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PRODUCT REVIEW

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Product review on the IMD serogroup B vaccine Bexsero®

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ABSTRACT

Bexsero® is a multicomponent vaccine composed of four major proteins of *Neisseria meningitidis*: the fHbp, NHBA, NadA and PorA. This vaccine was licensed against invasive meningococcal disease (IMD) due to serogroup B isolates. When administered alone, Bexsero® showed a safety profile similar to other childhood vaccines. It provides an excellent immunogenicity but that requires booster doses in infants and young children. Although the vaccine does not seem to impact on acquisition of carriage of serogroup B isolates, it confers protection against isolates of serogroup B harboring distinct but cross-reactive variants of fHbp, NadA and NHBA. Primary vaccination schemes in infancy underwent a rapid increase after a toddler booster suggesting an anamnestic response and the establishment of a memory response. As Bexsero® targets sub-capsular proteins that can be conserved regardless the capsule, the vaccine can be effective against non-B isolates such as isolates of serogroups W and X.

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1. Introduction

Neisseria meningitidis is a Gram-negative bacterium that is a major agent of invasive bacterial infections (IBI). These infections are mainly provoked by four major bacterial agents, *N. meningitidis, Haemophilus influenzae, streptococcus pneumoniae*, and *Streptococcus agalactiae*. IBI manifest by a wide range of diseases but are dominated by meningitis and septicemia although other clinical presentations are also reported. IBI are responsible for important morbidity and mortality worldwide and in different age groups. The World Health Organization (WHO) developed an ambitious road map to defeat invasive bacterial infections (including Invasive meningococcal dieseas, IMD) by 2030: Defeating meningitis by 2030.[1] This plan is based on 5 pillars, the first of which relies on the prevention and epidemic control that are mainly mediated by vaccines. Vaccination against *N. meningitidis* is a major challenge in fighting against IMD due to the presence of several serogroups of variable distribution in time and across the world.

2. Nature of the disease being prevented

IMD includes a wide range of diseases but most often presenting as meningitis and septicemia, represents a serious threat for health in all age groups but with variable incidence.[2–4] The onset is often sudden and difficult to distinguish from other febrile diseases notably in the very young.[5] The incidence varies from <1/100,000 per year in Europe, Oceania and North America to 10–1,000/100,000 per year in the "meningitis belt" of sub-Saharan Africa.[6,7] IMD is regarded by the population as a dramatic event due to the high risk of related permanent sequelae and death. Indeed, death may occur in 6–10% of cases despite the availability of antibiotics and nearly 20% of survivors experience permanent sequelae, including neurologic impairment, hearing loss, or limb amputation.[8] In spite of this notoriety, *N. meningitidis* (Nm) is usually hosted asymptomatically as part of the human nasopharyngeal microbiome without causing damage to the host.[9] The highest rates of nasopharyngeal carriage was reported in adolescents and young adults.[10] This has implications for the vaccination strategies that may be adopted to reduce infection and disease.[11]

The capsule is a major meningococcal virulence determinant, and non-capsulated meningococcus do not generally cause invasive disease but may be detected in immune-deficient subjects.[12] *N. meningitidis* is assigned to one of 12 established serogroups based on the immunochemical specificity of the capsular polysaccharides; that reflect genetic differences in their capsule loci. Only six of these serogroups (A, B, C, W, X, and Y) cause nearly all IMD worldwide.[13] The distribution of these serogroups causing IMD varies geographically and change over time as new strains emerge in susceptible populations. Serogroup B has become the leading cause of IMD in several industrialized countries in the absence of effective vaccine targeting this serogroup. Effective capsular polysaccharide conjugate vaccines against serogroups A, C, W, and Y are available but not for serogroup B.[14] The highest incidence of NmB is primarily observed in infants and young children.[15] A secondary IMD peak, observed during the adolescence, results from increased exposure to new strains linked to changes in social activities at this age group. A third peak is observed among older than 85 years.[6,16] The stumbling block preventing the development of a polysaccharide-based vaccine to serogroup B was the mimicry between α2-8 sialylated human glycoproteins found on the surface of many cells, in particular neuronal cells, and the serogroup B polysaccharide. Antibodies cross-reacting with these structures ran the risk of

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damaging human cells and disrupting the fetal development in immunized pregnant women.[17,18] In contrast to the other serogroups, immune tolerance to serogroup B polysaccharide has therefore been evolved rendering it poorly immunogenic without protective immune responses.[19–21] Alternative approaches to avoid the use of the potentially harmful B polysaccharide, were largely directed to the noncapsular antigens. The concept of OMV-based vaccine has been coined in 1970s by different research groups.[22–25] Outer membrane vesicles (OMVs) are 20–200 nm sized proteoliposome vesicles shed from the meningococcal surface as "blebs" rich in several surface-exposed potentially immunogenic proteins.[26–29] The outer membrane porin A (PorA) was identified as the most abundant component and the major immunogenic protein of the meningococcal OMVs.[30] Although, OMV-based vaccines were tailored for the containment of clonal NmB outbreaks in different geographical regions since 1990,[31–36] they displayed two major shortcomings that hinder their universal use. First, variation in PorA is substantial among various serogroup B strains making the OMV-based vaccine effective mainly against homologous strains to the reference one and little cross-protection was observed with heterologous strains.[36,37] It was suggested that more than 20 different PorA variants should be included in the vaccine to cover all *N. meningitidis* strains circulating worldwide.[38] The efforts to develop a multivalent OMV vaccines gathering distinct PorA subtypes remains an unsatisfied objective.[39] Second, their suboptimal immunogenicity in young children, the most frequently affected age group that may result from the alteration of epitope exposure following detergent-based preparation of OMVs.[40] The focus for development of a broadly protective meningococcal B (MenB) vaccine shifted therefore to the use of other surface-exposed subcapsular proteins.

