month after each of two doses of 4CMenB administered 1 month apart to adolescents aged 11–17 years. **b** Geometric mean titers. *Error bars* represent 95 % confidence intervals. Target antigens are indicated on the *x* axis. *fHbp* factor H-binding protein, *hSBA* human serum bactericidal assay, *NA* not available, *NadA* Neisseria adhesin A, *NHBA* Neisseria heparin-binding antigen, *PorA-OMV NZ* porin A as part of the New Zealand strain outer membrane vesicles

second major target group for meningococcal vaccination. The adolescent phase IIb/III study (V72P10; NCT00661713) enrolled 1,631 subjects in Chile from June 2008 to determine the number and schedule of doses necessary to establish immunogenicity, and the tolerability of those doses, as described by Santolaya et al. [29]. Subjects received three injections of either one, two, or three doses of 4CMenB or placebo, in a variety of schedules over 6 months (Table 2). At 1 month after one dose of 4CMenB, the proportion of adolescents with hSBA titers ≥4 ranged from 92 to 97 % for the three MenB indicator strains for PorA, fHbp, and NadA, increasing to 99–100 % after two doses given 1, 2, or 6 months apart (Fig. 3a).

At month 6 of this study (so 4 or 5 months after their last 4CMenB vaccination), 96–99 % of recipients given two doses of 4CMenB 1 or 2 months apart still had hSBA titers ≥4 against one or more vaccine antigens. In contrast, only 73–76 % of those given only one dose of 4CMenB had hSBA titers ≥4 against one or more vaccine components 6 months after vaccination. GMTs after three doses were

similar to those seen after two doses, showing that a third dose of 4CMenB provided no additional immunological benefit.

In this study, antibody titers against NHBA were initially measured by ELISA, and followed a pattern consistent with the GMTs of the other antigens investigated with reference to the increase from baseline and differences based on dosing regimen. When the M10713 was subsequently identified as an indicator strain against NHBA and subsets of sera were tested by hSBA, all subjects receiving two doses either 1 or 2 months apart achieved an hSBA titer ≥4 after the second vaccination. High pre-vaccination titers against M10713 were evident (80–96 % of subjects had hSBA titer ≥4 across groups), but robust responses were demonstrated by hSBA GMTs; pre-vaccination titers increased from 30–32 to 99–107 after the two-dose vaccination series (Fig. 3b). This study concluded that the preferred schedule in adolescents is two doses administered between 1 and 6 months apart.

In a continuation of this study (V72P10E1; NCT01148524), 666 subjects were enrolled to evaluate the persistence of these responses 18–24 months after the last vaccination with 4CMenB. Bactericidal titers ≥4 were still present in 77–94 % of recipients of two doses, a significantly higher level than after one dose, but not significantly less than in those who received three doses, apart from NZ OMV [30].

4.3 Safety—Infants

Common systemic reactions and adverse events (AEs) possibly related to 4CMenB vaccination for the infant studies are summarized in Table 3. Rates of solicited local and systemic AEs among infants who received 4CMenB concomitantly with routine vaccines in 2-, 4-, 6-month or 2-, 3-, 4-month schedules were similar, and were higher than those experienced by infants who received routine vaccines alone or routine vaccines concomitantly with MenC [27, 28]. In all these schedules, systemic and local reactions were transient and mostly of mild to moderate severity (Fig. 4). Rates of such reactions did not increase after the administration of subsequent doses of 4CMenB vaccine.

4.3.1 Local Reactions

All injection site reactions were more frequent with 4CMenB than with either of the routine vaccines (Fig. 4a), but most notably for tenderness, although erythema was also frequent. A higher proportion of 4CMenB vaccinations was associated with severe tenderness, but as with the other local reactions, these effects were transient and usually resolved within 24 h.

4.3.2 Systemic Reactions

When comparing separate injections of 4CMenB with routine vaccines in the intercalated schedule in study V72P12, 4CMenB was associated with higher rates of systemic reactions, particularly sleepiness and changes in eating habits (Fig. 4b). The most clinically relevant systemic reaction, fever ≥38 °C, was slightly more frequent with 4CMenB (38 %) than with routine vaccines alone (32 %) after the first dose at 2 months of age. These rates were lower than when the vaccines were administered concomitantly (Fig. 5) [27]. In the larger phase III study (V72P13), overall rates were higher than in V72P12, but the same pattern was observed—higher rates when 4CMenB was given with routine vaccines than routine vaccines alone, or in addition to MenC [28].

