Vaccine: Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

New recombinant vaccines for the prevention of meningococcal B disease

This article was published in the following Dove Press journal:
Vaccine: Development and Therapy
26 June 2012
Number of times this article has been viewed

Muhamed-Kheir Taha
Ala-Eddine Deghmane

Institut Pasteur, Unit of Invasive Bacterial Infections and National Reference Center for Meningococci, Paris, France

Abstract: Meningococcal disease is a life-threatening invasive infection (mainly septicemia and meningitis) that occurs as epidemic or sporadic cases. The causative agent, *Neisseria meningitidis* or meningococcus, is a capsulated Gram-negative bacterium. Current vaccines are prepared from the capsular polysaccharides (that also determine serogroups) and are available against strains of serogroups A, C, Y, and W-135 that show variable distribution worldwide. Plain polysaccharide vaccines were first used and subsequently conjugate vaccines with enhanced immunogenicity were introduced. The capsular polysaccharide of meningococcal serogroup B is poorly immunogenic due to similarity to the human neural cells adhesion molecule. Tailor-made, strain-specific vaccines have been developed to control localized and clonal outbreaks due to meningococci of serogroup B but no "universal" vaccine is yet available. This unmet medical need was recently overcome using several subcapsular proteins to allow broad range coverage of strains and to reduce the risk of escape variants due to genetic diversity of the meningococcus. Several vaccines are under development that target major or minor surface proteins. One vaccine (Bexsero®; Novartis), under registration, is a multicomponent recombinant vaccine that showed an acceptable safety profile and covers around 80% of the currently circulating serogroup B isolates. However, its reactogenicity in infants seems to be high and the long term persistence of the immune response needs to be determined. Its activity on carriage, and therefore transmission, is under evaluation. Indirect protection is expected through restricting strain circulation and acquisition. This vaccine covers the circulating strains according to the presence of the targeted antigens in the circulating isolates as well as to their levels of expression. The coverage rate should therefore be updated and the surveillance of circulating isolates should include typing schemes for the antigens of the future vaccines. We review the recent available data for these upcoming protein-based vaccines and particularly Bexsero®.

Keywords: *Neisseria meningitidis*, serogroup B, vaccine, typing, epidemiology

Introduction

Neisseria meningitidis, or meningococcus, is a Gram-negative bacterium surrounded by a capsule composed of polysaccharides that determines the serogroup of the meningococcus. This bacterium is known to be restricted to humans; its natural habitat is the upper respiratory tract, where it establishes a commensal relationship (carriage) that involves around 10% of the general population.[1] *N. meningitidis* is acquired and transmitted through respiratory droplets during inter-human contact. It first colonizes the mucosa of the human nasopharynx. *N. meningitidis* is also an important cause of invasive bacterial disease in infants, children, adolescents, and young adults.[2] Despite the increasing availability of potent antimicrobials and sophisticated

Correspondence: Muhamed-Kheir Taha
Institut Pasteur, Unit of Invasive Bacterial Infections and National Reference Center for Meningococci, 28 rue du Dr Roux, 75724 Paris Cedex 15, France
Tel +331 456 884 38
Fax +331 456 883 38
Email mktaha@pasteur.fr

intensive care units, invasive meningococcal disease is associated with case–fatality rates ranging between 6% to 14%, even with appropriate antibiotic treatment.[3–7] Among survivors, up to 20% endure subsequent neurologic and disabling sequelae.[3,8]

Meningococcal disease occurs as sporadic cases in Europe, North America, Australia, and New Zealand with localized outbreaks. The reported incidence of the disease in Europe ranges between 0.3 and 4 cases per 100,000 population.[9] The incidence is much higher in countries within the meningitis belt in sub-Saharan Africa where major periodic epidemics occur with an incidence approaching 1000 cases per 100,000 inhabitants.[4] Meningococcal disease affects mainly children and around half of the cases in Europe are reported in children younger than 10 years old. The highest rate is usually reported in infants younger than 1 year. In Europe, the incidence in this age group was 18.3 in 2008 and 15.9 cases per 100,000 population in 2009.[9]

Encapsulated isolates cause the majority of invasive infections. The capsule represents the major virulence factor responsible for evasion of opsonophagocytosis and complement-mediated killing.[10,11] Meningococcal capsules can be classified into 12 serogroups, based on chemically and antigenically distinct capsular polysaccharides, but only six serogroups are responsible for almost all meningococcal diseases worldwide: A, B, C, Y, W-135, and X with large variations in the global epidemiology.[7,12,13] Serogroup B predominates in Europe, North America, South America, Australia, and New Zealand.[4,14,15] The capsule is composed of the polymerization of units of sialic acid (for serogroups B, C, Y, and W-135) and the polymerization of units of mannosamine (for serogroup A).[16,17]

In addition, the meningococcus is naturally competent for transformation. The frequent horizontal DNA exchanges and recombination between isolates are responsible for the high genetic diversity encountered in the meningococcus. This diversity affects all surface proteins that undergo significant variations and complicates the development of universal vaccines against the meningococcus. The meningococcus is also subject to selective pressure by the host immune response. Typing of meningococcal isolates is crucial for the development of vaccination strategies. The phenotypic typing determines the serogroup (capsule), serotype (immunospecificity of the outer membrane protein, PorB) and serosubtype (immunospecificity of the outer membrane protein, PorA). The genotyping analyzes the DNA polymorphism of the meningococcus and allows clustering of the isolates into genetic lineages (clonal complexes). A clonal complex is a subset of isolates (clones) that are close enough to recognize a common origin (ancestor).