3. Origin and research basis for the design of the product

Since the sequencing of the first bacterial pathogen, *H. influenzae* in 1995 [41] and the arrival of the era of microbial genomics, the idea to mine genomic data with the aim to identify genes encoding new vaccine antigens for pathogens, such as MenB, started to emerge. The annotation of the first MenB strain (MC58) whole genome and the identification of a large number of novel surface-bound antigens marked a major tuning in the birth of 'reverse vaccinology.'[42–44] This conceptual shift broke from the traditional Pasteur's principles on conventional vaccinology which consist on isolation, inactivation/attenuation and injection of the disease-causing agent or purified one of its subunits.[45] Reverse vaccinology uses a bottom-up (rather than a top-down) approach exploiting genomic (instead of cellular) information provided by sequencing to develop vaccine candidates.[46,47] Bioinformatic tools available for protein structure, function and localization were used for *in silico* screening of the MC58 genome with the aim to identify potential vaccine candidates.[46] The potential candidates need to be surface exposed and induce serum bactericidal antibodies and/or promote passive protection in animal models. Furthermore, limited sequence variation is required to cover a broad diversity of circulating meningococcal strains.[47] Pizza *et al.* identified 570 out of 2158 open reading frames (ORFs) predicted to have features of surface-exposed or secreted proteins based on their coding sequence and homology with known virulence factors.

From these, 350 candidate antigens were successfully cloned, expressed in *Escherichia coli* and purified as histidine- or glutathione *S*-transferase (GST)-tagged proteins. Each purified recombinant protein was used to immunize mice and the antibody response was analyzed by a panel of immunological assays including immunoblotting using whole bacterial lysates and purified outer membrane proteins to verify antigen expression at the predicted molecular weight; enzyme-linked immunosorbent assay (ELISA) and fluorescence-activated cell sorting (FACS) using intact whole bacteria to address the surface localization of the target antigen. Subsequent screening of the mice sera for immunogenicity and surface localization revealed 91 novel surface-exposed proteins of which 28 elicited complement-mediated bactericidal antibodies. Testing of these antigens against a collection of MenB strains isolated from cases of disease and carriage showed that a single component would be insufficient to induce broad coverage and underlined the need of multiple antigens for the future "universal" vaccine.[48] The interrogation of a panel of MenB genomes and the cross-protective ability assayed by SBA, led to the identification of five antigens, namely, genome-derived *Neisseria* antigens: (GNA)2132, GNA1870, GNA1994, GNA2091 and GNA1030. The most promising antigens identified were GNA2132, GNA1870, and GNA1994 that were thereafter given the names NHBA (*Neisseria* heparin binding antigen), fHbp (factor H binding protein) and NadA (*Neisseria* adhesin A), respectively, based on their functional activity.

- NHBA is a surface-exposed lipoprotein that is expressed by most meningococcal strains.[49,50] NHBA-mediated binding of heparin facilitates adherence to host tissues by binding to heparan sulfate proteoglycans and seems to be associated with increased serum resistance *in vitro*.[51] Based on sequencing data, numerous NHBA peptide variants have been identified, and showed a high cross-protection in preclinical studies. The multicomponent vaccine includes (NHBA) peptide 2. NHBA is recognized by sera from patients convalescing from IMD and induces protective antibody response that correlates with the level of NHBA expression.[51]

- fHbp is a surface lipoprotein that binds human factor H (fH), a key inhibitor of the complement alternative pathway, and enables the meningococcus to evade killing by the innate immune system.[52,53] fHbp induces a complement-mediated protective antibodies that may also prevent factor H binding by the meningococcus, leading thereby to increased susceptibility to alternative complement-mediated killing.[54] Based on the sequence polymorphism of *fhbp* gene, three distinct variants of fHbp have been described, namely variants 1, 2, and 3 that are immunologically distinct and do not induce cross-protective antibodies, although some low cross-reactivity between variants 2 and 3 has been shown.[55,56] These variants can be further divided into subvariants.

The multicomponent vaccine includes the recombinant form of *N. meningitidis* fHbp peptide variant 1, subvariant 1 (fHbp 1.1). Crystallographic data were available for all the three main variants, and the X-ray structure of the complex between fHbp and the domains 6 and 7 of human factor H has allowed fine characterization of the residues involved in factor H binding.[57,58]

- NadA is an autotransporter surface-localized adhesin thought to promote nasopharyngeal colonization.[59] Furthermore, it can bind and activate macrophages and dendritic cells, bind human beta-1 integrins and the extracellular chaperone human heat shock protein Hsp90.[60–62] Unlike *fHbp* and *nhba*, the *nadA* gene was found in only 22.3% of European MenB isolates [63] and the expression level varies markedly between isolates by as much as or more than 100-fold [64,65] and is regulated by the repressor protein NadR.[66] Four clusters of homology-based variants (NadA-1, NadA-2/3, NadA-4/5, and NadA-6) have been described for NadA peptide sequences. The NadA-1 and NadA-2/3 variants have similar lengths and homologies (~60 to 80%), while the NadA-4/5 and NadA-6 variants are shorter and divergent (~20 to 40% similarity). The NadA1, NadA2, and NadA3 are highly immunogenic and induce cross-reactively protective antibodies.[59,67–69] The multicomponent vaccine includes the recombinant form of NadA peptide 8 (variant NadA-2/3).

