Review Article

Indian J Med Res 138, December 2013, pp 835-846

New versus old meningococcal Group B vaccines: How the new ones may benefit infants & toddlers

D. Panatto, D. Amicizia, PL. Lai, ML. Cristina, A. Domnich & R. Gasparini

Department of Health Sciences, University of Genoa, Genoa, Italy

Received June 17, 2013

Invasive disease caused by *Neisseria meningitidis* is associated with high mortality and high disability rates and mainly affects children under one year of age. Vaccination is the best way to prevent meningococcal disease, especially in infants and toddlers. The introduction of massive meningococcal serogroup C vaccination has drastically reduced the incidence of disease caused by this serogroup, and serogroup B has now become the main causative agent in several industrialized countries. The first serogroup B vaccines, which were used for more than two decades, were based on outer membrane vesicles and proved to be protective only against specific epidemic strains in Cuba, Norway, Brazil and New Zealand. Moreover, these often elicited a scant immune response in young children. Innovative genomics-based reverse vaccinology subsequently enabled researchers to identify genes encoding for surface proteins that are able to elicit a strong immune response against several B strains. This important discovery led to the development and recent approval in Europe of the four-component meningococcal serogroup B (4CMenB) vaccine. Large clinical trials have shown high immunogenicity and tolerability and acceptable safety levels of 4CMenB in infants and toddlers. This vaccine is expected to cover a large number of circulating invasive strains and may also be efficacious against other serogroups. Young children are particularly vulnerable to the devastating consequences of meningococcal disease. Given the high performance of 4CMenB and its non-interference with routine vaccinations, this age-group will be the first to benefit from the introduction of this vaccine.

Key words Four-component meningococcal serogroup B (4CMenB) vaccine - infants - meningococcal B vaccines - meningococcal disease - *Neisseria meningitides* serogroup B - outer membrane vesicle (OMV) vaccines - toddlers

Introduction

Neisseria meningitidis (Nm) is a major causative agent of invasive bacterial infections throughout the world[1]. The abrupt onset of meningococcal disease is associated with high mortality and long-term morbidity in spite of the availability of effective anti-meningococcal antibiotics[2]. Moreover, meningococcal disease is often misdiagnosed on admission to hospital because physicians have difficulty in identifying it[3].

Invasive meningococcal disease (IMD) mainly affects infants aged 3-12 months in whom case fatality rates (CFR) are high, followed by teenagers[4,5].

However, during epidemics, incidence rates can also rise among older children and young adults[5]. Other than age, risk factors include crowded living conditions, low socio-economic status, travel to highly endemic zones, frequenting crowded places such as pubs or discotheques, active or passive smoking, drug abuse, persistent complement component deficiencies (C5-C9, properdin, factors H and D), functional or anatomic asplenia and human immunodeficiency virus (HIV) infection[6,7].

The clinical presentation of IMD is usually classified as meningitis without septic shock (mortality rate 1-5%), meningitis associated to septic shock, and shock without meningitis, which is mostly associated with the development of *purpura fulminans* (Waterhouse-Friderichsen syndrome) and carries a mortality rate of up to 25 per cent[8].

IMD has a significant impact on public health, particularly in the developing world, owing to both its high mortality rates and the long-term disability that it can cause. It has been estimated that in a developing country such as Gambia, 2 per cent of all newborns will die of meningitis before the age of 5 yr and that up to 20 per cent of survivors will suffer permanent neurological sequelae such as epilepsy or mental retardation[4].

In developed countries, the impact of IMD is also considerable, particularly owing to the high costs engendered by permanent disabilities. About 11-19 per cent of subjects affected by IMD suffer permanent disabilities[9]. Sequelae may be neurological, physical and psychological[10]. Edmond *et al*[11] have estimated that the risk of sequelae in children aged less than 5 yr is twice as high as in other age groups.

The costs of IMD sequelae include at least the following components: treatment costs, the direct costs of caring for a disabled child, loss of productivity of parents or caregivers and the future loss of productivity of the disabled subject in adulthood. However, it is noteworthy that the financial burden that families incur in caring for disabled individuals is often unassessed or underestimated[11,12].

Until recently, there was no vaccine for the global prevention of meningococcal disease caused by serogroup B; conversely, vaccines against Nm of the four serogroups A, C, W-135 and Y have been licensed and widely used. The progress of vaccine development and the recent approval of a universal meningococcal B vaccine are expected to reduce cases of IMD and their sequelae drastically[5].

Epidemiology of meningococcal disease

One of the defining characteristics of invasive meningococcal disease is substantial cyclical fluctuation in its epidemiology. The high variability of the disease is in accordance with its geographical and serogroup distribution[13]. The traditional approach to classifying Nm is based on serological typing into at least thirteen groups [A, B, C, E-29, H, I, K, L, W-135, X, Y, Z and Z' (29E)] with distinct features in terms of immunological reactivity and structure of the capsular polysaccharide; however, only serogroups A, B, C, W-135, X and Y can cause life-threatening disease[14].

The world's highest incidence of meningococcal disease occurs in the Sahel and sub-Sahel African regions (the so-called "meningitidis belt"); in this biogeographic zone a large number of epidemics have been caused by serougroup A, accounting for nearly 200,000 cases in 1996[15]. To contain this dramatic epidemiologic situation, a group A conjugate vaccine specifically designed for Africa (MenAfriVac) has been developed[16]. The vaccine is highly efficacious; no cases caused by serogroup A have been recorded among vaccinees[16] and group A carriage among both vaccinated and unvaccinated people has disappeared, which indicates vaccine-induced herd immunity[17]. However, other serogroups also play an important role in the epidemiology of meningococcal disease in Africa, with outbreaks caused by serogroup C, W-135 and, more recently, X[18-20]. In Asia, serogroups A and C are responsible for the majority of cases; in some countries, other serogroups are playing an increasing role[21].

Endemic meningococcal infection in industrialized countries displays a relatively stable and sporadic background of incidence[22]. However, prolonged epidemics have also been described[23,24]. In Europe, the overall incidence of IMD diminished from 1.9 per 100,000 in 1999 to 0.73 per 100,000 in 2010, thanks to the widespread introduction of the Nm serogroup C (NmC) conjugate vaccine; Nm serogroup B (NmB) has, therefore, become by far the most frequent causative agent of the disease[25]. A similar serogroup distribution has also been reported in Australia and New Zealand[5]. Serogroup Y strains are relatively common in the United States (US) (accounting for more than 30% of cases) and other countries of the American continent[26].

Moreover, cases caused by this serogroup have increased in some parts of Europe[27].

Importance of carriage in the transmission of the disease

Nm has its own unique survival niche in humans[28]; it is considered a normal commensal of the upper respiratory tract, although it can lead to serious invasive disease. The reservoir of Nm is substantially constituted by healthy carriers. The proportion of carriers varies in the different stages of life; it is low among infants and school children, and then increases during adolescence and early adulthood. The relationship between carrier status and the development of IMD is a subject of research and is not yet fully understood[29].