In the large phase III study (V72P13), rates of medically attended fever were low across all treatment groups, both after the primary series and following the booster dose (1–3 %) [28]. An analysis comparing medically attended fever rates in infants receiving their routine vaccines with either 4CMenB or MenC showed that rates of medically attended fever in both groups were similar in the open-label subsets (1.4 and 1.8 %, respectively). In contrast, in the observer-blind subset, rates of medically attended fever were higher in the 4CMenB + routine vaccines group than in the MenC + routine vaccines group (overall, 5.3 vs. 2.8 %) [28]. A possible explanation for this difference is that awareness of parents and investigators as to which vaccines the child had received in the open-label groups modified the management of fever in such settings. In infants receiving 4CMenB, the highest rates of fever occurred within 6 h post-vaccination and usually resolved after the second day.

Approximately 1 % of all infants experienced fever ≥40 °C after vaccination [21, 22, 27, 28]. In one study where a study arm was dedicated to specifically assess this question, prophylactic paracetamol administration was effective in reducing fever without affecting immunogenicity of either the 4CMenB or concomitant vaccines [31].

As previously noted, some infant vaccinees also received rotavirus vaccinations concomitant with 4CMenB and other recommended infant vaccines. Analysis of the 303 vaccinated subjects in the V72P12 and V72P13 studies revealed comparable reactogenicity profiles in those who did or did not receive rotavirus vaccine. A systemic reaction was reported in 80.5 % of infants after receiving any dose of 4CMenB co-administered with rotavirus and other routine vaccines, with 19.5 % having a severe systemic reaction. Comparable rates in the much larger numbers (>4,000) who did not receive rotavirus vaccine were 75.3 and 24.7 %, respectively. Rates of fever, particularly high fever, were also comparable when

Table 3 Summary of key safety findings for pivotal studies

Study, *ClinicalTrials.gov no.*	Age at first vaccination	Participants, n (countries)	Concomitant vaccines administered	General safety findings
Vesikari et al. [28], *NCT00657709*	2 months	3,630 infants in primary series; 1,555 toddlers enrolled in booster study (Finland, Czech Republic, Germany, Austria, Italy)	Participants received DTaP-HBV-IPV/Hib (Infanrix Hexa: GlaxoSmithKline) and PCV7 (Prevenar; Pfizer/Wyeth Pharmaceuticals). Some participant groups received MenC conjugate vaccine (Menjugate®, Novartis Vaccines and Diagnostics) as comparator. Rotavirus vaccine (Rotateq® and Rotarix® administered per local recommendations)	Rectal temperature ≥38.5 °C reported in 65 % of infant 4CMenB recipients within 6 h of vaccination vs. 32 % receiving routine vaccines alone. Fewer toddlers experienced temperature ≥38.0 °C (4CMenB, 32 %; 4CMenB/MMRV, 31 %) Overall, the most frequent injection site reaction was tenderness (87 % of 4CMenB recipients, 29 % of which was reported as severe [crying when limb was moved]). Tenderness was reported for 79 % of PCV7 recipients, 24 % of which was reported as severe Rare events possibly related to 4CMenB included: 2 cases of seizures associated with fever, 1 case of leg convulsions, 1 case of seizure of right arm, 4 cases of Kawasaki Disease, and 1 case of pyrexia
Gossger et al. [27], *NCT00721396*	2 months	1,885 infants (Belgium, UK, Czech Republic, Germany, Italy, Spain)	Participants received DTaP-HBV-IPV/Hib (Infanrix Hexa®: GlaxoSmithKline) and PCV7 (Prevnar®; Wyeth Pharmaceuticals). Rotavirus vaccine (Rotateq® and Rotarix® administered per local recommendations)	The most frequent solicited systemic reaction was irritability, with the highest frequency (11 %) reported in the concomitant group. Fever of ≥39·0°C after any vaccination was reported for 15–17 % of the concomitant and accelerated groups vs. 6 and 12 % in the routine and intercalated groups, respectively. The injection site reaction of severe local tenderness was reported for 12–16 % of 4CMenB recipients in the concomitant and accelerated groups. Rare events possibly related to 4CMenB included 6 cases of hospitalization for fever within 2 days of vaccination, 4 cases of seizures and 1 case each of the following events: aseptic meningitis, hypotonic hyporesponsive episode, Kawasaki disease, retinal dystrophy (believed to be congenital), transient hearing loss, and transient synovitis of the right hip
Santolaya et al. [29], *NCT00661713*	11–17 years	1,631 adolescents (Chile)	Concomitant vaccines were not administered as part of the study protocol	The most common systemic reactions were malaise (4CMenB, 51 %; placebo, 30 %) and headache (4CMenB, 42 %; placebo, 27 %) The most common local reaction was pain (4CMenB, 86 %; placebo, 60 %) Events judged as possibly and probably related to 4CMenB injections occurred in 16 % of subjects. Two cases of juvenile arthritis, assessed as possibly and probably related to 4CMenB, were reported 170 and 198 days after the third dose of 4CMenB

