***Neisseria meningitidis* Factor H Binding Protein fHbp: A Key Virulence Factor and Vaccine Antigen**

Kate L. Seib^a, Maria Scarselli^b, Maurizio Comanducci^{b^}, Daniela Toneatto^b, Vega Massignani^{b*}

^a Institute for Glycomics, Griffith University, Southport, Queensland, Australia, 4215

^b Novartis Vaccines and Diagnostics, Via Fiorentina 1, 53100 Siena, Italy

[^] Current address: Crucell, Leiden, The Netherlands

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*Address correspondence to:

Vega Massignani, Novartis Vaccines, Via Fiorentina 1, 53100 Siena, Italy

Phone: +39-0577-243319 Fax: +39-0577-243564

vega.massignani@novartis.com

Abstract

Neisseria meningitidis is a leading cause of meningitis and sepsis worldwide. The first broad-spectrum multi-component vaccine against serogroup B meningococcus (MenB), 4CMenB (Bexsero[®]), was approved by the European Medical Agency in 2013, for prevention of MenB disease in all age groups, and by the FDA in January 2015 for use in adolescents. A second protein-based MenB vaccine has also been approved in the USA for adolescents (rLP2086, Trumenba[®]). Both vaccines contain the lipoprotein factor H binding protein (fHbp). Preclinical studies demonstrated that fHbp elicits a robust bactericidal antibody response that correlates with the amount of fHbp expressed on the bacterial surface. fHbp is able to selectively bind human factor H, the key regulator of the alternative complement pathway, and this has important implications both for meningococcal pathogenesis and for vaccine design. Here we review the functional and structural properties of fHbp, the strategies that led to the design of the two fHbp-based vaccines, and the data generated during clinical studies.

Introduction

Neisseria meningitidis is an obligate human pathogen that, despite available antibiotic therapy, remains a major cause of morbidity and mortality worldwide, mainly as a result of sepsis and meningitis. Of the 12 known meningococcal capsular groups, 5 are associated with the majority of disease (A, B, C, Y and W) [1]. More recently, serogroup X has also started to cause considerable disease in sub Saharan Africa [2]. Whereas several capsular polysaccharide-based vaccines against serogroups A, C, Y, and W have been developed and licensed [3-5], the similarity of the serogroup B capsular polysaccharide to human tissues, including the fetal neural cell adhesion molecule N-CAM [6], and its poor immunogenicity have hampered efforts to develop a glycoconjugate vaccine against meningococcus serogroup B (MenB).

In an attempt to explore new avenues for the design of effective MenB vaccines, the reverse vaccinology strategy was applied to a pathogenic serogroup B strain, MC58. This approach used computer-based prediction methods to screen the full bacterial genome sequence in order to identify novel potential vaccine candidates [7]. fHbp was identified, from 570 potential open reading frames (ORFs) that were predicted to encode outer membrane proteins, as a *Neisseria*-specific putative surface-exposed lipoprotein of unknown function, and named GNA1870 (genome-derived *Neisseria* antigen 1870) [7, 8]. Subsequent studies have shown that although fHbp is universally present in meningococci, its surface expression varies significantly between strains. Furthermore, *fHbp* gene sequencing in a panel of meningococcal isolates revealed marked sequence variation, consistent with classifications of fHbp into two subfamilies (A and B) [9] or three main variant groups (1, 2 and 3) [8], each including several subvariants. In infant rats, antibodies against recombinant variant 1 fHbp protein conferred passive protection by eliciting

complement-mediated bacterial killing of strains of different serogroups expressing fHbp subvariants from the same main variant group 1, but not of strains expressing fHbp from different variant groups [8].

Investigation of the biological role of fHbp led to the discovery that this antigen binds human complement factor H [10], a negative regulator of the alternative complement pathway [11, 12]. fHbp binds fH to the bacterial surface, enabling the pathogen to evade alternative complement-mediated killing by the host innate immune system and to survive in human serum and blood [10, 13, 14]. Additional proposed functions for fHbp include resistance to the antimicrobial peptide LL-37 [13] and binding to enterobactin, suggesting a potential role of fHbp in iron uptake [15].