Evaluating carriage is relevant to comprehending both the dynamics of carriage and disease and the potential effect of vaccination on the transmission of Nm. For example, the NmC Conjugate Vaccination Programme in the United Kingdom (UK) was successful because the vaccine not only protected against the disease, but was also able to prevent the acquisition of carriage, thereby enhancing herd immunity[30,31]. This explains how strengthening the immune system by means of appropriate vaccines can lead to marked reductions in the incidence of meningococcal disease[28,30].

Old meningococcal B vaccines (outer membrane vesicle vaccines)

Unlike other serogroups, NmB cannot be prevented by polysaccharide vaccines. The reason for this lies in the chemical structure of the NmB capsule, which contains α2-8–linked di- and trisyalosil units; these units are identical to some human polysaccharides and, therefore, determine immunological tollerance[32,33]. Consequently, research into an effective NmB vaccine has focused on subcapsular antigens, outer membrane vesicles (OMV) and individual antigens[34]. OMV are spherical particles with a diameter of 50-200 nm, and are released by many bacterial species. These vesicles contain a phospholipid bilayer with outer membrane proteins (OMP), lipopolysaccharide (LPS) and a lumen with periplasmic constituents[35]. Several proteins have been identified in the outer membrane, including the porin A protein (PorA), porin B protein (PorB), reduction-modifiable protein (Rmp), opacity-associated proteins (Opc and Opa), *Neisseria* surface protein A (NspA) and others. Some of these proteins, such Opc/Opa[36], NspA[37] and especially PorA[38,39], have been shown to induce protective antibodies and have, therefore, been considered as vaccine candidates, while others (for example, Rmp[40]) have not. However, it is important to note that the PorA protein is very heterogenic among NmB strains, a feature that has complicated further vaccine development[41].

Preparation of the first wild-type OMV vaccines included the process of detergent extraction, with the aims of removing lipopolysaccharide, which is highly toxic, and increasing vesicle release. However, it is important to note that approximately 1 per cent of lipopolysaccharide is needed to maintain the OMV structure and to adjuvate the immune response against PorA[42].

One of the most important successes of OMV vaccines was achieved in Cuba. The incidence of meningococcal disease in Cuba had been rising since 1976 and peaked between 1983 and 1984. During the epidemic, infants under one year of age were particularly affected and disease incidence in this age-class exceeded 120 per 100,000, the chief culprit being serogroup B[43]. In response to this epidemic, a candidate OMV vaccine was developed (VA-MENGOC-BC)[44]. The Cuban vaccine consisted of 50 μg of OMV from NmB (B:4:P1.19,15:L3,7,9 strain) and the same amount of the purified capsular polysaccharide of NmC (C11 strain) adsorbed on $Al(OH)_3$ gel. The vaccine contained thimerosal as a preservative[43].

A large-scale clinical trial (106,000 school children aged 10-14 yr) carried out between 1987 and 1989 demonstrated the high level of efficacy (83%) of a 2-dose schedule (0, 6-8 months). Another large trial among subjects aged 5 months-24 years in a high-incidence Cuban province confirmed the efficacy of the vaccine by comparing vaccinated and unvaccinated populations. In 1989, after the successful results of these trials, the Cuban Ministry of Public Health initiated an immunization programme targeting children aged 3 months-6 years in the most seriously affected provinces[45]. The effectiveness of the immunization programme was later confirmed by a significant decrease in the epidemic NmB strain among carriers[46]. Post-marketing surveillance of adverse events revealed a predominance of local over general reactions; serious adverse events were rare and accounted for less than 1 per 1 million doses administered[43]. However, in a Brazilian case-control study, the Cuban OMV vaccine displayed lower efficacy, especially among the youngest subjects: -37 per cent (95% CI: 100-73) in those under

24 months of age, 47 per cent (95% CI: 72-84) in those aged 24-47 months and 74 per cent (95% CI: 16-92) in those aged 48 months or older[47].

Another epidemic caused by NmB started in Norway in 1975[48] and compelled the Norwegian Institute of Public Health to develop another OMV vaccine. The vaccine (MenBvac) was prepared from strain 44/76 by means of fermenter growth and extraction of the bacteria with a detergent. OMVs were purified by ultracentrifugation and adsorbed on $Al(OH)_3$[49]. A double-blind, placebo-controlled, efficacy trial of this vaccine conducted among Norwegian secondary school students in 1988-91 found an efficacy of 57.2 per cent after two doses, a value which was not considered high enough to implement a national immunization campaign[38]. It was later established that three, rather than two, doses were needed to achieve long-lasting protective levels of serum bactericidal activity (SBA). After the third vaccine dose, the geometric mean titre of human-SBA (h-SBA) rose from 2.7 to 62.3[50]. Another placebo-controlled double-blind study, conducted among 374 Norwegian adolescents aged 12-17 yr, evaluated the safety and immunogenicity of the Norwegian vaccine when administered at 0, 6 and 12 wk and as a booster 10 months after the third dose. h-SBA titres of ≥4 against the vaccine strain were 53 per cent after the second dose, 65 per cent after the third and 93 per cent after the booster. Immunogenicity towards heterologous strains was also evaluated. For example, the vaccines showed similar bactericidal activities towards a French isolate of NmB (LNP20404) that contained the same PorA antigen as the vaccine strain, but differed from PorB. The vaccine was found to be safe, in that the majority of local and systemic reactions were mild or moderate in intensity[51].

The effectiveness of MenBvac was confirmed in Normandy (France), where it proved to be efficacious during an outbreak caused by a genetically close strain (B:14:P1.7,16). The disease incidence rate in vaccinees after three doses was 5.9, while in unvaccinated subjects it reached 31.6 per 100,000[52].

However, neither the Norwegian nor the Cuban OMV vaccines proved protective, especially in infants, during an epidemic caused by a heterologous strain, as was shown in a Chilean trial[53]. An OMV vaccine based on the Chilean epidemic strain 15:P1.3 was evaluated in Inquiqe (Chile) in 1992. This vaccine displayed 70 per cent efficacy among 5-21 year olds, while children under 5 yr remained unprotected[54]. This failure can be explained by the inadequate vaccination schedule, which comprised only two doses, the insufficient concentration of lipopolysaccharide, and the fact that the vaccine did not present its OMPs as proteoliposome vesicles[53].

Another important experience took place in New Zealand, where an epidemic caused by NmB (strain B:4:P1.4), which started in 1991[55], was seen to have reached an incidence rate of 17.4 per 100,000 by 2001[56]. However, the incidence in infants, especially in some geographic zones such as the Pacific islands, exceeded 300 per 100,000[56]. In response to this epidemic, a vaccine (MenZB) prepared from a B:4:P1.7-2,4 strain by means of the technology used for the Norwegian vaccine was developed[57]. This vaccine proved efficacious in toddlers aged 16-24 months. After three doses, administered at 0, 6 and 12 wk, a 4-fold or greater rise in h-SBA titres against the NZ98/254 outbreak strain was recorded in 75 per cent (95% CI: 69-80%) of vaccinees. The vaccine was well-tolerated and caused no serious adverse events[58].