DTaP-HBV-IPV/Hib combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio, and *Haemophilus influenzae* type b vaccine; *PCV7* 7-valent pneumococcal glycoconjugate vaccine

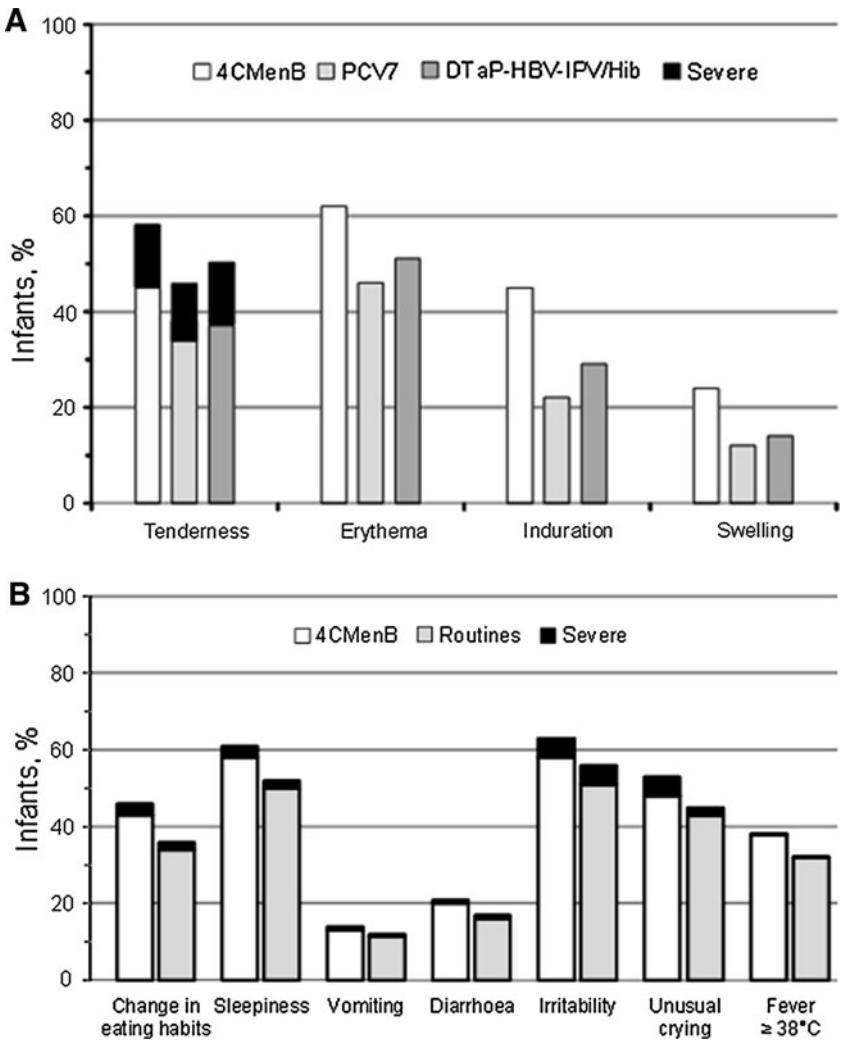

Fig. 4 4CMenB tolerability in infants. **a** Solicited local reactions after dose 1. Injection site data are provided for 624 infants given 4CMenB, PCV7, and DTaP-HBV-IPV/Hib concomitantly. Erythema, swelling, and induration were characterized as severe if local reaction was >50 mm. Tenderness was categorized as severe if subject cried when injected limb was moved. **b** Solicited general reactions after dose 1. Reactogenicity rates are given for infants who received 4CMenB and routine vaccines separately, 4CMenB at 2, 4, 6 months and routines at 3, 5, 7 months. Data are shown for the first dose of 4CMenB and routines (at 2 and 3 months, respectively). Reactions were categorized as severe if subject was unable to perform normal daily activities; fever was severe if temperature was ≥40 °C. Routine vaccines were DTaP-HBV-IPV/Hib (combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio, and *Haemophilus influenzae* type b vaccine) and PCV7 (7-valent pneumococcal glycoconjugate vaccine)

rotavirus vaccine was administered concomitantly. In study V72P12, only 3/135 (2.2 %) subjects who received concomitant rotavirus vaccine had fever ≥39.5 °C within 7 days after first vaccination, compared with 37/1,435 (2.6 %) who did not receive rotavirus vaccine. In study V72P13, 4/95 (4.2 %) and 107/2,384 (4.5 %) had fever ≥39.5 °C with concomitant and no concomitant rotavirus vaccine, respectively. Of note, in the latter study, fever was mostly assessed via the rectal route versus axillary route in the former study.

In summary, although these analyses were not prospectively planned, and limited numbers of subjects receiving rotavirus vaccination are available, they are reassuring that reactogenicity was not impacted in the 4CMenB phase III infant studies by concomitant administration of rotavirus vaccination, supporting its concomitant use in routine practice.

Overall, the safety and tolerability profiles for 4CMenB were similar to OMV vaccines, particularly relevant as the NZ OMV strain was used as MeNZB™ in New Zealand, where local and systemic AEs were common but generally mild to moderate in severity and did not preclude widespread use of the vaccine [32].

4.4 Safety—Adolescents

The low rate of withdrawals from the V72P10 study and the similarity of the reactogenicity profiles between 4CMenB and placebo indicate that 4CMenB was generally well tolerated in adolescents [29]. Although 4CMenB vaccinations were associated with higher reactogenicity than placebo injections, local and systemic reactions were mostly of mild or moderate severity. Transient pain at the injection site was the most commonly reported severe reaction among 4CMenB or placebo recipients. Fever ≥38 °C was uncommon (4 and 2 % for 4CMenB and placebo, respectively) and fever ≥39 °C was rare (1 and <1 % for 4CMenB and placebo, respectively). Common systemic reactions and events possibly related to 4CMenB vaccinations are summarized in Table 3.