The rationale behind combining different antigens was to increase the spectrum of vaccine coverage, minimizing the possibility of bacterial immune evasion through mutations or loss.[70] With the aim of inducing better and broader protection while simplifying large-scale manufacturing, these antigens were combined into a multicomponent vaccine named 5CVMB in which four of the five antigens were included as two fusion proteins: NHBA was fused with GNA1030 and fHbp was fused with GNA2091. These fusions were the best to be tested, in terms of stability and immunogenicity, among more than 30 different combinations tested.[70] The antigen NadA, less immunogenic in chimeric state with other antigens, was left as a single antigen.[70]

4. Regulatory issues

Licensure of anti-meningococcal vaccines is not and cannot be based on clinical efficacy trials as such trials are not feasible due to the low incidence of IMD. That is why efficacy trials are not used in meningococcal vaccine development and licensure.[71] The licensure is based on serological correlates of protection that link a threshold titer of serum bactericidal activity (SBA) to the protection. The threshold of ≥1:4 is the internationally accepted correlate of protection against MenB IMD.[26,72,73] Human complement should be used in serum bactericidal activity testing (hSBA). The assay scores the highest reciprocal dilution of serum that allows 50% killing after 60 minutes of incubation.[72] Comparison of proportions of subjects achieving the titer ≥1:4 before and after the vaccination as well as the geometric mean (GMT) of titers are used to evaluate MenB vaccines. For subjects who have preexisting titers of ≥1:4, at least a 4-fold increases in hSBA titers are scored.[71]

5. Preclinical studies

5.1. Immunogenicity

The 5CVMB was formulated with aluminum hydroxide ($Al(OH)_3$) and the first pre-clinical studies were conducted in mice, guinea pigs, rabbits, juvenile baboons and infant rhesus macaques to assess the immunogenicity of the individual antigenic components and the full clinical formulation of the vaccine (Table 1).[74] Active protection was not assessed due to the lack of reliable animal models of infection for *N. meningitidis*, an exclusive human pathogen. The studies measured antibody generation by ELISA and the functional activity of these antibodies by serum bactericidal activity (SBA) following single and repeated doses. SBA titer ≥ 1:4 has been correlated with protection.

Antisera obtained from mice immunized intra-peritoneally with the individual vaccine antigens or the multicomponent vaccine, were used to assess the potency of 5CVMB by analyzing the density of antibodies on the surface of immunogold-labeled whole meningococcal cells. While antibodies raised against individual antigens stained the bacterial surface with different densities, the multicomponent vaccine 5CVMB greatly increased the density of antibodies to a level comparable to anti-PorA, which are known to promote bacterial clearance suggesting synergism of binding between the antibodies. The functional activity of the immune response was then performed by bactericidal assays, using exogenous complement source from baby rabbit, against a panel of 85 MenB clinical isolates from USA, UK, Europe, Australia, and other countries. Almost 66% of these isolates belonged to four hypervirulent clonal complexes: cc32, cc41/44, cc8, and cc11. 5CVMB formulated with aluminum hydroxide ($Al(OH)_3$) adjuvant was found to induce effective murine bactericidal antibodies against 78% of the tested panel. Strain coverage increased to greater than 90% by using the alternative

Table 1. Doses of 5CVMB in pre-clinical immunogenicity studies.

Species	Treatment (route)	Dose of NHBA-953, 936-fHbp, and NadA each	Dose of OMV	Dose of $Al(OH)_3$	Volume	Study no.
Mouse	Days 0, 21, 35 (IP[1])	20 µg	10 µg (NZ[4])	600 µg	200 µL	263903–01
Guinea pig	Days 0, 21, 35, 48 (SC[2])	25 µg	12.5 µg (NZ)	750 µg	250 µL	
Juvenile baboon (9–20 months)	Day 0, months 1 + 2 (IM[3])	50 µg	25 µg (NW[5])	1.5 mg	0.5 mL	283886–01
Infant rhesus macaque (2–3 months)	Day 0, weeks 4 and 18 (IM)	50 µg	25 µg (NZ)	1.5 mg	0.5 mL	283887–01

[1]Intraperitoneal; [2]Subcutaneous; [3]Intramuscular; [4]New Zealand OMVs; [5]Norwegian OMVs.

adjuvants including cytosine-guanine (CpG) oligonucleotides plus $Al(OH)_3$, oil-in-water emulsion MF59 or Freund's complete adjuvant.[70] The widely used adjuvant $Al(OH)_3$ was finally selected for the final formulation. Representative sera from mice immunized with 5CVMB also provided effective passive protection against meningococcal disease by preventing meningococcemia in 100% of infant rats tested in a challenge model.[70] Pre-clinical studies also indicated that immunization with individual protein antigens, protein–protein fusions or the 5CVMB vaccine formulation with and without OMV/NZ, was immunogenic in guinea pig model.[75] OMV/NZ is the previously successful MeNZB™ OMV vaccine component of the New Zealand epidemic strain NZ98/254. The MeNZB™ OMV has been used since 2004 in the control of MenB epidemic diseases dominated by a single serosubtype (P1.4) in New Zealand and its safety and efficacy had been demonstrated.[76,77] The study conducted in juvenile baboons evaluated the immunogenicity of three consecutive doses of 5CVMB (50 µg per dose) formulated with $Al(OH)_3$ alone or in association with OMVs from the Norwegian meningococcal strain H44/76 that were administered intramuscularly (IM). Both formulations were immunogenic and elicited high geometric mean bactericidal titers (GMTs) and percentages of positive SBA. Similar results were obtained in infant rhesus macaques.[74,75]

5.2. Local tolerance

Data generated from the repeat-dose toxicity and the reproduction and developmental toxicity studies performed in rabbits (Table 2), were also used to assess local tolerance by macroscopic and microscopic examinations and cytotoxicity Draize scores of skin reactions. Injection site reactions, characteristic of an aluminum-adjuvanted vaccine, increased marginally in incidence and severity in treated rabbits that recovered partially after 14 days of injection. All toxicologic test scores were low and within acceptable limits.[74,75]

