

The long and winding road towards a MenB vaccine with broad strain coverage

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Review paper

The Minister of Health for the UK was advised by an expert committee meeting in February 2014 to include a new, broad-spectrum meningococcal serogroup B (MenB) vaccine, 4CMenB (Bexsero[®]) into the childhood immunization program. This new vaccine which recently received regulatory approval in Europe, Canada and Australia combines a conventional wild-type outer membrane vesicle (wtOMV) vaccine and antigens identified through reverse vaccinology. Strain coverage estimates from different parts of the world are in the range of 70% to 90%, depending on the local epidemiological situation. Following implementation of this vaccine, monitoring should focus on effectiveness data for various circulating strains and potential vaccine effects on carriage and herd immunity. From use of this new MenB vaccine on a larger scale and good monitoring in UK and other countries that are likely to follow shortly, the international vaccine community will learn a number of lessons. Such insights will be important for further improvement towards later generations of MenB vaccines and other protein-based vaccines against various diseases. Herein sights gained from more than 35 years of development and use of MenB vaccines are presented. The novel vaccine, 4CMenB represents a new time horizon in protein-based vaccine formulation, evaluation and value. Importantly, 4CMenB was developed with "cutting edge" joined with conventional vaccine technology, including experience from previous wtOMV vaccines, which have been successfully used since the late 1980s to prevent clonal outbreaks. Data from large clinical studies and retrospective statistical analyses give effectiveness estimates of at least 70% and a consistent pattern of moderate reactogenicity during the use of >80 million doses of three different wtOMV vaccine formulations. The key limitation of these wtOMV vaccines is the immunodominant response against the hypervariable PorA protein (especially in infants) and their likely inability to control disease in a population where the circulating strains are highly diverse. In New Zealand from 2004 to 2008, the wtOMV vaccine MeNZB[®] was used to control a clonal MenB epidemic. This public health intervention provided a number of new insights regarding international and public-private collaboration, vaccine safety surveillance, vaccine effectiveness-estimation and communication to the public. Thus, 4CMenB marks a new paradigm and represents the use of historical knowledge at the same time. Finally, the world now has the possibility to use a vaccine which is designed to give more comprehensive protection in epidemiological situations where circulating strains are very heterogeneous with respect to the genetic and antigenic properties. The historical integration of knowledge represented by 4CMenB will also prove important for other vaccine development in the time to come.

Dug i trnovit put prema sveobuhvatnom cjepivu protiv meningokoka grupe B

Pregledni rad

Stručno povjerenstvo je na sastanku u veljači 2014. godine savjetovalo ministra zdravlja Ujedinjenog Kraljevstva da u imunizacijski program za djecu uključi novo sveobuhvatno cjepivo za meningokoknu serogrupu B (MenB), 4CMenB (Bexsero[®]). Ovo novo cjepivo, koje je nedavno službeno odobreno u Europi, Kanadi i Australiji, kombinira konvencionalno cjepivo dobiveno iz vanjske membrane vezikula divljeg tipa meningokoka (wtOMV) i antigene koji su identificirani kroz reverznu vakcinologiju. Procjena obuhvaćenosti sojeva meningokoka iz različitih dijelova svijeta ovim cjepivom je između 70% i 90%, ovisno

o lokalnoj epidemiološkoj situaciji. Nakon provedene primjene ovog cjepiva, treba usmjeriti promatranje na podatke o učinkovitosti za različite cirkulirajuće sojeve i potencijalne učinke cjepiva na kliconoštvo i stečeni imunitet. Dio međunarodne zajednice koja se bavi cjepivima naučit će brojne lekcije iz šire upotrebe ovog novog MenB cjepiva i dobrog praćenja djelotvornosti cjepiva u Ujedinjenom Kraljevstvu kao i ostalim zemljama koje bi uskoro mogle slijediti ovaj primjer. Takve spoznaje bit će važne za daljnje poboljšanje kasnijih generacija MenB cjepiva i ostalih cjepiva protiv raznih bolesti koja se baziraju na proteinima. Rad donosi nove spoznaje stečene tijekom 35 godina razvoja i upotrebe MenB cjepiva. Novo cjepivo, 4CMenB, predstavlja novu epohu u formulaciji, procjeni i vrijednosti cjepiva koja se baziraju na proteinima. Značajno je spomenuti da je 4CMenB razvijeno spajanjem "cutting edge" i konvencionalne cjepne tehnologije, uključujući iskustva od ranijih wtOMV cjepiva koja se uspješno koriste od 1980-ih u svrhu prevencije klonalnih epidemija. Podaci velikih kliničkih studija i retrospektivne statističke analize daju procjenu učinkovitosti od najmanje 70 % i

dosljedno prikazuju umjerene reaktogenosti cjepiva tijekom uporabe od >80 milijuna doza za tri različite formulacije wtOMV cjepiva. Glavno ograničenje ovih wtOMV cjepiva je imunodominantni odgovor prema hipervarijabilnom PorA proteinu (posebno u djece) i vjerojatna nemogućnost da se bolest kontrolira u populaciji gdje postoje različiti cirkulirajući sojevi. Na Novom Zelandu je u razdoblju od 2004. do 2008. godine korišteno wtOMV cjepivo MeNZB[®] kako bi se kontrolirala klonalna MenB epidemija. Javnnozdravstvena intervencija je pružila mnogo novih spoznaja u pogledu međunarodne i javno-privatne suradnje, nadzora sigurnosti cjepiva, procjene učinkovitosti cjepiva i komunikacije s javnošću. Stoga, 4CMenB obilježava novu paradigmu i predstavlja korištenje povijesnog znanja istodobno. Konačno, svijet sada ima mogućnost koristiti cjepivo koje bi trebalo biti namijenjeno sveobuhvatnijoj zaštiti u epidemiološkim situacijama gdje su cirkulirajući sojevi vrlo heterogeni u pogledu genskih i antigenskih osobina. Povijesna integracija znanja koju predstavlja 4CMenB će se pokazati važnom i za razvoj drugih cjepiva u budućnosti.

Introduction

A vaccine against serogroup B has long been considered as part of the 'final frontier' of meningococcal disease prevention [1–3]. Recently, a novel vaccine, 4CMenB, was recommended by the Joint Committee of Vaccines and Immunisation (JCVI) in the UK as an addition to the routine childhood immunization schedule [4, 5]. This article discusses the history and lessons learned, as well as the difficulties surmounted to achieve this long sought-after goal.

Meningococcal septicemia and meningitis were feared even before the meningococcus was identified in the early nineteenth century [6–10]. Meningococcal infection is associated with rapid onset of severe disease, often following initial mild unspecific symptoms, and can often result in high case fatality rates or permanent disability. Hence there is a high level of anxiety concerning the possibility for epidemic disease caused by virulent clones or more sporadic, endemic incidences, which can occur suddenly in otherwise healthy individuals [1, 11–14]. Before the antibiotic era, the mortality rate was 70–90 %, and this has remained between 5 and 15 % despite the advent of modern antibiotics and advanced intensive hospital care [5, 9, 13, 15]. Permanent disabilities affect approximately 10–20 % of survivors [16, 17]. Case fatality rates are higher than average in patients with septicaemia, during epidemics, among adolescents and the elderly [18–20]. Infants and children under five years of age are most commonly affected by invasive meningococcal disease, and adolescents are also vulnerable to the disease especially during epidemic waves [12, 21]. Traditionally, the seasonal outbreaks of serogroup A disease in the Sub-Saharan "meningitis belt" in Africa has been considered to have the most significant global impact [22].

Starting in the late twentieth century, routine vaccination against meningococcal disease has become increas-

ingly widespread. This was possible because of important advances in technology, including the principle of using the capsular polysaccharide as a vaccine antigen, which was discovered and developed by Drs. Emil C. Gotschlich, Irvin Goldschneider and colleagues at the Walter Reed Army Institute of Research, USA, in the late 1960s [23, 24]. Their efforts produced highly effective vaccines against serogroup A and C disease. Serogroup Y and W vaccines were developed using the same strategy, and a quadrivalent ACYW polysaccharide vaccine was licensed in 1981 [25, 26]. Although these relatively inexpensive vaccines are effective, they have some important limitations. They employ T-cell independent antigens, which do not induce immunological memory, are in general not effective in children below 2 years of age and may induce hyporesponsiveness after multiple immunizations [27–29]. In the early 21st century, safe and effective conjugate vaccines against serogroups A, C, Y and W were introduced in a number of countries to protect all age groups by 2004 [30–34] and a low-cost conjugate vaccine against serogroup A disease was developed for use in Africa a few years later [15, 35, 36]. Hence, control of meningococcal serogroup B (MenB) became the remaining challenge for the overall prevention of meningococcal disease worldwide [22, 30].