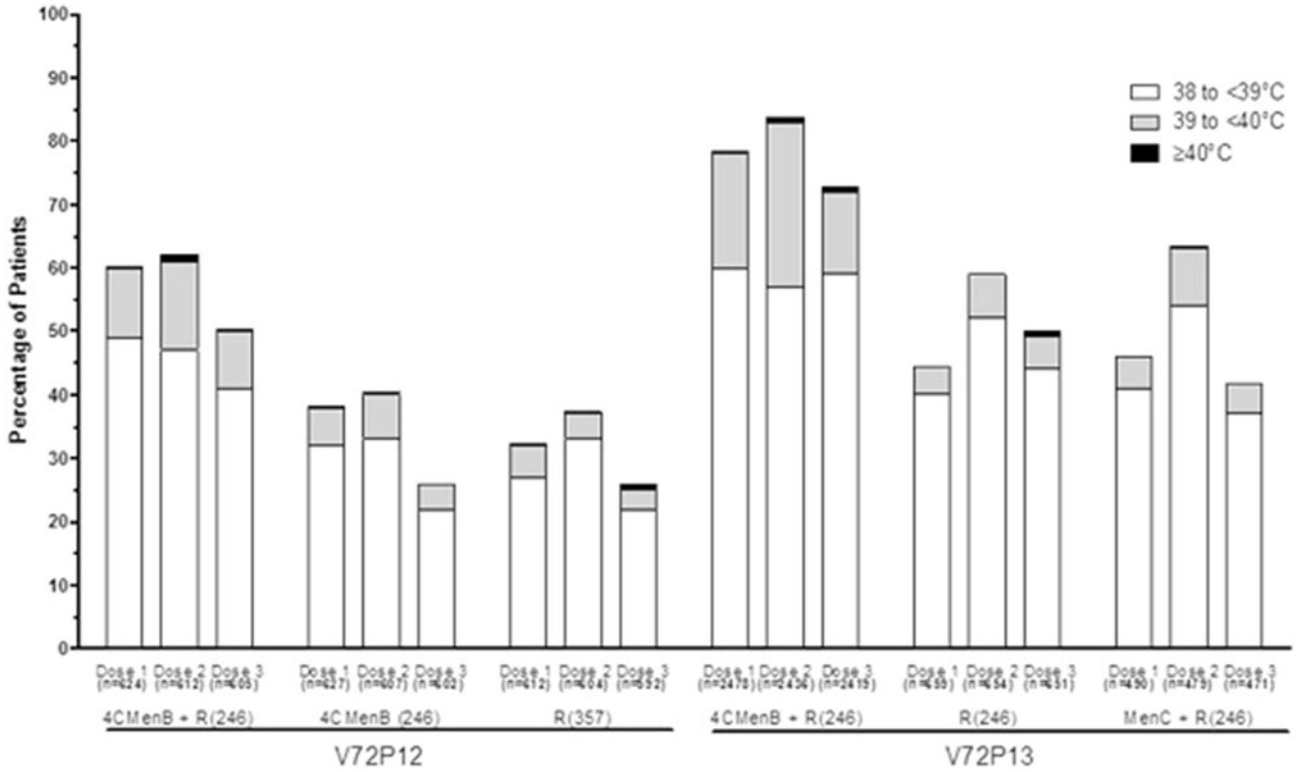

Fig. 5 Fever rates for 4CMenB with concomitant routine vaccines vs. routine vaccines alone by dose in infants. Fever rates are given for infants who received 4CMenB, routine vaccines, or routines + MenC. *Numbers in parentheses* indicate the months in which the infant received the vaccine. For study V72P12, axillary temperatures were measured in 63–64 % of subjects and rectal temperatures in 35–37 % of subjects. For study V72P13, fever was measured rectally in almost all subjects. *R* routine vaccines, namely DTaP-HBV-IPV/Hib (combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio, and *Haemophilus influenzae* type b vaccine) and PCV7 (7-valent pneumococcal glycoconjugate vaccine)

4.4.1 Rare Adverse Events

Additional analyses integrating all phase III studies in infants, children, adolescents, and adults have been performed. Although comparable in size to many pre-licensure vaccine clinical development programs, the sample size for 4CMenB exposures remains insufficient to give a definitive understanding of causal associations with rare AEs. A list of the serious AEs (SAEs) recorded in the clinical trials of 4CMenB assessed as at least possibly related to study vaccine, are summarized in Table 3. Several types of AEs have been observed in the clinical studies that have received more attention.

Due to increased rates of fever when 4CMenB was administered with routine vaccines, evidence of seizures in young children has been closely assessed. During the primary infant series (over 20,000 vaccination visits across all groups), febrile seizures on the day of or day following vaccination were rare, with a rate of 0.1 events/1,000 vaccination visits in the 4CMenB study arms. No events were observed in infants in the control study arms. Among toddlers aged 12–24 months, across over 11,000 vaccination visits with the same window of safety follow-up, febrile seizures for those receiving 4CMenB (with or without routine vaccines) were similarly rare (0.4 events/1,000 visits [95 % CI 0.05–1.46]) versus those receiving routine vaccines alone (0.3 event/1,000 visits [95 % CI 0.04–1.05]).

