**Review**

**Meningococcal B vaccine and the vision of a meningitis free world**

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**Key words**

Meningococcal B vaccine • MATS • Cross protection

**Summary**

A century of traditional vaccinology lost the fight against meningococcus serogroup B (MenB). However, thanks to an innovative genome-based approach, the first broadly effective MenB vaccine, Bexsero® (GSK Vaccines), was developed and has been licensed for use in various age groups by the European Commission and other regulatory authorities. Genes encoding for the main meningococcus B antigens were identified and screened in order to achieve a broadly protective vaccine, taking into account the fact that meningococcus B has many different subtypes whose membrane proteins may be different. Since the antigens selected for Bexsero® are also harbored by meningococci belonging to other serogroups there may be the potential for Bexsero® to offer a certain level of protection against non-B serogroups. Therefore preliminary studies were carried out to investigate the potential of the vaccine to also provide a degree of cross protection against non-B serogroups. Here we review the potential for Bexsero® to offer a certain level of protection against the diversity of meningococcus type B subtypes and its potential ability to offer some cross protection from non-B serogroups. Lastly, we describe the future perspectives in pentavalent meningococcal vaccine (ABCWY) development which hopefully will result in a vaccine able to help prevent Invasive Meningococcal Diseases (IMD) from the majority of currently circulating meningococcal strains.

**Introduction**

Two centuries of meningococcal infection have ‘pushed’ the scientific and medical community to search for vaccines in order to harness the ingenious and almost infinite and dynamic survival/infective strategies of meningococci: from live-attenuated vaccines (1900) to subunit polysaccharide vaccines (1970) to glycoconjugated vaccines (1990) to OMV vaccines, to quadrivalent vaccines ACWY (2003) and most recently to reverse vaccinology and the licensure of Bexsero® in the EU for individuals above the age of 2 months on 14 January 2013.  

At the beginning of the twentieth century, the majority of bacterial meningitis in children was caused by *Haemophilus influenzae* type b, pneumococcus, and meningococcus. Available vaccines for the first two bacteria have led to a dramatic incidence reduction of the disease leaving *N. meningitidis* as the major cause of bacterial meningitis worldwide [1-3].

Considering the panel of meningococcal vaccines now available, the prevention of serogroup B-related IMD placed unique challenges in the development of a MenB vaccine: firstly, the inability to use the Men B capsule because its structure resembles a self-antigen and secondly the high variability of the antigenic membrane protein mix [3, 4]. Glycoconjugate vaccines against serogroups A, C, W, and Y exploit the antigenicity of the capsular polysaccharides that characterize each one of the four serogroups: however, for MenB this approach was not feasible due to the similarity of the capsule to a self-antigen; if used in a vaccine the capsular polysaccharide would be very poorly immunogenic [3]. An alternative approach was devised when Outer Membrane Vesicles (OMVs) were successfully used to control specific outbreaks. OMVs are proteoliposomes that contain several different molecular moieties, out of which the porin protein, PorA, is the principle antigenic source of bactericidal antibodies. The limitations of these vaccines are well recognized: effectiveness tends to be limited to strains containing the same PorA protein (serosubtype-specific), limiting its use to strain-specific outbreaks [5]. Thus, advancements toward MenB IMD protection using OMPs (Outer Membrane Proteins) were limited, since multivalent OMPs-based vaccines may not be effective in preventing the majority of endemic disease. Indeed data from Tondella et al. showed that a large number of serosubtypes (20 or more) might have to be included in a multivalent OMPs-based vaccine to target 80% of sporadic disease caused by meningococcus B in US [6]. Therefore, due to the inability to use either the capsule or the OMVs, MenB vaccine development was significantly impaired. The challenge was to develop a MenB vaccine demonstrated to have acceptable safety and immunogenicity profiles in all age groups, particularly in infants, who represent the major group at risk of IMD, and able to induce immunity against the majority of circulating serosubtypes [4]. An innovative genome based approach was then devised, so to identify genes encoding for the main Men B antigens. These antigens were screened to select molecules possessing a good immunogenicity and being...
surface exposed. Once the candidate antigens were finally selected, a four component formulation was tested in clinical trials, showing a good immunogenicity and safety profile for the developmental vaccine [7].

The four main components of Bexsero® have a major role for the virulence of N. meningitidis, from adherence to colonization of the nasopharynx, to survival in blood stream and cerebrospinal fluid. The first component, factor H binding protein (fHbp), fused with GNA2091 protein, binds human factor H on its surface; once the bacteria is “covered” with factor H it can evade the host immune response by mimicking a self-antigen. The second component, NadA, is a heparin adhesion protein involved in colonization, invasion, and induction of pro-inflammatory cytokines. The third component, NHBA, is a heparin-binding protein that increases resistance against the bactericidal activity of human serum and is virtually present in all strains. NHBA is fused with protein GNA1030. The fourth antigen, OMV NZ98/254, has several antigenic components the major of which is PorA, and has successfully demonstrated tolerability and effectiveness when used to help control the New Zealand serogroup B outbreak [8].