Another phase II trial showed no negative interference of MenZB when administered with routine immunizations at 1.5, 3 and 6 months of age and a booster dose at 10 months. h-SBA titres of ≥4 were achieved in 53 and 69 per cent of infants after the third and booster doses, respectively, with no serious vaccine-related adverse events[59]. The good immunogenicity and safety profile of MenZB revealed by phase I and II clinical trials and previous trials on the parent Norwegian vaccine permitted the New Zealand public health authorities to give provisional consent for its use in that emergency situation, without undertaking phase III trials. An extensive immunization campaign comprising three doses was, therefore, started in 2004 and targeted people aged 0.5-20 yr[60]. Following the nationwide vaccination campaign, a prospective observational study found that MenZB yielded an efficacy rate of 73 per cent (95% CI: 52-85)[61]. Moreover, the vaccine proved particularly effective in the paediatric population, yielding rates of 80.0 per cent (95% CI: 52.5-91.6) among children aged 0.5-5 yr and 84.8 per cent (95% CI: 59.4-94.3) among those aged 0.5-3 yr[62].

An OMV vaccine was also developed in the Netherlands. The monovalent PorA-based vaccine (MonoMen) was constructed by expressing the P1.7-2,4 subtype, which was the most prevalent subtype in the Netherlands. This vaccine proved immunogenic in toddlers, with over 90 per cent and over 95 per cent of immunized toddlers showing h-SBA titres ≥4 on 2+1

and 3+1 schedules, respectively. The vaccine was well tolerated[63].

However, a great disadvantage of the aforementioned vaccines is that the single PorA-containing OMV vaccines provide limited coverage, and are therefore useful only during epidemics caused by a corresponding strain[64]. Another drawback to wild-type OMV vaccines is the alteration of OMPs and/or exposure of epitopes by detergent extraction. This could explain the scant or absent immune response in young children[65], a phenomenon that was particularly marked in the case of the Cuban vaccine[47]. An alternative approach consisting of the use of intact OMVs, *i.e.* not exposed to detergents, has, therefore, been suggested[65]. On the other hand, intact lipopolysaccharide is very toxic[42]. The problem of toxicity was solved in the Netherlands by the discovery of *lpxL1* mutant strains; disabling the *lpxL1* gene attenuates endotoxin activity while preserving adjuvant activity[66]. This discovery enabled native OMVs to be used without removing lipopolysaccharide by means of detergent extraction, thus leading to the development of the next-generation OMV vaccines[67].

New meningococcal B vaccines

Dutch OMV vaccines

A Dutch hexavalent OMV-based vaccine (HexaMen) consisted of OMV of two recombinant engineered strains, each of which expressed three different PorA subtypes (P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.19,15-1; P1.7,16; and P1.5-1,2-2)[67,68]. In a UK study, 103 infants received HexaMen at 2, 3 and 4 months, together with routine vaccines, and a booster dose was administered at 12–18 months. Good immune responses against two of the six NmB strains which expressed PorA contained in the vaccine were observed after the three-dose course. After the booster dose, higher h-SBA responses were observed, suggesting that the primary course had primed memory lymphocytes and that revaccination stimulated a booster response[39]. In another Dutch study, which involved toddlers aged 2-3 yr, HexaMen was administered through a three-dose scheme at different vaccine doses. The percentage of subjects showing a four-fold increase of h-SBA titres against the specific serosubtype varied from 28 to 98 per cent, with no statistically significant difference between higher and lower doses of the vaccine[68]. In both studies, HexaMen was seen to be well tolerated and safe[39,68].

The theoretical coverage of HexaMen, based on the exact match of vaccine subtypes, was estimated to be 50 per cent of NmB in the Netherlands[69]. To ensure a broader level of protection, a third recombinant OMV was added; the result was a nonavalent vaccine (NonaMen)[67]. On the basis of European PorA subtype data from 1999 to 2004, NonaMen was estimated to have a potential coverage of 80 per cent[70].

One problem of OMV-based vaccines lies in the ability of meningococcal OMPs to undergo antigenic shift or gene deletion, as seen with PorA, thus rendering the vaccines ineffective[71]. Another limitation of OMV vaccines is that the immunity elicited rapidly wanes. To overcome the limitations of old and new OMV vaccines, an alternative strategy (*i.e.* reverse vaccinology) has been undertaken.

Meningococcal B vaccine by means of reverse vaccinology

Thanks to the steady progress of bioinformatics, an innovative approach to the development of an NmB vaccine has been implemented and optimized. Unlike conventional methods, this approach begins by defining the genome sequence of the pathogen and continues with the computer-assisted prediction of more promising antigens for the new vaccine[72]. The first vaccine designed by means of the reverse vaccinology approach was rMenB[73]. Initial scrutiny of the NmB genome (MC58 virulent strain) turned up about 600 antigens, approximately 350 of which were expressed in *Escherichia coli*; these were used to immunize mice. Subsequent analysis of serum from immunized mice uncovered 91 previously unknown surface proteins that were able to induce antibodies *in vivo*, 29 of which induced bactericidal antibodies *in vitro*. Later research identified five antigens, four of which were expressed as fusion proteins [Genome-derived *Neisseria* Antigen 1030 (GNA1030) with GNA2132 and GNA2091 with GNA1870], while the fifth, *Neisseria* adhesin A (NadA), was not fused[74-76].

GNA1870 (factor H binding protein - fHbp) is a surface-exposed lipoprotein that binds factor H. As this is an effective inhibitor of the alternative complement pathway, it protects the pathogen from complement-mediated killing[77]. Early research found that all hypervirulent B strains contained fHbp[78,79]. More recently, however, Lucidarme *et al*[80] have described isolates from patients with IMD which do not express fHbp. As fHbp can differ from strain to strain, two approaches to classifying this antigen have been developed. The first approach divides fHbp into two subfamilies: A and B; within a subfamily, the amino

acid sequence displays 83 per cent homology or more, while between subfamilies, homology is approximately 60-75 per cent[81]. The second approach, which was proposed by Masignani *et al*[79], identifies three variants of fHbp (1, 2 and 3), with homology of 91.6-100 per cent within variants and 62.8 per cent between variants. Subfamily A corresponds to variants 2 and 3, while subfamily B corresponds to variant 1[82].

NadA is a member of non-fimbrial adhesins, or rather oligomeric coiled-coil adhesins; these antigens are all trimeric autotransporter adhesins. NadA is of vital importance to Nm, as it mediates binding and the subsequent invasion of human epithelial cells; indeed, antibody binding to NadA results in the killing of Nm, even in the presence of its polysaccharide capsule[83]. The recombinant NadA contained in the meningococcal B vaccine conserves the functional features of native NadA and is able to induce high levels of bactericidal antibodies in various models; moreover, it is recognized by serum from convalescent children[84]. It has been also shown that NadA is mostly associated with disease isolates rather than with isolates from carriers[85].