Meningococcal vaccines licensures

The licensure of meningococcal vaccines requires immunogenicity and safety studies, which provide data in age groups that will be targeted by the vaccination strategies. Once vaccine licensure is obtained, the recommendation may differ among countries according to local epidemiology (mainly incidence, serogroup, and age distributions). Immunogenicity studies essentially measure serum bactericidal titers in sera in vaccinated subjects and compared titers before and after vaccination. Titers in serum bactericidal assays (SBAs) are defined by the serum dilution with the capacity to reduce 50% of bacterial viability. Interpretation and validation of immunogenicity studies are based on the "surrogate of protection" that is based on the correlation between protection against invasive meningococcal disease and SBA titers of at least 4 using human complement in the SBAs.[18,19] During the development of meningococcal vaccines, immunogenicity studies must take into account three variables:

- The percentage of vaccinated subjects with a bactericidal titer of at least 4 (the values before and after vaccination are compared).
- The percentage of subjects with an increase in bactericidal titer after vaccination. Usually a fourfold increase in the SBA titers is considered to be correlated with vaccine effectiveness.
- The geometric mean of bactericidal titers in vaccine recipients (values before and after vaccination are compared).

Polysaccharide meningococcal vaccines

The rapid progression to serious illness or death and long-term morbidities associated with meningococcal disease, in spite of appropriate antibiotic treatment, argue for preventive vaccination to reduce the burden of the disease.[20]

The capsule is a suitable vaccine structure because of its immunogenicity, its abundance, and its surface accessibility. Vaccines based on the capsular polysaccharides of *N. meningitidis* have been developed since the 1960s.[21–23] Plain polysaccharide-based vaccines are widely used with relative success in mass immunizations during outbreaks. They are efficacious in older children and in adults, but are much less immunogenic in children younger than 2 years old.[24] The development and the use of meningococcal polysaccharide

conjugate vaccines are an optimal alternative as they induce a T-dependent immune response and result in the development of an immunological memory and can be used in children younger than 2 years old.[25,26] Moreover, conjugate vaccines do not induce hyporesponsiveness, a phenomenon defined by the absence of an increase in the antibody response against meningococci upon subsequent doses of meningococcal plain polysaccharide vaccines.[27] In addition, polysaccharide conjugate vaccines induce "herd immunity" through the restriction of circulation of isolates among people.[7,28] Vaccines exist to protect against meningococcal A, C, W-135, and Y disease. Development of an effective serogroup B capsular polysaccharide vaccine is hindered by the poor immunogenicity of the B polysaccharide[29] and by concerns over the possible induction of autoimmune antibodies.[30] Indeed, the serogroup B polysaccharide structure is similar to the neural-cell adhesion molecules.[31] Since the majority of cases in some parts of the developed countries are caused by serogroup B isolates, with a disproportionate burden in infants, a vaccine against this group would be highly desirable and represents a major unmet medical need.

Alternative strategies for a meningococcal B vaccine

The absence of a vaccine composed of capsular polysaccharide against serogroup B strains may be circumvented by the development of vaccines based on subcapsular antigens. These vaccines are expected to be directed against the meningococcal strains regardless of serogroup.

Outer membrane vesicles (OMVs)

Detoxified extracts of outer membrane vesicles are prepared from one or several strains and used as vaccines.[32] These vesicles are immunogenic and the immune response is mainly directed against the PorA outer membrane protein. Due to the high variability of PorA among isolates, this type of vaccine is strain-specific (tailor-made).[33,34] OMV vaccines are adequate to control hyperendemic or epidemic situations caused by a particular strain (clonal event).[33] This strategy was tested in three countries in epidemic conditions with three different strains: Cuba, Norway, and New Zealand.[33] However, these vaccines generate only little cross-immunity between strains, particularly in young children,[35] and do not offer protection against heterologous strains.[36] The immune response is largely directed against surface-accessible loops on the porin protein, PorA,[35,37] which is antigenically variable.[37] Moreover, the duration of the immune response is limited and repeated doses are required. A three-dose regimen was used in New Zealand in people less than 20 years old and over one million doses were administered (MeNZB®; Novartis, Basel, Switzerland). In children under one year, a fourth dose is required.[38] Another recent experience using an OMV vaccine (MenBvac®; The Norwegian Institute of Public Health, Oslo, Norway) was in the Seine Maritime in France among subjects between 1 and 24 years old with two doses at 6-week intervals and a booster dose 6 months after the second dose.[39] These vaccines are well tolerated. Side effects are usually local (pain, erythema, induration) and severe side effects (myelopathy, Guillain-Barré syndrome, and demyelinating diseases) are very rare.[39,40]