5.3. Cytotoxicity studies

A range of *in vitro* studies investigated potential effects of the 5CVMB vaccine proteins and OMVs that might be related to the pathogenicity of *N. meningitidis*. The protein antigens alone or combined with OMVs and $Al(OH)_3$ were not cytotoxic to primary human umbilical vein endothelial cells (HUVEC), although some binding occurred at high concentrations. The vaccine protein antigens did not affect cell monolayer permeability, prothrombin time (PT), and activated partial thromboplastin time (aPTT) in human plasma, nor activated protein C activity, platelet activation, or platelet-leukocyte aggregation. The protein antigens induced some cytokines in HUVECs and human whole blood, although the increases were similar to those induced by some registered aluminum-adjuvanted vaccines such as Prevnar® and Infanrix®. Vaccine recombinant antigens modestly increased expression of pro-inflammatory cytokines including IL-1β, IL-6, IL-8, interferon gamma (IFN-γ) and TNF-α in cultured human whole blood, similarly to Prevnar, Infanrix, Act-HIB, and Pneumovax 23. OMVs substantially increased levels of these pro-inflammatory cytokines.[75,78] Furthermore, small but significant increase in fibrinogen, globulin, and leukocyte and neutrophil counts were observed in New Zealand White rabbits injected IM with five consecutive doses of the clinical dose of 5CVMB, or twice the clinical dose of antigens. These results are consistent with an inflammatory response to the vaccine. The vaccine protein antigens, with or without OMVs, were immunogenic in rabbits without any evidence of systemic toxicity. Injection site reactions, characteristic of an aluminum-adjuvanted vaccine, were slightly more frequent or severe in vaccine-treated rabbits compared to controls, but were within acceptable levels and partially resolved within 14 days.[75]

5.4. Reproductive and developmental toxicity

A series of reproductive and developmental toxicity studies were performed in female rabbits that were injected with different formulations of the vaccine (summarized in Table 2). No overt hazard of vaccination to maternal animals, fetuses, or 4-week-old pups were raised. Injection site reactions, observed in both control and treated rabbits, were marginally increased in incidence and severity in treated rabbits that showed partial recovery 14 days post-dose. These data suggest local tolerance of 5CVMB vaccine.[75]

In 2008, early clinical trials were started by Novartis Vaccines and Diagnostics to evaluate the immunogenicity and reactogenicity of a 3-dose course of 5CVMB administered alone (also named recombinant Meningococcal B vaccine, rMenB) or in combination with OMV/NZ (named rMenB_OMV). The rMenB_OMV formulation has been shown to impart greater immunologic benefit and broader strain coverage, particularly against strains expressing homologous PorA, compared with rMenB alone in infants, an age group which would be expected to provide better discriminatory value than older age groups. OMV provides also an

Table 2. Toxicity studies with different vaccine formulations.

Study type	Treatment days	Dose of NHBA-953, 936-fHbp, and NadA each	Dose of OMV	Dose of $Al(OH)_3$	Study no. (date)
Rabbit single and repeat-dose toxicity study	1, 15, 29, 43, 57	50 µg 100 µg	25 µg (NW[1]/NZ[2]) 25 µg (NW)	1.5 mg 3 mg	1228–102 (2005)
Rabbit reproductive + developmental toxicity (dose-finding)	Premating 1, 15, 29, GD[3] 7, 20	50 µg 100 µg	25 µg (NZ) 50 µg (NZ)	1.5 mg 3 mg	UBA00041 (2008)
Rabbit reproductive + developmental toxicity (pivotal)	Premating 1, 15, 29, GD 7, 20	50 µg	25 µg (NZ)	1.5 mg	UBA00044 (2009)

[1]Norwegian OMV; [2]New Zealand OMV; [3]Gestation day.

immunoadjuvant effect thanks to the immunomodulatory properties of the residual LOS and other OM components.[79] This multivalent vaccine formulation that combines rMenB with MeNZB™ OMV was named four-component Meningococcal B vaccine, 4CMenB (trade name Bexsero®, GSK Biologicals).[54] The final formulation of the Bexsero is shown in the Table 3.

6. Clinical studies

Immunogenicity studies are usually used on the basis of a surrogate of protection.[80] The early clinical studies (see above) allowed determining the final formulation of the vaccine. The tolerance, safety, and immunogenicity of this final formulation were assessed in infants, toddlers and adolescents. Several clinical studies allowed the licensure of the vaccine in 2013 with the initial therapeutic proposition of the Bexsero® that was to immunize children (from 2 months of age), adolescents and adults against IMD due to serogroup B meningococci. The clinical studies for the licensure and their characteristics are depicted in Table 4.

6.1. Doses

The primary schedule in infants with three doses was evaluated at 2, 3, 4, or at 2, 4, 7 months of age (V72P12).[81] A booster dose after three doses in infant vaccinations was evaluated in V72P6, V72P13E1, and V72P12E1 (Table 4).

For older infants 6–8 months, a 2-dose schedule given 2 months apart (VP72P9), was evaluated. Similar schemes were studied for toddlers and older children (VP72P13E1, VP72P6E1, and V72P9E1).

For adolescents (11–17 years of age) and adults schedules with 1, 2 or three doses given at least 1 month apart were studied (VP72P10 and VP72P5 respectively).[81,82]

In 2018, the licensure was updated with a simplification of the primary vaccination schemes in infants between 2 and 5 months of age from 3 doses (in 2013) to 2 doses since July 2018. This 2 + 1 scheme was used in England since 2015, while it was recently recommended in France in 2021. The Bexsero® is administered intramuscularly.

6.2. Co-administration with other routine childhood vaccines

The use of Bexsero® in young infants required clinical studies on co-administration with other childhood vaccines. This co-administration is necessary due to the crowded vaccination calendar under the age of 1 year in many countries. This concomitant administration with routine infant vaccinations was addressed in infants and toddlers V72P12, V72P13, and V72P13E1 studies (see also paragraph 6.4.1).

