CLINICAL INVESTIGATION

The early clinical development of a multicomponent vaccine against meningococcal serogroup B

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The development of meningococcal serogroup B vaccines has been a worldwide public health priority based on continuing disease burden, combined with the scientific challenges associated with antigen identification. A new multicomponent vaccine, 4CMenB, is currently being evaluated for global licensure. The multicomponent strategy accounts for multiple surface antigens and, therefore, provides varied opportunities to induce bactericidal antibodies. 4CMenB contains four components: outer membrane vesicles from the New Zealand MenB outbreak strain, fHbp, NadA and NHBA. In early clinical studies protective antibody levels with acceptable tolerability outcomes were observed in persons who received 4CMenB starting at as young as 2 months of age. A meningococcal antigen-typing system has been developed to bridge clinical trial data with circulating strains. This article describes the early clinical development program and the rationale for Phase III study design and effectiveness evaluations.

Keywords: fHbp • MenB vaccine • meningococcal disease • meningococcal serogroup B • NadA • NHBA • PorA

The development of vaccines against *Neisseria meningitidis* serogroup B (MenB) has long been viewed by experts as a global public health priority, based on continuing disease burden combined with the scientific challenges associated with antigen identification [1–3]. Invasive MenB disproportionately affects infants in the developed world and has been associated with extended epidemics and outbreaks. MenB is the leading cause of meningococcal meningitis and sepsis in European infants, and attack rates in the UK have long been comparable to those that led to universal vaccination against meningococcal serogroup C (Figure 1) [4–12]. With the recent introduction of a low-cost serogroup A conjugate vaccine (MenAfriVac®) for use in Africa [13,14], experts note that MenB remains the last major hurdle to controlling invasive meningococcal disease (IMD) globally [2,3]. A multicomponent MenB vaccine, 4CMenB, has been submitted for consideration by licensing bodies [1,15–17]. We consider a recent body of literature on the topic of MenB vaccines, including recent review work, to provide a broader context and discussion about the early development program and the design of the late-stage clinical trials of 4CMenB, including plans for bridging clinical trial data against current regional epidemiology [1,15–21].

Rationale for vaccination against MenB

Several factors inform the rationale for establishing immunization programs against MenB disease, including symptoms, epidemiology, persistent case fatality rates and other undesirable outcomes [1,18,22,23]. IMD generally presents as rapidly progressive meningitis and/or septicemia, although small numbers of meningococcal pneumonia cases and other rare syndromes are reported in the literature

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Figure 1. Regions and countries with reports of serogroup B meningococcal disease.

[7,22,24]. Initial symptoms are indistinguishable from mild respiratory illnesses and progression from nonspecific signs to permanent disability or death is generally rapid and difficult to control once sepsis or shock develop. The advent of antibiotic therapies reduced the case fatality rate from IMD to approximately 10% overall, although the rates in septicemia patients (which can exceed 30%), or in the developing world or during epidemics, are often higher. The case fatality rate has remained relatively stable despite advances in therapeutic options [6,25] and the risks of death and disability in the developing world are higher relative to those in the developed world [26]. Delays in differential diagnosis can contribute to negative outcomes in some patients, particularly if culture confirmation is required to refine treatment choices [23,27]. Most people who develop IMD are otherwise healthy and have no commonly recognized risk factors, which contributes to public anxiety and bolsters epidemiologic and clinical rationales for preventive measures, such as vaccination [27].

While chemoprophylaxis of close contacts of IMD cases can be effective in limiting outbreaks [27,28,201], vaccination is the primary prevention strategy [7,23,29]. Conjugate vaccines against serogroups A, C, W-135 and Y have robust safety and immunogenicity profiles in all age groups and have been included in routine immunization programs in many countries for infants, children, adolescents and those at risk of developing meningococcal disease [30–33], In North America, adolescents, a primary reservoir for asymptomatic meningococcal carriage, commonly receive quadrivalent conjugate vaccine, which is also increasingly recommended for Hajj and Umrah [9,18,34]. Investigations into universal or broad-coverage MenB vaccines have been hampered by the poor immunogenicity of the capsular polysaccharide [2,7]. Three licensed wild-type (wt) outer membrane vesicle (OMV) vaccines were tailor-made to address specific pathogenic strains, and have successfully limited clonal outbreaks and epidemics [1,3,35–38]. Cuba has the only routine recommendation for serogroup B IMD immunization [7,18,38], using a tailor-made wtOMV vaccine that protects against strains bearing a specific PorA serosubtype [7,36,37]. However, the epidemiology and incidence of sporadic disease and the

possibility of new epidemics spurred the development of vaccines to address MenB more comprehensively [1,15,16,35].

MenB vaccines

The poor immunogenicity of the MenB capsular polysaccharide results because it is identical to the polysialic acid in the fetal neural cell-adhesion molecule [19,35–37]. Although this has raised concerns about the possibility that autoimmune responses could be induced by MenB conjugate vaccines, no immunogenic formulation has been developed to confirm or disprove this hypothesis [1,2,7,19,39]. All ongoing clinical vaccine development includes subcapsular antigens that exhibit considerable antigenic diversity and, therefore, pose significant challenges for vaccine development [1–3,7,39–41], including concerns about poor efficacy and the development of escape variants or mutants [41]

The three licensed wtOMV vaccines (VA-MENGOC-BC®, MenBVac® and MeNZB®) employ detergent-extracted OMV or culture-released blebs [1,36]. In contrast, recombinant OMVs are produced through genetic modification. To maintain the surface expression of proteins in a stable membrane, lipopolysaccharide (LPS), a known reactogen, is necessary to ensure the integrity of the OMV phospholipid bilayer. Various factors, such as detergent or aluminum adjuvant use, are known to limit LPS reactogenicity in vaccines. Furthermore, membrane-bound LPS is much less reactogenic than the free form [36,42].