The story of vaccine development against MenB disease has been long, complicated and full of challenges [2, 3, 37, 38]. Due to molecular mimicry between the MenB capsular structure [$\alpha(2\rightarrow8)$ -linked N-acetylneuraminic acid residues or polysialic acid] and glycoproteins in human tissue (especially in fetal, neural structures as, the neural cell-adhesion molecule, N-CAM) the MenB capsule is non-immunogenic [39, 40]. Should a vaccine formulation successfully break this immunologic tolerance, the scientific community and the regulators have been afraid that it might lead to auto-immunologic damages [41,

42]. Thus, for MenB vaccine development, sub-capsular structures have been the choice as vaccine candidates [2, 3]. Several wild-type outer membrane vesicle (wtOMV) vaccines have been successfully used to control clonal MenB outbreaks [43, 44].

Progress in Meningococcal Vaccine Development

A graphical representation of the development of meningococcal vaccines with broad strain coverage can be seen in Figure 1. Vaccines that use the capsular polysaccharide as the target cover all organisms with the same chemically and immunologically defined capsule, which is generally designated as the serogroup. In contrast, protein-based vaccines against MenB consist of a combination of selected antigens that aim for broad strain coverage. For decades, the "tailor-made" wtOMV vaccines against particular outbreak strains were the only vaccines with documented efficacy and effectiveness against MenB disease [2, 3, 45]. Vaccines based on the OMV concept were pioneered during the 1970s by Dr. Wendell D. Zollinger of Walter Reed Army Institute of Research, USA, Dr. Torstein B. Helting of Behringwerke, Germany and Dr. Carl E. Fraschof of the US Food and Drug Administration, USA and their coworkers [43, 46–51]. The research activity in these, and other laboratories, led to the development of two vaccine formulations for clinical protection trials in Cuba and Norway in the late 1980s [43]. Since they were designed to target specific epidemic strains, there was no expectation that they would be suitable for general use [43, 45, 52].

The initial wtOMV vaccine in general use was VA-MENGOCOC-BC[®], developed at the Finlay Institute in Cuba [53]. The second wtOMV formulation was MenBvac[®], developed at the Norwegian Institute of Public Health (NIPH). Efficacy estimates of 83 % and 57 % were found for the Cuban and Norwegian trials in adolescents, respectively [45, 53, 54]. The major difference between the efficacy estimates in the two trials was due to a longer observation period for the Norwegian trial (29 months versus 16 months in Cuba). Reanalysing the Norwegian clinical data for a 10-month observation period, following a two-dose schedule, showed 87 % efficacy [43, 45, 55]. A separate immunogenicity trial in Norway confirmed that adding a booster dose about one year after the primary immunization resulted in better persistence of protective antibodies, thus potentially providing longer lasting protection and greater effectiveness [43, 45, 55–57].

Two immunogenicity and reactogenicity trials [58, 59] sponsored by the Ministry of Health in Iceland, the US Centers for Disease Control and Prevention (CDC), the World Health Organization (WHO) and the Pan-American Health Organization (PAHO) compared the Cuban and Norwegian wtOMV MenB vaccines. In the two studies performed (one in Reykjavik, Iceland and the other in Santiago, Chile), both vaccines induced good functional immune responses as measured in a serum bactericidal activity test, using human complement (hSBA) against the respective, homologous MenB strains that were the basis for the vaccines. Neither of the two vaccines gave a sufficiently convincing immune response against heterologous MenB strains (i.e. strains with a different PorA serosubtype). When considering the MenB epidemic in Chile (on-

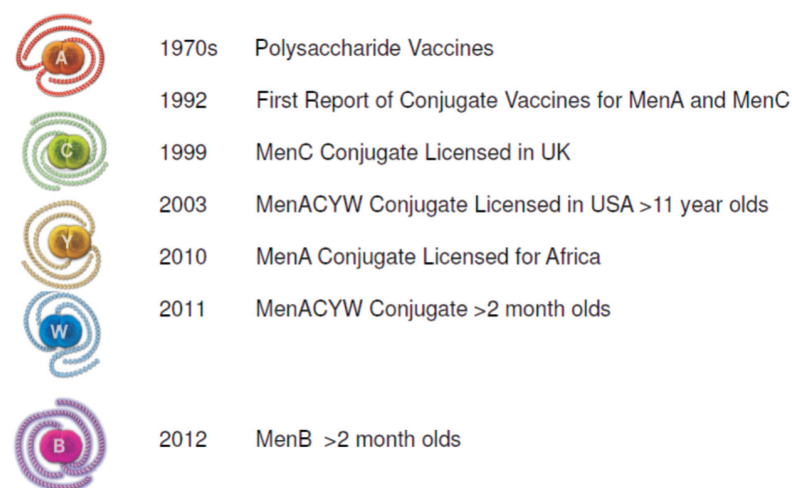


Figure 1. A dream coming through; in 2012 it became possible to prevent meningococcal disease caused by all major serogroups. (Note that the first wtOMV vaccines against MenB disease came in 1988/89 giving mainly serosubtype specific protection.)

Slika 1. Ostvarenje sna; 2012. godine postalo je moguće spriječiti meningokoknu bolest uzrokovanu najčešćim serogrupama meningokoka (treba naglasiti da su prva wtOMV cjepiva protiv bolesti uzrokovane MenB iz 1988/89 pružala uglavnom zaštitu protiv određenih subtipova)

going at that particular time), the monitoring committee judged that neither of the wtOMV vaccines would impact the local MenB clonal epidemic, especially not in infants, because the causative organism was heterologous to both vaccine strains. Immune responses to wtOMV vaccines in infants are largely directed towards the PorA protein; only about 10 % of infants mounted a protective antibody response against the Chilean epidemic strain following vaccination with either the Cuban or Norwegian wtOMV vaccine [59]. In contrast, approximately half of adult vaccinees had a protective antibody response against the Chilean epidemic strain after either of the two wtOMV vaccines, indicating broader immune response and thus, a less restricted protection in this age group [59]. Reassuringly, both wtOMV vaccines demonstrated good functional immunity; approximately 98 %, against their respective vaccine production strain in infants and older age groups, which suggested that [43, 59] a protein based, "tailor-made" vaccine for a defined clonal outbreak was likely to be successful in all age groups [43, 60]. Another important lesson from these pioneering clinical trials was that primary immunization with two doses of a wtOMV vaccine is likely to be insufficient to maintain long term protection against MenB disease [43, 45, 55].

Further Development and Use of "Tailor-Made" Vaccines

In 1991, a substantial clonal MenB outbreak was acknowledged in New Zealand [61]. This outbreak was later found to be caused by a strain with a PorA protein that was heterologous to that in the Cuban and Norwegian wtOMV vaccines. The magnitude and ongoing nature of this outbreak made it necessary to develop a new wtOMV vaccine [61–64]. The MeNZB[®] vaccine, which was based on a typical isolate, strain NZ98/254, from the clonal outbreak in New Zealand [65–69], was used between 2004 and 2008 to limit the MenB epidemic.

The experience from New Zealand is particularly important in the context of MenB vaccine development because extensive safety and effectiveness evaluations were undertaken in more than one million vaccine recipients [44]. In the present review, lessons learned during the development and use of wtOMV vaccines and the significant role that the experience played in the formulation of a multi-component MenB vaccine with broad strain coverage is summarized. Particular emphasis should be given to the history of MeNZB[®] where public health intervention was used to fight the devastating MenB epidemic occurring in New Zealand from the early 1990s to mid-2000s [44].

Since control of the epidemic was the primary objective of the MeNZB[®] program, vaccine effectiveness was assessed in an observational manner. Initial effectiveness, estimated using two different methodologies, was 80 % (95 % CI 52.5–91.6 %) for children 6 months to less than 5

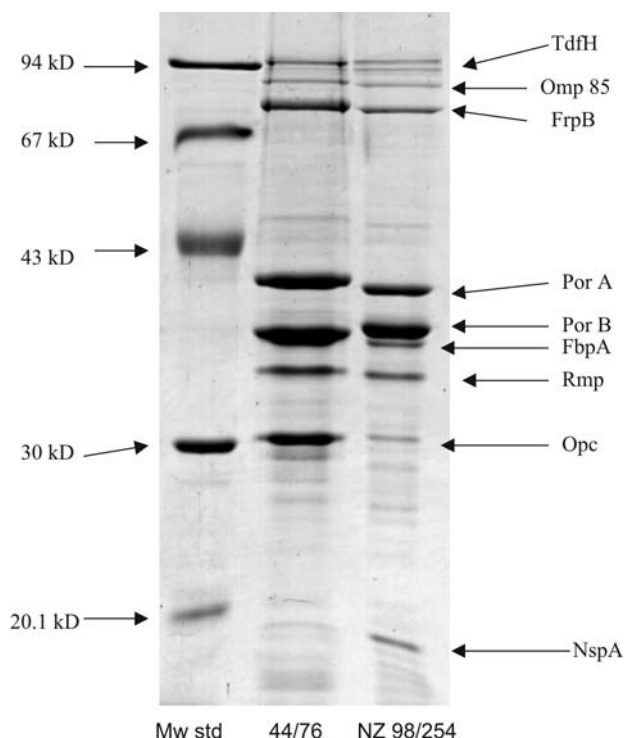


Figure 2. Main Protein Composition of wtOMVs from MenBvac[®] (44/76) and MeNZB[®] (NZ 98/254), visualized by CBB staining after SDS-PAGE. (Please note that Rmp and Opc is synonymous with "class 4" and "class 5" proteins, respectively; indicated in Fig. 4.)

