Review
Bexsero® chronicle

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Neisseria meningitidis causes globally 1.2 million invasive disease cases and 135 000 deaths per year, mostly in infants and adolescents. A century of traditional vaccinology had failed the fight against the serogroup B meningococcus (MenB), mostly prevalent in developed countries.
Eighteen years after the publication of the first complete genome sequence from a living organism, thanks to an innovative genome-based approach named 'reverse vaccinology', the first broadly effective MenB vaccine was licensed for use by the European Medical Agency and other authorities, and is being implemented worldwide. Here we review this long and passionate journey, from the disease epidemiology to novel antigen discovery, from vaccine clinical development to public health impact: two decades of scientific and technological innovation to defeat one of the most sudden and devastating invasive diseases.

Keywords: Bexsero, 4CMenB, *Neisseria meningitidis*, Serogroup B meningitis, Meningococcal, Antigen Typing System (MATS)

Global Epidemiology

Annually, vaccines prevent globally about six million deaths and save approximately 386 million years of life.[1] Still there are more than 20 major global diseases for which vaccines do not currently exist, including hepatitis C, Streptococcus groups A and B, leishmaniasis, HIV, and tuberculosis.[2] Even for diseases that prevention via vaccination does currently exist — as in the case of meningococcal meningitis — the global epidemiology is not very promising.

Neisseria meningitidis, an aerobic Gram-negative diplococcus, is a human-restricted opportunistic pathogen that accounts annually for 1.2 million cases of meningitis and 135 000 deaths globally,[3] even after counting more than two centuries, that human beings first became aware of meningococcal infection.[4] It is worth noting that the bacterium is part of the commensal flora that colonizes the upper respiratory tract of healthy individuals.

N. meningitidis strains are divided into 12 serogroups (serogroup D capsule is now classified as an unencapsulated serogroup C variant) on the basis of the immunochemistry of their capsular polysaccharides,[5] five of which, namely, A, B, C, W-135, and Y, are causing most of invasive disease cases worldwide; serogroup B is the prevalent cause of meningococcal meningitis in Europe (90%), New Zealand (82%), Australia (80%), Argentina (67%), Japan (57%), and Canada (53%).[6–14]

With the exception of rabies, meningococcal disease has the highest fatality rate (among other vaccine preventable diseases)[15] reaching up to 10%,[16] while in the case of meningococcal septicemia, it is even higher (40%).[17] Meningococcal disease can cause death in 24 hours and can be easily misdiagnosed.[18] Up to 20% of people who survive exhibit permanent life-long disabilities, such as brain damage, deafness, kidney failure, and limb amputation.[19] All age groups are susceptible to meningococcal disease; however, infants are 17 times more likely to be infected compared to the general population.[20] Ten percent of the general population is asymptomatic carriers of *N. meningitidis*,[21] while in the first 30 years of life each person is expected to become 10 times carrier of meningococcus.[22]

The Challenge

Capsular polysaccharides are key virulence factors, although initially geared towards a benign host–pathogen interaction during colonization of the nasopharynx, since only encapsulated forms of bacteria are likely to cause disease.[23] Intra-host, capsules enhance the resistance to bactericidal activity, in most cases via anti-opsonization,[24] leading to immune evasion; inter-host, most likely capsules evolved to play a pivotal role of anti-mutating shield against UV exposure of the bug during aerosol transmission among hosts.[25]

Four (B, C, W, and Y) of the five main serogroups are composed of sialic acid derivatives; serogroup A is an exception to this rule, composed of repeating units of O-acetylated (alpha1→6)-linked *N*-acetyl-D-mannosamine-1-phosphate.[26] Serogroups W and Y

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express alternating sequences of [*D*-galactose or *D*-glucose] and sialic acid. In serogroup C, the capsule has 2–9 linkages, whereas in serogroup B, the homolinear polymer of alpha (2→8) *N*-acetyl neur-aminic acid[27] is identical to human glycoproteins such as N-CAM found on human tissues (especially in foetal neuronal tissue),[28] leaving open the possibility of potential immunological cross-reactivity with human self-antigens that play pivotal role in neuronal development and psychiatric self-integrity,[29] leading to poor immunogenicity.