All OMVs contain various proteins that can theoretically contribute to immune responses. Such proteins include PorA, PorB, RmpM and OpcA invasin (Figure 2), each of which has been suggested as a possible vaccine antigen candidate [1,15,16,35,43]. Iron-regulated proteins may also be evident on wtOMVs. Based on the results of clinical studies, PorA is considered to be the immunodominant constituent of OMV in infants, who are the target population for MenB disease [36,37,44–46]. Nevertheless, it has been suggested that additional proteins, such as PorB may also elicit antibody formation [46].

■ Licensed wtOMV vaccines

The wtOMV vaccines were developed to limit long-term clonal MenB epidemics in Cuba, Norway and New Zealand (Table 1). MeNZB, which was implemented in New Zealand, was associated with marked reductions in IMD incidence during a serosubtype B:4:P1.7b,4 ST-41/44 (strain NZ 98/254) outbreak that reached an incidence of 17.4/100,000 of the population overall and more than 200/100,000 among some indigenous populations [47]. Initial vaccine effectiveness was 70–80% [48] and declined over time [49,50]. VA-MENGOC-BC and MenBVac have been used to prevent or control epidemic MenB IMD in Brazil, Chile and France caused by epidemic strains carrying PorA serosubtypes matched to the vaccines [36,50–53]. In infants, clinical data show that wtOMV vaccines do not provide cross protection against strains expressing heterologous PorA [7,36,37]. Furthermore, while these wtOMV vaccines may interrupt the acquisition of new carriage, only limited effects have been observed on existing carriage, suggesting that wtOMV vaccines will not provide similar herd-protection effects compared with conjugate vaccines, even for the target strains [4,23,36].

Millions of doses of wtOMV vaccine have been administered, yielding extensive safety outcomes data. Self-limited reactogenicity has been observed with wtOMV vaccines. Adults tend to report headache, injection site pain and swelling and generalized arm pain, while transient fever lasting 48 h or less and irritability has been noted in infants and young children. Results of several placebo-controlled studies suggest that aluminum-containing adjuvants may contribute to these reactogenicity outcomes [36,54–57].

The wtOMV vaccines have also been investigated in combination with additional components. For example, MeNZB has been studied in combination with a serogroup C conjugate vaccine and with MenBVac [51]. Recombinant OMVs designed to overexpress relevant surface proteins or purified proteins derived from OMVs have also been developed as a means to help overcome some limitations of wt vaccines (Table 2) [1,15,35–37,44,46,58]. However, PorA proteins, an important feature of the recombinant OMV vaccines, exhibit great variability, which can limit their potential usefulness as vaccine antigens [40]. In the USA, 20 different PorA variants would be needed to cover 80% of circulating MenB strains. In addition, preliminary clinical trials of hexavalent and nonavalent PorA vaccines have shown stronger immunogenicity for some PorA variants than others [59–61]. The addition of different antigens is a means of broadening protection and a vaccine with six PorA and five FetA variants has been suggested to address hypervirulent lineages [40,59].

■ Investigational vaccines against MenB

Most experimental MenB vaccines against serogroup B continue to use one or more OMV component, in part because the wtOMV vaccines are the only products to have demonstrated effectiveness. In addition, OMV may provide immunomodulatory or adjuvant effects beyond the primary immunogenicity contributed by PorA, as observed with a combination