The fHbp protein was selected as a target for development. In fact, two vaccines have been developed that contain recombinant fHbp, either as a fusion protein with a second meningococcal antigen in the multicomponent 4CMenB vaccine [16, 17], or as two subvariants of recombinant lipidated fHbp in the bivalent rLP2086 vaccine [9, 18]. While various aspects of fHbp have been reviewed in the past [19-21], in this review, we describe the structural and functional characteristics of the fHbp vaccine candidate, the impact of sequence variability on vaccine design, the features of the two vaccines containing recombinant fHbp, and the clinical studies of these vaccines.

fHbp discovery, classification and distribution in MenB strains

fHbp was initially identified as a surface-exposed lipoprotein (GNA1870) using reverse vaccinology, and selected as a putative meningococcal vaccine antigen. Subsequent studies

confirmed the surface localization of fHbp by flow cytometry in all meningococcal strains analyzed, and demonstrated that anti-fHbp antisera elicit complement-mediated bacterial killing of meningococci in the serum bactericidal activity (SBA) assay [8]. Another group also identified fHbp from meningococcal bacterial membranes using a traditional extraction/fractionation proteomic approach [9]. The products of this process were used for mouse immunization and the sera tested for bactericidal activity against a panel of strains. Each time a product displayed a positive SBA response, it was identified, expressed and purified in a recombinant form, and used for bactericidal data confirmation. The broadest bactericidal response was conferred by a lipoprotein named LP2086, which was subsequently confirmed to be fHbp [9].

Sequencing of the *fHbp* gene in diverse MenB strains revealed the presence of three main variants (var1, var2, var3) [8, 22], or two subfamilies (A and B: corresponding to variants 2/3 and 1, respectively) [9, 23, 24]. A significant degree of intravariant *fHbp* sequence diversity exists, with more than 760 subvariant polypeptides identified so far and submitted in the public fHbp database (<http://pubmlst.org/neisseria/fHbp/>). An fHbp nomenclature system has been proposed in the fHbp database in which new protein subvariants are assigned a sequential numerical identifier alongside a prefix corresponding to the variant designation, *e.g.*, fHbp-1.x, fHbp-2.x and fHbp-3.x, where x denotes the specific peptide subvariant. fHbp proteins from different variant groups do not induce cross-protection (*i.e.*, immunization with one fHbp subvariant is not able to raise protective antibodies against strains harboring a subvariant from a different variant group), although some residual cross-reactivity exists between fHbp variants 2 and 3 [8, 9]. A modular architecture has also been proposed for fHbp, consisting of 9 modular groups, in which

combinations of 5 variable segments derived from either variant 1 or 3 *fHbp* genes are flanked by invariable residues [25, 26].

Members of variants 1, 2, and 3 are present in approximately 65%, 25%, and 10% of the MenB global population, respectively [27-30]. In contrast, the proportion of variant 1 is different in other meningococcal serogroups, ranging from 39% in MenC to 3% in MenY strains isolated in the United States (US) [31]. In the US, the proportion of fHbp variants 1 and 2 in carriage isolates are similar (37%–54%), while variant 3 is rare (3%–9%) [32].

The *fHbp* gene is also present in strains of commensal *Neisseria* species that are closely related to *N. meningitidis*. In particular, *Neisseria cinerea* strains contain *fHbp-1* whereas *Neisseria polysaccharea* encodes *fHbp-3*, although the gene is frame-shifted in most strains [33]. In contrast, no fHbp gene was identified within a broad strain collection of *Neisseria lactamica*, a common childhood commensal [33, 34]. Finally, although a *fHbp-3* gene is present in the genome of 16 *Neisseria gonorrhoeae* strains, it contains a frameshift within the N-terminal sequence, which results in the loss of the lipoprotein motif [33]. Recent investigation has demonstrated that the *N. gonorrhoeae* fHbp homologue is not expressed on the bacterial surface and that is not able to bind fH [35].

Key findings for the discovery, classification, and distribution of fHbp are summarized in **Table 1**.

Expression and regulation of fHbp

Most *N. meningitidis* strains studied to date express fHbp, with levels of expression that vary

significantly between isolates [8, 9, 24, 28]. Variation in fHbp expression by *N. meningitidis* strains allows classification into low, medium and high expressing strains, however a few invasive strains have been reported to lack *fHbp* expression, due to a frameshift mutation [58].

fHbp is expressed throughout the *in vitro* growth cycle [36], however regulatory pathways are not fully understood. Early studies suggested that this gene might be regulated by iron, as a putative Fur-box motif was identified within its promoter region [8]. More recent investigations demonstrated that *fHbp* gene transcription is regulated by iron availability, but the mechanism of regulation varies between genetic lineages [37]. Oriente and colleagues showed that fHbp is expressed from two different transcripts, one of which is under the control of a promoter that contains a FNR-box motif, and that expression is induced during growth in low oxygen conditions, thus underlining the important role that fHbp plays in oxygen limited microenvironments in the host, such as the submucosa and the bloodstream [38]. The finding that fHbp expression is induced during growth in human blood provides further support for its crucial role in invasive meningococcal disease [39, 40].

