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# Les vaccins méningococciques : état des lieux et perspectives

## Meningococcal vaccines: current state and future outlook

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## Résumé

Les infections à *Neisseriameningitidis* sévissent dans le monde entier et restent un véritable problème de santé publique. Toutefois, la vaccinologie conventionnelle n'a pas permis le développement d'un vaccin contre le méningocoque de sérotype B. Une nouvelle technique, la vaccinologie inverse, a alors ouvert de nouvelles perspectives. Récemment, un vaccin universel antiméningococcique B, Bexsero<sup>®</sup>, a été autorisé en Europe, en Australie et aux États-Unis, suite à des études cliniques d'immunogénicité et d'innocuité concluantes. La mise à disposition d'un tel vaccin peut apporter une réelle contribution à l'amélioration de la santé humaine, pouvant réduire significativement l'incidence des infections méningococciques. Cependant, la vaccination systématique n'est pas recommandée actuellement, en raison d'une analyse coût-efficacité défavorable. Un second vaccin antiméningococcique B, Trumemba<sup>®</sup>, a également été récemment autorisé aux États-Unis. Comme tout médicament, ces deux vaccins nécessiteront une surveillance étroite, permettant d'étudier leur impact sur l'épidémiologie méningococcique.

**Mots clés :** *Neisseriameningitidis*, vaccin, Bexsero<sup>®</sup>, vaccinologie inverse

## Abstract

*Neisseria meningitidis* infections are a major public health problem worldwide. Although conventional approaches have not led to development of a serogroup B meningococcal vaccine, a new technique based on genome sequencing has created new perspectives. Recently, a universal serogroup B meningococcal vaccine, Bexsero<sup>®</sup>, was licensed in Europe, Australia and United States, following several clinical studies demonstrating its immunogenicity and safety. Availability of this vaccine could contribute positively to human health, by significantly reducing the incidence of meningococcal infections. However, unfavorable cost-effectiveness analysis means that routine vaccination is not currently recommended. Another serogroup meningococcal vaccine, Trumemba<sup>®</sup>, was also recently licensed in United States. Like any drug, Bexsero<sup>®</sup> and Trumemba<sup>®</sup> will require close observation to assess their impact on meningococcal epidemiology.

**Keywords:** *Neisseria meningitidis*, vaccine, Bexsero<sup>®</sup>, Trumemba<sup>®</sup>, reverse vaccinology

Invasive meningococcal disease (IMD) is perceived as a serious threat by the public, who are very influenced both by the media, and by the medical professions.[1] These infections have different clinical consequences (meningitis, septicemia, arthritis, pneumonia, and pericarditis), which can occur simultaneously, sometimes inducing misdiagnosis.[2,3]

Meningococcal infections remain a public health problem throughout the world.[2,4] Indeed, in the context of the development and accessibility of relatively new vaccines (especially against *Haemophilus influenzae* type b and *Streptococcus pneumoniae*), *Neisseria meningitidis* is considered one of the most frequent infectious causes of death outside the neonatal period.[5-7] The diversity of meningococcal strains is correlated to the antigenic variability of the bacterial capsule.[8,9]

In accordance with serological methods based on the composition of the polysaccharide capsule, an international nomenclature was established to identify and to monitor precisely

epidemic clones.[10] This classifies *Neisseria* into 13 distinct serogroups, of which 6 (A, B, C, W135, X and Y) are distinguished by their high virulence for humans.[3,8,9,11]

Since the introduction of polysaccharide conjugate vaccines against serogroups A, C, W-135 and Y, serogroup B has emerged as the basic cause of IMD, particularly in Europe and North America, where it creates hyperendemic or epidemic situations, especially due to the lack of prophylaxis for this serogroup.[4,11]

Thus, the best strategy against IMD remains primary prevention via meningococcal vaccination, including the development of a vaccine targeting serogroup B.[3,10,12,13]

## 1. Development of a meningococcal group B vaccine

### 1.1. The need for meningococcal group B vaccine

The epidemiology of meningococcal disease is dynamic: all serogroups vary temporally and geographically.[6,10,14] This marked disparity can mainly be explained by differences in immunity among ethnic groups, and by the influence of environmental factors.[3,6]