Slika 2. Proteinski sastav wtOMVs u MenBvac[®] (44/76) i MeNZB[®] (NZ 98/254), prikazan bojanjem s CBB pomoću SDS-PAGE (Rmp i Opc su sukladni proteinima "klase 4" i "klase 5", kao što je označeno na Slici 4.)

years of age [68] and 73 % (95 % CI 52–85 %) for all ages, [68, 70–72]. However, since this was a large-scale introduction rather than a clinical trial, interpretation of effectiveness was complicated by secular disease trends. In an analysis of disease prior to the vaccine campaign in 2004 showed a steady decrease in incidence between 2001 and 2004, which accelerated following implementation of the vaccination program, indicating a vaccine effect [71]. Arnold and colleagues estimated overall vaccine effectiveness using Poisson-regression models adjusted for year, age, season, region, ethnicity and socioeconomic status [71]. They also tested for a relationship between the number of doses and effectiveness, and for possible waning effectiveness one year after vaccination. Their approach allowed the vaccine program effect to be differentiated from a secular decrease in disease incidence. Arnold *et al.* estimated vaccine effectiveness of 77 % (95 % CI 62–85 %) over an average period of 3.2 years following the three-dose primary series, but only 68 % when potential residual confounding was considered. In partially vaccinated individuals, effectiveness was estimated to be 47 % (95 % CI 16–67 %) after two doses of MeNZB[®]. No evidence of waning protection after one year with the full three-dose

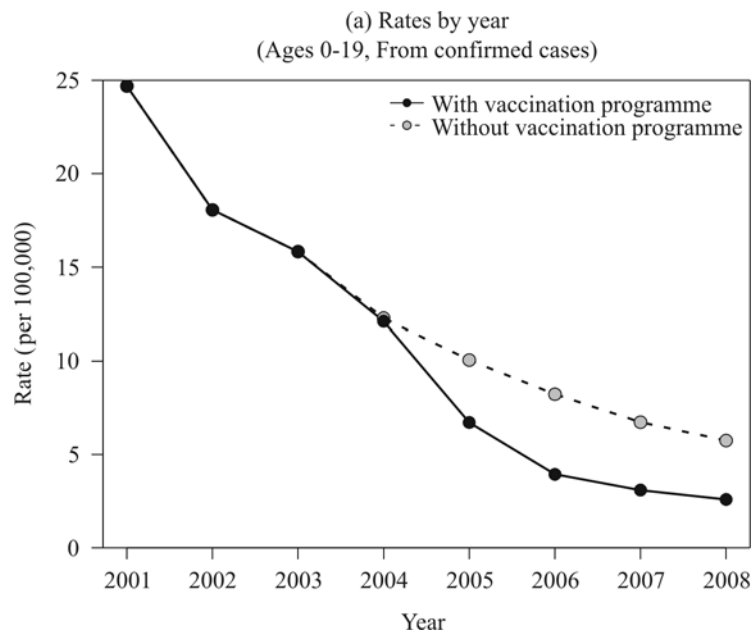


Figure 3. Rates of Meningococcal B Disease in New Zealand. (Adopted from a Figure originally designed by Richard Arnold, New Zealand; and presented in a slightly different format in [44])

Slika 3. Incidencija meningokokne bolesti grupe B u Novom Zelandu (preuzeta i djelomično promijenjena originalna slika autora Richarda Arnolda, Novi Zeland [44])

immunization series could be detected [71]. The adjustments for residual confounding resulted from a test for "protection" against pneumococcal disease by MeNZB[®]. An observed dose-response relationship in the level of protection (not attributed to the vaccine itself) was interpreted as a combination of program effects and some degree of residual confounding [71]. The correlation of protection with the number of doses further supports the conclusion that the observed effectiveness is vaccine related.

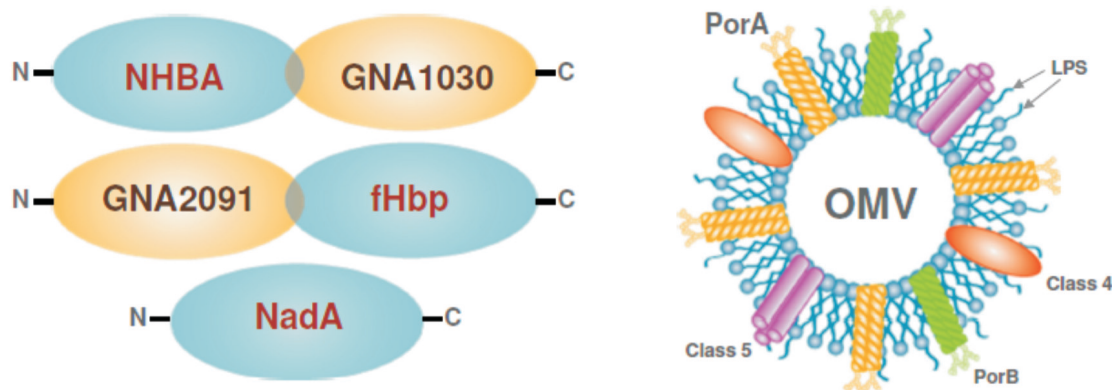
Figure 3 illustrates the decline of the MenB disease among the vaccinated and unvaccinated parts of the population below 20 years of age in New Zealand. As can be seen from the graph, the epidemic was on decline before the vaccine campaign started. However, a significantly more rapid decline was demonstrated among the vaccinated individuals. The effect of introducing the vaccine appeared even more dramatic; analysing the cumulative cases of meningococcal disease over the years from 2002 to 2010 in the Northern region (having the highest incidence, and it was also here where the vaccination started). Within a year the drop in meningococcal cases was significant and by 2007 it was down to pre-epidemic rates in that region [44].

It is worth noting that some protection was also observed against MenB strains other than the outbreak clone (i.e. non-P1.4) with an effectiveness of 54 % (41 % including the correction for potential residual confounding). Since the wtOMV component is not serogroup- (i.e. capsular polysaccharide) specific, effectiveness was also calculated against meningococcal disease caused by additional

serogroups where effectiveness was found to be 56 %, (or 43 % corrected for residual confounding) [71, 72]. These observations are important when considering the role of the New Zealand strain wtOMV in the new multi-component vaccine 4CMenB. Another recent experience is the regional use of MenBvac[®], originally designed for Norway, in the Normandy district in France [52, 73–76]. This unique undertaking provided new data to expand the use of the OMV concept [73].

Combining "Cutting-Edge" Technology with Conventional Vaccinology

To provide broad strain coverage against the substantial diversity of MenB organisms circulating worldwide, vaccine candidates require well-conserved antigens and a combination of multiple surface proteins, which can induce bactericidal antibodies against a majority of circulating strains [77]. Inspired by whole genome sequencing of *Haemophilus influenzae* in 1995 by Dr. J. Craig Venter and colleagues at TIGR [78], Dr. Rino Rappuoli and the research group at Chiron Vaccines in Siena (later Novartis Vaccines) started on the endeavour of sequencing the whole genome of one particular MenB strain (MC58). From the digitally available genome there could be searched for potential vaccine candidates *in silico*; clone these proteins in *E. coli* and immunize mice, search for expression by flow cytometry and study the capacity of specific antibodies to kill meningococcus (bactericidal activity). This approach was called "reverse vaccinology" [79–



■ 4CMenB is a suspension for injection

Dose	NHBA- GNA1030	fHbp- GNA2091	NadA	OMV	Al ³⁺
0.5 ml	50 µg	50 µg	50 µg	25 µg	0.5 mg

Figure 4. The Composition of 4CMenB, Bexsero[®]. Two recombinant fusion proteins and one single recombinant vaccine antigen (NadA); the three main active components visualized red letters on a blue background. The fourth active ingredient is the wtOMV manufactured from the strain NZ 98/254 with PorA P1.4 as the immunodominant protein. The final vaccine is formulated with Al-hydroxide as an adjuvant; making it a colloidal suspension.

Slika 4. Sastav cjepiva 4CMenB, Bexsero[®]. Dva rekombinantna fuzijska proteina i jedan rekombinantni cjepni antigen (NadA); prikazane su tri glavne aktivne komponente uz četvrti aktivni sastojak wtOMV proizveden iz soja NZ 98/254 imunodominantnog proteina PorA P1.4. Cjepivo je napravljeno kao koloidna suspenzija dodatkom Al-hidroksida kao adjuvanta.