6.3. Immunogencity and persistence of immune responses

Several studies addressed the immunogenicity with responses against the four reference strains used to evaluate the immunogenicity against the four components of the vaccine. Bactericidal titers were high one month after the completeness of both schedules (ranging from 85 to 100%).[81,83] The persistence of antibody response after vaccination was evaluated 24 to 36 months after the last dose in infants who received 3 + 1 (priming vaccination in infancy and a toddler booster). Globally, these studies showed a drop in bactericidal titers regardless the age of the booster according to the proportions of subjects achieving SBA titers of ≥5. However, this drop was variable according to the vaccine antigens. The proportions of subjects with hSBA titers of ≥5 were NadA (89–100%)> NHBA (53–80%)> fHbp (12–35%)> PorA (8–12%). Similar data were also observed for GMTs.[84] The persistence of the 2 + 1 schedule (priming vaccination in infancy and a toddler booster) also declined 2 years later and showed similar kinetics as for the 3 + 1 schedule. The proportions of subjects with hSBA titers of ≥5 were NadA (100%)> NHBA (79%)> fHbp (36%)> PorA (14%) with similar data also observed for GMTs.[85] However, these titers (in both 3 + 1 and 2 + 1) underwent a rapid increase suggesting an anamnestic response and the establishment of a memory response.[84–86] These data clearly underscore the requirement of a toddler booster regardless the priming schedule in infancy. Real-world data from the use of Bexsero® in England suggested a duration of protection of 4 years in infants and toddlers.[87]

The persistence of the immune responses (as proportions of hSBA titers equal or higher the threshold that is correlated with protection) was evaluated in adolescents and young adults. Several studies showed higher hSBA titers in these age groups than in toddler and young children and, in particular, for fHbp and PorA. Subjects of 18 and 24 years old who were primed according to a 2-dose schedule showed 4 to 7.5 years later hSBA titers that remained significantly higher than in vaccine-naive participants at baseline except for NHBA. Indeed, the proportions of hSBA titers of ≥4 were, respectively: for fHbp (44% vs 13%); NadA (84% vs 24%) and for PorA (29% vs 14%)) but for NHBA (81% vs 79%).[88] Interestingly, 93–100% of the primed subjects achieved after booster, hSBA titers for all the four antigens suggesting an anamnestic memory response.[88]

Table 3. Components of the Bexsero Vaccine.

Component	Amount (per dose of 0.5 ml)	Relevant characteristics/function
FHbp (*Factor H binding protein* in fusion with the accessory protein GNA2091	50 µg	Negative regulatory protein of the alternative pathway of the complement
NHBA (*Neisserial Heparin Binding Antigen* in fusion with the accessory protein GNA1030	50 µg	Complement dependent Bacterial lysis
NadA (*Neisserial Adhesin A*	50 µg	Binding to epithelial cells
Vaccin OMV rMenB (strain NZ98/254, vaccin Men-ZB®)	25 µg	Mainly PorA outer membrane protein
Al(OH)3	1.5 mg	Adjuvant

Table 4. The major clinical studies for licensure.

Study	Phase	Type	Population at enrollment	N° of arm(s) of the study*	N enrolled	Schedule (month)
V72P5	1	Observer blind, randomized single center	Adult 18–40y	1	28	0,1,2
V72P4	2	Open multicenter	Adult 18–50y	1	54	0,2,6
V72P6	2	Open multicenter randomized controlled	Infants 2 mo	2	50 24	2,4,6,12 12
V72P6E1	2	Open label single-center extension	Infants 2 mo, childfren 40 mo	3	19 8 43	2,4,6,12,40 12,40,42 40,42
V72P9	2	Single-blind, randomized, single-center	Infants (6–8mo)	1	30	6,8,12
V72P9E1	2	Open label single-center extension	Infants 6–8mo Childfen 40 mo Children 60 mo	3	14 41 49	6,8,12,40 40,42 60,62
V72P12	2	Open multicenter randomized	Infants 2 mo	3	627† 318† 628‡	2,4,6 2,3,4 2,4,6
V72P12E1	2	Open label multicenter extension	Toddlers 12, 18, 24 mo 4th booster dose at 12, 18 or 24 mo; 2 catch up doses at 12–14, 18–20 mo (naive) and in naive children (24–26 mo)	3	1588 246 51 + 56	Booster at 12, 18 or 24 months of age in subjects who received 3 doses of at 2,4,6 + Routine at 2,3,4 or 2,4,6 months in V72P12 at 12 and 14 months (in subjects who received routine at 2,3,4) at 18, 20 and at 24, 26 months of age
VP72P16	2	Partially observer-blind, randomized multicenter controlled	Infants (2mo)	8	Total 1507	2,3,4 8 vaccine groups injected with either different composition or formulation process of the meningococcal B antigens (groups I–VI), or with concomitant administration of paracetamol (Par+B+ OMV, group VIII), or receiving the control vaccine (MenC, group VII)
V72P10	3	Observer-blind multicenter randomized controlled	Adolescents (11–17y)	8	Total 1631	1 dose, or 2 doses [0,1 or 0,2], or 3 doses [0,1,2 or 0,2,6 or 0,1,6]
V72P13	3	Partially blinded randomized multicenter controlled	Infants (2mo)	3	Total 2481	2,4,6 Concomitant with routine Routine alone Routine+MenC
V72P13 E1	3	open label randomized multicenter extension	Toddlers (12 mo)	6	Total 2249	A booster 12 month for the groups of VP7213 (with MMRV) concomitantly or A mo aftewards
V72P13E2	3	Open label randomized multicenter extension	Toddlers 23 mo (naives)	3	Total 508 305 86 116	12mo persistence 3rd dorse to 2-dose cach-up 2 doses in nive children 24mo and 26 mon

*Only the arms that used the final formulation (rMenB+OMVNZ) are mentioned in the Table.
† concomitant with routine vaccines, ‡ intercalated with routine vaccines at 3,5,7mo.