Each year, IMD estimates rise by 1.2 million cases worldwide, including 135,000 deaths.[12] In response to these alarming statistics, the World Health Organization (WHO) has for example introduced specific vaccination campaigns against serogroup A in Africa where it is predominant to limit large meningococcal epidemics.[14,15]

However, the threat persists, especially in industrialized countries where serogroup B is involved in most cases of IMD (84% of IMD in Australia, 83% in New Zealand, 76% in Europe and 35% in the United States). In Europe, the annual incidence of serogroup B is 1 case per 100,000 population.[14]

According to epidemiology data, some populations are more sensitive to infection. In the United States, nearly 60% of IMD in infants under the age of 59 months is caused by serogroup B.[16]

In France, the highest incidence is observed in infants aged 0-12 months, with the annual incidence reaching 11.1 cases per 100,000 population. This rate is correlated to the immaturity of their immune system.

A high incidence is also observed later, in young adults aged 15-24: at 19 years, the incidence rises to 2.89 cases per 100,000 population.[17,18] The social behavior of young adults

can facilitate exposure to strains against which they have no immunity.[17,19]

IMDs are characterized by their global distribution, their epidemic potential, their unpredictability (their potential to alter the health of a healthy individual without any history of illness), their rapid progression and their minor and non-specific symptoms.[3,6,8,20,21] All these factors explain the severity of these infections, and why rapid diagnosis and immediate initiation of treatment are vital.[2]

Despite technical and therapeutic advances, the overall mortality rate remains between 10% and 15%, and up to 19% of survivors have disabling sequelae such as hearing loss, cognitive dysfunction, motor nerve deficits, seizure disorders, amputation.[16,20]

Given the severity of meningococcal infections, developing a meningococcal vaccine that can control the most of these devastating epidemics has become a real priority for medical research.[17,22]

## 1.2. Special features of serogroup B

Meningococcal vaccines targeting serogroups A, C, W-135 and Y have demonstrated their effectiveness.[6,23] However, there are a number of reasons why the immunological approach to traditional vaccine development, conjugate or polysaccharide, is out of the question for serogroup B.[6,13,24,25]

First, the heterogeneity of intra-serogroup proteins, especially the dominant protein, Porin A (PorA), seriously complicates universal vaccine design.[26,27]

Up to now, only specific vaccines prepared from vesicles on the bacterium surface, *outer membrane vesicle* (OMV), were developed and used as the need arose. They were used

for disease control in epidemic situations in Brazil, Chile, Cuba (VaMencog-BC®), Norway (MenBVac® vaccine), New Zealand (MenZB® vaccine) and recently in France (Norwegian MenBVac® vaccine because of epidemic strain similarity).[4,22,28-35] The composition of these vaccines was determined by a particular bacterial clonal strain, so their effectiveness was limited to certain epidemic conditions.[24,27] For example, the Cuban vaccine, which showed an effectiveness rate of about 83% in Cuban children aged above 4, was less effective in Brazilian children, with an effectiveness rate of 74%.[28,31,36] Moreover, Cuban vaccine effectiveness in Brazilian newborns appeared to be considerably lower than that reported for Cuba newborns.[28] These vaccines, termed *tailor-made*, are poorly immunogenic in children less than 4 years old, and the duration of protection is limited.[4,10,37] These protein vaccines do not therefore constitute a global vaccination strategy against serogroup B.[25,28,38]

Secondly, the polysaccharide capsule of serogroup B contains polysialic acid, as do all the capsules of meningococcal virulent serogroups, with the exception of serogroup A which is composed mainly of N-acetylmannosamine-1-phosphate.[3] The main difference between serogroups B and C is their linkage patterns: the group B capsule has 2-8 linkage while group C has 2-9 linkage.[38]