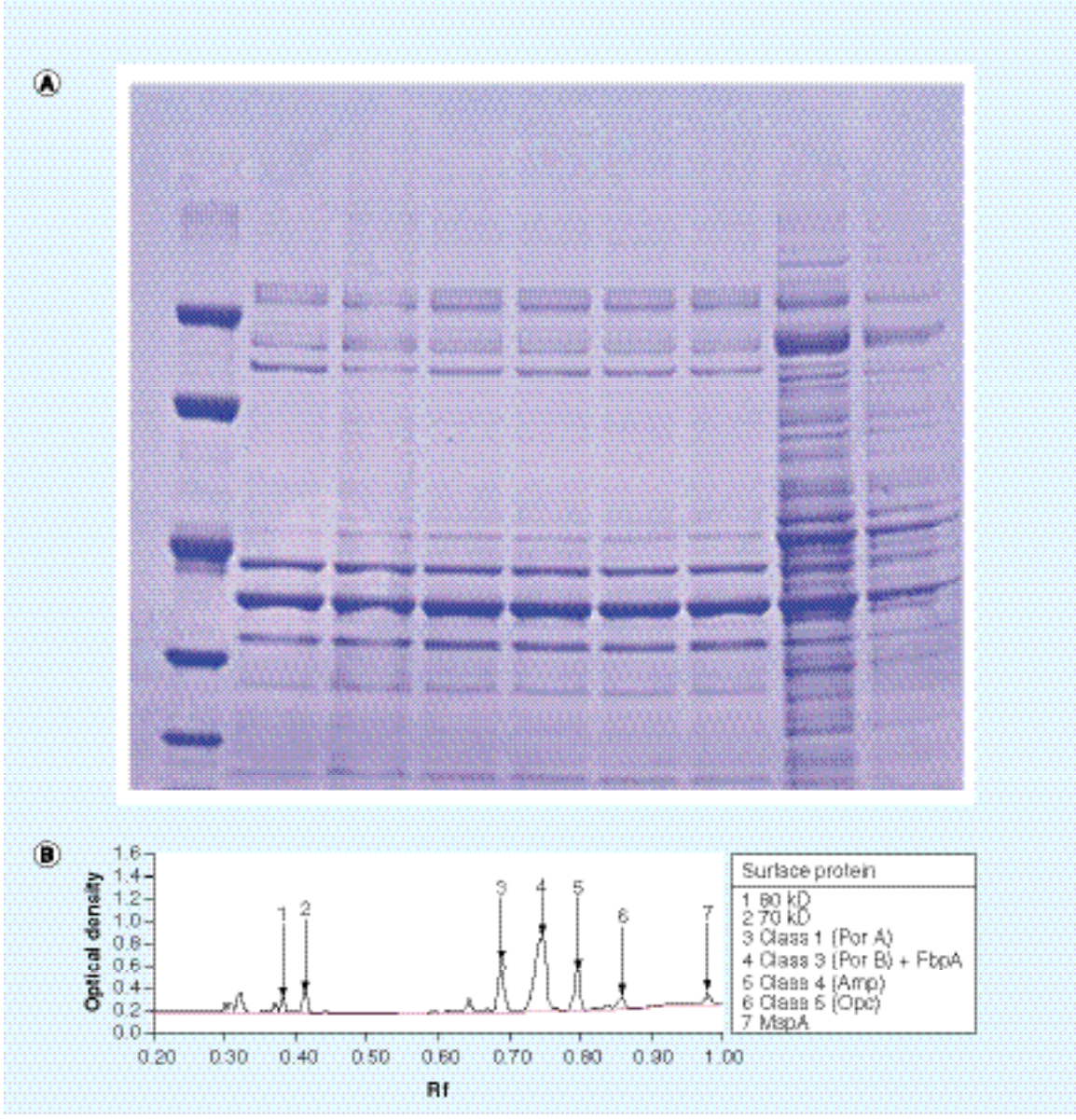

Figure 2. Identification of various proteins within a typical wild-type outer membrane vesicle used in vaccine development. (A) Shows a representative Coomassie gel and a representative lane electropherogram, shown with the corresponding gel lane for a single protein. **(B)** Protein pattern composition, relative ratio of outer membrane proteins and total purity are monitored by SDS-PAGE. A typical SDS-PAGE separation for outer membrane vesicle is presented in **(A)**. Evaluation of the presence and relative quantities of eight proteins was performed by densitometric evaluation of gel lane electropherograms.

Table 1. Outer membrane vesicle vaccines licensed for clinical use.

OMV type	Trade name	Countries employing	Number of doses administered
Finlay 4:P1.19, 15	VA-MENGOC-BC®	Cuba, Chile and Brazil	2
NIPH 15:P1.7, 16	MenBVac®	Norway and France	3
NIPH/Novartis 4:P1.7–2,4	MeNZB®	New Zealand	3

OMV: Outer membrane vesicle.

Table 2. Examples of subcapsular proteins under investigation for use in meningococcal serogroup B vaccines.

Protein(s)	Type of vaccine	Latest research stage initiated
Single PorA	wtOMV	Licensed for use against specific clonal groups
Multiple PorA	Recombinant OMV	Early clinical trials
OpcA	OMV	Early clinical trials
Bivalent PorA with fHbp and NadA	Multicomponent	Early clinical trials
Bivalent fHbp	Multicomponent	Advanced clinical trials
fHbp with NadA and NHBA (rMenB)	Multicomponent	Early clinical trials
rMenB with Norway strain OMV	Multicomponent	Early clinical trials
4CMenB (rMenB with New Zealand strain OMV)	Multicomponent	Application for licensure

MenB: Neisseria meningitidis serogroup B; OMV: Outer membrane vesicle; wtOMV: Wild-type OMV.

OMV and outer membrane protein vaccine, which provides an additional rationale for inclusion in novel formulations [62].

Bivalent, hexavalent and nonavalent vaccines based on recombinant OMVs that express multiple different PorA serosubtypes have entered trials in adults [63,64,202] and are described based on the number of PorA variants, not necessarily the number of OMV components [60,61,65]. The hexavalent and nonavalent vaccines contain two or three OMVs, respectively, each expressing three different PorA variants. The nonavalent vaccine has also been tested in combination with a pneumococcal conjugate vaccine [61]. Additional OMV vaccines, including formulations containing OpcA with or without overexpressed fHbp, have also been studied [66,67]. Vaccines based on OMV from genetically detoxified or mutant lipooligosaccharides that eliminate the detergent extraction step that can affect protein conformation, have also entered clinical trials [15,16,36]. It was hoped that an OMV vaccine derived from *Neisseria lactamica*, which has surface proteins similar to those on the meningococcus, would be able to induce cross-protective antibodies for both species, but this has not progressed to late-stage clinical development [1,68]. Additional vaccines have induced bactericidal activity against OpcA and fHbp with good tolerability in adults [2,35,66,67].

Vaccine formulations containing fHbp, either alone, in combination with additional recombinant antigens, or in multicomponent formulations containing OMV, have been shown to generate immune responses [15,16,35,66,67,69]. As mentioned above, one multicomponent vaccine, 4CMenB, has completed late-stage clinical trials and is under consideration for licensure. It contains four components: OMV from the New Zealand outbreak strain, fHbp, NadA and NHBA.

Reverse vaccinology: antigen identification for novel MenB vaccines

The fHbp, NadA and NHBA included in 4CMenB were identified via reverse vaccinology. Whole genome sequences represent a list of virtually all the proteins that a pathogen can express at any time and reverse vaccinology uses bioinformatic algorithms to 'mine' these sequences for potential vaccine antigens. The first group of targets was identified for MenB strain

Table 3. Major antigens included in 4CMenB.

Antigen	Full name	Number of variants	Biological function (example)	Presented as
fHbp	Factor H binding protein	Three major non-crossreactive variants†	Recruits host molecules to the bacterial surface, facilitating survival in host tissues	Fusion protein with GNA 2091
NadA	Neisserial‡ adhesin A	Five major variants; variants 1–3 are crossreactive	Mediates adhesion and invasion of host cells	Self (nonfused)
NHBA	Neisserial‡ heparin binding antigen	Over 24 crossreactive peptides	Recruits host molecules to the bacterial surface, aiding survival	Fusion protein with GNA 1030
PorA	Porin A	Multiple non-crossreactive serosubtypes	Participates in transport into and out of the cell membrane	OMV

†An alternate nomenclature groups variants 2 and 3 into subfamily A and terms variant 1 subfamily B, based on genetic information.

‡Alternative spellings have appeared in the literature.

OMV: Outer membrane vesicle.