81]; this in contrast to the classical search for vaccine candidates where the investigation starts with specific antibodies and/or the vaccine candidate itself. Through reverse vaccinology, the research team in Siena discovered a number of new and previously unknown vaccine candidates that, in only a few years, surpassed the efforts of conventional vaccine research by the previous three decades [82–85]. Initially, seven proteins were identified as promising vaccine candidates and later, a recombinant vaccine was formulated with three main active ingredients: Neisserial adhesin A, (NadA), factor H-binding protein (fHbp) and Neisserial heparin-binding antigen (NHBA) [15, 86–88]. The two latter proteins were manufactured as fusion proteins with genome-derived Neisserial antigen (GNA) 2091 and GNA1030, respectively, to impart greater stability and immunogenic properties [89]. The three active components, NadA, fHbp, NHBA have been identified as important virulence factors; NadA being an adhesin, fHbp a lipoprotein binding the complement regulating protein, human factor H, with substantial importance for survival of the bacteria in blood. Finally, NHBA has demonstrated properties for binding heparin or highly sulphated glycosaminoglycan analogues and other highly negative charged biomolecules (anions). The full extent of the pathophysiology of NHBA is under investigation. Various studies have provided data showing importance for adhesion and survival in the human bloodstream [90]. The whole story of vaccine development for 4CMenB has

been thoroughly described by Drs. Marzia M. Giuliani, Mariagrazia Pizza and Davide Serruto in various publications [89, 91–93].

Early preclinical and clinical data for the properties and performance of the three recombinant antigens showed substantial promise. However, it became evident that the strength and breath of immunogenicity could be improved with the addition of OMVs [94, 95]. A formulation where the recombinant proteins were combined with OMVs from strain NZ 98/254 (the active ingredient in MeNZB[®], with PorA P1.4 as the immunodominant protein), performed much better than the recombinant proteins alone [94, 95]. The choice with the addition of MeNZB[®] was fortunate since this type of strain (cc41/44), with fHbp-1.14 in general seems to be more difficult to kill with antibodies raised by the recombinant proteins [89, 94, 95]. The reason for this is possibly due to a low degree of expression and surface availability of the three antigens. For fHbp some sophisticated studies illustrating this have been done by the research group led by Dr. Dan M. Granoff at CHORI, Oakland (CA), USA; showing that antibodies raised against fHbp modular group I (for example fHbp-1.1 as in the Novartis vaccine) are not very effective in killing bacteria with fHbp modular group IV (for example fHbp-1.14 or fHbp-1.55). This effect is most pronounced when the strain tested in hSBA is a low or medium expresser of fHbp [96, 97]. For the main components and formulation of 4CMenB, Bexsero[®]; see Figure 4.

The 4CMenB vaccine was granted Marketing Authorization by the European Medicines Agency in January 2013 and later in Canada and Australia [87, 89, 91, 98]. In April 2014, 4CMenB received FDA Breakthrough Therapy designation in the US, and in June 2014 Novartis submitted an application for 4CMenB to help protect US adolescents and young adults [99, 100]. After MenB outbreaks at Princeton University and University of California Santa Barbara [101, 102], nearly 30,000 doses of Bexsero[®] were distributed among students and staff under an Investigational New Drug (IND) designation in the period from December 2013 to April 2014 [100]. Estimates for global strain coverage performed by the meningococcal antigen typing system (MATS) for 4CMenB, vary from about 70 % to over 90 %, depending on the regional epidemiologic situation [103], a substantial improvement from the wtOMV vaccines (about 50 % in adults and less than 10 % among infants against some heterologous strains) [44, 59]. The recently developed method, MATS is a way to measure the degree of total expression for each of the three recombinant vaccine antigens (NadA, fHbp and NHBA) and cross reactive variants of these proteins [104]. The MATS assay can be seen as a correlate to the well-established bactericidal activity test with human complement (hSBA), that for a long time has been accepted as a correlate of protection [23, 24, 105, 106]. How well 4CMenB really performs clinically and in real life awaits practical use and prospective effectiveness studies. Of note, MATS evaluation accounts separately for each individual antigen, which eliminates any accounting for synergistic effects between antibodies against different antigens. In some preclinical studies, such an effect has been shown (cooperation between anti-fHbp and anti-NHBA antibodies) [107, 108]. How these observations translate into clinical performance of the 4CMenB among ordinary population groups worldwide, only post-licensure evaluations and implementation studies can tell.

Introducing a New Vaccine in Current Childhood Immunization Programs

In developed countries, especially the UK, a MenB vaccine has been high on the priority list since the control of serogroups A, C, W and Y by conjugate vaccines [109, 110]. After decimation of MenC disease, 90 % of the cases in the UK are MenB and this disease remains the last challenge in the area of meningococcus (as in most of the other European countries). In the past 20 years, no other infectious disease has claimed more lives than meningococcal disease in the UK. Currently there are between 600 to 1,400 cases each year in England and Wales, generally in infants less than 6 months of age [111]. Although 4CMenB has the desired broad strain coverage, a substantial hesitation to implement the vaccine in the routine childhood immunization has so far dominated the situation.

The precedence of using cost effectiveness studies started with the introduction of pneumococcal conjugate vaccines in the US, early in the 21st century [112]. In June 2013 the Joint Committee on Vaccination and Immunisation (JCVI) in UK presented a preliminary, non-favourable advice to the Ministry of Health of introducing the 4CMenB vaccine [113]. A number of doubts and problems were presented, of which unfavourable cost-effectiveness estimation was judged as the most important issue. This interim JCVI recommendation started a large debate in newspapers and scientific publications [112, 114–120]. Various aspects of the estimation were discussed, including the role of economic evaluation in such decision processes, the lack of sophistication for the cost-effectiveness models *per se* and the relative accuracy of several parameters involved in the calculations.

Various stakeholders were invited to submit more data and arguments, which were evaluated and discussed by JCVI in February 2014 [121]. Key points from the updated recommendations are: introduction of 4CMenB in the childhood immunization program with a "2+1" schedule at 2, 4 and 12 months. The vaccine is planned to be given together with the ordinary childhood vaccines. Infants who have passed their 2 and 4 month visits will receive one dose at 6 months and the booster dose at 12 months of age. No other "catch-up" program will be offered [4, 122]. It is important to note that a prerequisite for the proposed introduction of 4CMenB may be negotiations about the price with the manufacturer. Involved parties are aiming for a start of vaccination using 4CMenB by the autumn 2014 [111].

Important points to note include:

- i) Strain coverage is estimated to be 88 % for UK based on the hSBA of 40 UK strains, not the 72 % predicted by MATS [103, 123], which is known to underestimate strain coverage [123].
- ii) Vaccine efficacy is defined to be 90 %, with duration of 18 months after the two primary immunizations and 36 months following the booster. Duration of 10 years protection might be expected if the vaccine is to be used among adolescents; however, only infants are included in the current plan because of a lack of cost effectiveness in older persons.
- iii) The issue of possible influence on carriage and herd immunity is currently under discussion. A recent carriage study in UK by Dr. Robert Read *et al.* (Lancet 2014, in press) and data from the use of MenBvac[®] in Normandy, France [76] suggest a possible impact. Reliable carriage data and the true effect on herd immunity would require implementation of the vaccine in the population, followed by specific studies and overall good surveillance.
- iv) The current cost-effectiveness models were questioned, particularly the value of vaccines for serious diseases with a fairly low incidence. It was decided to

establish a working group for study of some of these aspects in greater detail.

Discussion

Over the past 50 years, our understanding has evolved regarding the importance of MenB disease *per se*, the social impact of fear caused by the devastating effects of the disease and the role of OMV vaccines in providing protection. We now know that wtOMV-based vaccines are most effective when used against epidemics due to a homologous or clonal outbreak caused by bacteria carrying the same PorA as present in the vaccine. When used against endemic disease or outbreaks due to a number of different strains, (a heterologous epidemiologic situation), the level of effectiveness will generally be too low to rely on a conventional wtOMV vaccine alone for protection. Multiple doses of these vaccines are required for primary protection and a booster dose is needed to assure long term protection, especially in those who receive an initial vaccine series as young infants.

More than 6,000 cases and around 250 deaths were caused by meningococcal disease in New Zealand between 1991 and 2006, with approximately 80 % of cases due to the epidemic clone targeted by the MeNZB[®] vaccine [64, 124]. However, following the concerted efforts of an extensive international and national collaboration, including the WHO, Chiron and NIPH; a vaccine, the MeNZB[®] was developed to control this specific outbreak. A substantial national mobilization in New Zealand, involving complex logistics, monitoring and various communication exercises were successfully carried out to handle the public health challenge represented by the particular meningococcal epidemic. The mass vaccination campaign that started in July 2004 and ended in June 2006, targeted the population below 20 years of age (approximately 1.2 million persons) resulted in a vaccine uptake of 81 % [68, 70, 71]. It has been estimated for the period between July 2004 and December 2008 that 210 cases, six deaths and 15–30 cases of severe sequelae were avoided thanks to the MeNZB[®] vaccine [71].