GNA2132 (*Neisseria* Heparin Binding Antigen - NHBA) is another surface-exposed lipoprotein discovered by reverse vaccinology, and is able to induce bactericidal antibodies in humans. This antigen is an important virulence factor that binds heparin, thereby promoting Nm resistance in blood. Genetically diverse B strains present variable segments of NHBA; however, C- and N-terminal regions are highly conserved[86,87].

The new vaccine was called 5CVMB (five component vaccine against NmB) and included 20 µg of each of the two protein-protein fusions (GNA1030-GNA2132 and GNA2091-GNA1870) and 20 µg of NadA, adsorbed to $Al(OH)_3$[88]. In a preclinical study, the bactericidal activity of 5CVMB was tested against 85 different strains of NmB; 5CVMB was also compared with two OMV vaccines in terms of the bactericidal activity. Sera from mice vaccinated with OMV vaccines made from the Norwegian strain H44/76 and the New Zealand strain NZ98/254 were able to kill 20 and 21.2 per cent of the strains, respectively, while sera from mice immunized with 5CVMB killed 77.7 per cent of the strains[88]. The final formulation was called four component meningococcal serogroup B (4CMenB) vaccine and consisted of recombinant NmB NHBA fusion protein (50 µg), recombinant NmB NadA protein (50 µg), recombinant NmB fHbp fusion protein (50 µg), and OMV from NmB strain NZ98/254, measured as the amount of total protein containing PorA P1.4 (25 µg). OMV-NZ was added to achieve broader strain coverage and to reduce the risk of escape mutants. The vaccine contained 0.5 mg of $Al(OH)_3$ as an adjuvant; other excipients were sodium chloride, histidine, sucrose, and water for injection[89]. On January 14, 2013, the Committee for Medicinal Products for Human Use of the European Medicines Agency (EMA) authorized 4CMenB, commercially named Bexsero, for use in subjects from two months of age[90].

Clinical trials in infants and toddlers

The successful results of preclinical studies and phase 1 studies on adults were followed by a series of clinical trials in infants and toddlers. To evaluate immunogenicity in infants, a phase II, single-blind, randomized trial was conducted in the UK. Infants aged 6-8 months were randomized in a 1:1 ratio to receive recombinant meningococcal serogroup B (rMenB) or rMenB+OMV vaccines on day 0, day 60 and at the age of 12 months. h-SBA was evaluated against seven different NmB strains. After three doses of rMenB+OMV, h-SBA titres ≥4 against five NmB strains were found among ≥90 per cent of participants, and 70 per cent of participants also showed h-SBA titres ≥4 against the sixth NmB strain. When the infants who received rMenB alone were evaluated, it was found that 88 per cent of them showed an h-SBA titre ≥4 against only three of the seven strains tested. Both vaccines were found to have acceptable safety and tolerability profiles[91].

Another phase II clinical trial demonstrated good immunogenicity in infants after three vaccine doses at 2, 4 and 6 months of age (rMenB or rMenB+OMV). rMenB+OM Velicited h-SBA titres ≥4 against 5/99 (anti-NadA response), 44/76-SL (anti-fHbp response), NZ 98/254 (anti-PorA response), and M00 242922 (anti-PorA response) strains in 95, 87, 85 and 63 per cent of subjects, respectively. The rMenB vaccine alone displayed comparable immunogenicity to that of rMenB+OMV, except against NZ 98/254 and M00 242922. Furthermore, the fourth dose of rMenB+OMV at 12 months of age elicited a good anamnestic response, with h-SBA titres ≥4 against 44/76-SL, NZ 98/254, 5/99, and M00 242922 strains in 100, 93, 96 and 78 per cent of subjects, respectively. Both vaccine formulations were well-tolerated; however, local reactions of induration and tenderness were more frequent after rMenB+OMV than after rMenB[92].

A Phase 2b, multicenter, open-label, parallel-group, randomized controlled study of 1885 infants was

conducted in Europe between 2008 and 2010. Infants aged 2 months were randomized in a 2:2:1:1 ratio to receive: *(i)* 4CMenB at 2, 4, and 6 months together with their routine vaccines; *(ii)* 4CMenB at 2, 4, and 6 months and routine vaccines at 3, 5, and 7 months; *(iii)* 4CMenB administered together with routine vaccines at 2, 3, and 4 months; and *(iv)* a control group in which only routine vaccines were administered at 2, 3, and 4 months. In all three groups receiving three doses of 4CMenB, h-SBA titres ≥ 5 against 44/76-SL (anti-fHbp response) and 5/99 (anti-NadA response) were found in 99.1-100 per cent of the infants. h-SBA titres ≥ 5 against NZ98/254 (anti-PorA response) ranged from 79.0 to 86.1 per cent across three groups. No clinically significant interaction with routine vaccines was found. Local reactions of erythema, swelling or induration were observed in less than 1 per cent of all infants. Fever was noted in 80, 71, 76 and 51 per cent of infants in the first, second, third and control groups, respectively[93].

The first phase III clinical trial evaluated the immunogenicity and safety of 4CMenB when administered with routine vaccinations at 2, 4 and 6 months of age. After the third dose, 100 per cent of infants had h-SBA titres ≥5 against 44/76-SL (anti-fHbp response) and 5/99 (anti-NadA response) strains, and 84 per cent had h-SBA titres ≥5 against NZ98/254 (anti-PorA response) and M10713 (anti-NHBA response) strains. After a booster dose, h-SBA titres ≥5 against all four strains were achieved in 95-100 per cent of infants. This trial also found that 4CMenB did not interfere with routinely administered vaccines. Concomitant vaccination was associated with increased reactogenicity. In particular, concomitant vaccination more frequently produced fever; in the majority of cases, however, this resolved in one day[94].

Bivalent recombinant lipoprotein 2086 (rLP2086)

Another vaccine currently under clinical development is based on fHbp [also called lipoprotein 2086 (LP2086)][73]. According to the above-described division of NmB strains into two subfamilies on the basis of fHbp[81,82], this vaccine (rLP2086) is bivalent, as it is composed of a representative variant of each subfamily (A05 and B01). The immunogenicity, safety and tolerability of this vaccine were investigated in a randomized controlled trial in infants aged 18-36 months. Specifically, 99 healthy toddlers were subdivided into three dose cohorts – dose cohort 1: 20 µg rLP2086, dose cohort 2: 60 µg rLP2086 and dose cohort 3: 200 µg rLP2086. Each cohort was matched with a control group of subjects, who received hepatitis A virus (HAV) vaccine in a 2-dose schedule and a saline placebo administered at the second of the 3 vaccination time-points (0, 1 and 6 months). After dose 3, seroconversion (h-SBA ≥ 4-fold rise from baseline) against NmB strains expressing LP2086 variants homologous to the vaccine antigens was found in 61.1-88.9 per cent of toddlers (rate dependent on dose-level) and against NmB strains expressing heterologous LP2086 variants in 11.1-44.4 per cent. This study indicated that the rLP2086 vaccine had an acceptable safety profile and was well tolerated[95].