This caveat of serogroup B 'humanization' put on-hold early efforts towards the development of a safe and immunogenic conjugate polysaccharide vaccine against B group since the polysaccharide does not elicit serum bactericidal antibodies and *in vitro* anti-capsule B antibodies recognize neural cell adhesion molecules in fetal brain tissue; on the contrary, for the remaining four serogroups, the route to success was less 'curvy' enabling the development of mono-valent or polyvalent conjugate vaccines with proven safety, immunogenicity, and effectiveness profiles in real-life, massive vaccination campaigns in human populations; serogroup C infections and deaths were reduced by more than 90% after the 1999 deployment of a vaccination campaign in the UK with a new conjugate vaccine.

Moreover, meningococcus B has a very dynamic pan-genome[30] with antigenic diversity extending beyond the level of clonal complexes, involving both core and accessory gene pools, thus making the discovery of a broadly protective vaccine recipe for serogroup B even more challenging; the idea of a multi-component vaccine, with both core and accessory antigenic components (pan-genomic), quickly became an interesting topic of discussion and further exploration.

Outer membrane vesicle (OMV) Vaccines

OMVs are proteoliposomes machineries made of outer membrane phospholipids, lipopolysaccharides and outer membrane and periplasmic proteins, deprived of any inner membrane and cytosolic protein/lipid content.[31]

OMVs are shaped when the outer membrane bulges and pinches off, encapsulating soluble peri-plasmic material, most likely at sites of weak linkage between the outer membrane and peptidoglycan.[32–34]

OMVs play a pivotal role in host–pathogen interaction and communication of Gram-negative bacteria, more profoundly during infection. Among others, OMVs, as secretory vehicles for bacterial proteins and lipids, are involved in niche coloniza-tion, modification of host immune response, delivery of virulence factors into host cells, and overall disease progression;[31] notably, meningococcal OMVs have

been detected in asymptomatic carriers and cere-brospinal fluid or blood of patients.[35–37]

The concept of developing OMV component vaccines to control specific outbreaks in certain geographical regions was firstly coined in 1970s by different research groups lead by Zollinger WD, Frasch C, and Helting TB, respectively.[38–41]

Following the pioneer research in the 1970s, three different vaccine formulations were independently developed to control regional outbreaks in Cuba (two-dose scheme), Norway (three-dose scheme), and New Zealand (four-dose scheme) with an estimated effectiveness of 83%, 87%, and 73%, respectively,[42–44] counting already over 60 million administered doses.[45,46]

The first formulation (VA-MENGOC-BC®) was developed by the Finlay Institute in Cuba (1987–1989),[43] the second (MenBVac®) was developed at the Norwegian Institute of Public Health (NIPH) (1988–1991),[44] and the third (MeNZB®) was devel-oped by a five-tier consortium between NIPH, WHO, New Zealand Government, Auckland University, and Chiron to control the 2004–2008 epidemic.[42]

The major limitation of OMV vaccines lies on the trade-off between specificity and sensitivity, i.e. effectiveness and coverage, rendering these formula-tions effective mainly against homologous strains to the reference one.[47] It is worth noting, however, that in the New Zealand formulation, a moderate effec-tiveness (54%) was observed against heterologous strains. Furthermore, since OMV components are not serogroup-specific, some non-B effectiveness is expected and observed (56%) against other ser-ogroups.[42] Finally, efforts to develop effective multi-valent OMV vaccines combining distinct and diverse PorA subtypes into a single formulation have reached half the way to success.[48]

The Solution

For more than two centuries, following up the pioneer work of Edward Jenner on smallpox virus in 1796,[49] empirical approaches such as killed or live-attenuated microorganisms, subunit vaccines, detoxified toxins, and polysaccharides have enabled the harnessing or even elimination of many devastating diseases. In the last third of our century, new technologies have progressed further the field of vaccinology, enabling the control of many more previously unpreventable diseases; such advances include recombinant DNA technology, glyconjugation, reverse vaccinology, and structural vaccinology.[50]

The first successful full-genome sequencing project (*Haemophilus influenzae*) in 1995[51] enabled for the first time ever the mining of the entire gene-repertoire of a living organism with the aim to identify genes encoding potential candidate antigens, leading to the

era of genomic vaccinology, namely, Reverse Vaccinology.[52]

In conventional vaccine development, pathogenic strains are grown by sequential passages in vitro to develop live attenuated (or killed) strains that are harmless to the host but retain the ability to trigger a protective immune response. Alternative approaches have involved using antigens as a basis for subunit vaccines. Although promising, conventional vaccine approaches are not applicable to pathogens that cannot be grown *in vitro* (for example, hepatitis B and C viruses) or to pathogens in which immunodominant cellular components resemble components of human tissues (for example, the serogroup B meningococcus). Moreover, conventional vaccine approaches are time-consuming (5–15 years) and can only identify and exploit antigens that are highly expressed and immunogenic during disease.[52]