The New Zealand epidemic was waning before and during the roll-out of MeNZB[®]. However, the staggered introduction of the vaccine enabled year-by-year comparison of rates in vaccinated and unvaccinated populations that allowed estimating the effectiveness of the vaccine. Simultaneous modeling of invasive pneumococcal disease and the clonal outbreak strain of MenB disease suggests a degree of residual confounding that reduces the effectiveness estimate from 77 % to 68 % [71]. Following the (cumulative) number of MenB cases in the area with the highest incidence in New Zealand (the northern region) from 2002 to 2010 also demonstrate the vaccine impact from one year (2004) to the next (2005) [44]. There was also

found some evidence for (lesser) cross-protection against other MenB strains [71]. This observation is consistent with the findings of Dr. Jordan Tappero *et al.* in Santiago, Chile where they found an age-dependent, but clear functional immune response (hSBA) against non-vaccine type strains [59].

The extensive general experience with wtOMV vaccines, and in particular the thorough evaluation of MeNZB[®] in more than one million individuals, provides vital information regarding the safety and acceptability of wtOMV vaccines for widespread use. By the end of 2013 more than 80 million doses of the wtOMV vaccine type have been administered worldwide [20, 125]. Although these vaccines are moderately reactogenic, in New Zealand local and systemic reactions such as fever were common, but predictable and transient; moreover did not interfere with widespread acceptance of vaccination. A very effective education program to inform parents and recipients regarding the nature of these events likely contributed to the high levels of public acceptance of this vaccine.

Unlike MeNZB[®], which was designed to provide protection against a clonal outbreak, 4CMenB was formulated to provide protection against the majority of circulating MenB strains, which enable it to be used for routine immunization in various regions of the world. The three recombinant protein components, active in this vaccine, were identified through a process called reverse vaccinology, starting with the bacterial genome instead of microbial pathogenicity factors inducing dominant immune responses in convalescents. A multi-component vaccine approach was considered necessary for MenB because of the labile nature of the meningococcal genome, differences in protein sequences and surface expression among various MenB strains for the proteins selected as vaccine antigens. The intrinsic ability of the meningococcus to change both through recombination and variability in the degree of surface expression of proteins creates a situation in which any single component vaccine, even if effective initially, would likely become ineffective over time as meningococcus could adapt and become resistant to that particular vaccine. A multi-component strategy severely reduces the ability of the organism to circumvent all antibodies elicited by the vaccine. Based on these observations and insights the novel multi-component vaccine 4CMenB contains four major active ingredients, including the same wtOMV as used in MeNZB[®]. It is important to recognize that this is a new class of vaccine, which has employed reverse vaccinology in the design of a vaccine against MenB disease suitable for more general use.

Apart from the implications of the MeNZB[®] experience for newer OMV containing vaccines such as 4CMenB, the program also provides a number of other important broadly applicable public health lessons. Key factors that contributed to the success of the program were the

willingness of New Zealand and international parties to collaboratively support the goal of epidemic control. Lengthy negotiations and discussions built trust and understanding between parties. Those leading the project from the New Zealand government and Chiron Vaccines were given enough autonomy to enable timely progress. The overall process from recognition of the outbreak in New Zealand, to the final implementation of the vaccine program was much faster than the normal process of vaccine development and introduction. Despite this, it took several years, and some critics have indicated the need to act more expediently during similar situations in the future [126]. One key lesson might be that all countries should be prepared, with regulatory mechanisms already in place, to anticipate the possible rapid evaluation and introduction of a new vaccine? The recent H1N1 influenza pandemic is a case in point, but this lesson might be especially relevant for developing countries where new vaccines for malaria, typhoid and other diseases for which no prior experience in Europe or the US exists, will become available. Such situations will also require local oversight and evaluation, active surveillance, adequate epidemiology and sufficient strain characterizations. In Cuba, Norway and New Zealand [45] the basis for using the concept of wtOMV vaccines was the selection of a manufacturing strain that matched the clone causing the epidemic. In each case, a measured approach to vaccine evaluation and introduction was undertaken. In considering approaches to any public health emergency, there will always be a tension between the need to introduce a new intervention quickly and the need to ensure that the intervention is safe and effective. The extent and success of the post-introduction evaluation in New Zealand could provide the impetus to develop protocols for earlier introduction of interventions for public health emergencies which are associated with contemporaneous evaluation, thus reducing the need for extensive, time consuming pre-introduction evaluations. This will most likely happen when the vaccine is of a type or form about which much is already known.

Learning Points

A number of learning points can be extracted from the long and challenging voyage towards a MenB vaccine with broad strain coverage. These points might also be used in other vaccine development programs in times to come.

1. Recombinant proteins are often inferior immunogens when compared to their native counterparts produced by pathogenic bacteria. Thus, a strong need for better adjuvants and/or more optimal ways of formulating vaccines continues to exist.
2. Preclinical immunization procedures and screening methods do not always translate 1:1 in the clinic (for

example, the proteins GNA1030 and GNA2091 that early on were shown to induce functional immunity, but later proved to contribute little to hSBA when sera from clinical trials were tested).

3. Even minor outer membrane proteins that might not be under strong immunological selection ("pressure") may vary much more than originally anticipated (for example, fHbp versus PorA; PorA has a long standing reputation of being "hypervariable" and antibodies induced by one fHbp variant do not cross-react as well as hoped for. This effect seems to be most pronounced in the case where the expression level is low to moderate and the difference is fairly large between the fHbp variant in the vaccine and the one harboured by the target strain used in the hSBA test).
4. For development of protein based bacterial vaccines, one is likely to reduce the risk for "escape mutants" by choosing the strategy of using a multicomponent vaccine; especially with a dynamic and adaptive organism as *N. meningitidis*.
5. Thorough epidemiological surveillance and strain characterization is essential on a global level. Access to well-curated strain collections is paramount for proper vaccine evaluation.
6. Proper and well justified evaluation and judgement of the actual value of a vaccination program requires continuing scrutiny and improvement of the models used for evaluating them.
7. It is a constant and growing need for a balanced and respectful collaboration between private industry (as vaccine developers) and various public, governmental bodies (as responsible for disease surveillance, implementation of vaccination programs and vaccine evaluation from the perspective of society).

Conclusion

Meningococcal wtOMV vaccines have been employed for decades and administered to millions of individuals. These vaccines have been effective and documented a well characterized and acceptable safety profile. The major limitation of these wtOMV vaccines is that their immune response provides protection mainly against strains that are homologous (i.e. harbouring the same PorA, sero-subtype protein) to the outbreak strain used to develop the vaccine [45, 59]. This shortcoming has restricted the utility of wtOMV vaccines to large ongoing epidemics, and public health benefits have been limited due to the long delay in formulation. To address these concerns and make management of MenB disease a routine rather than an episodic event, a multi-component vaccine (4CMenB), which includes the wtOMV used in MeNZB[®], has recently been designed for widespread use and coverage against multi-

ple strains and diverse epidemiological situations globally [86, 87, 89, 91, 98]. Thus, even novel technologies in this field draw on previous experience with wtOMV vaccines. Additional knowledge and experience for use of the wtOMV concept can also be gleaned from the handling of a localized clonal outbreak in Normandy, France [52, 73, 74] and from preclinical and clinical studies using so-called native OMV vaccines; where the LPS has been genetically detoxified (*lpxI*-mutants), avoiding the need for detergent extraction and with over expressed vaccine antigens naturally folded in the membrane [127–133]. These different and promising vaccine approaches owe much to the pioneering experiences gained by using wtOMV vaccines, and in particular such large scale public health interventions as the one that took place in New Zealand with the MeNZB[®] vaccine.

The first universal MenB vaccine is about to be implemented in UK and in the coming years the whole vaccine community will learn numerous important lessons; also applicable to development and use of other protein-based bacterial vaccines against other diseases. How well 4CMenB will perform we do not know. Available data and a number of years with clinical experience give substantial hope for success. However, it is also evident that there are still room for improvement. The present vaccine is unlikely to protect against all circulating strains. Somewhere between 70 % and 90 % might be a reasonable guess for clinical straincoverage. Real sustainability of the protection in field situations and the true effect on carriage and herdimmunity will be evaluated as more information is gathered.

The introduction of 4CMenB marks a paradigm shift in vaccinology. It is the first protein-based vaccine against a bacterial disease that does not depend on some sort of toxin neutralization. In years to come, a number of similar vaccines will be implemented in various immunization programs [134, 135]. Thus, lessons learnt from the pioneering achievement will be paramount for making needed vaccines faster available to those who are in need.

The heated and extended discussion from the UK, following the interim JCVI decision from July 2013, brought out important and thoughtful arguments and views. One important example was Professor Steven Black's comment on the use of cost-effectiveness analyses as a "gating criteria" to decide which vaccines should be developed or routinely used; runs the risk of transforming vaccines into primarily "a tool for achieving cost savings within the health care system rather than a public health intervention targeting human suffering, death and disability" [112]. It is realistic to hope for an increased understanding of the true and comprehensive value of vaccines and vaccination following the recent debate in UK. From various initiatives it can also be anticipated an increased refinement in various models used for estimating cost effectiveness and a better understanding of the limitations of such calculations.