The final structure is sufficiently different to induce specific antibodies for each serogroup.[6] The derivative of the polysialic acid contained in the serogroup B capsule is found in many human tissues, especially in the central nervous system, and particularly in adhesion cells (*Neural cell Adhesion Molecule* N-CAM), highly expressed in the fetus

and the young child in development.[26,39,40] Cross-reactivity between N-CAM embryonic isoforms and sera from patients with serogroup B meningococcus was also demonstrated while this reaction was absent in healthy subjects.[39] Molecular mimicry induces low immunogenicity, attributed to immunological tolerance by the host to the sialylated glycopeptides, physiologically contained in some body zones.[25] Consequently, if this antigen was included in a vaccine, T-cells, particularly T-lymphocytes, might recognize the self-antigen.[25,39] Antibodies secreted by the immune system would be directed against a molecule physiologically present in the human body, and might cause autoimmune disorders, particularly in the brain. Despite various attempts, particularly chemical modification of the N-acetyl group, there has been no real success.[41]

The real challenge is therefore to develop a multivalent vaccine to expand vaccine coverage and to avoid the risk of selecting for vaccine escape variants against a pathogen that mimics host molecules.[27]

### 1.3. Reverse vaccinology, an innovative and promising technique

The conventional approach to vaccine development proceeds via the *in vitro* culture of the pathogenic organism, allowing potential antigenic components to be individually identified by biochemical, immunological and microbiological methods.[37,42] Finally, each isolated antigen is analyzed to determine its ability to induce protective immunity. Although this long and tedious technique, termed "classical vaccinology", has led to many vaccines commonly used today, it still presents some limitations.[42] It cannot be applied to all micro-organisms, excluding all bacteria

difficult to cultivate or non-cultivable and also antigenically variant, especially meningococcal serogroup B. Moreover, antigens expressed only during *in vivo* infection are not produced under laboratory conditions and therefore not analyzed.[42]

Scientists have recently proposed a new concept, called "reverse vaccinology".[39] This new strategy is based on the complete sequencing of the bacterial genome, to identify potential, highly immunogenic antigens, regardless of their expression level.[37,42-45] This technique presents the advantage of applying to all germs, pathogenic or commensal, cultivable or not. However, it does not detect non-protein antigens such as capsular polysaccharides and glycolipids, which nevertheless remain promising as components for future vaccines.[44] Reverse vaccinology was applied for the first time in 2000, on a virulent strain of meningococcal group B (MC58).[45,46] The entire sequence of the bacterial genome was analyzed: nearly 600 candidate genes encoding for new surface-exposed proteins were identified.[42,44] The predicted proteins were cloned and expressed in *Escherichia coli*. [37,42,44,46]

Of these, 350 putative surface proteins were successfully expressed, purified and then tested on mice. The sera were analyzed, in particular by Western Blot to confirm the expression of these proteins *in vivo*. Furthermore, enzyme-linked immunosorbent assay (ELISA) and fluorescence-activated cell sorting (FACS) were used to determine the exact location of these proteins and their function.[37,42,44-46] Protein antigens selected were classified according to their serum bactericidal activity (SBA) against the target strain. Among the 91 proteins inducing bactericidal antibodies in animals, about 25, not detected by conventional

techniques, were selected for their surface location.[42,45,46] Moreover, these antigens were recognized by the serum of patients who had developed meningococcal disease. Finally, only 3 protein antigens were selected for their characteristic properties, allowing the development of the recombinant protein (rMenB). This was the first example of real success, resulting in a universal vaccine against meningococcal group B.[37,44] The new strategy was then applied to the development of vaccines against other pathogens, such as *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Chlamydia pneumoniae* ...[37,46,47]

## 2. The marketing of the first universal meningococcal serogroup B vaccine

### 2.1. The formulation of the new vaccine

To ensure that protection is the most universal possible and to avoid the emergence of mutants, the ideal solution is to develop a multi-antigenic meningococcal vaccine.[27,45,48] Reverse vaccinology yielded 3 vaccine antigens, commonly called *Neisseria genome-derived antigens* (GNA).[44,45]

- *Factor H binding protein* (fHBP), also named GNA 1870 or rLP 2086 fused to GNA 2091: A surface-exposed lipoprotein expressed by most strains of meningococcal group B, its expression is highly variable.[6,27,45,49,50] Meningococcal B vaccine includes the sub-variant 1 or the subgroup B, which is the most common. Secreted antibodies are specific and exclusively protect the host against strains presenting this variant. For the development of a universal vaccine, it is therefore advisable to add at least one other antigen, to expand coverage.[49] This fHbp protein is implicated in the regulation of the alternative complement

pathway essential for bacterial lysis.[6,45] Blocking this protein through a vaccine, would help the host's immune system to clear the bacteria from the body.