Reverse vaccinology uses a bottom-up (rather than a top-down), genomic (instead of cellular) approach and has been successfully applied to the development of vaccines against pathogens that were previously recalcitrant to such development. The only requirement for this new process is the genome sequence (or sequences) of the target pathogen. Such genome sequences are used as the input material for *in silico* algorithms that make predictions about putative antigens that are likely to be successful vaccine candidates. The key steps in the Reverse Vaccinology workflow are: gene prediction, cellular localization prediction, *in vivo* expression, immunogenicity testing, and prediction of coverage. Reverse vaccinology is fast (1–2 years, depending on the availability of high-throughput screening systems); can identify virtually all potential antigens, irrespective of their concentration, time of expression and immunogenicity; and can be used against all pathogens, including those that cannot be grown *in vitro*. However, this methodology cannot currently be used to develop vaccines that are based on non-protein-coding antigens, such as lipopolysaccharides.

There are three 'flavors' of reverse vaccinology:[50] (1) the *classical* reverse vaccinology approach consists in mining a genome sequence for the identification of putative surface-exposed antigens that could be used as vaccine candidates; (2) the *pan-genome* reverse vaccinology approach compares different genome sequences of different strains to increase the coverage and to avoid the escape of the microorganism by antigen variability; and (3) the *subtractive* reverse vaccinology approach consists in comparing pathogenic and nonpathogenic genome sequences in order to select those antigens that could be directly involved in pathogenesis.

Reverse vaccinology has recently been successfully applied to the development of universal vaccines against group B Streptococcus[53] and meningococcus serogroup B.[54] Reverse vaccinology, which is now a routine approach, has also been applied to other life-threatening pathogens, including staphylococci and streptococci.[52]

Pizza *et al.*[54] were the first to successfully implement the concept of reverse vaccinology by using the complete genome sequence of a virulent *N. meningitidis* serogroup B strain as input to prediction algorithms for the identification of putative vaccine candidates. In less than 2 years, the authors had predicted 600 putative surface-exposed proteins, more than half of which were cloned and expressed in *E. coli* and purified for use in the immunization of mice. A quarter of these proteins were novel antigens that were exposed on the surface of the bacterial cell, and almost one-third (25 proteins) induced a bactericidal antibody response. The novelty of this approach was that the identified antigens were well conserved at the sequence level, and so were ideal for the development of vaccines that offer protection against a wide range of homologous or even heterologous serogroup B strains. Reverse vaccinology is a promising method for the high-throughput discovery of putative population-wide, rather than strain- or serotype-specific vaccine candidates that have the potential to mirror the variability, dynamics, and diversity of entire microbial populations. This milestone pioneering research project made it successfully all the way from research, to development and product launch with the European Medicines Agency approval on the 14 January 2013, under the commercial name Bexsero.

Bexsero: The First Multi-component, Protein-based Vaccine against *N. meningitidis* Serogroup B

Genome mining and subsequent reverse vaccinology analysis discovered three protein antigens, two of which were fused with two additional proteins (also discovered via reverse vaccinology).[55] These proteins were combined with the previously successful New Zealand OMV vaccine component used to protect against the epidemic strain (NZ98/254).

The four components of Bexsero were 'surgically' selected, in order to play major role in all steps of *N. meningitidis* lifecycle in human host, from colonization in nasopharynx, to survival, function, and virulence in blood stream and cerebrospinal fluid. The first component, namely factor H binding protein (fHbp), fused with GNA2091 protein, binds human factor H, a negative regulator of the alternative pathway of complement activation. The gene encoding fHbp is *nmb1870*. The second component, NadA, encoded by gene *nmb1994*, is a major adhesion protein involved in colonization, invasion, and induction of pro-inflammatory cytokines. The third component, NHBA, encoded by gene *nmb2132*,

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 forth component, namely, OMV NZ98/254, has several antigenic components, the major of which is PorA and has successfully demonstrated tolerability and effectiveness in actual use (in the case of the New Zealand serogroup B outbreak).