Over the past 20 years, particularly in the UK, tremendous amounts of effort and many resources have been spent on improving outcomes from meningitis and septicaemia by the government, scientists and health professionals by raising awareness and promoting early recognition of disease in addition to developing rapid life-saving procedures in hospitals. This great cooperative effort has been important and has made a difference. However, it has also become painfully evident that prevention by an effective vaccine is the only sustainable solution to the challenge posed by the devastating consequences of meningococcal disease. Even in situations like the current low incidence of MenB disease in countries like Norway and Croatia one might very well argue that a good and safe vaccine should be used. A new "tool" in the fight against meningococcal disease is now available and should be used in the best possible way to prevent the maximum amount of death and suffering in the future.

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Affiliations and disclosed potential conflicts of interest

JH: Employee of NIPH and have been performing consulting activities for Wyeth Vaccines Research (now Pfizer) and Novartis Vaccines and Diagnostics; in addition served as a temporary advisor on a number of occasions for WHO and PAHO.

Literature

- [1] Stephens DS. Conquering the meningococcus. *FEMS Microbiol Rev* 2007; 31: 3–14.
- [2] Granoff DM. Review of meningococcal group B vaccines. *Clin Infect Dis* 2010; 50 (Suppl 2): S54–65.
- [3] Sadarangani M, Pollard AJ. Serogroup B meningococcal vaccines—an unfinished story. *Lancet Infect Dis* 2010; 10: 112–24.
- [4] Pollard AJ, Riordan A, Ramsay M. Group B meningococcal vaccine: recommendations for UK use. *Lancet* 2014; 383: 1103–4.
- [5] Andrews SM, Pollard AJ. A vaccine against serogroup B *Neisseria meningitidis*: dealing with uncertainty. *Lancet Infect Dis* 2014; 14: 426–34.
- [6] Vieusseux M. Memoire sur le maladie qui a regne a Geneva au printemps de 1805. *J Med Clin Pharm* 1805; 11: 163–82.
- [7] Danielson L, Mann E. A history of a singular and very noted disease, which lately made its appearance in Medfield. *Medical and Agricultural Register* 1806; 1: 65–9.
- [8] Weichselbaum A. Ueber die aetiologie der akuten meningitis cerebro-spinal. *Fortschr Med* 1887; 5: 573–83, 620–6.
- [9] Flexner S. The results of the serum treatment in thirteen hundred cases of epidemic meningitis. *J Exp Med* 1913; 17: 553.
- [10] Greenwood M. The outbreak of cerebrospinal fever at Salisbury in 1914–15. *Proc Roy Soc Med* 1917; 10: 44–60.
- [11] Stephens DS. Uncloaking the meningococcus: dynamics of carriage and disease. *Lancet* 1999; 353: 941–2.
- [12] Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. *Lancet* 2007; 369: 2196–210.
- [13] de Souza AL, Seguro AC. Two centuries of meningococcal infection: from Vieusseux to the cellular and molecular basis of disease. *J Med Microbiol* 2008; 57: 1313–21.
- [14] Tyler KL. Chapter 28: a history of bacterial meningitis. *Handb Clin Neurol* 2010; 95: 417–33.
- [15] Sow SO, Okoko BJ, Diallo A, et al. Immunogenicity and safety of a meningococcal A conjugate vaccine in Africans. *N Engl J Med* 2011; 364: 2293–304.
- [16] Goldacre MJ, Roberts SE, Yeates D. Case fatality rates for meningococcal disease in an English population, 1963–98: database study. *BMJ* 2003; 327: 596–7.
- [17] Sorensen HT, Steffensen FH, Schonheyder HC, et al. Trend in incidence and case fatality of meningococcal disease over 16 years in Northern Denmark. *Eur J Clin Microbiol Infect Dis* 1998; 17: 690–4.
- [18] Pollard AJ, Frasch C. Development of natural immunity to *Neisseria meningitidis*. *Vaccine* 2001; 19: 1327–46.
- [19] Pollard AJ. Global epidemiology of meningococcal disease and vaccine efficacy. *Pediatr Infect Dis J* 2004; 23 (Suppl 12): S274–9.
- [20] Holst J, Nøkleby H, Bettinger JA. Considerations for Controlling Invasive Meningococcal Disease in High Income Countries. *Vaccine* 2012; 30 (Suppl. 2): B57–62
- [21] Gardner P. Clinical practice. Prevention of meningococcal disease. *N Engl J Med* 2006; 355: 1466–73.
- [22] Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. *N Engl J Med* 2001; 344: 1378–88.
- [23] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* 1969; 129: 1307–26.
- [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: 1367–84.
- [25] Hankins WA, Gwaltney JM, Jr., Hendley JO, Farquhar JD, Samuelson JS. Clinical and serological evaluation of a meningococcal polysaccharide vaccine groups A, C, Y, and W135. *Proc Soc Exp Biol Med* 1982; 169: 54–7.
- [26] McLeod Griffiss J, Brandt BL, Altieri PL, Pier GB, Berman SL. Safety and immunogenicity of group Y and group W135 meningococcal capsular polysaccharide vaccines in adults. *Infect Immun* 1981; 34: 725–32.
- [27] MacDonald NE, Halperin SA, Law BJ, Danzig LE, Granoff DM. Can meningococcal C conjugate vaccine overcome immune hyporesponsiveness induced by previous administration of plain polysaccharide vaccine? *JAMA* 2000; 283: 1826–7.
- [28] Harris SL, Finn A, Granoff DM. Disparity in functional activity between serum anticapsular antibodies induced in adults by immunization with an investigational group A and C *Neisseria meningitidis*-diphtheria toxoid conjugate vaccine and by a polysaccharide vaccine. *Infect Immun* 2003; 71: 3402–8.
- [29] Poolman J, Borrow R. Hyporesponsiveness and its clinical implications after vaccination with polysaccharide or glycoconjugate vaccines. *Expert Rev Vaccines* 2011; 10: 307–22.
- [30] Tan LK, Carlone GM, Borrow R. Advances in the development of vaccines against *Neisseria meningitidis*. *N Engl J Med* 2010; 362: 1511–20.
- [31] Lewis S, Sadarangani M, Hoe JC, Pollard AJ. Challenges and progress in the development of a serogroup B meningococcal vaccine. *Expert Rev Vaccines* 2009; 8: 729–45.
- [32] Gasparini R, Panatto D. Meningococcal glycoconjugate vaccines. *Hum Vaccin* 2011; 7: 170–82.
- [33] Broker M, Cooper B, Detora LM, Stoddard JJ. Critical appraisal of a quadrivalent CRM(197) conjugate vaccine against meningococcal serogroups A, C W-135 and Y (Menveo) in the context of treatment and prevention of invasive disease. *Infect Drug Resist* 2011; 4: 137–47.
- [34] Keyserling H, Papa T, Koranyi K, et al. Safety, immunogenicity, and immune memory of a novel meningococcal (groups A, C, Y, and W-135) polysaccharide diphtheria toxoid conjugate vaccine (MCV-4) in healthy adolescents. *Arch Pediatr Adolesc Med* 2005; 159: 907–13.
- [35] Bilukha OO, Rosenstein N. Centers for Disease Control and Prevention. Prevention and control of meningococcal disease. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2005 27; 54: 1–21.
- [36] LaForce FM, Okwo-Bele J-M. Eliminating epidemic group A meningococcal meningitis in Africa through a new vaccine. *Health Affairs* 2011; 30: 1049–57.
- [37] Wyle FA, Artenstein MS, Brandt BL, et al. Immunologic response of man to group B meningococcal polysaccharide vaccines. *J Infect Dis* 1972; 126: 514–21.
- [38] Bruge J, Bouveret-Le Cam N, Danve B, Rougon G, Schulz D. Clinical evaluation of a group B meningococcal N-propionylated polysaccharide conjugate vaccine in adult, male volunteers. *Vaccine* 2004; 22: 1087–96.