- *Neisseria adhesin A* (NadA) or GNA 1994: This protein is present in only 50% of the tested strains, but is found in most clonal hypervirulent serogroup B strains in Europe.[46,51-53] In contrast to the 2 other principal antigens of the vaccine, this trimeric protein is not associated with an accessory protein.[24,45,53] This protein allows host cell adhesion and invasion, so it would have an indispensable function in asymptomatic carriers.[51,53] The induced antibodies should have the potential to stop bacterial colonization and thus to prevent invasive disease, but this hypothesis was not confirmed by scientific evidence.[27] In contrast, the secretion of protective antibodies in laboratory animals was proven, and the sera of patients who reported a meningococcal disease recognize the protein: this shows that NadA plays a major role not only in the virulence of meningococci but also in immunogenicity in humans.[27,51,52]

- *Neisseria Heparin Binding Antigen* (NHBA) or GNA 2132, fused to GNA1030: This is a ubiquitous lipoprotein which is specific for *Neisseria* species with a variety of proteins.[50] In mice, antibodies developed showed bactericidal activity mediated by the complement, and the sera of patients with a history of meningococcal disease also recognize the protein.[45,54]

The 2 accessory proteins, GNA 2091 and GNA 1030, respectively fused to fHbp and NHBA, reinforce the immune responses and enhance the stability of the final recombinant protein.[27,45] An outer membrane vesicle (OMV) derived from a serogroup B New Zealand strain (strain NZ98 / 254)

is firstly added to these 3 principal proteins in order to enhance immunogenicity, followed by an adjuvant, aluminum hydroxide, currently controversial.[55-58]

This is the formulation of the new multicomponent vaccine 4CMenB (for 4 components against meningococcus B) recently marketed under the name Bexsero<sup>®</sup>. It obtained marketing authorization in Europe in January 2013 as the first broad spectrum vaccine for *Neisseria meningitidis* serogroup B.[6,12,38] In August 2013, this vaccine was also approved in Australia,[12] and in January 2015 in United States.[59]

In October 2014, another serogroup meningococcal vaccine, Trumemba<sup>®</sup>, was licensed in United States. This vaccine consists of two fHBP variants immunologically distincts (A05 and B01).[60] Although clinical trials for this second vaccine were conducted in the United States, Australia but also in Europe, Trumemba<sup>®</sup> is not yet licensed in Europe.

## 2. 2. The effectiveness of 4CMenB

The relatively low incidence of IMD ruled out performing efficacy studies of the new vaccine prior to marketing authorization. Thus, 4CMenB was basically awarded marketing authorization on evidence of immunogenicity and safety data rather than on a formal demonstration of its effectiveness.[4,13,61] To estimate the effectiveness of a new vaccine, immunogenicity and immunization coverage must be taken into account, and there are standardized analytical methods for a true consensus.[6,23] Goldschneider demonstrated in 1969 the inverse correlation between the incidence of a disease and the presence of bactericidal antibodies in human sera, meaning that the probability of contracting the disease

depends on the amount of circulating antibodies.[23,62] Further to these observations, circulating bactericidal antibodies were accepted as true markers of protection against IMD.[61,62] The SBA assay is considered the most relevant immunological indicator, the one most closely correlated with clinical efficacy.[61,63,64] This test consists in introducing a viable *Neisseria meningitidis* suspension in different serum dilutions in the presence of a human complement (hSBA) and then determining the amount of surviving bacteria.[63] The dilution at which more than 50% of bacteria are immediately killed was for the 3 vaccine antigens: fHbp, NadA and PorA[6,23,35]. This assay could not be performed for NHBA protein, which was analyzed by ELISA.[65] An SBA titer of  $\geq 1:4$  is considered a surrogate of protection, although some clinical trials in infants have set the threshold at 5, to be on the safe side.[66,67] However, this technique requires many SBA assays for various endemic strains for each geographic region; in practice, this is impossible, especially with respect to infants, whose serum volumes are very limited.[56,68]