To enlarge strain coverage by OMV-based vaccines, a new generation of OMV-based vaccines was tailored using an engineered capsule deficient strain that also lacked the lacto-N-neotetraose structure on their lipooligosaccharide. Two or three strains were therefore used, each expressing three different PorA genes to produce a recombinant PorA OMV vaccine with six or nine different PorA subtypes (HexaMen® or NonaMen®, respectively).[41,42] This vaccine was developed by the National Vaccine Institute in The Netherlands and was aimed to cover the majority of circulating isolates in that country. However, the main drawback in these vaccines is still the coverage of circulating isolates. Moreover, the induced immune response was variable against PorA subtypes included in the vaccine and the immune response was of short duration.[43]

Reverse vaccinology era paving the way towards a recombinant vaccine against serogroup B

Traditionally, vaccines have been developed by isolating and purifying antigenic components from the pathogen of interest, which typically has been heat-killed or chemically inactivated. The need to find highly conserved antigens for a universal meningococcal B vaccine has led to a pioneering approach called reverse vaccinology that takes advantage of the availability of the complete genome sequences of *N. meningitidis* to help define novel antigens as vaccine candidates (in silico analysis to define conserved surface-located meningococcal proteins). Subsequently, these vaccine candidates can be confirmed (or not) experimentally through cloning, expression of the corresponding genes, and immunization with purified proteins.[44] Indeed, genomic analysis allowed the identification of a "cocktail" of several proteins that can be used for a universal vaccine against *N. meningitis* and in particular those belonging to serogroup B.[45,46] Using this approach, the Chiron Company (now Novartis, Basel, Switzerland)

exploited the data of the complete genome of serogroup B MC58 meningococcal strain generated by Tettelin et al[47] to identify several proteins as vaccine candidates. They were used to develop a new recombinant vaccine that can target meningococcal isolates of serogroup B. This vaccine went through several stages of development.[46] Five components were first identified (GNA1870, GNA2132, NadA, GNA1030, and GNA2091) and were evaluated for their immunogenicity alone or taken together in a five-component vaccine against MenB (5CVMB). In preclinical studies, these proteins have shown the potential to protect against a broad range of disease-causing meningococcal B strains using sera obtained from immunized mice.[48] Three of these proteins were found to be immunogenic and induced a bactericidal immune response: GNA1870 (also known as factor-H binding protein), GNA2132 (also known as *Neisseria* heparin-binding antigen [NHBA]), and the meningococcal adhesion, NadA.[48] Subsequently, three proteins were evaluated: a fusion protein GNA2091–1870, another fusion protein GNA2132–1030, and the NadA protein. The OMV of the strain NZ98/254 was then added to increase strain coverage through the PorA protein and to enhance immunogenicity.[49,50] This four-component vaccine against MenB (4CMenB/Bexsero) was thereafter used in the subsequent clinical studies.[49] The final formulation of the vaccine is 50 µg each of factor H binding protein 1 (fHbp), NadA, and NHBA fusion proteins, 25 µg of detoxified OMV from *N. meningitidis* strain NZ98/254, and 1.5 mg of aluminum hydroxide.

Immunogenicity and safety of the Bexsero/4CMenB vaccine

For capsular polysaccharide-based vaccines, SBA is a well-established surrogate assay for protection. Works on OMV-based vaccines also showed that protection after vaccination correlated with SBA, and suggested that SBA can also serve as a surrogate assay for protection.[51] The selection of target strains for SBA is uncomplicated for polysaccharide vaccines as the capsule is highly expressed on the meningococcal surface (in particular in invasive isolates) and the structure of the capsule is shared by isolates belonging to the same serogroup regardless of their genotypes. For protein-based vaccines, the choice of isolates for the SBA is less straightforward. Ideally, the selected isolates should allow testing the immunogenicity of each component of the vaccine separately and evaluating the interference/synergism of the antibodies targeting several components.

The first immunogenicity studies with the 4CMenB vaccine in infants were performed with three doses and used six different serogroup B isolates to evaluate the immunogenicity. Several of these six isolates shared antigens with the vaccines but others were unmatched or even missed vaccine components.[52,53] These studies analyzed in infants the immunogenicity of two formulations of the vaccine (with or without OMV). The immune response was evaluated by measuring the geometric mean of the SBA titers, the percentage of responders (fourfold increase in SBA titer), and the percentage of subjects achieving at least a bactericidal titer of 4 (correlated with protection). Immune response was low or even absent against unmatched strains. The results were clearly in favor of enhancement of the immunogenicity by the addition of the OMV component to the vaccine.[52,53] The immune response declined 6 months after the third dose but a booster dose administered at 12 months of age elicited an anamnestic response.[53] These studies unraveled the requirement of estimating the coverage of the circulating isolates by the vaccine using representative collections of isolates. Moreover, the level of expression of theses antigens and their surface accessibility should also be addressed (see the next paragraph).