These better responses can be explained by a more mature immune system but also by priming and boosting through pharyngeal carriage that is more frequent among adolescent and young adults.[89]

6.4. Safety and tolerance testing

The safety profile of the vaccine was evaluated in infants, toddlers, adolescents, and adults through several studies involving a total of >6000 subjects. This evaluation was mainly comprising infants from 2 months of age (75% of the total enrolled subjects). Side effects with frequencies around 1/1000 can therefore be identified but more rare effects may require post-marketing studies.

The first safety and reactogenicity profiles of 4CMenB came from the original and extension clinical studies in which individuals received at least one dose of 4CMenB.[79,81,83,90–92] Events occurring within 6 days after the day of injection and assumed to be at least possibly related to the administration of the vaccine were solicited and used as indicators of reactogenicity. These events included local reactions (tenderness, erythema, swelling, and induration), systemic reactions (change in eating habits, sleepiness, unusual crying, vomiting, diarrhea, irritability, and rash) and other reactogenicity indicators (fever ≥38.0°C, and use of analgesic/antipyretics). Overall, the safety and tolerability profiles for 4CMenB were similar to that of several other routine infant vaccines, and did not preclude widespread use of the vaccine.

6.4.1. Infants and toddlers

The comparators in the "patient exposure" analysis in infants were infants receiving routine childhood vaccination including MenC vaccines (V72P12 and V72P13). The comparators were placebo groups for adolescents and adults (V72P10) and the safety profile was globally acceptable in these age groups.

Infants receiving 4CMenB reported mild or moderate local and systemic reactions that resolved within 7 days of observation window.[85,93,94] The rates of these reactions were higher among infants who received 4CMenB concomitantly with routine vaccines than those in infants who received routine vaccines alone or routine vaccines concomitantly with MenC-conjugate vaccine.[79,81,83,90,91] Severe local tenderness ranging between 12 and 29% were also reported.[79,83,85,91] As with the other local reactions, these effects were transient and usually resolved within 24 h. Systemic reactions, mostly sleepiness and irritability, were reported in 81–92% of infants after any primary dose of 4CMenB administered with routine vaccinations.[95] Furthermore, pyrexia was reported in 23–36% of cases when the vaccine is administered alone and increased to 51–61% when 4CMenB was co-administered with routine vaccines.[83] Fever (≥38.5°C) was the most strikingly relevant systemic reaction. In the early Phase II studies, less than 20% of infants experienced fever after receiving 4CMenB alone.[79] In a Phase IIb study, the rate of fever (≥38°C) was reported in 26–41% of infants and was slightly more frequent than those immunized with routine vaccines alone (23–36%) after the first dose at 2 months of age.[83] Administration of 4CMenB concomitantly to routine vaccinations rose the rate of fever to 76–80% of infants.[83,91] The phase III study reported 65.3% of infants that experienced fever within 6 h of vaccination. The duration of the fever was transient, resolving within 48 hours after vaccination. This pattern was consistent for all three 4CMenB doses and the booster dose at 12 months.[91] The use of different routine vaccines, variations between the lots of the 4CMenB vaccine and the route of measuring temperatures (axillary *vs*. rectal routes) as well as the lower number of recruited persons in the early phase studies, may explain the discrepancy in the rate of fever between the different studies. In all these studies, infants who experienced fever ≥40°C after vaccination were infrequently reported.[79,83,91] The OMV vaccine component, shown to be pyrogenic,[96] is likely a contributing factor to 4CMenB reactogenicity.

Prophylactic administration of antipyretics, such as paracetamol, diminished the rate of these reactions, in particular the development of tenderness, from 56–66% to 37–47% and the rate of fever (≥38.5°C) from 70.3 to 39.1%, without compromising the immunogenicity of either 4CMenB or routine vaccines (V72P16).[95,97]

There were a number of adverse events reported in the infant studies. These included febrile and non-febrile seizures, Kawasaki disease and hypotonic hyporesponsive episode (HHE).[83,91] However, the few cases that occurred in the 4CMenB clinical studies did not allow a definitive assessment of the causal relationship between administration of 4CMenB and increased risk of these adverse events in infants and toddlers.[98] Indeed, the pivotal studies V72P12 and V72P13 that include about 4000 subjects exposed to Bexsero®, 3 cases of seizures were reported within 2 days of vaccination. This frequency was too low to be evaluated as possibly related to vaccination. However, this was initially a matter of concern as these cases were observed in a younger age that usually described for febrile seizures in infants (≥6 months). It is noteworthy here that the management of seizures in infants <6 months requires hospitalization and lumbar puncture.[99] The evaluation of this risk awaited post-marketing and real-world data that did not reveal any specific increase of risk for seizures post Bexsero® vaccination. Concerning Kawasaki Disease (KD), few cases were reported in the clinical studies V72P12, V72P13, and V72P13E1 in both Bexsero® vaccinated and control subjects. However, the low frequency of KD was in this study (<1/1000) and the incidence rates that did not differ between the two groups argue against a causal relationship between Bexsero® vaccination and KD and in agreement with post-marketing and real-world.[100]

6.4.2. Adolescents and adults

In the adolescents, the safety profile of 4CMenB was mainly based on the Phase IIb/III study NCT00661713. 4CMenB-injected adolescent group developed injection-site reactions (mainly pain) in 86% of cases, with 17% reported as severe, comparing to 60% among placebo-receiving group with 4% of severe cases. The most common systemic reactions were malaise reported in 51% of 4CMenB recipients (*vs*. 30% of placebo recipients) and headache reported in 42% of 4CMenB recipients (*vs*. 27% of placebo recipients). The frequency of local and systemic reactions did not increase with the second dose of 4CMenB, and the majority of the reactions were transient. Fever is less common than in infants (4% of 4CMenB *vs*. 2% of placebo injected adolescents).[81]

In adults, 4CMenB showed an acceptable tolerability profile. Indeed, most of the subjects reported local and systemic adverse reactions which were mild to moderate in severity and resolved spontaneously within few days. The most commonly reported local reactions were local pain, followed by erythema. Myalgia and headache were still the mainly reported systemic reactions.[90,101]