The resulting vaccine, Bexsero® was the first broad-coverage MenB vaccine based on recombinant proteins approved for use in individuals 2 months of age and above by the European Commission in January 2013, and was approved for use in individuals from 10 to 25 years of age by the US FDA in January 2015. Bexsero® has also been approved for use in individuals of varying ages in Australia, Canada, Brazil and Uruguay among other countries [9, 10].

A further analysis was needed in order to predict the ability of the vaccine to be broadly protective against a variety of Men B subtypes: therefore a specific Meningococcal Antigen Typing System was devised (MATS) [9].

According to conventional genotyping and other preliminary studies the antigens contained in Bexsero® are not only pan genomic, i.e. present in the majority of circulating serogroup B strains, but in addition are evolutionarily conserved in the meningococcal population leading to the fact that Bexsero® may also offer a certain level of protection against non-B serogroups meningococci [8, 11-13].

The Meningococcal Antigen Typing System: MATS

Since the 1960s immunogenicity of meningococcal vaccines has been evaluated by means of complement-mediated killing of bacteria in the serum bactericidal antibody assay with human complement (hSBA) [14-17]. However, since protein antigens may vary in their presence, sequence and level of expression, evaluating the effectiveness of protein-based vaccines such as Bexsero® would require testing many different meningococcal subtype strains directly in hSBA, an impractical undertaking especially when the tests are done for infants, because serum volumes are very limited [5]. Due to frequent recombination in the MenB subtypes genotyping-based methods such as multilocus sequence typing are not suitable either [18, 19]. An alternative means of measuring the presence of surface-based antigens was needed and the Meningococcal Antigen Typing System (MATS) was developed to meet that need [9].

MATS evaluates the degree to which circulating serogroup B strains express each of the vaccine antigens, fHbp, NadA, NHBA, and PorA1.4, and helps determine the probability that strains will be killed in hSBA by antibodies obtained from individuals immunized with Bexsero® [20, 21]. Positive results in this type of test (MATS) are obtained if antigens are: (1) expressed to a sufficient degree; and (2) similar enough in terms of structure and sequence to the antigens in the vaccine so that the antibodies generated by Bexsero® will kill the bacteria. Good expression of at least one Bexsero® antigen is sufficient for a strain to be killed. MATS has been validated and standardized and is used by national reference laboratories around the globe to estimate the predictive effectiveness of Bexsero®. MATS has already been used to estimate strain coverage in the following countries: Australia, Brazil, Canada, Czech Republic, England and Wales, France, Germany, Greece, Italy, Norway, Spain, and the United States, with predicted coverage ranging from 66% in Canada (95% CI, 43–78%) to 91% in US (95% CI, 72–96%) [8].

In particular Vogel and colleagues assessed all MenB isolates from 5 European countries that were submitted to reference laboratories over a full epidemiological year from July 2007 to June 2008 [20]. Overall, 1052 MenB strains were collected in England and Wales (n = 535), France (n = 200), Germany (n = 222), Italy (n = 54), and Norway (n = 41). The predicted Bexsero® coverage in individual countries ranged from 73% (95% CI, 57-87%) in England and Wales to 87% (95% CI, 70-93%) in Italy. Importantly, 50% of all strains and 64% of covered strains could be targeted by antibodies against more than one Bexsero® antigen, thus ensuring redundancy: this is an important factor to help reduce the risk of the emergence of escape mutants not covered by the vaccine [9]. Although very important to evaluate potential effectiveness generated by Bexsero®, MATS is an in vitro test which was designed to provide a proxy of the ability of Bexsero® to help protect against the diversity of MenB subtypes [9]. Of course the results obtained can only be used as an indicator of vaccine effectiveness, whereas the true vaccine effectiveness will only be available after extensive use of the vaccine, i.e. in vaccination campaigns. However, if countries intend to use currently available MATS data, they should be aware that the current estimation obtained by MATS may be an undersatimation of the true ability of Bexsero® to help protect against circulating Men B subtypes for several reasons [5]. In fact MATS does not account for the activity of antibodies from non-PorA OMV antigens nor it can take into account synergistic effects among the multiple vaccine components [21] or differential expression of antigens when expressed in vivo rather than in vitro: for example NadA expression is repressed under in vitro growth conditions used in both MATS and hSBA and NHBA expression is temperature-
regulated and is reduced at 37°C, the temperature at which MATS is performed [22, 23].

Recently, a new study aiming at experimentally validating the accuracy of MATS predictions tested strains isolated from England and Wales between 2007 and 2008 in the hSBA assay with pooled sera from infant and adolescent vaccinees, and compared these results with MATS. The results showed that 66% of the strains predicted not covered by MATS were killed in the hSBA assay (false negatives). Only one of the 28 strains predicted positive by MATS was resistant to killing in the hSBA assay. The authors concluded that MATS is a conservative predictor of the strain coverage of Bexsero® in infants and adolescents [24]. The same conclusion was reached in a second study conducted in Spain in which pooled sera from adolescents and infants have been tested by hSBA assay against 10 meningococcal group B strains that were negative or had very low levels of the 3 antigens by MATS. It was found that all strains were killed by sera from adolescents and that 5 out of 10 strains were also killed, although at a low titer, by sera from infants [25].