MC58 [70] and yielded 28 proteins that could be expressed as recombinant proteins in *Escherichia coli*, were surface-expressed and also induced bactericidal antibody responses in animals. The most immunogenic of these fHbp, NadA and NHBA were selected for inclusion in a multicomponent vaccine (Table 3) to provide broad strain coverage and minimize the potential for immune evasion and the development of escape mutants. To enhance immunogenicity and facilitate large-scale manufacturing, NHBA was fused to a meningococcal accessory protein (GNA1030) and fHbp was fused to GNA2091. These fusion proteins, formulated with aluminum hydroxide, induced more potent bactericidal antibodies than those induced by the individual antigens [71]. NadA was included as a single protein because it did not perform well in fusion, which may have altered its native trimeric organization [72]. These antigens were adsorbed to aluminum hydroxide [71] for use in clinical trials [15,16,70,73].

fHbp

Meningococcal fHbp is a 27 kDa membrane-bound lipoprotein that binds human factor H, a down-regulator of the alternative complement pathway. Thus, fHbp aids bacterial survival in human blood by permitting complement evasion [20,74,75]. Surface expression of fHbp can be high, intermediate or low [76,77]; only a few strains (1% in the USA) lack fHbp expression altogether [78]. Such strains may employ alternative systems for recruiting human factor H, such as NspA [79]. Three fHbp sequence variants, with intravariant conservation of 91.6–100% and between-variant conservation as low as 62.8%, have been identified. An alternate classification system divides fHbp into subfamilies A (variants 2 and 3) and B (variant 1), based on genetic relationships between the variants [80]. A bivalent fHbp vaccine is being developed based on the hypothesis that cross-reactivity from two subvariants corresponding to the subfamily classification, would be sufficiently cross-reactive to provide protection against a majority of pathogenic strains; limited clinical data have been published describing the effects of this vaccine [81].

The three fHbp variants (termed 1, 2 and 3) induce bactericidal antibodies that exhibit evidence of intra- and inter-variant cross reactivity that appears to vary by age (Figure 3). Within all variants, crossprotection by anti-fHbp antibodies is strongest for strains carrying homologous alleles and weaker against isolates harboring distantly related variants or subvariants [76]. The fHbp subvariant 1.1 fusion protein included in 4CMenB has been shown to induce bactericidal antibodies in 90–100% of infant, adolescent and adult vaccinees [82–86]. When tested against a panel of isogenic serogroup B strains engineered to express ten different fHbp variant 1 subvariants [87], sera from adults who received two to four doses of 4CMenB and 13-month-old toddlers vaccinated at 2, 4, 6 and 12 months of age were crossreactive against all ten subvariants. However, sera from 7-month-old infants who received three doses of 4CMenB were cross protective for closely related subvariants only [87].

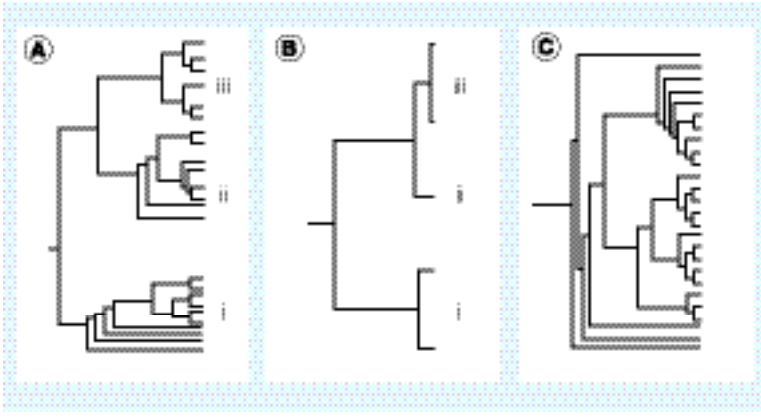

Figure 3. Simplified dendrograms showing the major variants and subvariants of fHbp, NadA and NHBA. (A) Three variants exist for fHbp; fHbp-induced antibodies are crossprotective against strains carrying homologous, but not distantly related alleles. **(B)** Five variants have been identified for NadA. The three main variants (NadA-1, NadA-2 and NadA-3) are crossprotective regardless of sequence variations. **(C)** NHBA is structured into a substantial number of peptides. Antibodies induced by NHBA peptide 2 are crossprotective against most strains tested thus far. Courtesy of S Bambini (Novartis Vaccines, Siena, Italy).

NadA

NadA is an 'Oca' (oligomeric coiled-coil adhesin) bacterial trimeric autotransporter adhesin [88]. These proteins characteristically form trimers on the bacterial surface and mediate meningococcal adhesion to, and entry into, human epithelial cells [72]. NadA is commonly considered a meningococcal protein. No known strains of *Neisseria gonorrhea* or the commensal species *N. lactamica* and *Neisseria cinerea* harbor the *nadA* gene [88]. Recent analysis using a noninvasive *Yersinia enterocolitica* mutant engineered to express NadA revealed that β-1 integrin is a likely NadA receptor [89].

Unlike fHbp and NHBA, which occur on nearly all meningococcal strains, NadA is found primarily in a substantial subset of pathogenic strains and is associated with three of the four known hypervirulent lineages of MenB and MenC. In the USA, the *nadA* gene was identified in 39% of strains in a panel of 650 MenB isolates collected between 2000 and 2008 [90]. In this panel, NadA was more common in pathogenic strains

than in strains associated primarily with isolates from asymptomatic carriers or 'carriage strains.'

In clones where it is present, NadA is well conserved. Five variant alleles have been identified [88,91,92] and the three most common variants (NadA-1, NadA-2 and NadA-3) induce crossreactive antibody activity in animals and humans, regardless of sequence variations (Figure 3). NadA-4 occurs more rarely and is strongly associated with 'carriage strains' [77,91], while NadA-5 is generally associated with strains from a single clonal complex [92].