A recent Phase IIb immunogenicity study,[54] randomized 1885 2-month-old infants to receive three doses of Bexsero/4CMenB together with routine infant vaccines (7-valent pneumococcal conjugate vaccine and a combined diphtheria, tetanus, acellular pertussis, inactivated polio, hepatitis B, and *Haemophilus influenzae* type b vaccine), or given separately. Immune responses against fHbp, NadA, or PorA antigens were measured using the human serum bactericidal antibody (hSBA) assay measured 30 days after the third dose of 4CMenB against three reference strains expressing each of the specific antigens. A titer ≥ 5 was used to ensure that SBA titers in sera form vaccinated infants reached with statistical confidence the threshold of 4 that is the surrogate of protection. Moreover, response against NHBA antigen was measured using a specific enzyme-linked immunosorbent assay (ELISA), as a specific reference strain was still missing at the time of the study. At least 99% of participants had hSBA titers of ≥ 5 for strains of fHbp and NadA, and 79% for the OMV reference strain after immunization with 4CMenB/Bexsero and routine vaccines together either at 2, 4, and 6 months, or at 2, 3, and 4 months.[18,54] No major difference in SBA titers was observed when Bexsero/4CMenB was administered alone or with the routine vaccines. Furthermore, responses to routine vaccines given with Bexsero/4CMenB were non-inferior to routine vaccines alone for almost all antigens.

The data also showed that Bexsero/4CMenB, when administered alone, had a reactogenicity profile that was similar

to those of the routine vaccines. However, higher systemic reaction in infants, notably an increased fever was experienced in co-administration schedules of Bexsero/4CMenB with other routine infant vaccines (51% to 61%). This finding could become an important issue with routine use. Fever mirrors the inflammatory process that is usually associated with immunization and antipyretic drugs such as paracetamol could be prescribed preventively, however, such prescription may reduce antibody responses and should not be used routinely.[55] This Phase IIb randomized controlled study found supporting evidence to use this vaccine in various vaccination schedules in the first year of life, when the likelihood of contracting this often-deadly disease is the highest.

Another recent Phase IIb/III randomized, observer-blind, placebo-controlled study was also conducted among adolescents in Chile. Bexsero/4CMenB was used at 1-, 2-, and 3-dose schedules with no coadministration of other vaccines.[56] The study concluded with an acceptable safety profile in adolescents and suggested an optimal dosing schedule of two doses administered 1 to 6 months apart.[56] Interestingly, rates of fever (≥38°C) among adolescents were low and reports of fever 39°C or higher were rare and no evidence of increasing rates of reactions with subsequent doses of Bexsero/4CMenB was identified. However, two cases of juvenile arthritis, individually assessed as probably related to Bexsero/4CMenB, were reported 170 and 198 days, respectively, after the third dose (3-doses scheduled at 0, 1, 2 months) among the 303 per-protocol subjects. These cases require further investigations due to the known association between invasive meningococcal infection and arthritis.[57]

At 6 months, seroresponse rates reached 99%–100% for each of the three strains expressing one of the three antigens included in the vaccine (NadA, fHbp, and NHBA) after second or third doses. Moreover, preliminary data were reported in this work for hSBA against a strain indicative of NHBA responses. These data were consistent with the ELISA results and therefore allowed to address directly the bactericidal role of antibodies against NHBA.

Targeting infants by the vaccination is required as the incidence is the highest in this group with a high proportion of serogroup B isolates.[9] Targeting adolescents may represent an additional advantage in conferring a "population-based immunity." Indeed, carriage and circulation of meningococcal isolates is the highest among adolescents and young adults.[1] High coverage rate of vaccination among adolescents may therefore limit the circulation of isolates and hence conferring an indirect protection of the population in addition the direct protection of the adolescents.[58] The impact of the vaccine on the carriage is currently under evaluation in an ongoing, multicenter, controlled study to evaluate the effect of the 4CMenB vaccine and MenACWY conjugate vaccines on pharyngeal carriage of *N. meningitidis* in young adults (clinicalTrials.gov identifier NCT01214850).