How the new meningococcal B vaccines can be used in infants and toddlers

Immunization schedules of 4CMenB (Bexsero) in infants and toddlers

Bexsero has been approved for active immunization against disease caused by NmB in subjects aged ≥ 2 months. In those aged 2-5 months, the primary immunization schedule comprises 3 doses, with an interval of at least 1 month between doses. The primary schedule may be boosted at 12-23 months of age. The schedule for unvaccinated infants aged 6-11 months comprises 2 doses, with an interval of at least 2 months, and a subsequent booster dose in the second year of life. In toddlers aged 12-23 months, the primary schedule recommends two doses, administered at least two months apart; a booster dose may be administered 12-23 months after the primary course[89].

4CMenB (Bexsero) may be co-administered with one or more of the infants (monovalent or combination) vaccines, such as a cellular pertussis, diphtheria, *Haemophilus influenzae* type b, hepatitis B, heptavalent pneumococcal conjugate, inactivated poliomyelitis, measles, mumps, rubella, tetanus and varicella[89,94].

Potential coverage of 4CMenB (Bexsero) against invasive NmB disease

The potential coverage of 4CMenB has recently been assessed in seven European countries by means of the Meningococcal Antigen Typing System (MATS)[96]. MATS is a standardized and reproducible vaccine antigen-specific ELISA, which has been developed to measure the amount of each antigen expressed by a strain and its immunological cross-reactivity with the antigen present in the 4CMenB vaccine[97]. In this large epidemiological survey (1052 hypervirulent strains collected) MATS analysis showed that 4CMenB could cover a significant proportion of European strains that

cause invasive disease. Overall predicted coverage was 78 per cent, with some variation between single countries (73% in England/Wales, 85% in France, 82% in Germany, 87% in Italy, 85% in Norway, 74% in the Czech Republic and 69% in Spain)[96]. In a recent study, the potential coverage of 4CMenB against Canadian hypervirulent strains circulating from 2006 to 2009 was estimated by applying MATS analysis to 157 isolates from adults and children. Overall, MATS predicted a strain coverage of 66 per cent (95% CI: 46-78%), with 26, 29 and 11 per cent of strains covered by one, two and three vaccine antigens, respectively[98].

Potential efficacy of 4CMenB (Bexsero) against serogroups other than B

Given that the 4CMenB antigens may be present in the external membrane of all pathogenic Nm, this vaccine has the potential to prevent disease caused by different serogroups. In this regard, interesting results have recently been published by Hong *et al*[99], who estimated the potential coverage of 4CMenB against serogroup X isolates from several African countries. Using MATS, the authors concluded that 4CMenB could cover African isolates, since the strains tested expressed at least one vaccine antigen (in particular fHbp).

Potential coverage of rLP2086 against NmB disease and against serogroups other than B

Although the bivalent rLP2086 vaccine has not completed all clinical phases, studies on the potential efficacy of this vaccine against NmB and other serogroups have been carried out. In a recent study, Anderson *et al*[100] reported that rLP2086 was able to provide broad protection against invasive NmB strains. They tested the sera of vaccinees against different isolates from Europe and the US. The proportion of vaccinees with h-SBA titres ≥4 against hypervirulent NmB strains with different variants of fHbp ranged from 75 to 100 per cent.

As fHbp is expressed by other Nm serogroups, the anti-fHbp antibodies elicited by rLP2086 might exert a bactericidal effect on meningococci, regardless of the serogroup. On the basis of this assumption, Harris *et al*[101] tested some invasive NmC isolates in a preclinical study. They demonstrated that all NmC isolates had the *fhbp* gene, and the non-human serum showed bactericidal antibody activity against the NmC tested.

Conclusions

Vaccination is the best way to prevent meningococcal disease in infants and toddlers, as well as in other age groups. The broad use of meningococcal conjugate serogroup C vaccine has dramatically reduced the incidence of the disease, particularly in Europe and the US[25,26]. NmB has now become the main causative agent of the disease in several areas, including Europe, the Americas and Australia[5,25]. Until 2013, there was no universally available vaccine against NmB. The development and recent approval of 4CMenB constitute an important step forward in the prevention of invasive disease caused by NmB. Unlike OMV vaccines, which can only prevent disease caused by a specific meningococcal B strain[64], the new vaccine provides broad protection against several NmB strains[96]. Studies conducted on potential coverage have shown that 4CMenB will cover a large number of circulating invasive strains[96]. However, further research is needed to evaluate the real effectiveness of the vaccine when it is widely used. Moreover, as we have seen, old-generation OMV vaccines induced either an inadequate and short-lived immune response or no response in young children[47,54]. Therefore, infants and children will be the first to benefit from the introduction of 4CMenB. Indeed, clinical trials have shown good immunogenicity, tolerability and safety in these subjects, who are the most vulnerable age-class to meningococcal disease[91-94].

The benefit of the new 4CMenB vaccine will be even greater once its effectiveness on carriage has been proved; this will yield indirect benefits in all age groups (herd protection). The importance of this issue prompted Novartis Vaccines & Diagnostics to conduct a study aimed at investigating meningococcal carriage status following immunization with 4CMenB. The Novartis researchers are currently evaluating the results of this study, which recruited 2,978 young adults vaccinated in the UK. Their findings should become available soon[102].

Before the introduction of a new vaccination programme using new vaccines, it is important to evaluate the health benefits and cost-effectiveness of routine vaccination. Therefore, pharmaco-economic and Health Technology Assessment evaluations are needed to help policy decisions. Two pharmaco-economics studies have recently been carried out in Europe[103,104]. Christensen *et al*[103] implemented two models for introducing 4CMenB in the UK[103]. The first model evaluated the impact of vaccination on invasive disease alone: vaccinating a cohort of infants at 2, 3, 4 and 12 months could reduce cases of disease by 27 per cent over the lifetime of the subjects. The second

transmission dynamic model also evaluated the impact on carriage; in this perspective, a substantial reduction (71%) in cases could be achieved 10 years after the introduction of routine vaccination for infants in combination with a broad catch-up campaign[103].

Given that invasive disease often causes permanent impairment of health and places an economic burden on healthcare services, social security institutions and society at large, it is difficult to assess the health benefits and financial savings that might be achieved through the introduction of vaccination for infants and toddlers. Only by considering all these variables can we construct decisional and pharmaco-economic models that can really help decision-makers to choose vaccination strategies to improve the health of a country. Furthermore, each country needs to consider the epidemiological data and socio-demographic and other characteristics of its own population.