Because of the diversity of intra-serogroup antigens, a standardized method specific to the 4CMenB vaccine, the Meningococcal Antigenic Typing System (MATs) was developed to estimate vaccination coverage. MATs indicates the probability that the analyzed meningococcal strain will be killed by the serum of an immunized patient. This method allows the characterization and quantification, via immune-enzymatic ELISA analysis, of the concentration of each vaccine antigen.[13,68] Then it compares the expression obtained from the analyzed strain with standard rates from reference strains.[68,69] This comparison determines the relative potency (RP) essential to define the positive



bactericidal threshold (PBT). PBT represents the minimum level of expression of each vaccine antigen to ensure sufficient bactericidal activity.[68-70] The objective is to estimate the percentage of circulating strains with a satisfactory PBT, equivalent to immunization coverage. The presence of the gene encoding the protein PorA is detected by PCR, and the sub-type is determined by sequencing.[68] A strain is considered sensitive to 4CMenB vaccine if it expresses the P1.4 subtype of PorA protein or if 1 of the 3 antigens has a sufficient level of expression.[70] Studies have shown that if a strain exceeds the positive threshold for only 1 of the 3 vaccine antigens, the probability of being killed by the host antibodies is 80%. On the other hand, if a strain exceeds the positive threshold for at least 2 vaccine antigens, the probability of being killed by the antibodies is 96%.[68] The MATS test, individually evaluating vaccine antigens, neglects potential synergy for strains expressing more protein antigens and underestimates immunization coverage.[70,71]

The 4CMenB vaccine would cover 66% of the pathogenic strains in Canada, 76% in Australia, 78% in Europe, 85% in France and Norway.[69,70,72,73] Data from clinical trials on 4CMenB vaccine prove its immunogenicity in infants, adolescents and adults.[48,65] In infants, whatever the vaccination schedule (primary vaccination at 2, 4, and 6 months or 2, 3 and 4 months), more than 99% have protective titers to fHbp and NadA and 79 to 87% for the OMV component, 1 month after the last dose.[74] Moreover 4CMenB immunogenicity is not affected by co-administration of other routine childhood vaccines. Thus, it can be administered in association with vaccines against diphtheria, tetanus, *Haemophilus influenzae* type b, hepatitis

B, measles, mumps, rubella and chickenpox.[12,48,75] The co-administration of 4CMenB with inactivated polio vaccine, pneumococcal conjugate vaccine and acellular pertussis vaccine caused a weakened immune response without suggesting clinically significant interference.[75] Simultaneous injection of 4CMenB with other meningococcal vaccines has not yet been analyzed.[76] This new vaccine therefore constitutes a major advance in the prevention of meningococcal disease. However its promising immunogenicity needs to be considered in the light of its safety profile.[48]

### 2. 3. 4CMenB tolerance

Several clinical trials have evaluated the innocuousness and safety of the 4CMenB vaccine. In 6,427 subjects (4,843 infants from 2 months, 1,503 adolescents and 81 adults), adverse effects were detected at a rate of up to 1/1000.[66,76]