In the future MATS could be useful in post implementation programs to monitor the effectiveness and coverage of Bexsero® over time [9].

**Potential coverage of Bexsero® vaccine on non-B meningococci**

Bexsero® main antigens are not exclusive to serogroup B because the genes encoding for the antigens fHbp, NHBA and NadA can be present and expressed also in the other serogroups suggesting that the immunization with Bexsero® could potentially offer a certain level of protection also against non-B strains [12]. Some investigations have been carried out in order to explore the potential impact of MenB vaccination against non serogroup B disease in different geographic areas (Australia, Europe and Brazil) [12, 13, 26].

In a study designed to estimate Bexsero® coverage in Australia, 108 meningococcal non-B isolates (serogroups C [n = 50], W [n = 27], Y [n = 30] and X [n = 1]) were tested using MATS. Of the non-B strains tested, 56% (39-76%) exceeded thresholds for at least one Bexsero® antigen [C, 64% (46-86%); W, 63% (41-93%); Y, 37% (27-43%)]. These preliminary results using MATS with non-B strains indicate that non-B strains circulating in Australia express significant levels of Bexsero® antigens [26]. However it should be noted that the MATS thresholds established for fHbp, NHBA and NadA were derived on serogroup B strains and their use to predict non-B strain coverage has not yet been validated [9].

Due to this, the ability of pooled sera from infants and adolescents immunized with Bexsero® to kill meningococcal C, W and Y strains isolated in Europe and Brazil has been recently described. In this study, a subpanel of 147 non-B meningococci isolates, representative of the genetic diversity of non B strains isolated in UK, Germany, France and Brazil, was collected and tested in serum bactericidal assay using human complement. The results showed that sera of subjects immunized with Bexsero are able to induce complement mediated killing of MenC, MenW and MenY in a range from 45% to 90%, suggesting that Bexsero® could potentially have an impact on meningococcal disease caused by non B serogroups [13].

It is noteworthy that the first investigation about the potential ability of Bexsero® to cover non-B serogroups meningococci was a pilot evaluation on the possibility of controlling the emerging Neisseria meningitidis capsular group X causing some recent outbreaks in Africa [27].

These preliminary results can represent an indication that Bexsero® may potentially have an impact on prevention of the meningococcal disease caused by non B serogroups [12, 26, 27].

The UK Joint Committee on Vaccination (JCVI) has stated that “the multicomponent MenB vaccine Bexsero® would likely provide some protection against other serogroups of meningococci, including serogroup C” thus suggesting the possibility to remove the dose of meningococcal C vaccine at 3 months of age in the immunizations infants calendar, after the introduction of MenB vaccine as a universal vaccination for infants in the UK schedule [28, 29].

**Future perspective**

Even if the circulation of *N. meningitidis* serogroups is typically dynamic and diverse in its geographic distribution, 5 serogroups are accountable for the majority of invasive meningococcal disease: A, B, C, Y and W135. Since the year 2000 many European states, as well as Canada, have experienced substantial declines in the incidence of serogroups C disease after the extended use of glycoconjugate polysaccharide monovalent serogroup C vaccine [30, 31].

Most recently also tetravalent glycoconjugate vaccines including serogroups A,C,W,Y are available to help prevent the disease in Europe, North and Latin America and Asia and lastly the first broadly effective MenB vaccine, Bexsero®, has been licensed in the EU, USA, Canada, Australia, Brazil and Uruguay among other countries for various age groups [8, 10].

Efforts are currently ongoing to develop a combination meningococcal vaccine including antigens against the 5 meningococcal serogroups: A, B, C, Y and W135 [32, 33].

Investigational formulations of a meningococcal ABCWY vaccine, containing oligosaccharides from meningococcal serogroups ACWY conjugated to a CRM197 carrier protein, as well as MenB vaccine components, have been administered in healthy adolescents in clinical trials in the USA and Latin America [32, 33]. Studies results showed that the investigational MenABCWY formulations are able to elicit a robust immune response against ACWY serogroups and serogroup B test strains with an acceptable reactogenicity and safety profile. If confirmed and approved by regulatory agencies, this approach could lead to the availability of a pentavalent vaccine capable of helping to prevent invasive meningococcal diseases from the majority of circulating strains [32, 33].
With the availability of the existing glycoconjugates, the protein-based multi-component MenB vaccine and the potential pentavalent combination aiming at offering protection to human populations against the five major serogroups (A, B, C, Y, and W-135), the world might reach the milestone of being for the first time ever capable of preventing the majority of meningococcal meningitis, adding a new chapter in medical history [3].

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