References

1. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. *N Engl J Med* 2011; *344* : 1378-88.
2. Ladhani SN, Flood JS, Ramsay ME, Campbell H, Gray SJ, Kaczmarski EB, *et al*. Invasive meningococcal disease in England and Wales: Implications for the introduction of new vaccines. *Vaccine* 2012; *30* : 3710-6.
3. Thompson MJ, Ninis N, Perera R, Mayon-White R, Phillips C, Bailey L, *et al.* Clinical recognition of meningococcal disease in children and adolescents. *Lancet* 2006; *367* : 397-403.
4. Girard MP, Preziosi MP, Aguado MT, Kieny MP. A review of vaccine research and development: meningococcal disease. *Vaccine* 2006; *24* : 4692-700.
5. World Health Organization (WHO). Meningococcal vaccines: WHO position paper, November 2011. *Wkly Epidemiol Rec* 2011; *86* : 521-39.
6. MacNeil J, Cohn A. Meningococcal disease. CDC. *Manual for the surveillance of vaccine-preventable diseases*. 5th ed. Atlanta, GA: Centers for Disease Control and Prevention; 2011.
7. MacLennan J, Kafatos G, Neal K, Andrews N, Cameron JC, Roberts R, *et al*. Social behavior and meningococcal carriage in British teenagers. *Emerg Infect Dis* 2006; *12* : 950-7.
8. Miller F, Lécuyer H, Join-Lambert O, Bourdoulous S, Marullo S, Nassif X, *et al*. *Neisseria meningitidis* colonization of the brain endothelium and cerebrospinal fluid invasion. *Cell Microbiol* 2013; *15* : 512-9.
9. Black SB, Plotkin SA. Meningococcal disease from the public health policy perspective. *Vaccine* 2012; *30* (Suppl 2): B37-9.
10. Buysse CM, Vermunt LC, Raat H, Hazelzet JA, Hop WC, Utens EM, *et al*. Surviving meningococcal septic shock in childhood: long-term overall outcome and the effect on health-related quality of life. *Crit Care* 2010; *14* : R124.
11. Edmond K, Clark A, Korczak VS, Sanderson C, Griffiths UK, Rudan I. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; *10* : 317-28.
12. Griffiths UK, Dieye Y, Fleming J, Hajjeh R, Edmond K. Costs of meningitis sequelae in children in Dakar, Senegal. *Pediatr Infect Dis J* 2012; *31* : e189-95.
13. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine* 2009; *27* (Suppl 2): B51-63.
14. Rouphael NG, Stephens DS. *Neisseria meningitidis*: biology, microbiology, and epidemiology. *Methods Mol Biol* 2012; *799* : 1-20.
15. Greenwood B. Manson Lecture. Meningococcal meningitis in Africa. *Trans R Soc Trop Med Hyg* 1999; *93* : 341-53.
16. Frasch CE, Preziosi MP, LaForce FM. Development of a group A meningococcal conjugate vaccine, Men AfriVac(TM). *Hum Vaccin Immunother* 2012; *8* : 715-24.
17. Kristiansen PA, Diomandé F, Ba AK, Sanou I, Ouédraogo AS, Ouédraogo R, *et al*. Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity. *Clin Infect Dis* 2013; *56* : 354-63.
18. Boisier P, Nicolas P, Djibo S, Taha MK, Jeanne I, Maïnassara HB, *et al*. Meningococcal meningitis: unprecedented incidence of serogroup X-related cases in 2006 in Niger. *Clin Infect Dis* 2007; *44* : 657-63.
19. Broome CV, Rugh MA, Yada AA, Giat L, Giat H, Zeltner JM, *et al*. Epidemic group C meningococcal meningitis in Upper Volta, 1979. *Bull World Health Organ* 1983; *61* : 325-30.
20. Decosas J, Koama JB. Chronicle of an outbreak foretold: meningococcal meningitis W135 in Burkina Faso. *Lancet Infect Dis* 2002; *2* : 763-5.
21. Vyse A, Wolter JM, Chen J, Ng T, Soriano-Gabarro M. Meningococcal disease in Asia: an under-recognized public health burden. *Epidemiol Infect* 2011; *139* : 967-85.
22. Caugant DA. Population genetics and molecular epidemiology of *Neisseria meningitidis*. *APMIS* 2009; *106* : 505-25.
23. Diermayer M, Hedberg K, Hoesly F, Fischer M, Perkins B, Reeves M, *et al.* Epidemic serogroup B meningococcal disease in Oregon: the evolving epidemiology of the ET-5 strain. *JAMA* 1999; *281* : 1493-7.
24. Baker MG, Martin DR, Kieft CE, Lennon D. A 10-year serogroup B meningococcal disease epidemic in New Zealand: descriptive epidemiology, 1991-2000. *J Paediatr Child Health* 2001; *37* : 13-9.
25. European Centre for Disease Prevention and Control (ECDC). *Annual epidemiological report 2012. Reporting on 2010 surveillance data and 2011 epidemic intelligence data.* Stockholm: ECDC; 2013.
26. Harrison LH. Epidemiological profile of meningococcal disease in the United States. *Clin Infect Dis* 2010; *50* (Suppl 2): S37-44.
27. Bröker M, Jacobsson S, DeTora L, Pace D, Taha MK. Increase of meningococcal serogroup Y cases in Europe: a reason for concern? *Hum Vaccin Immunother* 2012; *8* : 685-8.