Evidence of *in vivo* expression of fHbp comes from a number of serologic studies of anti-fHbp antibodies in humans. Litt and coworkers observed antibodies against fHbp (referred to as Antigen 741 / NMB locus 1870) in children convalescing after meningococcal disease [41]. Jacobsson and colleagues demonstrated that titers increase with age, supporting the age-related increase of meningococcal acquisition and carriage [42]. Furthermore, anti-fHbp antibodies were detected in subjects with invasive meningococcal disease both at the time of hospital admission

and during convalescence, as well as in healthy individuals with no history of meningococcal disease either with or without meningococcal carriage/ colonization at the time of testing [42, 43].

Key findings for the expression and regulation of fHbp are summarized in **Table 1**.

fHbp is one of the many meningococcal factors involved in immune evasion

The complement system is a key component of the innate immune defense against invading pathogens, including meningococci. Healthy humans can clear meningococcal infection via both the classical and alternative complement pathways, and patients with complement deficiencies are more susceptible to developing meningococcal disease [44, 45]. Microbial pathogens have evolved several sophisticated mechanisms to limit complement activation on their surface and evade complement-mediated killing. One such mechanism relies on binding of the bacteria to fH, the main inhibitor of the alternative complement cascade (**Figure 1**). In 2006, pioneering work investigating the interaction of fH with the meningococcus by Madico and coworkers demonstrated that GNA1870 (*i.e.*, fHbp) is the principal fH-binding meningococcal protein [10]. Strains with the *gna1870* /*fHbp* gene deletion were unable to bind fH and showed reduced serum resistance. Furthermore, all three variants displayed fH binding, and the level of binding correlated with the level of GNA1870 expression [10]. To reflect the critical function of this protein, GNA1870 was renamed factor H binding protein (fHbp). Interestingly, fHbp binds only human fH, which may be one explanation of the species-specificity of meningococci for the human host [46]. Indeed, increased bacteremia was seen in human fH transgenic rats infected with *N. meningitidis* compared to normal human fH-negative control rats [47].

The ability of fHbp to bind human fH with high affinity has implications not only for its role as a virulence factor *in vivo* but also as a target antigen for vaccination. Anti-fHbp antibodies can provide protection by two mechanisms: i) direct complement-mediated killing of the bacterium, and ii) blocking fH binding to the bacteria to increase the susceptibility of the bacterium to killing by the alternative complement pathway [10]. Several studies have shown that inhibition of fH binding to fHbp results in increased susceptibility of the bacteria to complement-mediated bactericidal activity [48-51]. Furthermore, since the interaction of fHbp with fH could impair its immunogenicity by decreasing access to epitopes, several studies have focused on investigation of nonfunctional fHbp antigens as vaccine candidates. It is interesting to note that normal mice whose fH did not bind to the fHbp antigen had higher serum bactericidal responses than human fH transgenic mice, and a fHbp variant 1 mutant with a single amino acid substitution, R41S (Arg at residue 41 was replaced by Ser), that is no longer able to bind human fH, is more immunogenic than wild-type molecule in human fH transgenic mice. The fHbp R41S mutant induced higher serum bactericidal antibody responses than the wild-type fHbp, either when tested as a recombinant protein or when natively expressed in OMVs, and these antibodies had increased ability to block fH binding to wild-type fHbp [52, 53]. Similarly, mutants of fHbp variant 2 (T221A or D211A) [54, 55] and variant 3 (T286A or E313A) [56] with decreased fH binding also induced higher serum bactericidal antibody responses in the hfH transgenic mice. In another study, immunization of wild-type and fH transgenic mice with 4CMenB resulted in weaker SBA responses in transgenic mice against a serogroup B strain with all of the antigens mismatched to the 4CMenB vaccine except fHbp, suggesting that presence of human fH negatively affects fHbp antigen presentation [57]. These studies on fHbp highlight the way in which many different lines of research (*i.e.*, studies on bacterial pathogenesis, host-pathogen interactions and vaccinology)

have converged and are simultaneously revealing new information about meningococcal pathogenesis and antigen function, and driving advances in rational design of vaccine antigens [reviewed in 19].