Seven suspected cases of Kawasaki Disease were reported across all 4CMenB studies, six of which occurred in subjects after receiving a 4CMenB-containing vaccine regimen. All cases were adjudicated by a panel of outside experts. Several cases were clustered in time and location in the clinical studies. The onset varied from 1 day to 5.5 months after vaccination; three occurred within 1 month and three occurred beyond 1 month post-4CMenB vaccination. Due to the nature of the study designs, the safety follow-up was approximately four-fold higher for 4CMenB recipients (6,917 vs. 1,794 person-years for 4CMenB and routine vaccines, respectively). Excluding the case with onset on the day of vaccination, the estimated annual incidence post-vaccination is 72 (95 % CI 23–169) per 100,000 subject-years after 4CMenB versus 56 (95 % CI 1–311) after control vaccines. These rates are similar to

rates of other rare AEs observed in other pre-licensure clinical programs. With the given clinical study database sample size, a direct causal link to the study vaccine cannot be excluded for seizures or for Kawasaki disease, and post-licensure safety surveillance will be important.

5 Novel Methodology for Estimating Vaccine Coverage against Multiple MenB Strains Circulating in Different Regions and Time Periods

For several traditional bacterial vaccines that target fixed and highly conserved antigenic epitopes, antibody titers correlate with protection against the majority of circulating strains. For MenB, antigenic variability and level of surface expression of outer membrane proteins presents a challenge for determining vaccine coverage against the myriad of circulating strains. The use of hSBA (as an immunologic correlate) enables detection of functional antibodies in serum, and, by extension, clinical protection. However, a limitation of hSBA is that it does not define how killing is mediated (i.e., which specific components the bactericidal activity is directed against). Nor can hSBA detect whether antibody-mediated killing against different antigens expressed on the bacterial surface can exert synergistic effects, which may influence vaccine efficacy [33]. Effectiveness of a protein-based vaccine against MenB will therefore depend on the presence and quantity of target antigens on the circulating strain. Any method predicting vaccine effectiveness must account for the genetic diversity of MenB strains, which, through gene regulation, phase variation, and sequence diversity, can affect the levels of expression of these surface antigens [33].

The meningococcal antigen typing system (MATS) uses polyclonal antibodies against fHbp, NadA, and NHBA in an ELISA to quantify the expression and amounts of these individual antigens, which cross-react with the corresponding antigen used in the vaccine. The PorA component is determined using PCR, as this is universally expressed and is only dependent upon the presence of the PorA 1.4 gene [33]. 4CMenB is expected to provide protection against strains that meet a minimum threshold of reactivity in the MATS ELISA and/or contain the PorA 1.4 antigen. MATS coverage estimates were developed using pooled sera from infants who received a three-dose series of 4CMenB with an additional dose at 12 months. MATS provides an estimate of vaccine coverage against a panel of strain isolates from a given region. In a large panel of invasive MenB isolates from across Europe, MATS determined that 78 % of MenB strains would be covered by vaccination with 4CMenB [34]. Predicted strain coverage rates in Europe using MATS are shown by country in Fig. 6, with additional data for non-EU countries.