- [39] Lackie PM, Zuber C, Roth J. Polysialic acid and N-CAM localisation in embryonic rat kidney: mesenchymal and epithelial elements show different patterns of expression. *Development* 1990; 110: 933–47.
- [40] Rougon G, Dubois C, Buckley N, Magnani JL, Zollinger W. A monoclonal antibody against meningococcus group B polysaccharides distinguishes embryonic from adult N-CAM. *J Cell Biol* 1986; 103: 2429–37.
- [41] Finne J, Leinonen M, Makela PH. Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. *Lancet* 1983; 2: 355–7.
- [42] Finne J, Bitter-Suermann D, Goridis C, Finne U. An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. *J Immunol* 1987; 138: 4402–7.
- [43] Holst J, Feiring B, Nass LM, et al. The concept of "tailor-made", protein-based, outer membrane vesicle vaccines against meningococcal disease. *Vaccine* 2005; 23: 2202–5.
- [44] Holst J, Oster P, Arnold R, et al. Vaccines against meningococcal serogroup B disease containing outer membrane vesicles (OMV): – Lessons from past programs and implications for the future. *Hum Vaccin* 2013; 9: 1241–53.
- [45] 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): B3–12.
- [46] Helting TB, Guthohrlein G, Blackkolb F, Ronneberger H. Serotype determinant protein of *Neisseria meningitidis*. Large scale preparation by direct detergent treatment of the bacterial cells. *Acta Pathol Microbiol Scand C* 1981; 89: 69–78.
- [47] Tsai CM, Frasch CE, Mocca LF. Five structural classes of major outer membrane proteins in *Neisseria meningitidis*. *J Bacteriol* 1981; 146: 69–78.
- [48] Frasch CE. Meningococcal Vaccines: Past, Present and Future. In: Cartwright K, editor. *Meningococcal Disease*. New York: John Wiley & Sons, 1995: 245–83.
- [49] Frasch C, van Alphen L, Holst J, Poolman J, Rosenqvist E. Outer membrane protein vesicle vaccines for meningococcal disease. In: Pollard AJ, Maiden MC, editors. *Meningococcal vaccines: methods and protocols*. Totowa, New Jersey: Humana Press, 2001: 81–107.
- [50] Zollinger WD, Boslego J, Froholm LO, Ray JS, Moran EE, Brandt BL. Human bactericidal antibody response to meningococcal outer membrane protein vaccines. *Antonie Van Leeuwenhoek* 1987; 53: 403–11.
- [51] Zollinger WD, Mandrell RE, Altieri P, Berman S, Lowenthal J, Artenstein MS. Safety and immunogenicity of a *Neisseria meningitidis* type 2 protein vaccine in animals and humans. *J Infect Dis* 1978; 137: 728–39.
- [52] Taha MK, Zaranonelli ML, Alonso JM, et al. Use of available outer membrane vesicle vaccines to control serogroup B meningococcal outbreaks. *Vaccine* 2007; 25: 2537–8.
- [53] Sierra GV, Campa HC, Varcacel NM, et al. Vaccine against group B *Neisseria meningitidis*: protection trial and mass vaccination results in Cuba. *NIPH Ann* 1991; 14: 195–207; discussion 208–10.
- [54] Bjune G, Høiby EA, Grønnesby JK, et al. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. *Lancet* 1991; 338: 1093–6.
- [55] 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: 734–7.
- [56] Rosenqvist E, Høiby EA, Wedege E, et al. Human antibody responses to meningococcal outer membrane antigens after three doses of the Norwegian group B meningococcal vaccine. *Infect Immun* 1995; 63: 4642–52.
- [57] Feiring B, Fuglesang J, Oster P, et al. Persisting immune responses indicating long-term protection after booster dose with meningococcal group B outer membrane vesicle vaccine. *Clin Vaccine Immunol* 2006; 13: 790–6.
- [58] Perkins BA, Jonsdottir K, Briem H, et al. Immunogenicity of two efficacious outer membrane protein-based serogroup B meningococcal vaccines among young adults in Iceland. *J Infect Dis* 1998; 177: 683–91.
- [59] 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: 1520–7.
- [60] Wenger JD. Serogroup B meningococcal disease: new outbreaks, new strategies. *JAMA* 1999; 281: 1541–3.
- [61] Martin DR, Walker SJ, Baker MG, Lennon DR. New Zealand epidemic of meningococcal disease identified by a strain with phenotype B: 4: P1.4. *J Infect Dis* 1998; 177: 497–500.
- [62] O'Hallahan J, Lennon D, Oster P, et al. From secondary prevention to primary prevention: a unique strategy that gives hope to a country ravaged by meningococcal disease. *Vaccine* 2005; 23: 2197–201.
- [63] O'Hallahan J, Martin D, Oster P. An epidemic of group B meningococcal disease controlled by a vaccine – the final chapter. 15th International Pathogenic *Neisseria* Conference; 2006 September 10-15, 2006; Cairns, Australia; 2006. Abstract page 42.
- [64] Loring BJ, Turner N, Petousis-Harris H. MeNZB vaccine and epidemic control: when do you stop vaccinating? *Vaccine* 2008; 26: 5899–904.
- [65] Holst J, Aaberge IS, Oster P, et al. A "tailor made" vaccine trialled as part of public health response to group B meningococcal epidemic in New Zealand. *Eurosurveillance* 2003; 7: 030724 <http://www.eurosurveillance.org/ew/030724.asp#5>
- [66] Martin DR, Ruijine N, McCallum L, O'Hallahan J, Oster P. The VR2 epitope on the PorA P1.7-2,4 protein is the major target for the immune response elicited by the strain-specific group B meningococcal vaccine MeNZB. *Clin Vaccine Immunol* 2006; 13: 486–91.
- [67] Thornton V, Lennon D, Rasanathan K, et al. Safety and immunogenicity of New Zealand strain meningococcal serogroup B OMV vaccine in healthy adults: beginning of epidemic control. *Vaccine* 2006; 24: 1395–400.
- [68] Galloway Y, Stehr-Green P, McNicholas A, O'Hallahan J. Use of an observational cohort study to estimate the effectiveness of the New Zealand group B meningococcal vaccine in children aged under 5 years. *Int J Epidemiol* 2009; 38: 413–8.
- [69] Jackson C, Lennon DR, Sotutu VT, et al. Phase II meningococcal B vesicle vaccine trial in New Zealand infants. *Arch Dis Child* 2009; 94: 745–51.
- [70] Kelly C, Arnold R, Galloway Y, O'Hallahan J. A prospective study of the effectiveness of the New Zealand meningococcal B vaccine. *Am J Epidemiol* 2007; 166: 817–23.
- [71] Arnold R, Galloway Y, McNicholas A, O'Hallahan J. Effectiveness of a vaccination programme for an epidemic of meningococcal B in New Zealand. *Vaccine* 2011; 29: 7100–6.

- [72] McNicholas A, Galloway Y, Martin D, Sexton K, O'Hallahan J. Surveillance of vaccine breakthrough cases following MeNZB vaccination. *N Z Med J* 2008; 121: 38–46.
- [73] Caron F, du Chatelet 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: 455–63.
- [74] Caron F, Delbos V, Houivet E, et al. Evolution of immune response against *Neisseria meningitidis* B:14:P1.7,16 before and after the outer membrane vesicle vaccine MenBvac. *Vaccine* 2012; 30: 5059–62.
- [75] Delbos V, Lemee L, Benichou J, Berthelot G, Taha MK, Caron F. Meningococcal carriage during a clonal meningococcal B outbreak in France. *European journal of clinical microbiology & infectious diseases* : official publication of the European Society of Clinical Microbiology 2013; 32: 1451–9.
- [76] Delbos V, Lemee L, Benichou J, et al. Impact of MenBvac, an outer membrane vesicle (OMV) vaccine, on the meningococcal carriage. *Vaccine* 2013; 31: 4416–20.
- [77] Zollinger WD, Poolman JT, Maiden MC. Meningococcal serogroup B vaccines: will they live up to expectations? *Expert Rev Vaccines* 2011; 10: 559–61.
- [78] Fleischmann RD, Adams MD, White O, et al. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* 1995; 269: 496–512.
- [79] Tettelin H, Saunders NJ, Heidelberg J, et al. Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. *Science* 2000; 287: 1809–15.
- [80] Pizza M, Scarlato V, Masignani V, et al. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science* 2000; 287: 1816–20.
- [81] Nassif X. Microbiology. A furtive pathogen revealed. *Science* 2000; 287: 1767–8.
- [82] Rappuoli R. Reverse vaccinology. *Current opinion in microbiology* 2000; 3: 445–50.
- [83] Rappuoli R. Reverse vaccinology, a genome-based approach to vaccine development. *Vaccine* 2001; 19: 2688–91.
- [84] Rappuoli R. The application of reverse vaccinology, Novartis MenB vaccine developed by design. In: van Alphen L, van Ley P, van den Dobbelsteen G, editors. 16th International Pathogenic *Neisseria* Conference. Rotterdam, The Netherlands, 2008. Abstract O65, page 81.
- [85] Donati C, Rappuoli R. Reverse vaccinology in the 21st century: improvements over the original design. *Ann N Y Acad Sci* 2013; 1285: 115–32.
- [86] Bai XF, J, Borrow R. Recombinant protein meningococcal serogroup B vaccine combined with outer membrane vesicles. *Expert Opinion Biol Ther* 2011; 11: 969–85.
- [87] Su EL, Snape MD. A combination recombinant protein and outer membrane vesicle vaccine against serogroup B meningococcal disease. *Expert Rev Vaccines* 2011; 10: 575–88.
- [88] Bambini S, Muzzi A, Olcen P, Rappuoli R, Pizza M, Comanducci M. Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus. *Vaccine* 2009; 27: 2794–803.
- [89] Giuliani MM, Adu-Bobie J, Comanducci M, et al. A universal vaccine for serogroup B meningococcus. *Proc Natl Acad Sci U S A* 2006; 103: 10834–9.
- [90] Serruto D, Spadafina T, Ciocchi L, et al. *Neisseria meningitidis* GNA2132, a heparin-binding protein that induces protective immunity in humans. *Proc Natl Acad Sci U S A* 2010; 107: 3770–5.
- [91] Pizza M, DeTora L, Wassil J. Advances in meningococcal vaccines. *Clin Pract* 2012; 9: 101–17.
- [92] Pizza M, Donnelly J, Rappuoli R. Factor H-binding protein, a unique meningococcal vaccine antigen. *Vaccine* 2008; 26 (Suppl 8): 146–8.
- [93] Serruto D, Bottomley MJ, Ram S, Giuliani MM, Rappuoli R. The new multicomponent vaccine against meningococcal serogroup B, 4CMenB: immunological, functional and structural characterization of the antigens. *Vaccine* 2012; 30 (Suppl 2): B87–97.
- [94] 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: 1127–37.
- [95] Snape MD, Dawson T, Oster P, et al. Immunogenicity of two investigational serogroup B meningococcal vaccines in the first year of life: a randomized comparative trial. *Pediatr Infect Dis J* 2010; 29: e71–9.
- [96] Pajon R, Beernink PT, Harrison LH, Granoff DM. Frequency of factor H-binding protein modular groups and susceptibility to cross-reactive bactericidal activity in invasive meningococcal isolates. *Vaccine* 2010; 28: 2122–9.
- [97] Konar M, Granoff DM, Beernink PT. Importance of inhibition of binding of complement factor H for serum bactericidal antibody responses to meningococcal factor H-binding protein vaccines. *J Infect Dis* 2013; 208: 627–36.
- [98] Bai X, Findlow J, Borrow R. Recombinant protein meningococcal serogroup B vaccine combined with outer membrane vesicles. *Expert opinion on biological therapy* 2011; 11: 969–85.
- [99] Althoff E, Power L. Novartis Press Release "Novartis meningitis B vaccine Bexsero[®] receives FDA Breakthrough Therapy designation in the US". Novartis International AG 2014 April 7. <http://www.novartis.com/newsroom/media-releases/en/2014/1774805.shtml>
- [100] Althoff E, Power L. Novartis Press Release "Novartis submits application to the FDA for meningitis B vaccine candidate Bexsero[®] to help protect US adolescents and young adults". Novartis AG 2014 June 17. <http://www.novartis.com/newsroom/media-releases/en/2014/1793710.shtml>
- [101] Centers for Disease Control and Prevention. Princeton University Meningococcal Disease Outbreak. CDC Media Statement 2014 March 18. <http://www.cdc.gov/meningococcal/outbreaks/princeton.html>
- [102] Centers for Disease Control and Prevention. University of California, Santa Barbara Meningococcal Disease Outbreak. CDC Media Statement 2014 April. <http://www.cdc.gov/meningococcal/outbreaks/ucsb.html>
- [103] Vogel U, Taha MK, Vazquez JA, et al. Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. *Lancet Infect Dis* 2013; 13: 416–25.
- [104] 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: 19490–5.