In one study involving 4CMenB vaccine *versus* a placebo, it was found that reactogenicity was lower among subjects who received *verum*. [65] The transient local reactions most frequently described irrespective of age were: pain, often described as severe, more frequent in the 4CMenB arm (from 79 to 93%, compared to the placebo from 49 to 86%), erythema (48 to 56% against 29 to 40% in the placebo group) and induration at the site of injection. The most common systemic reactions in infants and young children were: fever, irritability and sleepiness.[48,65,72,75] In clinical studies, fever appeared more often during 4CMenB administration than with other routine vaccines.[77] 5.2% of infants vaccinated with 4CMenB were taken to the pediatrician for febrility, against only 2.7% for a routine

vaccine.[70] Fever rarely exceeded 39°C (40% of infants showed fever  $\geq 38.5^\circ\text{C}$  after 1 and 2 doses of vaccine 4CMenB, 20% after the third dose, and less than 1% had a fever  $\geq 40^\circ\text{C}$ ).[65,72] The febrile episodes, although usually mild and spontaneously resolved, caused anxiety among parents and health professionals, especially where they involved infants and possible febrile seizures were feared.[70,77,78] Prophylactic administration of phenacetin can be recommended to reduce the incidence and severity of fever.[77] This prophylaxis does not affect 4CMenB immunogenicity.[78,79] In adolescents and adults, frequently observed systemic reactions were discomfort (44 to 58% against 22 to 48% in the placebo) and headache (37-50% compared to 21-37% in the placebo).[74] The general safety profile of 4CMenB vaccine is similar to that of MenZB, used in New Zealand to control a local epidemic.[65] This new vaccine appears to have an acceptable safety profile: no participant discontinued the trial because of troublesome adverse effects.[55] Local and systemic reactions are similar to those reported from other vaccines.[6] Other milder and less common adverse effects reported in infants include changes in eating habits, unusual crying, vomiting, diarrhoea and rash.[66,75]

Some cases potentially attributable to 4CMenB vaccine have been reported: [48,72,75]

- 1 case of febrile seizures within 48 hours following vaccination (evolution was favorable)
- 2 cases of Kawasaki syndrome with a favorable evolution within 30 days of vaccination with 4CMenB in combination with other routine vaccines,
- 2 cases of idiopathic juvenile arthritis after vaccination

In clinical studies on co-administration of the new meningococcal vaccine with routine pediatric vaccines (vaccines against pneumococcal disease, diphtheria, tetanus, pertussis, hepatitis B, polio and *Haemophilus influenzae* type b), an increased risk of systemic reactions was noted, especially fever. In fact, 32.2% of infants were reported to experience hyperthermia following administration of Bexsero®; and 65.3% when infants received Bexsero® simultaneously with routine vaccines. Thus, 4CMenB vaccine appears less reactogenic when administered alone,[48,75] and separate vaccinations are recommended whenever possible. When administration is simultaneous, it is preferable to inject in separate places.[80] Like all vaccines containing aluminum salts, 4CMenB may have additional adverse effects. According to some experimental studies, the aluminum used as an adjuvant in vaccines, could cause a number of disorders in humans, such as immunological disorders and neurological complications.[81,82] According to some reports, it may notably trigger a neuromuscular disease, macrophagic myofasciitis, characterized by an infiltration of non-epithelioid histiocytic cells.[58]

### 3. Place of 4CMenB in the immunization schedule

#### 3. 1. Current recommendations

4CMenB vaccine was granted European authorization for active immunization against invasive meningococcal disease caused by *Neisseria meningitidis* group B. In view of the epidemiology of this pathogen, the authorization recommends the first injection from the age of 2 months, in order to optimize the vaccine's effectiveness.[12] After 1 to 2 doses administered at 1 month minimum intervals, some

protection is already assumed.[55] However, one of the first strategies used in clinical trials, injecting the vaccine at 2, 4 and 6 months accompanied by a booster, may not be fully effective, particularly at 3-5 months. Thus, it is preferable to adopt an optimal immunization schedule at 2, 3, and 4 months accompanied by a booster dose between 12 and 23 months.[75] Adolescents, another population at risk, could also be vaccinated in the interests both of reducing nasopharyngeal carriage, and of creating collective immunity. Despite accreditation, this vaccine is not currently on the national immunization program in several countries including France, Spain, England, and Australia, for different reasons: either due to lack of efficacy data, or due to lack of profitability.[22,66,83-86] This failure to commit to the vaccine is a source of controversy and divided public opinion.[83]

