

Review Article

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

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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 meningitidis* serogroup B - outer membrane vesicle (OMV) vaccines - toddlers

Introduction

Neisseria meningitidis (Nm) is a major causative agent of invasive bacterial infections throughout the world¹. The abrupt onset of meningococcal disease is associated with high mortality and long-term morbidity in spite of the availability of effective anti-

meningococcal antibiotics². Moreover, meningococcal disease is often misdiagnosed on admission to hospital because physicians have difficulty in identifying it³.

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 adults⁵. 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 cent⁸.

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 retardation⁴.

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 disabilities⁹. Sequelae may be neurological, physical and psychological¹⁰. 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 drastically⁵.

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 distribution¹³. 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 disease¹⁴.

The world's highest incidence of meningococcal disease occurs in the Sahel and sub-Saharan African regions (the so-called "meningitis belt"); in this biogeographic zone a large number of epidemics have been caused by serogroup A, accounting for nearly 200,000 cases in 1996¹⁵. To contain this dramatic epidemiologic situation, a group A conjugate vaccine specifically designed for Africa (MenAfriVac) has been developed¹⁶. The vaccine is highly efficacious; no cases caused by serogroup A have been recorded among vaccinees¹⁶ and group A carriage among both vaccinated and unvaccinated people has disappeared, which indicates vaccine-induced herd immunity¹⁷. 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, X¹⁸⁻²⁰. In Asia, serogroups A and C are responsible for the majority of cases; in some countries, other serogroups are playing an increasing role²¹.

Endemic meningococcal infection in industrialized countries displays a relatively stable and sporadic background of incidence²². 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 disease²⁵. A similar serogroup distribution has also been reported in Australia and New Zealand⁵. Serogroup Y strains are relatively common in the United States (US) (accounting for more than 30% of cases) and other countries of the American continent²⁶.

Moreover, cases caused by this serogroup have increased in some parts of Europe²⁷.

Importance of carriage in the transmission of the disease

Nm has its own unique survival niche in humans²⁸; 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 understood²⁹.

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 α 2-8-linked di- and trisyalosil units; these units are identical to some human polysaccharides and, therefore, determine immunological tolerance^{32,33}. Consequently, research into an effective NmB vaccine has focused on subcapsular antigens, outer membrane vesicles (OMV) and individual antigens³⁴. 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 constituents³⁵. 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 as Opc/Opa³⁶, NspA³⁷ and especially PorA^{38,39}, have been shown to induce protective antibodies and have,

therefore, been considered as vaccine candidates, while others (for example, Rmp⁴⁰) 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 development⁴¹.

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 PorA⁴².

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 B⁴³. In response to this epidemic, a candidate OMV vaccine was developed (VA-MENGOC-BC)⁴⁴. The Cuban vaccine consisted of 50 μ g 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 preservative⁴³.

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 provinces⁴⁵. The effectiveness of the immunization programme was later confirmed by a significant decrease in the epidemic NmB strain among carriers⁴⁶. 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 administered⁴³. 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 older⁴⁷.

Another epidemic caused by NmB started in Norway in 1975⁴⁸ 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)₃⁴⁹. 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 campaign³⁸. 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 62.3⁵⁰. 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 intensity⁵¹.

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 100,000⁵².

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 trial⁵³. An OMV vaccine based on the Chilean epidemic strain 15:P1.3 was evaluated in Inquique (Chile) in 1992. This vaccine displayed 70 per cent efficacy among 5-21 year olds, while children under 5 yr remained unprotected⁵⁴. 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 vesicles⁵³.

Another important experience took place in New Zealand, where an epidemic caused by NmB (strain B:4:P1.4), which started in 1991⁵⁵, was seen to have reached an incidence rate of 17.4 per 100,000 by 2001⁵⁶. However, the incidence in infants, especially in some geographic zones such as the Pacific islands, exceeded 300 per 100,000⁵⁶. 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 developed⁵⁷. 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 events⁵⁸.

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 events⁵⁹. 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 yr⁶⁰. 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 yr⁶².

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 tolerated⁶³.

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 strain⁶⁴. 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 children⁶⁵, a phenomenon that was particularly marked in the case of the Cuban vaccine⁴⁷. An alternative approach consisting of the use of intact OMVs, *i.e.* not exposed to detergents, has, therefore, been suggested⁶⁵. On the other hand, intact lipopolysaccharide is very toxic⁴². 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 activity⁶⁶. 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 vaccines⁶⁷.

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 response³⁹. 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 vaccine⁶⁸. 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 Netherlands⁶⁹. 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 cent⁷⁰.

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 ineffective⁷¹. 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 vaccine⁷². The first vaccine designed by means of the reverse vaccinology approach was rMenB⁷³. 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 killing⁷⁷. 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 cent⁸¹. 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 1⁸².

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 capsule⁸³. 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 children⁸⁴. It has been also shown that NadA is mostly associated with disease isolates rather than with isolates from carriers⁸⁵.

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)₃⁸⁸. 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 strains⁸⁸. 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 injection⁸⁹. 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 age⁹⁰.

Clinical trials in infants and toddlers

The successful results of preclinical studies and phase I 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 profiles⁹¹.

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+OMV elicited 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 rMenB⁹².

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, respectively⁹³.

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 day⁹⁴.

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 μ g rLP2086, dose cohort 2: 60 μ g rLP2086 and dose cohort 3: 200 μ g 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 tolerated⁹⁵.

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 course⁸⁹.

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 vaccine⁹⁷. 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, respectively⁹⁸.

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 strain⁶⁴, the new vaccine provides broad protection against several NmB strains⁹⁶. Studies conducted on potential coverage have shown that 4CMenB will cover a large number of circulating invasive strains⁹⁶. 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 disease⁹¹⁻⁹⁴.

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 soon¹⁰².

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 UK¹⁰³. 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 campaign¹⁰³.

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.

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