In post-marketing surveillance from Australia, England, and Italy, no safety concerns have been raised with >1,000,000, doses distributed worldwide. The adverse effects (AE) were reported at the rate of 26.5 per 100,000 doses. The majority of these AEs were reported as not serious (18 per 100,000 does, 67.8%) in Apulia region, Italy between 2014 and 2019.[102] AEs appear to be consistent with that established in clinical trials.[78,100,102–106]

7. Production and assays for releasing and characterizing 4CMenB

4CMenB contains four active components: three meningococcal recombinant antigens, two of which are fused to accessory proteins: NHBA (peptide 2) fused to GNA1030 (also called antigen 953), fHbp (variant 1.1) fused to GNA2091 (also known as antigen 936) and NadA peptide 8 (variant NadA2/3) present as a single antigen. The protein antigen sequences were derived from three meningococcal strains: NZ98/254 (antigen NHBA), strain 2996

(antigens 953, 936, and NadA) and strain MC58 (antigen fHbp). These protein antigens are produced separately via bacterial fermentation by standard recombinant DNA technology methods in *E. coli* using a plasmid vector system. While the recombinant protein NadA is expressed and secreted into the culture supernatant, the other two recombinant fusion proteins NHBA-953 and 936-fHbp are expressed intracellularly. The recombinant proteins are separately harvested and are separated from the bacteria by centrifugation and filtration steps, then purified through a series of chromatography columns and concentrated by filtration. The fourth active component corresponds to detoxified OMVs from *N. meningitidis* NZ98/254 strain (B:4:P1.7–2,4), expressing the immunodominant protein PorA serosubtype P1.4 and other minor outer membrane proteins. This strain representative of the circulating meningococcal serogroup B strain responsible for an epidemic in New Zealand, has been selected for its good yield in production of OMVs. OMV is obtained through culture expansion of the NZ 98/254 strain, inactivation and subsequent purification and filtration. During the manufacturing, most of the LOS content of OMV is substantially reduced by detergent-treatment prior to incorporation into the vaccine to reduce the pyrogenicity of OMVs. The bioavailability of LOS is further reduced by adsorption of the OMVs to $Al(OH)_3$ adjuvant.[36,76,107,108] The residual LOS present in the vaccine is required to maintain the structural integrity of the proteins. The filtered OMVs pre-bulk is finally sterile filtered to yield the OMV sterile bulk concentrate that is stored at 2–8°C. A thorough work has been made by the manufacturer, including an adapted *in vivo* rabbit pyrogen test (RPT), to demonstrate the safety of released batches, both in clinical studies and commercial experience.[78] The released specifications for both the recombinant proteins and the OMV sterile bulk concentrate include: purity, protein content and concentration, identity, osmolality, endotoxin, sterility, pH, conductivity, DNA, and process related impurities.

The final product is available as 0.5 ml-prefilled syringe ready for intramuscular injection. Each dose contains 50 µg each of the purified *N. meningitidis* recombinant antigens and 25 µg of OMVs. All these components (the protein antigens and OMVs) are adsorbed on 1.5 mg of aluminum hydroxide ($Al(OH)_3$) adjuvant to achieve adequate immunogenicity of all antigens.[75] In addition, 10 mM histidine buffer (to adjust the pH, the antigen adsorption and the stability of the final product) and 6.25 mg/mL sodium chloride, pH 6.5 with 3% sucrose solution (to obtain an isotonic preparation) are present as excipients in every dose.[109]

The release tests for 4CMenB vaccine include test for aluminum content (1.5 mg/ dose, which corresponds to 0.5 mg of elemental aluminum per vaccine dose), and the degree of antigens adsorption as well as other compendial tests (sterility, endotoxins, pyrogens, etc.). *In vivo* immunogenicity test in mice are performed to assure consistency of batches before release. The final product is proposed to be stable for 2 years when stored at 2–8°C protected from light. The claimed shelf-life at the recommended storage temperature is supported by clinical effectiveness of vaccine lots used beyond their proposed 24-month shelf-life in two clinical studies, V72P16 (vaccine median age of 27 months) and V72_41 (vaccine median age of 29 months).[75] The vaccine should be administered intramuscularly into the anterolateral aspect of the thigh (in infants) or the deltoid muscle (in all other groups), with separate injection sites being used if the vaccine is co-administered with other vaccines.

8. 4CMenB mechanism of action and strain coverage

Protection against IMD is conferred mainly by complement-mediated antibody-dependent killing of *N. meningitidis*. Killing requires that the binding of antibodies to the bacterial surface is sufficient to activate complement. Vaccination with 4CMenB leads to the production of antibodies directed against the major active components NHBA, NadA, fHbp, and PorA P1.4 (present in OMV). The presence of antigen-encoding genes by itself is not directly predictive of a strain susceptibility to antibody-mediated killing. The susceptibility of MenB strain to complement-mediated antibody-dependent killing after vaccination with 4CMenB is critically dependent on the similarity between the antigens expressed on the bacterial surface and the antigen variants included in the vaccine (cross-reactivity) in one hand, and the level of antigen expression on the surface of the invading strain. Indeed, different degrees of cross-reactivity among individual strain antigens to the antibodies elicited by the vaccine exist due to the qualitative (sequence variations) and quantitative (expression level) variations of expressed antigens on the surface of MenB strains. Moreover, performing human serum bactericidal assay (hSBA) of post-immunization sera against a large panel of disease-causing strains is challenging due to the requirement of a considerable amount of sera, ethical issues and inherent variability in the observed killing between strains. To mitigate the need to perform hSBA, a high-throughput *in vitro* assay, called Meningococcal Antigen Typing System (MATS), has been specifically developed to estimate the breadth of coverage of MenB strains by 4CMenB. MATS correlates information on the expression level of the antigens expressed by individual MenB strains and the potency of the immune response elicited by the vaccine based on bactericidal assays.[110] It combines conventional genotyping for PorA with binding of polyclonal antibodies in specialized sandwich ELISA assay against each of the three other antigens fHbp, NHBA, and NadA in a given MenB strain. The extent of binding of antibodies in a given strain is compared to a reference strain for each antigen. This metric is called the "relative potency" (RP). An antigen-specific positive bactericidal threshold (PBT) has been established for each antigen based on bactericidal activity using pooled sera obtained from 13-month olds having received 4 doses of 4CMenB against a panel of 57 serogroup B strains. A given strain is assumed to be covered if the RP is higher than PBT.[65] Accordingly, for a RP exceeding PBT of binding for

Table 5. Characteristics of the two protein-based vaccines targeting meningococci B.