### 3.2. The cost of 4CMenB vaccination

By definition, vaccines have the potential to reduce incidence of disease and thereby to reduce direct and indirect costs. Today, the cost-effectiveness of a new drug is considered when adopting a vaccination policy.[85] This ratio, also known as cost/performance ratio, is calculated in terms of quality-adjusted life years (QALY) gained.[66,85] Several vaccine strategies were considered and compared to absence of vaccination. Since irrespective of prophylactic strategy, the proportion of prevented cases remains lower than 30%.[66,87]

The absence of concrete proof of herd immunity, the need for a primary immunization with 3 doses followed by a booster dose, the limited duration of protection argue for relatively low epidemiological impact from vaccination. The

cost/effectiveness ratio is high: if each dose of vaccine costs €60, and the desired coverage is 80%, cost/performance ratio varies from €585,000 to €1,617,000/QALY.[66] According to one study, a unit price of approximately €9 for a routine childhood vaccine strategy would be worthwhile.[87]

### 3.3. 4CMenB vaccine acceptance

The introduction of a new vaccine requires careful communication from political decision-makers, health professionals, financing organizations and the media.[1] Public awareness of disease prevention through vaccine prophylaxis has been shown to lead to better adoption of immunization programs, which is essential to improve immunization coverage.[66,70,77] Health education is essential for the understanding of the new drug and particularly for its community acceptance.[88] Parents should know the benefits and risks of vaccination before making their decision. According to one study, the most important factor in determining the acceptance of vaccination is the recommendation of the attending physician.[77] In fact, 81.7% of parents said they would agree to vaccination if their doctor recommended it, 5.4% were undecided and 12.4% said the doctor's recommendations would not affect their decision.[77] Among arguments which can influence parents' choice, physicians cite the incidence, severity and unforeseen development of meningococcal disease. Moreover, according to another study, a lack of knowledge about disease can lead to poor adherence to the new vaccine.[88] According to an Australian study of 5,200 families, while 39.9% felt positive about this new drug, 31.8% were concerned about possible adverse effects. Furthermore, the financial aspect was a factor

in decisions: 74.1% of parents wishing to vaccinate their children were prepared to pay the price, however 8.7% were unwilling to pay and 16.6% remained undecided.[77]

Physicians need to inform parents of the potential adverse effects to guide their decisions and to reassure them in case these side effects occur. The safety of drugs remains a difficult and worrying subject, particularly because of the potential risk of adverse effects not detected during clinical studies.[77]

#### **4. Vaccination limitations**

##### 4.1. Immunity loss

According to current immunogenicity data, different vaccine schedules envisaged in the 4CMenB authorization require 3 doses of primary immunization to obtain sufficient protection. However, protection is not eternal: it decreases over time.[64,65] This immunological instability is generally observed with all other meningococcal vaccines, and especially with the first specific vaccines against serogroup B.[64,89,90] The decline seems clear from 2 months after the first dose. In fact, 69-81% of subjects receiving a single dose of vaccine still have seroprotective antibodies 6 months later.[65] These observations illustrate a probable relatively rapid seroprotection decrease over time. Booster doses are essential to maintain a certain quantity of serologic antibodies.[74] 12 months after the first vaccination, following booster dose administration a strong anamnestic response was observed. Antibody titers, higher than those observed after primary vaccination reflect the establishment of immunological memory.[72,90] However, this phenomenon does not seem sufficient, and a certain

quantity of bactericidal antibodies appears to be necessary for protection against meningococci. Therefore, if the quantity of circulating antibodies is not sufficient before infection, protective antibody synthesis will take too long to avoid bacterial invasion.[16] Thus, a booster dose 6 months after the second or third dose of 4CMenB restores the quantity of seroprotective antibodies in 99 to 100% of subjects.[65] But after the booster dose, the seroprotection titers decrease again.[66,72,90] Studies found that this serologic decrease differs according to the vaccine antigens of 4CMenB, and is more pronounced with NHBA and PorA proteins.[72]