The Rationale Behind a Multi-component Vaccine

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 over lengthy timeframes of at least half a century, according to a recent study of 165 pathogenic strains collected in the Netherlands over a period of 50 years.[56] This means that Bexsero has the ability to provide effective protection for long time against the constantly mutating, dynamic bacterial population of serogroup B meningococci, due to its multicomponent nature of distinct protein antigens encoded by genes localized in dispersed positions in the genome sequence of *N. meningitidis*. More specifically, Bexsero antigenic sub/variants fHbp-1.1 and NadA-3.8 persisted for 30 years and NHBA-2 for 50 years. Interestingly, throughout this half-century timeframe, the conservation and persistence of each and every antigen varies over time, but importantly, this variation happens asynchronously, i.e. each time at least one or two of the three sub/variants is conserved in the population. These data suggest that Bexsero will be able to provide long-term protection against populations of meningococci, as its antigens persist in time independently and combined.

The Clinical Compendium of Bexsero (Synopsis)

The safety of Bexsero was evaluated in 14 studies including nine randomized controlled clinical trials with 8776 subjects (from 2 months of age) who received at least one dose of Bexsero.[57] Among Bexsero recipients, 5849 were infants and children (less than 2 years of age), 250 were children (2–10 years of age) and 2667 were adolescents and adults. Of the subjects who received primary infant series of Bexsero, 3285 received a booster dose in the second year of life. Starting from 2 months of age, Bexsero offers several immunization schedule options that can fit with routine vaccination visits.

Clinical data showed[57] that Bexsero could be administered to infants as young as 2 months. In infants, Bexsero could be either: co-administered with other routine vaccines or given alone as part of a flexible vaccination schedule. In infants, Bexsero had a safety and tolerability profile similar to that of several other routine infant vaccines. The most common local and systemic adverse reactions observed were: tenderness and erythema at the injection site, irritability, and fever when given concomitantly.

In infants and children (less than 2 years of age), the most common local and systemic adverse reactions observed in clinical trials were tenderness and erythema at the injection site, fever, and irritability. In adolescents and adults, the most common local and systemic adverse reactions observed were pain at the injection site, malaise, and headache.

The efficacy of Bexsero has not been evaluated through clinical trials. Vaccine efficacy has been inferred by demonstrating the induction of serum bactericidal antibody responses to each of the vaccine antigens.[57]

Serum bactericidal antibody responses to each of the vaccine antigens NadA, fHbp, NHBA, and PorA P1.4 were evaluated using a set of four meningococcal group B reference strains. Bactericidal antibodies against these strains were measured by the Serum Bactericidal Assay using human serum as the source of complement (hSBA). Most of the primary immunogenicity studies were conducted as randomized, controlled, multicenter, clinical trials. Immunogenicity was evaluated in infants, children, adolescents, and adults.[57]

Bexsero has been shown in large Phase III studies to be highly immunogenic in infants (starting at 2 months of age), toddlers, and children. A protective immune response against all Bexsero antigens was demonstrated in infants after receiving three doses at 2, 4, and 6 or 2, 3, and 4 months of age, allowing for a flexible vaccination schedule.[57–60]

In infants and children >6 months of age, Bexsero was shown to be immunogenic when administered as a two-dose primary series 2 months apart, with a booster dose recommended for children receiving the primary series at <2 years of age.[57]

Bexsero is immunogenic in adolescents from 11 years of age and adults when administered as a two-dose series with no less than a 1-month interval between doses. High-level protective immune response rates for each of the four antigenic components were seen even after the first dose in adolescents.[57]

Bexsero can be given concomitantly with any of the following vaccine antigens, either as monovalent or as combination vaccines: diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type b, inactivated poliomyelitis, hepatitis B, heptavalent pneumococcal conjugate, measles, mumps, rubella, and varicella.[57]

Predicted and Actual Coverage of Bexsero

Estimation of the public health impact of vaccination with Bexsero requires the evaluation of strain

coverage in addition to immunogenicity.[61] Coverage of BEXSERO is predicted by an assay called the Meningococcal Antigen Typing System (MATS). 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, the well-established correlate of protection, by antibodies induced by vaccination with Bexsero.[61–63]

Antigens used in Bexsero can be found in circulating strains. For bacterial killing by antibodies induced by this vaccine, antigens have to be: (1) expressed to a sufficient degree; and (2) similar enough to the antigens in the vaccine such that the antibodies generated by Bexsero will kill the bacteria. 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 coverage of Bexsero.