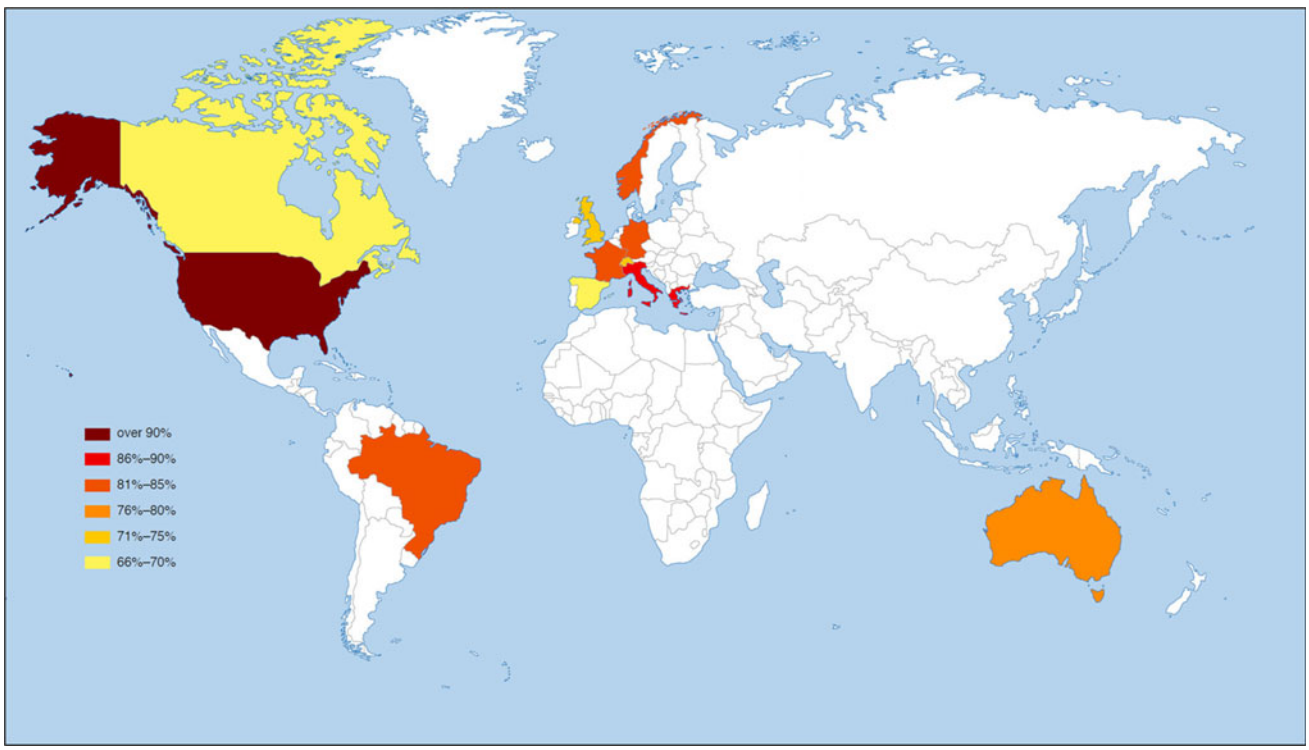

Fig. 6 Predicted capsular group B strain coverage of 4CMenB globally. The *color code* indicates the level of predicted coverage (no color indicates no MATS data available). *MATS* meningococcal antigen typing system

Coverage estimates provided by MATS may be an underestimation since MATS does not account for the activity of antibodies from non-PorA OMV antigens, or for synergistic effects among the multiple vaccine components [33]. Furthermore, in adults and adolescents, MATS is considered to provide a conservative estimate of strain coverage because the breadth of bactericidal activity is expected to be higher than predicted by MATS, due to greater levels of cross-reactivity observed at these older ages [33]. Some infant studies have shown hSBA activity against non-reference strains [25, 26], but the small amounts of sera available from infants precludes analyses of infant coverage against panels of strains, except through pooling of sera as used in the MATS analyses. MATS-based predictions were recently validated in an analysis of a representative MenB strain panel from England and Wales, which showed that whereas MATS predicted a coverage of 70 % (95 % CI 55–85), the actual coverage based on killing by pooled infant and adolescent immune sera in hSBA was observed to be 88 % (95 % CI 72–95) [35]. However, the method is dependent upon pooled sera, and thus misses an assessment of individual seroresponse levels. Indications of individual or proportional protection of a population comes from the observed responses in clinical trials of individuals, which show that almost 100 % of individual infants and adolescents produced hSBA titers $\geq$4 or 5 against three or more of the vaccine antigens, noting that the use of hSBA titers $\geq$4 or 5 as indicators of protective responses is itself a surrogate for protection [17–19]. However, data from the UK following the success of the polysaccharide-protein conjugate vaccines against meningococcal serogroup C, and the use of OMV serogroup B vaccines against outbreaks in Cuba, Brazil, and Norway, appear to confirm the utility of this surrogate.

MATS is a powerful tool that will allow the assessment of the likely effectiveness against individual outbreak strains or, where adequate surveillance is in place, strain coverage predictions for MenB endemic disease in a given country or geographic region. Information collected using MATS will be useful for ongoing global surveillance of pathogenic bacterial isolates through established reference laboratories via a public–private partnership [36]. MATS can provide ongoing data on temporal and geographic changes in MenB epidemiology, and potential development of escape mutant strains as a result of selective pressure from vaccination. Additional assessments are ongoing but it may be that MATS may also be applied to assess 4CMenB coverage rates against other (non-B) meningococcal serogroups. Lastly, MATS provides data that could assist in cost-effectiveness or cost/benefit analyses of 4CMenB if implemented in national immunization programs.