The *nadA* gene is both subject to complex regulatory controls and highly dependent on environmental signals, leading to highly variable expression in different conditions [88,93,94]. During bacterial growth, NadA is expressed maximally in stationary phase [88]. Phase variation occurs because of a tetranucleotide tract (TAAA) that is located upstream of the *nadA* promoter [93] and controlled by transcriptional regulator, NadR, which represses surface exposure *in vitro* [94]. 4-hydroxyphenyl acetate (4-HPA), aromatic amino acid catabolites secreted in human saliva, can de-repress NadA transcription *in vitro* [95], promoting surface expression at high levels and rendering strains highly susceptible to killing in a serum bactericidal assay [96]. In clinical studies, NadA formulated with fHbp and NHBA or in 4CMenB has been shown to induce high levels of bactericidal antibodies in infants, toddlers, adolescents and adults [82–86].

■ NHBA

NHBA is a *Neisseria*-specific surface-exposed lipoprotein with a predicted molecular weight of 50.5 kDa that binds heparin *in vitro*. This property correlates with increased survival of the unencapsulated bacterium in human serum [97]. Serum antibodies from mice immunized with recombinant NHBA elicited complement-mediated bactericidal activity against diverse MenB strains [70,71]. Anti-NHBA antibody also elicited deposition of human C3b on the bacterial surface and passively protected infant rats against bacteremia after challenge [98].

NHBA is present in all meningococcal strains tested [21,77,92,99] and is structured into a substantial number of peptides that have some association with clonal complexes and sequence types. While gene-sequence analysis of genetically diverse MenB strains reveals variable segments of NHBA, its amino- and carboxyl-terminal regions are highly conserved. 4CMenB contains NHBA peptide 2, which was shown to be the most common in a recent molecular epidemiology study (Figure 3) [95]. As with the other antigens included in 4CMenB, NHBA formulated with fHbp and NadA or in 4CMenB induced bactericidal antibodies in all age groups studied [82–86].

4CMenB: a multicomponent vaccine against meningococcal disease

To date, 4CMenB is the only broadly protective serogroup B vaccine that has completed Phase III trials and is under review by several regulatory authorities. The multicomponent approach might create the possibility of antigen cooperativity or synergy that could augment protection provided on the individual contributions of each vaccine component. Cooperative serum bactericidal activity between human antibodies raised against fHbp and NHBA has been detected [100]. Functional data on these antigens suggest that antibodies induced by 4CMenB could act in two ways: directly, by activating classical complement pathway, or indirectly, by interfering with adhesion and colonization and/or preventing fH binding on the bacterial surface, increasing susceptibility to killing by the alternative pathway.

■ Early clinical development

Evaluating novel vaccines requires extensive planning efforts, which can include the development or adaptation of end point measures for use in clinical trials. In disease states that occur at a relatively low incidence, such as meningococcal disease, clinical development programs must employ serological measures that correlate to clinical protection. For meningococcal disease, the original accepted correlate of protection, a titer ≥4 in the serum bactericidal assay using human complement (hSBA) was established by Goldschneider *et al.* and has subsequently been used widely in clinical trials, including studies of wtOMV vaccines [101,102]. Studies of 4CMenB have employed hSBA titers ≥4, ≥5 (which ensures that the lower bound of a 95% CI is ≥4), ≥8 (a more conservative measure) and fourfold rises in hSBA titer above pre-existing protective levels of antibody as measures of immunogenicity. ELISAs for antigen-specific antibody have also been obtained in some studies [82–86]. Although such measures would be considered sufficient for polysaccharide vaccines, conjugate vaccines and wtOMV vaccines, assessments of broad strain coverage by 4CMenB required additional measures to bridge clinical trial data to circulating strain epidemiology, as discussed below [58].

Early clinical studies of vaccine formulations containing fHbp, NadA and NHBA evaluated immunogenicity against strain panels that were selected in part to assess and identify possible reference strains for use in Phase III studies to evaluate the individual contribution of different vaccine antigens (Tables 4 &

Table 4. Early clinical results with various formulations containing fHbp, NadA and NHBA.

			Percentage of vaccinees with hSBA titers ≥4		
Strain	Phenotype	Sequence type	4CMenB	rMenB with Norway strain OMV	rMenB alone
5/99	B:2b:P1.5,2	1349	100	100	100
2996	B:2b:P1.5,2	540	>80†	>70	>50
M6190	B:2b:P1.5,2	1988	–	–	–
M01240013	B:2b:P1.5,2	11	>70	>60	>70
95N477	B:2b:P1.2	475	–	–	–
44/76	B:15:P1.7,16	32	>90	100	100
MC58	B:15:P1.17,16b	74	100	100	100
CU385	B:4:P1.15	33	100	100	100
M4105	B:4,7:P1.7,4	154	100†	>50	–
NZ98/254	B:4:P1.7–2,4	154	>90†	>70	>50
M1390	B:15:P1.7,4	41	>90	>90	>90
1000	B:NT:P1.5	20	>80	>70	>50
M4458	B:NT:P1.3	6161	70	>70	>75
M01240364	B:NT:P1.22,9	275	100	>90	>90
M3812	B:NT:P1.5	60	>70	70	>80

†Strains for which a possible immunogenic advantage was identified for 4CMenB relative to rMenB or rMenB with a different OMV component. Of note, both formulations with an OMV showed similar effects against the Norway strain 44/77.
Dashes indicate that fewer than half of participants generated protective hSBA titers
OMV: Outer membrane vesicle.

5) [73,84–86].

In a Phase I study in 70 healthy adults, Toneatto *et al.* observed that 4CMenB provided good evidence of immunogenicity against a panel of 15 genetically heterologous MenB strains, including three of the four strains later chosen for evaluation in Phase III studies (Table 5) [84]. Compared with rMenB alone or formulated with OMV from the Norwegian outbreak strain, 4CMenB induced protective hSBA titers against more strains, and also provided crossprotection in exceess of that expected based on PorA serosubtypes. For example, both vaccines seemed to induce similarly protective effects against strain 44/76, the source for the Norwegian OMV component. This finding may indicate the activity of another protein in the New Zealand strain OMV, synergystic effects among vaccine antigens, or crossprotection afforded by PorA 1.4 [84]. In the case of strain 44/76, the primary 4CMenB immunogen contributing to killing in the hSBA was later found to be fHbp [73].