Vaccine	Bexsero®	Trumenba®
Composition	fHbp variant1 (subfamily B): 50 µg NHBA 50 µg NadA 50 µg PorA P1.4 25 µg	Lipidated proteines of:fHbp variant1 (subfamily B): 60 µg fHbp variant3 (subfamily AB): 60 µg
Licensure	Europe (EMA) (2013): ≥2 mo USA FDA (2015): ≥10 y	Europe (EMA) (20173): ≥10 y mo USA FDA (2015) ≥ 10 y
Schedule <10 y)	2 Mo-2y: 2 + 1 > 2 y-10y: 2 doses (0–2mo)	-
Schedule ≥ 10 y	> 10 y: 2 doses (0–1mo); booster unknown	2 doses (0–6mo) or 3 doses (0–1/2-6mo); booster unknown
Persistence of the immune response	Infants and toddlers 24–36 mo after booster Adolescents 4–7.5 years	4–5 y
Estimation of strain converge	78% (CI 63–90)	91% (71.5–99.3)*
Impact on acquisition of carriage	No	No
Protection against non-B isolates	Yes	Yes

*Range of coverage against reference strains.

one antigen, a given strain is predicted to have at least 80% probability of vaccine coverage. This probability increased to 96% for isolates with two or more antigens with RP above the PBT.[63,65] In this regard, a strain expressing only one vaccine-matched antigen may be susceptible to killing. The protective capacity of 4CMenB is enhanced when the bactericidal antibodies elicited by 4CMenB bind simultaneously multiple targets. An isolate that expresses PorA P1.4 is considered to be covered by the OMV component of Bexsero.[111]

MATS was able to predict an overall coverage rate of 81% calculated for a sample size of 3912 invasive MenB isolates, collected from 17 different countries.[112] The coverage rate ranges from 68% to 89% in European countries and from 66% to 91% in Australia, Canada, Brazil, and the USA.[63,110,113–116] NHBA and fHbp were consistently the antigen components that contributed mostly to coverage.[112] However, MATS has recently been shown to underestimate the potential coverage of Bexsero. Indeed, while MATS predicted coverage of 70% of 40 MenB disease isolates from England and Wales, hSBA predicted 88% of strains to be killed.[117] Similar observations were made in a study conducted with sera from Spain.[118] Indeed, as MATS predicts coverage based on individual antigens testing, it does not reflect the synergistic aspects of binding of bactericidal antibodies. Moreover, the use of pooled versus individual sera in hSBA assays may explain the underestimation of seroprotection conferred by Bexsero® when predicted by MATS.[119]

As Bexsero® targets sub-capsular proteins that can be conserved regardless the capsule, the vaccine can be effective against non-B isolates. Several works provided evidence on the basis of hSBA and/or MATS on the coverage of non-B isolates by the Bexsero®.[120–123] Real-world data from England provided evidence of protection by Bexsero® against IMD serogroup W.[124]

9. Impact on the acquisition of carriage

Optimally, meningococcal vaccines are aimed to impact on the acquisition of carriage as its reduction has been shown to produce indirect protection in non-vaccinated subjects by reducing the transmission and the contamination among unvaccinated population. This has been shown for vaccination against serogroup A and serogroup C using polysaccharide-conjugate vaccines.[125,126] For example, the incidence of serogroup C IMD was reduced among infants < 1 year old that were not targeted by the vaccination in the Netherlands in addition to the reduction among vaccinated subjects (1–18 years old).[127] However, studies on Bexsero® did not show any significant reduction of the acquisition of carriage of serogroup B isolates following the vaccination.[128,129] This may be due to the high heterogeneity of carriage isolates, the difference in the alleles encoding the vaccine antigens among these isolates and their expression levels compared to invasive isolates.[130,131]

10. Comparison to bivalent rLP2086vaccine (Trumenba®)

Another protein-based vaccine targeting serogroup B isolates was also licensed. It is composed of two variants of fHbp and was developed by Pfizer (Trumenba®). The two vaccines differ by several aspects although they share the fHbp in their composition (Table 5). Only the Bexsero® is licensed in children <10 years of age but both vaccines seem to show similar immunogenicity data.

11. Conclusions

Developing vaccines against meningococci of serogroup B was a major challenge in vaccinology due to the difficulties in obtaining capsule-based vaccine. The reverse vaccinology was a major breakthrough allowing the development of the Bexsero® (a multicomponent protein-based vaccine). The lack of impact of the vaccine on acquisition of carriage of serogroup B meningococci and the need to estimate the effective duration of protection in childhood remain to be solved. However, the observational data now available showing real-world efficacy of the vaccine in England, Portugal, and Italy,[87,132,133] are also showing an impact of reducing the incidence of IMD due to serogroup W. One current research focus to be considered in the future is the potential development of combined MenABCWY vaccines that

may reduce the cost of stocking and administering separate meningococcal vaccines, avoid extra health-care visits, and facilitate the introduction of new vaccines into immunization programs.

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Authors' contribution

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Trademark statement

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