6 Future Developments

The 4CMenB clinical development program has established an acceptable safety and tolerability profile for the vaccine across all age groups. The post-vaccination increase in fever will require education of parents/caretakers on appropriate management. Nevertheless, as with all newly approved vaccines, additional long-term studies are needed to demonstrate a more comprehensive picture of the vaccine's safety profile. Post-licensure studies of 4CMenB will monitor long-term safety events across a large cohort of vaccine recipients. The full spectrum of clinical protection provided by 4CMenB against meningitis, invasive disease, and fulminant septicemia will be determined by well designed surveillance programs in place in countries before, during, and after introduction in national vaccine programs. Moreover, as new vaccines are developed, particularly for infants and young children, the safety and efficacy of 4CMenB used concomitantly in multi-vaccine regimens will require evaluation. Future efficacy considerations will involve monitoring the emergence of regional strain variants, since the selective pressure of 4CMenB could potentially drive the emergence of escape mutants and may reduce vaccine efficacy. MATS is expected to be an efficient tool for monitoring MenB strain variations. Currently, strain panels specific for defined geographical regions are being developed and are expected to hasten the prediction of strain coverage in these regions [35].

Herd immunity has been demonstrated to be an important factor in the success of the polysaccharide-protein conjugate vaccines against meningococcal serogroup C, as well as other bacterial causes of infant meningitis and septicemia (*Haemophilus influenza*e type b, *Streptococcus pneumoniae*). This will only become apparent when active surveillance programs in place (once the vaccine is widely used in specific populations) provide effectiveness data. A preliminary report of a recent study on the impact of 4CMenB and a quadrivalent MenACWY vaccine on nasopharyngeal carriage in university students in England (clinicaltrials.gov NCT01214850) demonstrated proof of principle of an effect on serogroup B carriage [37], but the results have yet to be fully reported and further evaluated.

Other protein-based MenB vaccines are in development using different approaches compared with 4CMenB [38]. The most advanced of these is the bivalent rLP2806 vaccine being developed by Pfizer, which has been through phase II and is currently being evaluated in phase III trials [39]. This vaccine is based on lipidated forms of two immunologically distinct sub-families (A and B) of the fHbp protein, which is expressed in the vast majority of MenB strains. For comparison, 4CMenB includes an fHbp fusion protein from the B sub-family. Phase I and phase II

data indicate that the rLP2806 vaccine elicits a strong immune responses in adolescents, 75–100 % achieving hSBA titers ≥4 against a panel of clinical isolates [39]. As of this writing, the trials in which the vaccine is being studied include subjects down to the age of 10 years, with no indication yet of age de-escalation to infants and toddlers, so it remains to be seen whether the vaccine will be appropriate for these important age groups.

In summary, 4CMenB is a multi-component vaccine containing three broadly conserved surface-expressed recombinant antigens and a specific OMV, thereby providing broad protection against circulating heterologous strains of MenB. The 4CMenB formulation has proven to be immunogenic in adults, adolescents, and young infants with a well characterized tolerability profile in clinical trials performed in the most susceptible age groups. Incremental increases in reactogenicity (including fever) compared with routine childhood vaccinations when 4CMenB is added to the routine schedule have been observed. Concomitant administration with routine vaccines (DTaP-HBV-IPV/Hib, PCV7, MMRV, and rotavirus vaccines) does not appear to interfere with the immunogenicity of any of the vaccines, but the use with more recently introduced vaccines, e.g., PCV13, or other routine vaccines such as MenC conjugates will need to be investigated. The pyrogenic profile observed after vaccination with 4CMenB in infants and toddlers tends to be of short duration, does not increase with successive doses, and few vaccinees experienced temperatures ≥40 °C. As with all new vaccines, post-marketing surveillance will be conducted to monitor for any other safety signals.

Estimating strain coverage of 4CMenB is crucial for public healthcare policy; MATS will provide valuable information for policy makers and healthcare workers and inform decisions relating to vaccination programs against MenB at a population level, which has important implications in shaping public health policy regarding IMD caused by MenB. For the first time, a vaccine has the potential to limit the devastating effects of IMD caused by heterologous serogroup B strains.

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