Strain coverage by the Bexsero/4CMenB vaccine

Due to the variability of *N. meningitidis*, the proteins included in the Bexsero/4CMenB vaccine show variations at the level of the sequences of these proteins as well as at the level of their expression among the different circulating isolates. Indeed, Brehony et al showed that the *fHbp* gene displays diversity in a representative meningococcal strains sample. Variants of this gene can be classified into two or three major groups of the meningococcal isolates, each displaying several alleles that have some association with meningococcal lineages and serogroups.[59] Furthermore, Murphy et al performed nucleotide sequencing of *fHbp* genes obtained from 1837 invasive meningococcal B isolates from the USA, Europe, South Africa, and New Zealand.[60] All sequences fell into two subfamilies or variants that each had a rather large number of alleles. Intersubfamily recombination may lead to the emergence of new fHbp sequences.

The NadA encoding gene also varies among isolates with variable levels of expression and may be absent in several genetic lineages of *N. meningitidis*.[61] Indeed, NadA seems to be linked to isolates belonging to the ST-32 clonal complex (mainly serogroup B) and ST-11 and ST-8 clonal complexes (hyperinvasive isolates that are mainly serogroup C but rarely serogroup B). This observation is of interest as antibodies against NadA may allow targeting of serogroup B isolates belonging to the ST-11 and ST-8 lineages that mismatch with the other components of the vaccine.[53,61]

SBA assays are still indispensable to the vaccine licensure. However, in order to overcome the heavy and complex SBA assays, high throughput laboratory methods should be developed to reliably link polymorphism/the level of expression of the vaccine candidates to bactericidal antibodies. The Meningococcal Antigen Typing System (MATS) was therefore developed to determine the presence, the diversity, the level of expression of the proteins included in the 4CMenB vaccine, and to estimate the potential coverage by the vaccine of the circulating isolates. MATS is a vaccine antigen-specific ELISA assay which can detect quantitative differences in the expressed antigens relative to a reference strain. MATS predicts killing (at ≥80% probability) of an isolate by SBA if the relative expression level value

(also called relative potency [RP]) of this isolate for any of the three vaccine antigens (NadA, fHbp, and NHBA) is higher than a threshold called the positive bactericidal threshold (PBT).[62] A collection of 124 serogroup B isolates from five European countries, the USA, Australia, and New Zealand were analyzed for both their level of expression by MATS and their killing by SBA. When pooled sera from 7-month-old infants given three doses of Bexsero/4CMenB were analyzed, 83% of strains, showing at least one antigen with a RP above the PBT, were killed and 73% at or below the PBT were not killed.[62] Works are now in progress to predict the coverage rate in large collections of currently circulating isolates of *N. meningitidis* of serogroup B in Australia, Europe, and the USA.

Other vaccines targeting meningococci B

While 4CMenB is the leading vaccine against meningococci B, other vaccines targeting this serogroup are under development. One of these vaccines consists of two variants of fHbp (under development by Pfizer). fHbp shows high levels of diversity among isolates.[60] However, fHbp induced bactericidal antibodies against strains of different phenotypes.[63] Combining two variants of this protein in the vaccine aims to produce cross immune reactivity against fHbp proteins of a large majority of isolates of serogroup B. A recent study presented data from a Phase II trial in adolescents with this bivalent vaccine containing two variants of fHbp (one representative of each subfamily) and concluded on good safety and immunogenicity profiles.[64] However, this vaccine still needs to be evaluated in children and infants. The SBA analysis used eight isolates that represent the most frequent alleles of fHbp. It is noteworthy here that as for the 4CMenB vaccine, the SBA prediction of killing of isolates was linked to the level of surface expression of fHbp.[65] It is therefore crucial to select isolates for immunogenicity studies that are relevant to the ongoing epidemiology of the disease, to evaluate the level of expression of fHbp and to correlate these levels of expression with bacterial killing.

The fHbp bivalent vaccine was well tolerated in adolescents and local pain was the most frequent reported reaction. However, one related case of anaphylactic reaction requires further exploration.[64,66]

Concluding remarks

The development of vaccines based on recombinant proteins and targeting meningococci B is a kind of quantum leap in meningococcal vaccine development and the effort to control serogroup B meningococcal disease. The main point is to use a "cocktail" of several components to achieve strain coverage and enhanced protection.[67] Reverse vaccinology should also allow vaccine development against other microbial agents and therefore enhance the control of infectious diseases. In December 2010, a Marketing Authorization Application for Bexsero/4CMenB was submitted in Europe and in other countries.[68] The dossier included clinical and epidemiological data supporting the safety profile and immunogenicity of Bexsero/4CMenB. Regulatory action is not yet undertaken but may be expected by the end of 2012 or early 2013. Nevertheless, many questions remain to be answered:

- What is the anticipated effectiveness of Bexsero/4CMenB in different countries or regions? The incidence of the serogroup B varies from country to country. Genetically diverse isolates may circulate in different regions. Implementation of recommendations will depend on country-specific incidence of serogroup B disease but also on the level of coverage of the circulating isolates by the vaccine. The recommendations may therefore vary from a routine use in the calendar to a targeted use of the vaccine to control outbreaks and clusters. Therefore, future immunogenicity studies should address the issue of the choice of the strains for hSBA that should reflect epidemiological distribution.
- Bexsero/4CMenB targets many antigens shared by the meningococcal isolates irrespective of their serogroup. Thus the effect of the vaccine on transmission, carriage, and prevention of disease caused by other serogroups (non-B serogroups) needs to be evaluated. This point is of interest in countries applying national strategies against other serogroups. The Bexsero/4CMenB vaccine may offer a potential unique strategy against meningococcal disease in counties where the incidence of serogroup C is now low after routine vaccination against *N. meningitidis* of serogroup C.
- Additionally, under future selective pressure, the emergence of escape variants should be monitored as isolates expressing variants of the antigens included in the vaccine might not be covered. The implementation of MATS approach may be required in different areas to monitor such escape variants and evaluate whether the vaccine requires "updating" according to the circulating isolates.

Finally, the persistence of protective SBA titers beyond 6 months after the third dose needs to be defined. This point is of interest notably for infants and young children where the incidence of the disease is the highest. It is also required to determine whether further booster doses are needed.

Disclosure

Conflict of interest: the laboratory of the authors conducts collaborations with Novartis and Pfizer on strain characterization related to vaccine coverage with no involvement in clinical trials.