##### 4.2. Impact on nasopharyngeal carriage

Although a vaccine is primarily designed for individual protection, its impact can also be measured in terms of an entire community. Indeed, if a vaccine is effective against asymptomatic carriage, then it reduces the probability of transmission, creating group immunity and indirectly protecting the unvaccinated.[91] The conjugate vaccine against meningococcal group C, widely used in the UK, led to decreased nasopharynx bacterial colonization, correlated to decreased meningococcal incidence due to this serogroup.[91,92] Since, some antigens (fHbp and NadA) are also found in other serogroups,[93] this multi-antigenic vaccine may induce a protective effect against other serogroups expressing enough of these antigens.[73,93] Further studies are currently underway. If their findings confirm this first observation, 4CMenB vaccine would be particularly beneficial for young children and adolescents, whose rate of bacterial carriage is higher.[17,70]

### 4.3. Harmful effects of vaccination

Meningococcus is a recombinant bacteria, inducing wide genetic variability. This pathogen is in fact able to incorporate into its chromosomes DNA captured from its environment, and frequently recombines DNA through the many repetitive sequences encoding components of the cell surface.[11,94] Similarly, the high frequency of an asymptomatic carriage of meningococcal strains in the commensal flora of the human nasopharynx can facilitate bacterial capsule switching, particularly in cases of simultaneous colonization of different meningococcal serogroups.[9] For example, in Canada and in Italy, cases of capsule switching between serogroups B and C have been observed.[95-97] The emergence of serogroup W135 connected with the pilgrims' return from Mecca, usually presenting a serogroup C clone, is another example.[96,98] These examples demonstrate the possibility of genetic exchange between endemic or epidemic strains. This diversity ultimately determines the virulence of the meningococcal strains, the ability of the host to defend itself and thus, indirectly, the immunization strategies to adopt.[97] Under these circumstances, scientists fear that a mass vaccination program could cause a selection of strains targeted by this vaccine. Indeed, there may be progressive emergence and the circulation of strains not covered by this vaccine, recalling past experience with pneumococcal vaccines.[9,70] So far, no capsular substitution has been demonstrated following the introduction of meningococcal vaccines. Nevertheless, post-implementation surveillance must be rigorously applied, in particular using the established typing method for

meningococci, termed multilocus sequence typing (MLST).

This method determines the molecular characterization of meningococcal strains, and identifies clones responsible for epidemics worldwide.[3,11] Such vigilance is aimed at rapidly identifying emerging strains not covered by this vaccine, so that immunization policies can be adjusted for maximum effectiveness.[12] The best way to prevent an infectious disease is to eradicate the etiological agent, as done for smallpox. In the case of meningococci, this aim seems achievable since the number of virulent serogroups is limited, and the only reservoir is human. However, despite the severity of meningococcal disease, eradication of all meningococci is undesirable.[21] Indeed, the majority of meningococci, including serogroup B meningococci, form commensal bacterial flora. Thus, their disappearance from the human nasopharynx could have severe consequences for natural immunity.[99] Human microbiome modification could increase the expression of pathogens of unknown virulence previously kept in check by the presence of meningococci.[21] The fight against the disease must therefore respect certain limits, primarily targeting encapsulated virulent meningitis.

The development of a universal vaccine against group B has proved a real clinical challenge, requiring decades of research. Finally, however, marketing authorization is bringing hope of control of meningococcal epidemics.[10,38] A post-implementation surveillance plan is required to monitor the evolution of bacterial clones and to enhance immunization programs.[12,35]

4CMenB was the first vaccinal prophylaxis proposed against *Neisseria meningitidis* serogroup B, but several vaccine candidate vaccines are currently under development. For example, Trumemba<sup>®</sup>, a bivalent recombinant lipoprotein 2086 vaccine based on two fHBP, was recently licensed in United States; and a meningococcal pentavalent vaccine (ABCWY) is under study.[100]

Other preventive strategies may also be worth considering, such as the passive protection given to children by transfer of maternal antibodies during pregnancy.[10,12]

By helping to reduce the global burden of meningococcal disease, these therapeutics advances should contribute to the progress of modern medicine.

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