- [105] Borrow R, Balmer P, Miller E. Meningococcal surrogates of protection--serum bactericidal antibody activity. *Vaccine* 2005; 23: 2222–7.
- [106] Borrow R, Carlone GM, Rosenstein N, et al. *Neisseria meningitidis* group B correlates of protection and assay standardization--international meeting report Emory University, Atlanta, Georgia, United States, 16-17 March 2005. *Vaccine* 2006; 24: 5093–107.
- [107] Beernink PT, Welsch JA, Bar-Lev M, Koeberling O, Comanducci M, Granoff DM. Fine antigenic specificity and cooperative bactericidal activity of monoclonal antibodies directed at the meningococcal vaccine candidate, factor H-binding protein. *Infect Immun* 2008; 76: 4232–40.
- [108] Vu DM, Wong TT, Granoff DM. Cooperative serum bactericidal activity between human antibodies to meningococcal factor H binding protein and Neisserial heparin binding antigen. *Vaccine* 2011; 29: 1968–73.
- [109] Campbell H, Borrow R, Salisbury D, Miller E. Meningococcal C conjugate vaccine: the experience in England and Wales. *Vaccine* 2009; 27 (Suppl 2): B20–9.
- [110] Donaldson L. Meningococcal ACWY and meningococcal B. 2007 Annual Report of the Chief Medical Officer On the State of Public Health, Department of Health UK 2008 (July) :38.
- [111] Wise J. Meningitis B vaccine to be introduced in UK after U turn on its cost effectiveness. *BMJ* 2014; 348: g2327.
- [112] Black S. The role of health economic analyses in vaccine decision making. *Vaccine* 2013; 31: 6046–9.
- [113] Joint Committee on Vaccination and Immunisation. JCVI interim position statement on the use of Bexsero meningococcal B vaccine in the UK, July 2013. Department of Health 2013. <https://www.gov.uk/government/publications/jcvi-interim-position-statement-on-the-use-of-bexsero-meningococcal-b-vaccine-in-the-uk>
- [114] Moxon R, Snape MD. The price of prevention: what now for immunisation against meningococcus B? *Lancet* 2013;382:369–70.
- [115] Mekalanos JJ. Vaccine economics: what price human life? *Science translational medicine* 2013; 5: 204ed16.
- [116] Head C. Immunisation against meningococcus B. *Lancet* 2013; 382: 935.
- [117] Taha MK. Immunisation against meningococcus B. *Lancet* 2013; 382: 936.
- [118] Rappuoli R. Immunisation against meningococcus B. *Lancet* 2013; 382: 935–6.
- [119] Holmes D. Report triggers quibbles over QALYs, a staple of health metrics. *Nat Med* 2013; 19: 248.
- [120] Holmes D. UK poised to make decision on 4CMenB vaccine. *The Lancet* 2014; 14: 192–3.
- [121] Joint Committee on Vaccination and Immunisation. JCVI Minute of the meeting on 11/12 February 2014. Department of Health 2014. <https://www.gov.uk/government/policy-advisory-groups/joint-committee-on-vaccination-and-immunisation>
- [122] Andrews SM, Pollard AJ. A vaccine against serogroup B *Neisseria meningitidis*: dealing with uncertainty. *Lancet Infect Dis* 2014; 14: 426–34.
- [123] Frosi G, Biolchi A, Lo Sapio M, et al. Bactericidal antibody against a representative epidemiological meningococcal serogroup B panel confirms that MATS underestimates 4CMenB vaccine strain coverage. *Vaccine* 2013; 31: 4968–74.
- [124] Martin D, Lopez L, Sexton K. The epidemiology of meningococcal disease in New Zealand in 2006. Report from Institute of Environmental Science and Research Ltd, Wellington 2007.
- [125] Sotolongo FP, Campa Huergo C, Casanueva VG, Diaz EMF, Valdespino IEC, Gotera NG. Cuban meningococcal BC Vaccine: experiences and contributions from 20 years of application. *MEDICC review* 2007; 9: 16–22.
- [126] Lennon D, Reid S, Stewart J, Jackson C, Crengle S, Percival T. Reducing inequalities with vaccines: New Zealand's MeNZB vaccine initiative to control an epidemic. *Journal of paediatrics and child health* 2012; 48: 193–201.
- [127] Koeberling O, Seubert A, Granoff DM. Bactericidal antibody responses elicited by a meningococcal outer membrane vesicle vaccine with overexpressed factor H-binding protein and genetically attenuated endotoxin. *J Infect Dis* 2008; 198: 262–70.
- [128] Pajon R, Fergus AM, Koeberling O, Caugant DA, Granoff DM. Meningococcal factor H binding proteins in epidemic strains from Africa: implications for vaccine development. *PLoS neglected tropical diseases* 2011; 5: e1302.
- [129] Fisseha M, Chen P, Brandt B, Kijek T, Moran E, Zollinger W. Characterization of Native Outer Membrane Vesicles from lpxL Mutant Strains of *Neisseria meningitidis* for Use in Parenteral Vaccination. *Infect Immun* 2005; 73: 4070–80.
- [130] Keiser PB, Biggs-Cicatelli S, Moran EE, et al. A phase I study of a meningococcal native outer membrane vesicle vaccine made from a group B strain with deleted lpxL1 and synX, over-expressed factor H binding protein, two PorAs and stabilized OpcA expression. *Vaccine* 2011; 29: 1413–20.
- [131] Zollinger WD, Babcock JG, Moran EE, et al. Phase I study of a *Neisseria meningitidis* liposomal vaccine containing purified outer membrane proteins and detoxified lipooligosaccharide. *Vaccine* 2012; 30: 712–21.
- [132] Moran EE, Burden R, Labrie JE, 3rd, et al. Analysis of the bactericidal response to an experimental *Neisseria meningitidis* vesicle vaccine. *Clin Vaccine Immunol* 2012; 19: 659–65.
- [133] van der Ley P, van den Dobbelaars G. Next-generation outer membrane vesicle vaccines against *Neisseria meningitidis* based on nontoxic LPS mutants. *Hum Vaccin* 2011 Aug; 7(8): 886–90.
- [134] Delany I, Rappuoli R, Seib KL. Vaccines, reverse vaccinology, and bacterial pathogenesis. *Cold Spring Harb Perspect Med* 2013; 3: a012476.
- [135] Delany I, Rappuoli R, De Gregorio E. Vaccines for the 21st century. *EMBO Mol Med* 2014; 6: 708–20.