References

1. Yazdankhah SP, Caugant DA. Neisseria meningitidis: an overview of the carriage state. *J Med Microbiol*. 2004;53(Pt 9):821–832.
2. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. *N Engl J Med*. 2001;344(18):1378–1388.
3. Bilukha OO, Rosenstein N; for National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). Prevention and control of meningococcal disease. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2005;54(RR-7):1–21.
4. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine*. June 24, 2009;27 Suppl 2:B51–B63.
5. National Advisory Committee on Immunization (NACI). An update on the invasive meningococcal disease and meningococcal vaccine conjugate recommendations. An Advisory Committee Statement (ACS). *Can Commun Dis Rep*. 2009;35(ACS-3):1–40.
6. Harrison LH, Pass MA, Mendelsohn AB, et al. Invasive meningococcal disease in adolescents and young adults. *JAMA*. 2001;286(6):694–699.
7. Harrison LH. Prospects for vaccine prevention of meningococcal infection. *Clin Microbiol Rev*. 2006;19(1):142–164.
8. Borg J, Christie D, Coen PG, Booy R, Viner RM. Outcomes of meningococcal disease in adolescence: prospective, matched-cohort study. *Pediatrics*. 2009;123(3):e502–e509.
9. European Centre for Disease Prevention and Control. *Surveillance of Invasive Bacterial Diseases in Europe 2008/2009*. Stockholm, Sweden: ECDC; 2011.
10. Harrison LH. Epidemiological profile of meningococcal disease in the United States. *Clin Infect Dis*. 2010;50 Suppl 2:S37–S44.
11. Al-Tawfiq JA, Clark TA, Memish ZA. Meningococcal disease: the organism, clinical presentation, and worldwide epidemiology. *J Travel Med*. 2010;17 Suppl:3–8.
12. Dull P, Rosenstein N. Meningococcal disease and vaccines. *Pediatr Ann*. 2001;30(6):358–361.
13. Stephens DS. Conquering the meningococcus. *FEMS Microbiol Rev*. 2007;31(1):3–14.
14. Rosenstein NE, Perkins BA, Stephens DS, et al. The changing epidemiology of meningococcal disease in the United States, 1992–1996. *J Infect Dis*. 1999;180(6):1894–1901.
15. Cartwright K, Noah N, Peltola H; for Meningococcal Disease Advisory Boad. Meningococcal disease in Europe: epidemiology, mortality, and prevention with conjugate vaccines. Report of a European advisory board meeting Vienna, Austria, October 6–8, 2000. *Vaccine*. 2001;19(31):4347–4356.
16. Bhattacharjee AK, Jennings HJ. Determination of the linkages in some methylated, sialic acid-containing, meningococcal polysaccharides by mass spectrometry. *Carbohydr Res*. 1976;51(2):253–261.
17. Bhattacharjee AK, Jennings HJ, Kenny CP, Martin A, Smith IC. Structural determination of the sialic acid polysaccharide antigens of Neisseria meningitidis serogroups B and C with carbon 13 nuclear magnetic resonance. *J Biol Chem*. 1975;250(5):1926–1932.
18. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med*. 1969;129(6):1307–1326.
19. Borrow R, Balmer P, Miller E. Meningococcal surrogates of protection – serum bactericidal antibody activity. *Vaccine*. 2005;23(17–18):2222–2227.
20. van Deuren M, Brandtzaeg P, van der Meer JW. Update on meningococcal disease with emphasis on pathogenesis and clinical management. *Clin Microbiol Rev*. 2000;13(1):144–166.
21. Grabenstein JD, Pittman PR, Greenwood JT, Engler RJ. Immunization to protect the US Armed Forces: heritage, current practice, and prospects. *Epidemiol Rev*. 2006;28:3–26.
22. Tyler KL. Chapter 28: a history of bacterial meningitis. *Handb Clin Neurol*. 2010;95:417–433.
23. Brundage JF, Ryan MA, Feighner BH, Erdtmann FJ. Meningococcal disease among United States military service members in relation to routine uses of vaccines with different serogroup-specific components, 1964–1998. *Clin Infect Dis*. 2002;35(11):1376–1381.
24. Gotschlich EC, Goldschneider I, Artenstein MS. Human immunity to the meningococcus. IV. Immunogenicity of group A and group C meningococcal polysaccharides in human volunteers. *J Exp Med*. 1969;129(6):1367–1384.
25. Ada G. Vaccines and vaccination. *N Engl J Med*. 2001;345(14):1042–1053.
26. Goldblatt D. Recent developments in bacterial conjugate vaccines. *J Med Microbiol*. 1998;47(7):563–567.
27. Poolman J, Borrow R. Hyporesponsiveness and its clinical implications after vaccination with polysaccharide or glycoconjugate vaccines. *Expert Rev Vaccines*. 2011;10(3):307–322.
28. Tan LK, Carlone GM, Borrow R. Advances in the development of vaccines against Neisseria meningitidis. *N Engl J Med*. 2010;362(16):1511–1520.
29. Wyle FA, Artenstein MS, Brandt BL, et al. Immunologic response of man to group B meningococcal polysaccharide vaccines. *J Infect Dis*. 1972;126(5):514–521.
30. Finne J, Bitter-Suermann D, Goridis C, Finne U. An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. *J Immunol*. 1987;138(12):4402–4407.
31. Eckhardt M, Mühlenhoff M, Bethe A, Koopman J, Frosch M, Gerardy-Schahn R. Molecular characterization of eukaryotic polysialyltransferase-1. *Nature*. 1995;373(6516):715–718.
32. Holst J, Martin D, Arnold R, et al. Properties and clinical performance of vaccines containing outer membrane vesicles from Neisseria meningitidis. *Vaccine*. 2009;27 Suppl 2:B3–B12.
33. Holst J, Feiring B, Naess LM, et al. The concept of "tailor-made", protein-based, outer membrane vesicle vaccines against meningococcal disease. *Vaccine*. 2005;23(17–18):2202–2205.
34. Granoff DM. Review of meningococcal group B vaccines. *Clin Infect Dis*. 2010;50 Suppl 2:S54–S65.
35. Tappero JW, Lagos R, Ballesteros AM, et al. Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines: a randomized controlled trial in Chile. *JAMA*. 1999;281(16):1520–1527.
36. Poolman JT. Development of a meningococcal vaccine. *Infect Agents Dis*. 1995;4(1):13–28.
37. Tondella ML, Popovic T, Rosenstein NE, et al. Distribution of Neisseria meningitidis serogroup B serosubtypes and serotypes circulating in the United States. The Active Bacterial Core Surveillance Team. *J Clin Microbiol*. 2000;38(9):3323–3328.
38. Oster P, O'Hallahan J, Aaberge I, Tilman S, Ypma E, Martin D. Immunogenicity and safety of a strain-specific MenB OMV vaccine delivered to under 5-year olds in New Zealand. *Vaccine*. 2007;25(16):3075–3079.
39. Caron F, du Châtelet IP, Leroy JP, et al. From tailor-made to ready-to-wear meningococcal B vaccines: longitudinal study of a clonal meningococcal B outbreak. *Lancet Infect Dis*. 2011;11(6):455–463.
40. Nøkleby H, Aavitsland P, O'Hallahan J, Feiring B, Tilman S, Oster P. Safety review: two outer membrane vesicle (OMV) vaccines against systemic Neisseria meningitidis serogroup B disease. *Vaccine*. 2007;25(16):3080–3084.
41. de Kleijn ED, de Groot R, Labadie J, et al. Immunogenicity and safety of a hexavalent meningococcal outer-membrane-vesicle vaccine in children of 2–3 and 7–8 years of age. *Vaccine*. 2000;18(15):1456–1466.