28. Gasparini R, Amicizia D, Lai PL, Panatto D. *Neisseria meningitidis*, pathogenetic mechanisms to overcome the human immune defences. *J Prev Med Hyg* 2012; *53* : 50-5.
29. Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; *10* : 853-61.
30. Trotter CL, Gay NJ, Edmunds WJ. Dynamic models of meningococcal carriage, disease, and the impact of serogroup C conjugate vaccination. *Am J Epidemiol* 2005; *162* : 89-100.
31. Bettinger JA, Deeks SL, Halperin SA, Tsang R, Scheifele DW. Controlling serogroup B invasive meningococcal disease: the Canadian perspective. *Expert Rev Vaccines* 2013; *12* : 505-17.
32. 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.
33. Wyle FA, Artenstein MS, Brandt BL, Tramont EC, Kasper DL, Altieri PL, *et al*. Immunologic response of man to group B meningococcal polysaccharide vaccines. *J Infect Dis* 1972; *126* : 514-21.
34. Granoff DM. Review of meningococcal group B vaccines. *Clin Infect Dis* 2010; *50* (Suppl 2): S54-65.
35. van de Waterbeemd B, Zomer G, van den Ijssel J, van Keulen L, Eppink MH, van der Ley P, *et al*. Cysteine depletion causes oxidative stress and triggers outer membrane vesicle release by *Neisseria meningitidis*; implications for vaccine development. *PloS One* 2013; *8* : e54314.
36. Rosenqvist E, Høiby EA, Wedege E, Kusecek B, Achtman M. The 5C protein of *Neisseria meningitidis* is highly immunogenic in humans and induces bactericidal antibodies. *J Infect Dis* 1993; *167* : 1065-73.
37. Cadieux N, Plante M, Rioux CR, Hamel J, Brodeur BR, Martin D. Bactericidal and cross-protective activities of a monoclonal antibody directed against *Neisseria meningitidis* NspA outer membrane protein. *Infect Immun* 1999; *67* : 4955-9.
38. Bjune G, Høiby EA, Grønnesby JK, Arnesen O, Fredriksen JH, Halstensen A, *et al*. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. *Lancet* 1991; *338* : 1093-6.
39. Cartwright K, Morris R, Rümke H, Fox A, Borrow R, Begg N, *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* : 2612-9.
40. Munkey A, Tinsley CR, Virji M, Heckels JE. Blocking of bactericidal killing of *Neisseria meningitides* by antibodies directed against class 4 outer membrane protein. *Microb Pathog* 1991; *11* : 447-52.
41. Peeters CC, Claassen IJ, Schuller M, Kersten GF, Rouppe van der Voort EM, Poolman JT. Immunogenicity of various presentation forms of PorA outer membrane protein of *Neisseria meningitidis* in mice. *Vaccine* 1999; *17* : 2702-12.
42. van de Waterbeemd B, Streefland M, van der Ley P, Zomer B, van Dijken H, Martens D, *et al*. Improved OMV vaccine against *Neisseria meningitidis* using genetically engineered strains and a detergent-free purification process. *Vaccine* 2010; *28* : 4810-6.
43. Sotolongo F, Campa C, Casanueva V, Fajardo EM, Cuevas IE, González N. Cuban meningococcal BC vaccine: Experiences & contributions from 20 years of application. *MEDICC Rev* 2008; *9* : 16-22.
44. Rodriguez AP, Dickinson F, Baly A, Martinez R. The epidemiological impact of antimeningococcal B vaccination in Cuba. *Mem Inst Oswaldo Cruz* 1999; *94* : 433-40.
45. Sierra GV, Campa HC, Varcacel NM, Garcia IL, Izquierdo PL, Sotolongo PF, *et al*. Vaccine against group B *Neisseria meningitidis*: protection trial and mass vaccination results in Cuba. *NIPH Ann* 1991; *14* : 195-207.
46. Martínez I, Sierra G, Núñez N, Izquierdo L, Climent Y, Mirabal M. Characterization of *Neisseria meningitidis* strains isolated from carriers in Cuba during 20 years. *Rev Cub Med Trop* 2006; *58* : 1-17.
47. deMoraes JC, Perkins BA, Camargo MC, Hidalgo NT, Barbosa HA, Sacchi CT, *et al*. Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. *Lancet* 1992; *340* : 1074-8.
48. World Health Organization (WHO). Control of epidemic meningococcal disease. WHO practical guidelines 2nd ed. Available from: *http://www.who.int/csr/resources/publications/meningitis/whoemcbac983.pdf*, accessed on June 12, 2013.
49. Fredriksen JH, Rosenqvist E, Wedege E, Bryn K, Bjune G, Frøholm LO, *et al*. Production, characterization and control of MenB-vaccine "Folkehelsa": an outer membrane vesicle vaccine against group B meningococcal disease. *NIPH Ann* 1991; *14* : 67-79.
50. Holst J, Feiring B, Fuglesang JE, Høiby EA, Nøkleby H, Aaberge IS, *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.
51. Feiring B, Fuglesang J, Oster P, Naess LM, Helland OS, Tilman S, *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.
52. Caron F, du Châtelet IP, Leroy JP, Ruckly C, Blanchard M, Bohic N, *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.
53. Tappero JW, Lagos R, Ballesteros AM, Plikaytis B, Williams D, Dykes J, *et al*. Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines: a randomized controlled trial in Chile. *JAMA* 1999; *281* : 1520-7.
54. Boslego J, Garcia J, Cruz C, Zollinger W, Brandt B, Ruiz S, *et al*. Efficacy, safety, and immunogenicity of a meningococcal vaccine group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile. *Vaccine* 1995; *13* : 821-9.
55. 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.
56. Holst J, Aaberge I, Oster P, Lennon D. A 'tailor made' vaccine trialled as part of public health response to group B meningococcal epidemic in New Zealand. *Euro Surveill* 2003; *7* : 2262.

57. Rosenqvist E, Bryn K, Harbak K, Holst J, Høiby EA, Kristiansen P, *et al*. Development of a tailor-made outer membrane vesicle vaccine against the group B meningococcal epidemic in New Zealand. In: Caugant DA, Wedege E, editors. *Abstracts of the 13th International Pathogenic Neisseria Conference*; 2002 September 1-6; Oslo, Norway. Norway: Nordberg Aksidenstrykkeri AS; 2002. p. 64.
58. Wong S, Lennon D, Jackson C, Stewart J, Reid S, Crengle S, *et al*. New Zealand epidemic strain meningococcal B outer membrane vesicle vaccine in children aged 16-24 months. *Pediatr Infect Dis J* 2007; *26* : 345-50.
59. Wong SH, Lennon DR, Jackson CM, Stewart JM, Reid S, Ypma E, *et al*. Immunogenicity and tolerability in infants of a New Zealand epidemic strain meningococcal B outer membrane vesicle vaccine. *Pediatr Infect Dis J* 2009; *28* : 385-90.
60. O'Hallahan J, McNicholas A, Galloway Y, O'Leary E, Roseveare C. Delivering a safe and effective strain-specific vaccine to control an epidemic of group B meningococcal disease. *N Z Med J* 2009; *122* : 48-59.
61. 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.
62. 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.
63. de Kleijn ED, de Groot R, Lafeber AB, Labadie J, van Limpt KC, Visser J, *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* : 1141-8.
64. Tan LK, Carlone GM, Borrow R. Advances in the development of vaccines against *Neisseria meningitidis*. *N Engl J Med* 2010; *362* : 1511-20.
65. 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.
66. Kaaijk P, van Straaten I, van de Waterbeemd B, Boot EP, Levels LM, van Dijken HH, *et al*. Preclinical safety and immunogenicity evaluation of a nonavalent PorA native outer membrane vesicle vaccine against serogroup B meningococcal disease. *Vaccine* 2013; *31* : 1065-71.
67. van der Ley P, van den Dobbelsteen G. Next-generation outer membrane vesicle vaccines against *Neisseria meningitidis* based on nontoxic LPS mutants. *Hum Vaccin* 2011; *7* : 886-90.
68. de Kleijn ED, de Groot R, Labadie J, Lafeber AB, van den Dobbelsteen G, van Alphen L, *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* : 1456-66.
69. Vermont CL, van Dijken HH, Kuipers AJ, van Limpt CJ, Keijzers WC, van der Ende A, *et al*. Cross-reactivity of antibodies against PorA after vaccination with a meningococcal B outer membrane vesicle vaccine. *Infect Immun* 2003; *7* : 1650-5.
70. Borrow R. Advances with vaccination against *Neisseria meningitidis*. *Trop Med Int Health* 2012; *17* : 1478-91.
71. Harrison LH, Jolley KA, Shutt KA, Marsh JW, O'Leary M, Sanza LT, *et al*. Antigenic shift and increased incidence of meningococcal disease. *J Infect Dis* 2006; *193* : 1266-74.
72. Rappuoli R. Reverse vaccinology. *Curr Opin Microbiol* 2000; *3* : 445-50.
73. Panatto D, Amicizia D, Lai PL, Gasparini R. *Neisseria meningitidis* B vaccines. *Expert Rev Vaccines* 2011; *10* : 1337-51.
74. Jones D. Reverse vaccinology on the cusp. *Nat Rev Drug Discov* 2012; *11* : 175-6.
75. Sette A, Rappuoli R. Reverse vaccinology: developing vaccines in the era of genomics. *Immunity* 2010; *33* : 530-41.
76. Boccadifuoco G, Brunelli B, Pizza MG, Giuliani MM. A combined approach to assess the potential coverage of a multicomponent protein-based vaccine. *J Prev Med Hyg* 2012; *53* : 56-60.
77. Seib KL, Serruto D, Delany I, Adu-Bobie J, Veggi D, Aricò B, *et al*. Factor H-binding protein is important for meningococcal survival in human whole blood and serum and in the presence of the antimicrobial peptide LL-37. *Infect Immun* 2009;*77* : 292-9.
78. Murphy E, Andrew L, Lee KL, Dilts DA, Nunez L, Fink PS, *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* : 379-89.
79. Masignani V, Comanducci M, Giuliani MM, Bambini S, Adu-Bobie J, Arico B, *et al*. Vaccination against *Neisseria meningitidis* using three variants of the lipoprotein GNA1870. *J Exp Med* 2003; *197* : 789-99.
80. Lucidarme J, Tan L, Exley RM, Findlow J, Borrow R, Tang CM. Characterization of *Neisseria meningitidis* isolates that do not express the virulence factor and vaccine antigen factor H binding protein. *Clin Vaccine Immunol* 2011; *18* : 1002-14.
81. Fletcher LD, Bernfield L, Barniak V, Farley JE, Howell A, Knauf M, *et al*. Vaccine potential of the *Neisseria meningitidis* 2086 lipoprotein. *Infect Immun* 2004; *72* : 2088-100.
82. McNeil LK, Zagursky RJ, Lin SL, Murphy E, Zlotnick GW, Hoiseth SK, *et al*. Role of factor H binding protein in *Neisseria meningitidis* virulence and its potential as a vaccine candidate to broadly protect against meningococcal disease. *Microbiol Mol Biol Rev* 2013; *77* : 234-52.
83. Linke DT, Riess T, Autenrieth IB, Lupas A, Kempf VA.Trimeric autotransporter adhesins: variable structure, common function. *Trends Microbiol* 2006; *14 :* 264-70.
84. Montanari P, Bozza G, Capecchi B, Caproni E, Barrile R, Norais N, *et al*. Human heat shock protein (Hsp) 90 interferes with *Neisseria meningitidis* adhesin A (NadA)-mediated adhesion and invasion. *Cell Microbiol* 2012; *14* : 368-85.
85. 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.
86. Serruto D, Spadafina T, Ciucchi L, Lewis LA, Ram S, Tontini M, *et al*. *Neisseria meningitidis* GNA2132, a heparin-binding