Two Phase II studies of 4CMenB were conducted in healthy infants, whose sera were tested in the hSBA against a six-strain panel, which included three of the four strains later chosen for evaluation in Phase III studies [15,16,82,83]. In these studies, infants received 4CMenB or rMenB at either 2, 4 and 6 months of age or at 6–8 months of age and 2 months later. All infants received a booster dose at 12 months of age. Infants enrolled in these studies were found to mount robust immune responses to genetically heterologous MenB strains. However, these antibody responses tracked closely to strains whose surface antigens were closely related to the vaccine variants. In other words, responses to genetically heterologous strains appeared to have resulted from the activity of multiple components as opposed to cross-protection afforded by individual

Table 5. Meningococcal serogroup B strains used for Phase III studies of 4CMenB.

Strain	Phenotype	Sequence type	PorA type	hSBA killing by antibodies against
NZ98/254	B:4:P1.7–2,4	42	P1.4	PorA
44/76-SL	B:15:P1.7,16	32	P1.16	fHbp
5/99	B:2b:P1.5,2	8	P1.2	NadA
M10713	B:NT:P1.17,16–3	136	P1.16–3	NHBA

components. Thus, the induction of protective antibodies was not evident against strains that expressed only antigen variants that were distantly related to the vaccine components. These findings were further supported by *in vitro* assessment of a genetically engineered meningococcal strain that expressed multiple fHbp variant 1 subvariants. In this study, post-vaccination sera from 7-month-old infants covered only closely related fHbp variants on the recombinant strain. However, the same sera were capable of killing wt strains harboring distantly related fHbp variant 1 subvariants, which was considered likely due to the contribution of the other components in the vaccine [87].

The Phase II studies in infants also include an evaluation of safety and tolerability parameters. The infants aged 2, 4 and 6 months of age at study immunization also received Pediacel® (diphtheria, tetanus, acellular pertussis, inactivated poliovirus, *Haemophilus influenzae* type B conjugate vaccine; Sanofi Pasteur) and Prevnar® (7-valent pneumococcal conjugate vaccine; Pfizer) [82]. In the study in older infants, Menitorix® (*H. influenzae* type B, *N. meningitidis* group C polysaccharide conjugate vaccine; GlaxoSmithKline) was concomitantly administered at the 12-month booster vaccination with 4CMenB [83]. The concomitant use of 4CMenB with these routinely used vaccines did not markedly affect immunogenicity or tolerability outcomes for any of the vaccines. In both studies, researchers observed that all vaccine regimens were generally well tolerated by the enrolled infants and that no unexpected side effects occurred [82,83].

■ Selection of reference strains for Phase IIb & III studies

The late-stage clinical development program for 4CMenB was designed to provide a means of evaluating the individual immunogenic contributions of each major vaccine component and then to bridge these findings against strain epidemiology to predict the likelihood that 4CMenB would provide adequate strain coverage to limit endemic MenB disease in a given area or population. Reference strains were chosen to evaluate the individual ability of the major antigenic protein in each 4CMenB component to induce bactericidal antibodies. Therefore, reference strains were selected because they strongly express only one 4CMenB antigen and either lack the gene, have very low surface expression of, or express a mismatched variant of the other antigens (Table 5). Researchers genetically characterized a large panel of MenB strains and then identified strains that are killed by antibodies against a single 4CMenB antigen using a competitive inhibition serum bactericidal assay [73]. The reference strains for fHbp, NadA and PorA P1.4 (the immunodominant protein in the OMV) have been described [73]. Of note, although an initial NHBA strain was proposed, logistical difficulties prevented its use in the hSBA and strain M10713 was later selected as the NHBA reference strain for pediatric studies [86].

■ Late-stage trials

Data from a large-scale study in 1885 healthy infants indicated a promising immunogenicity and safety profile for 4CMenB when administered at 2, 3 and 4, or 2, 4 and 6 months of age [85]. Prevnar® (7-valent pneumococcal conjugate vaccine) and Infanrix® hexa (diphtheria, tetanus, acellular pertussis, inactivated poliovirus, hepatitis B and *H. influenzae* type B combination vaccine) were administered to infants either with 4CMenB or alone. One schedule alternated routine vaccines with 4CMenB at separate study visits. Based on hSBA titers ≥5 against a panel of MenB reference strains, 4CMenB was immunogenic in all dosing schedules. No evidence of clinical interference was observed for the comparator vaccines with concomitant administration of 4CMenB [85]. Results of the booster dose in this study, as well as the results of a large-scale safety trial [103–105] and a toddler 'catch-up' schedule, are pending publication. Specific outcomes such as fever, which are commonly associated with wtOMV vaccines, warrant further consideration across the large dataset collected in these trials.

In adolescents, 4CMenB induced robust protective-antibody responses in the vast majority of vaccines, with acceptable safety, using various dosing schedules. Overall, 92–97% of subjects had hSBA titres ≥4 against a panel of three MenB reference strains after one dose of 4CMenB, as did 99–100% of those who received two or three doses. Evidence of waning of antibodies in persons without pre-existing titers against MenB strains was observed after a single dose, but not after two doses of 4CMenB [86].

In order to assess immunogenicity in adults (aged 18–50 years) who have increased exposure to *N. meningitidis* relative to the general population, a trial in laboratory workers was conducted at one center in Italy and one center in Germany (n = 54), 4CMenB was administered at 0, 2 and 6 months. Although baseline titres were high, as might be expected in such a population, fourfold rises in hSBA titres against a panel of MenB reference strains were observed in 64–88% of subjects after one dose, and 69–100% after three doses. A follow-up vaccination with conjugate quadrivalent meningococcal vaccine was also immunogenic. Pain was reported following 4CMenB administration by every participant, consistent with previous reports of pain following OMV vaccines in this age group. Three participants experienced transient fever [106]. Additional clinical studies of 4CMenB are ongoing.