42. de Kleijn ED, de Groot R, Lafeber AB, et al. Immunogenicity and safety of monovalent p1.7(h),4 meningococcal outer membrane vesicle vaccine in toddlers: comparison of two vaccination schedules and two vaccine formulations. *Vaccine*. 2000;19(9–10):1141–1148.
43. Cartwright K, Morris R, Rümke H, et al. Immunogenicity and reactogenicity in UK infants of a novel meningococcal vesicle vaccine containing multiple class 1 (PorA) outer membrane proteins. *Vaccine*. 1999;17(20–21):2612–2619.
44. Bambini S, Rappuoli R. The use of genomics in microbial vaccine development. *Drug Discov Today*. 2009;14(5–6):252–260.
45. Rappuoli R. Reverse vaccinology, a genome-based approach to vaccine development. *Vaccine*. 2001;19(17–19):2688–2691.
46. Su EL, Snape MD. A combination recombinant protein and outer membrane vesicle vaccine against serogroup B meningococcal disease. *Expert Rev Vaccines*. 2011;10(5):575–588.
47. Tettelin H, Saunders NJ, Heidelberg J, et al. Complete genome sequence of Neisseria meningitidis serogroup B strain MC58. *Science*. 2000;287(5459):1809–1815.
48. Giuliani MM, Adu-Bobie J, Comanducci M, et al. A universal vaccine for serogroup B meningococcus. *Proc Natl Acad Sci U S A*. July 18, 2006; 103(29):10834–10839.
49. Toneatto D, Ismaili S, Ypma E, Vienken K, Oster P, Dull P. The first use of an investigational multicomponent meningococcal serogroup B vaccine (4CMenB) in humans. *Hum Vaccin*. 2011;7(6):646–653.
50. Toneatto D, Oster P, deBoer AC, et al. Early clinical experience with a candidate meningococcal B recombinant vaccine (rMenB) in healthy adults. *Hum Vaccin*. 2011;7(7):781–791.
51. Holst J, Feiring B, Fuglesang JE, et al. Serum bactericidal activity correlates with the vaccine efficacy of outer membrane vesicle vaccines against Neisseria meningitidis serogroup B disease. *Vaccine*. 2003;21(7–8):734–737.
52. Snape MD, Dawson T, Oster P, et al. Immunogenicity of two investigational serogroup B meningococcal vaccines in the first year of life: a randomized comparative trial. *Pediatr Infect Dis J*. 2010;29(11):e71–e79.
53. Findlow J, Borrow R, Snape MD, et al. Multicenter, open-label, randomized phase II controlled trial of an investigational recombinant Meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy. *Clin Infect Dis*. 2010; 51(10):1127–1137.
54. Gossger N, Snape MD, Yu LM, et al. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. *JAMA*. 2012;307(6):573–582.
55. Prymula R, Siegrist CA, Chlibek R, et al. Effect of prophylactic paracetamol administration at time of vaccination on febrile reactions and antibody responses in children: two open-label, randomised controlled trials. *Lancet*. 2009;374(9698):1339–1350.
56. Santolaya ME, O'Ryan ML, Valenzuela MT, et al. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. *Lancet*. 2012; 379(9816):617–624.
57. Whittle HC, Abdullahi MT, Fakunle FA, et al. Allertic complications of meningococcal disease. I. Clinical aspects. *Br Med J*. 1973;2(5869):733–737.
58. Cohn AC, Messonnier NE. Inching toward a serogroup B meningococcal vaccine for infants. *JAMA*. 2012;307(6):614–615.
59. Brehony C, Wilson DJ, Maiden MC. Variation of the factor H-binding protein of Neisseria meningitidis. *Microbiology*. 2009;155(Pt 12): 4155–4169.
60. Murphy E, Andrew L, Lee KL, et al. Sequence diversity of the factor H binding protein vaccine candidate in epidemiologically relevant strains of serogroup B Neisseria meningitidis. *J Infect Dis*. 2009;200(3):379–389.
61. Comanducci M, Bambini S, Brunelli B, et al. NadA, a novel vaccine candidate of Neisseria meningitidis. *J Exp Med*. 2002;195(11):1445–1454.
62. Donnelly J, Medini D, Boccadifuoco G, et al. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proc Natl Acad Sci U S A*. 2010;107(45):19490–19495.
63. Fletcher LD, Bernfield L, Barniak V, et al. Vaccine potential of the Neisseria meningitidis 2086 lipoprotein. *Infect Immun*. 2004; 72(4):2088–2100.
64. Richmond PC, Marshall HS, Nissen MD, et al. Safety, immunogenicity, and tolerability of meningococcal serogroup B bivalent recombinant lipoprotein 2086 vaccine in healthy adolescents: a randomised, single-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis*. May 4, 2012. [Epub ahead of print.]
65. Jiang HQ, Hoiseth SK, Harris SL, et al. Broad vaccine coverage predicted for a bivalent recombinant factor H binding protein based vaccine to prevent serogroup B meningococcal disease. *Vaccine*. 2010;28(37):6086–6093.
66. Taha MK, Deghmane AE. Vaccines targeting serogroup B meningococci. *Lancet Infect Dis*. May 4, 2012. [Epub ahead of print.]
67. Zollinger WD, Poolman JT, Maiden MC. Meningococcal serogroup B vaccines: will they live up to expectations? *Expert Rev Vaccines*. 2011;10(5):559–561.
68. Gorringe AR, Pajón R. Bexsero: A multicomponent vaccine for prevention of meningococcal disease. *Hum Vaccin Immunother*. 2012;8(2):174–183.