protein that induces protective immunity in humans. *Proc Natl Acad Sci USA* 2010; *107* : 3770-5.

87. Esposito V, Musi V, de Chiara C, Veggi D, Serruto D, Scarselli M, *et al*. Structure of the C-terminal domain of *Neisseria* heparin binding antigen (NHBA), one of the main antigens of a novel vaccine against *Neisseria meningitidis*. *J Biol Chem* 2011; *286* : 41767-75.
88. Giuliani MM, Adu-Bobie J, Comanducci M, Aricò B, Savino S, Santini L, *et al*. A universal vaccine for serogroup B meningococcus. *Proc Natl Acad Sci USA* 2006; *103* : 10834-9.
89. European Medicines Agency (EMA). Available from: *http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002333/WC500137881.pdf*, accessed on June 12, 2013.
90. European Medicines Agency (EMA). Available from: *http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002333/human_med_001614.jsp&mid=WC0b01ac058001d124*, accessed on May 20, 2013.
91. Snape MD, Dawson T, Oster P, Evans A, John TM, Ohene-Kena B, *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.
92. Findlow J, Borrow R, Snape MD, Dawson T, Holland A, John TM, *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.
93. Gossger N, Snape MD, Yu LM, Finn A, Bona G, Esposito S, *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* : 573-82.
94. Vesikari T, Esposito S, Prymula R, Ypma E, Kohl I, Toneatto D, *et al*. Immunogenicity and safety of an investigational multicomponent, recombinant, meningococcal serogroup B vaccine (4CMenB) administered concomitantly with routine infant and child vaccinations: results of two randomised trials. *Lancet* 2013; *381* : 825-35.
95. Marshall HS, Richmond PC, Nissen MD, Jiang Q, Anderson AS, Jansen KU, *et al*. Safety and immunogenicity of a meningococcal B bivalent rLP2086 vaccine in healthy toddlers aged 18-36 months: a phase 1 randomized-controlled clinical trial. *Pediatr Infect Dis J* 2012; *31* : 1061-8.
96. Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, *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.
97. Donnelly J, Medini D, Boccadifuoco G, Biolchi A, Ward J, Frasch C, *et al*. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proc Natl Acad Sci USA* 2010; *107* : 19490-5.
98. Bettinger JA, Scheifele DW, Halperin SA, Vaudry W, Findlow J, Borrow R, *et al*. Diversity of Canadian meningococcal serogroup B isolates and estimated coverage by an investigational meningococcal serogroup B vaccine (4CMenB). *Vaccine* 2013; *32* : 124-30.
99. Hong E, Giuliani MM, Deghmane AE, Comanducci M, Brunelli B, Dull P, *et al*. Could the multicomponent meningococcal serogroup B vaccine (4CMenB) control *Neisseria meningitidis* capsular group X outbreaks in Africa? *Vaccine* 2013; *31* : 1113-6.
100. Anderson AS, Hao L, Jiang Q, Harris SL, Jones TR, Perez JL, *et al*. Potential impact of the bivalent rLP2806 vaccine on *Neisseria meningitidis* carriage and invasive serogroup B disease. *Hum Vaccin Immunother* 2013; *9* : 471-9.
101. Harris SL, Zhu D, Murphy E, McNeil LK, Wang X, Mayer LW, *et al*. Preclinical evidence for the potential of a bivalent fHBP vaccine to prevent *Neisseria meningitidis* serogroup C disease. *Hum Vaccin* 2011; *7* (Suppl): 68-74.
102. A Phase 3 observer blind randomized, multi-center, controlled study to evaluate the effect of Novartis vaccine's meningococcal B recombinant and MenACWY conjugate vaccines on pharyngeal carriage of *N. meningitidis* in young adults. Available from: *http://clinicaltrials.gov/ct2/show/study/NCT01214850*, accessed on June 12, 2013.
103. Christensen H, Hickman M, Edmunds WJ, Trotter CL. Introducing vaccination against serogroup B meningococcal disease: An economic and mathematical modeling study of potential impact. *Vaccine* 2013; *31* : 2638-46..
104. Pouwels KB, Hak E, van der Ende A, Christensen H, van den Dobbelsteen GP, Postma MJ. Cost-effectiveness of vaccination against meningococcal B among Dutch infants: Crucial impact of changes in incidence. *Hum Vaccin Immunother* 2013; *9* : 1129-38.

Reprint requests: Dr D. Panatto, Department of Health Sciences, University of Genoa, via Pastore 1-16132, Genoa, Italy
e-mail: panatto@unige.it