Strain coverage

Numerous genetically diverse MenB strains cause IMD each year; therefore, assessments consider protective antibodies for all circulating strains. However, performing hSBA against all circulating strains poses logistical and ethical hurdles, particularly in infants, as large volumes of sera would be required. Strain coverage could be predicted by examining antigens on circulating strains. Since established typing recommendations, such as multi-locus sequence typing, do not account for fHbp, NadA and NHBA, a new system was developed [107].

The meningococcal antigen typing system (MATS) was developed to assess the expression, degree of crossreactivity and antigenicity of fHbp, NadA, and NHBA in meningococcal strains and to estimate strain coverage based on those findings in conjunction with the PorA serosubtype of the strains [58]. The use of the MATS method to predict MenB global and national strain coverage by 4CMenB is underway.

In MATS, the genotype of the variable region 2 of PorA is determined using conventional PCR. For fHbp, NadA and NHBA, a sandwich ELISA is used to test expression and antigenicity. Strains are considered covered by 4CMenB if the relative potency of fHbp, NadA or NHBA in the MATS ELISA is above a value that predicts killing in the hSBA. This value, the positive bactericidal threshold, conservatively estimates the minimum level of expression and antigenicity for fHbp, NadA or NHBA above which at least 80% of strains will be killed in the hSBA. Any strain with a relative potency above the positive bactericidal threshold for fHbp, NadA or NHBA or a strain expressing PorA P1.4 is considered covered by 4CMenB.

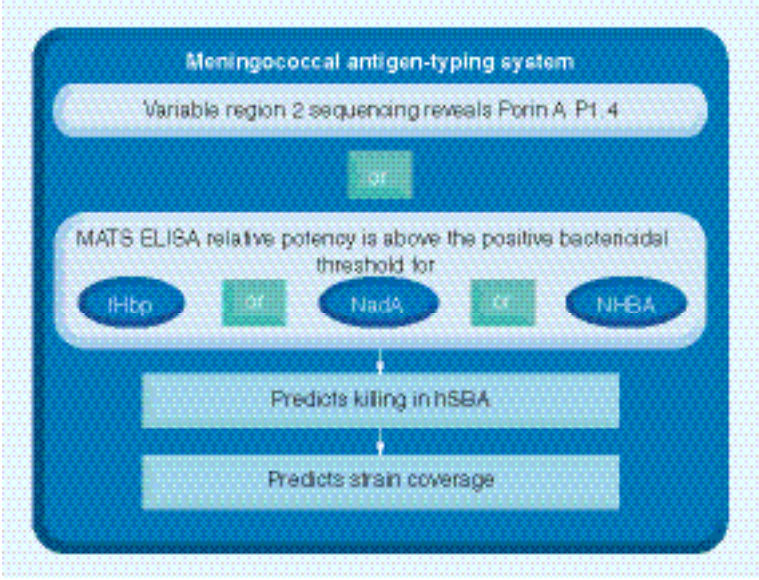

Figure 4. Overview of the MATS platform. Killing in the hSBA is predicted based on results of ELISAs for fHbp, NadA and NHBA where the expression level and immunologic crossreactivity of these proteins are determined, in addition to the PorA sequence obtained by PCR.

The initial MATS predictions were confirmed by hSBA testing against large strain panels designed to over-represent strains that did not strongly express the vaccine antigens. Confirmatory hSBA testing showed that MATS predictions were conservative for toddlers, adolescents and adults because a substantial proportion of strains predicted not to be covered were in fact killed according to the hSBA. Very few strains predicted to be killed by MATS survived confirmatory hSBA testing, showing that MATS had a high positive predictive value for strain killing and a moderate negative predictive value for strains that would not be killed in the hSBA (Figure 4) [58]. Additional work to confirm whether MATS results are conservative against epidemiologically representative strain panels is pending publication.

The MATS ELISA has been transferred to national reference laboratories in Europe, North America and Australia and may be transferred to other laboratories as well. MATS testing of MenB strain panels collected during the last full defined epidemiological year(s) for which strain collections were available have been undertaken. Based on presentations at scientific meetings, the overall conservatively predicted coverage of 4CMenB is between 70 and 90% for individual countries.

The antigens in 4CMenB can occur in all serogroups [69,81,90]. Most circulating serogroup C, Y and W-135 strains express or have the genes coding for fHbp (99%) and NHBA (>90%), and a majority of serogroup C strains also express NadA [81,90]. In the USA, approximately half of strains with fHbp express variant 1, and most of these harbor subvariant 1.1, which is included in 4CMenB. Many different subvariants of NadA, NHBA and fHbp were detected in circulating strains [90]. Assessment of 4CMenB coverage in additional serogroups is also of interest.

Expert commentary

Conjugate vaccines against meningococcal serogroups A, C, W-135 and Y are commonly used and becoming more widely available. One rate-limited factor in the implementation of these vaccines in the developing world was expense, thus, with the advent of the new low-cost vaccine MenAfriVac® (Meningitis Vaccine Project), to prevent disease caused by serogroup A in the African 'meningitis belt', vaccination against MenB has become the most important public health need for IMD. Since MenB disproportionately affects infants in the developed world, new vaccines should be designed to be included in existing routine immunization programs. Various investigational vaccines have been

proposed and one, 4CMenB, has completed Phase III trials and is being considered for approval in Europe and elsewhere.

The genetic diversity and mutability of *N. meningitidis* relies on various mechanisms, including horizontal gene transfer, for all meningococcal serogroups. The dynamic epidemiology of this organism poses special challenges for MenB, which lacks a universal antigen. The necessity for using subcapsular proteins is therefore well established. The multicomponent strategy is promising because it can address genetic diversity and the potential for future mutations. 4CMenB was developed to provide protection against circulating strains over time by employing surface antigens that are immunogenic and conserved across pathogenic and carriage isolates. Additional characterization of MenB strains over time and across geographic regions is ongoing.