Approximately 1000 different disease-causing serogroup B strains (collected between 2007 and 2008) have been analyzed in five European countries. Depending on the country of origin, between 73% and 87% of the serogroup B isolates had an estimated MATS antigen profile to be covered by Bexsero. Overall, 78% of the ~1000 strains were potentially susceptible to vaccine-induced antibodies.[57] These results support the potential for Bexsero to have a high impact on disease.

Recently, a new study[64] aiming at experimentally validating the accuracy of the 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) possibly owing to synergy of antibody raised against multiple antigens, each of which was independently below the antigen-specific threshold. 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.

Snapshot of Worldwide Current Vaccinations

Vaccinations with Bexsero have already commenced in Europe,[65] Canada,[66] Australia,[67] and two US universities[68,69] (with local serogroup B outbreaks). Many more countries are about to introduce the new vaccine, but a representative country-wide snapshot as of April 2014 is as follows: in the UK, the Joint Committee on Vaccination and Immunization (JCVI) recommends routine infant program (2 + 1 schedule) with funding and high-risk groups of all ages. Australia published clinical recommendation for infants, children, and adolescents. Austria recommends Bexsero in high-risk groups. Czech Republic published clinical recommendation without funding for infants and children from 2 months to 15 years of age and high-risk groups. France recommends Bexsero for high-risk groups and outbreaks. Germany published regional recommendation in Saxony for children from 2 months to 18 years and national recommendation for high-risk groups; furthermore, 43 sick funds provide voluntarily reimbursement (target population of ~32 million people). Italy issued clinical recommendation without funding for infants from 2 months of age and regional funding in Basilicata. Poland published clinical recommendation without funding. Finally, with special approval from Food and Drug Administration (FDA), around 30 000 doses have been administered to students at Princeton University and Santa Barbara University at California to control local outbreaks of serogroup B.[68,69] On 7 April 2014, Bexsero received a Breakthrough Therapy designation from FDA with plans to file for US licensure of Bexsero as early as Q2 2014. According to the FDA, Breakthrough Therapy designation is intended to expedite the development and review of new medicines that treat serious or life-threatening conditions. The designation includes all of the fast track program features, as well as more intensive FDA guidance.[70]

Cost-effectiveness Evaluation

On 21 March 2014, the JCVI revised its interim position and recommended Bexsero inclusion in National Immunization Program in a 2 + 1 scheme (2, 4, and 12 months), with 100% reimbursement.[71] JCVI accepted the potential non-B effect of Bexsero, and announced that is willing to revisit serogroup C vaccination in the UK, 2 years later, given that Bexsero will prove each effectiveness on serogroup C.

The current health economics framework, largely developed for therapeutic treatments, is not adequate for vaccines especially if the disease has low incidence and high fatality. Therefore, the challenge still remains open for a more adequate health economic analysis framework to assess vaccine cost-effectiveness. Recently, new initiatives towards that direction have started to emerge.[72]

The Joint Committee concluded, among others, that certain adolescent vaccination schedules against serogroup B were cost-effective, but further analysis and data collection is needed before any actions is taken towards that direction. Experts and charities have wholeheartedly welcomed the decision to include Bexsero in routine childhood vaccination schedule.

The Vision of a Meningitis-free World

Two centuries of meningococcal infection have 'pushed' the scientific and medical community to improvise and innovate constantly and frequently in order to harness the ingenious and almost infinite and dynamic armory of meningococci: from Vieusseux (1805) to live-attenuated (1900) to subunit vaccines (1970) to glycoconjugation (1990) to quadrivalent vaccines (2003) to genomics and recently to reverse vaccinology and the Bexsero EMA approval 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 bugs have led to the virtual eradication or massive incidence reduction of the disease,[73,74] leaving *N. meningitidis* the major cause of bacterial meningitis worldwide.

With the availability of the existing glycoconjugate and the new protein-based multi-component vaccine aiming at protecting human population against the five major serogroups (A, B, C, Y, and W-135), the world has reached the milestone of being for the first time ever capable of getting rid of meningococcal meningitis, adding a new chapter in medical history.[15]

However, the complete eradication of the meningococcus, even if possible, may not be desirable due to its potential negative impact on disease from other co-colonizing species by opening the 'gate' to other human pathogens.[15]

Today, with a full spectrum armory against all five serogroups, bacterial meningitis is a 100% vaccine preventable disease; the next big challenge is to make these existing and new vaccines broadly available to populations at risk via strong political will and genuine and widespread public awareness campaigns.

Disclaimer Statements

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