Clinical studies of 4CMenB demonstrated protective antibody levels with acceptable tolerability outcomes in persons as young as 2 months of age. In adults, two- and three-dose schedules generally provided similar outcomes. Data are needed to assess persistence over time and the publication of the major datasets for Phase III is pending. Published safety findings indicate that 4CMenB outcomes were generally similar to those in studies of the OMV component alone. Studies in infants also indicate robust immune effects with a primary series and a booster dose, and tolerability that was comparable to published data describing the OMV vaccine in that age group. The most common solicited reactions to 4CMenB in adolescents and adults were injection site pain and swelling, malaise and headache while infants and toddlers experienced tenderness and erythema at the injection site, fever and irritability. These reactions were generally transient and self-limiting, most commonly resolving within 48 h of onset (Table 6).

The assessment of strain coverage is of special interest for MenB. The MATS methodology, which accounts for genetic expression and antigenicity, may prove to be a valuable tool for assessing strain protection and surveillance efforts.

5-year view

Where it has been implemented, MenAfriVac is having a dramatic impact on serogroup A IMD and is revealing the impact of serogroup W-135 and X disease. Although these serogroups cause only a fraction of MenA reports before MenAfriVac was introduced, disease incidence warrants additional efforts to develop protective vaccines. Perhaps with the existing complement of quadrivalent conjugate vaccines, new low-cost vaccines against serogroups A, X and W-135 may become available. With rises in serogroup Y disease reported in some regions that currently recommend only serogroup C vaccines, quadrivalent vaccines may become more widely used in developed nations as well.

The substantial body of available data describing the effects of 4CMenB is currently being evaluated by the EMA and other regulatory agencies. If introduced into national immunization programs, 4CMenB could markedly reduce MenB-related morbidity and mortality. Post-licensure studies will be crucial to help define 4CMenB safety and effectiveness, the extent of herd protection, persistence of antibodies, need for a booster, degree of strain coverage and potential coverage of nonserogroup B meningococcal strains.

It is likely that, in addition to the usual immunogenicity and safety studies required to assess recently licensed products, ongoing epidemiologic surveillance of circulating strains using MATS will be required to monitor MenB. Regional epidemiology and surveillance will be important to address the possibility for strain replacement or serogroup replacement, which has been reported with pneumococcal vaccines. Possible influence on commensal species, such as *N. lactamica,* should also be considered. Effective databases that can track molecular epidemiology of IMD and nasopharyngeal flora worldwide are needed. Of particular interest is new information about the surface features of pathogenic and carriage meningococcal isolates, which could prove important in future public health decision making. Development of additional combination and multicomponent vaccines could draw on more comprehensive information on the characterization of pathogenic meningococcal strains, regardless of capsular serogroup.

Table 6. Solicited reactogenicity outcomes.

Study	n	Age	Local reactogenicity rate (mainly erythema and pain) (%)	Systemic reactogenicity rate (%)	Ref.
Snape *et al.* (2010)	60	Infants	10–26	1–18	[83]
Findlow *et al.* (2010)	147	Infants	20–100	12	[82]
Kimura *et al.* (2011)	54	Adults	40–100	10–50	[106]
Santolaya *et al.* (2012)	1631	Adolescents	40–85	5–50	[86]
Gossger *et al.* (2012)	1885	Infants	10–69	1–79	[85]

Future perspective

We anticipate that much of the work initiated to develop 4CMenB – the first broad coverage MenB vaccine to complete sufficient Phase III clinical trials – will likely contribute to the reduction of IMD worldwide. The multicomponent strategy, which accounts for multiple surface antigens and therefore provides varied opportunities to induce bactericidal antibodies, could prove a vital addition to the public-health armamentarium. In addition, the MATS method could provide a means of future MenB surveillance and could be adapted for use with additional pathogens. Should 4CMenB be licensed, it will likely result in a reduction of disease burden where implemented, particularly in areas with a high or relatively high incidence of MenB disease. Further investigation of the possible effects of this vaccine against other meningococcal serogroups could yield data to support additional applications for this vaccine. Overall, we hope that in the next 5 years, the development of 4CMenB will have provided valuable information for the community working to eliminate meningococcal disease.

Executive summary

Background

- The development of vaccines against *Neisseria meningitidis* serogroup B (MenB) has been a global public-health priority based on continuing disease burden combined with the scientific challenges associated with antigen identification.
- Several factors inform the rationale for establishing immunization programs against MenB, including disease characteristics, epidemiology and persistent case fatality rates.
- The poor immunogenicity of the MenB capsular polysaccharide arises from its structural similarity to the polysialic acid in the fetal neural cell-adhesion molecule.

MenB vaccines

- Licensed wild-type outer membrane vesicle (OMV) vaccines (VA-MENGOC-BC®, MenBVac® and MeNZB®) are protective against strains bearing the same PorA serosubtype.
- Further vaccine development includes the wild-type OMV vaccine combinations, recombinant OMVs containing multiple surface proteins and purified proteins.
- One multicomponent vaccine 4CMenB has completed late-stage clinical trials and is under consideration for licensure.
- 4CMenB contains four components: OMV from the New Zealand outbreak strain, fHbp, NadA and NHBA.

Reverse vaccinology: antigen identification for novel MenB vaccines

- Reverse vaccinology uses bioinformatic algorithms to 'mine' the genomic sequences for potential vaccine antigens.
- The first group of such targets was identified for MenB strain MC58 and led to the selection of purified proteins included in 4CMenB.

4CMenB: a multicomponent vaccine against meningococcal disease

- Clinical studies employed accepted correlates of protection and safety outcomes.
- Clinical data in infants, adolescents and adults support the immunogenicity of 4CMenB.
- Safety and tolerability findings for 4CMenB were promising in all studies and age groups.
- Strain coverage assessments will be based on a meningococcal antigen-typing system method that accounts for genotypic expression and antigenicity.

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