Although the ability of fHbp to bind fH undoubtedly has important implications both for meningococcal pathogenesis and for strains' susceptibility to fHbp-based vaccines, meningococci possess redundant mechanisms to enable immune evasion [45]. In some strains, the deletion of fHbp eliminates or greatly diminishes survival in human blood or serum [13], for example deletion of fHbp in the high fHbp expressing strain MC58 renders the bacteria unable to survive in human blood [13, 36]. However, in other strains the deletion of fHbp does not have such a strong impact on serum resistance [39], explaining why a few invasive meningococcal strains have been identified in which the *fHbp* gene was either absent or contained frameshift mutations that resulted in a non-functional fHbp protein [58-60]. The neisserial surface protein A (NspA) also binds hfH with high affinity [61] and has been shown to be important for complement evasion by naturally fHbp-deficient mutants [62]. In addition, fH interactions with lipooligosaccharide (LOS) sialylic acid [63] and the porin B2 (PorB2) protein [64] have been identified. A recent review highlights the numerous additional mechanisms and structures used by *N. meningitidis* to evade killing by human complement, including capsule, LOS, porin proteins, Opa and Opc [45]. As such, vaccination with the fHbp antigen alone may select for mutants that do not require fHbp for complement evasion.

Key findings regarding the role of fHbp in immune evasion and virulence are summarized in **Table 1**.

Structural characterization of fHbp

Partial or full length three-dimensional structures are available for fHbp proteins from each of the three main variant groups. The first structural data were obtained for fHbp var1 by nuclear magnetic resonance (NMR) in aqueous and micellar solutions [24, 65], and later by X-ray crystallography [66]. More recently, the atomic coordinates of the full length fHbp var3 in complex with human fH was also reported, highlighting the molecular mechanisms by which different variants engage with fH. In contrast, only the C-terminal domain of var2 could be crystallized, reflecting an intrinsic instability of the N-terminal region of this variant, as also confirmed by Differential Scanning Calorimetry (DSC) profiles [67]. Interestingly, despite the remarkable sequence diversity of the three main variants, the three-dimensional structures are almost perfectly superimposable (**Figure 2**).

The fHbp var1 and var3 molecules consist of two domains predominantly formed by β -strands arranged in a bi-lobed structure. While the C-terminal domain adopts a canonical beta barrel conformation, the N-terminus shows a more unusual taco-shaped beta-barrel fold characterized by higher intrinsic flexibility (**Figure 2, A–D**). This flexibility is more pronounced in fHbp var2, and may explain the difficulty in determining the crystal structure of the var2 N-terminal domain. All fHbp variants contain a glycine-rich N-terminal tail that anchors the protein to the bacterial cell by a lipid chain that is covalently linked to the first cysteine residue. This linker may act as a flexible spacer that orients the portion of the protein surface that includes the fH binding site towards the extracellular milieu. As a consequence, this face of the protein is more exposed to antibodies, and consequently highly populated by variable residues. On the contrary, the

conserved 20 N-terminal residues following the glycine linker form a patch that is likely to be accommodated *in vivo* in the lipo-oligosaccharide layer that surrounds the outer membrane of the bacteria, and is therefore more shielded from the immune system [68]. This model implies that the N-terminus of mature fHbp is the most suitable candidate for any modification aimed at enhancing its immunogenicity. In Trumenba^R, the antigen is lipidated at its N-terminal cysteine, in Bexsero^R fHbp is fused at the N-terminus to the GNA2091 antigen. In both cases, the modified proteins elicit higher bactericidal responses compared to the unmodified recombinant forms [9, 16], on one hand reflecting the propensity of lipidation to enhance the immunological response to the proteins [69], on the other hand suggesting that N-terminal capping could be beneficial for the overall structural stabilization of the protein and of its flexible conformational epitopes. Despite the high degree of sequence variability between strains, the hydrophobic cores and domain interface are highly conserved. In contrast, highly variable amino acids are oriented toward the extracellular space and are mainly located on one side of the molecule (**Figure 3A**). This side holds the binding site for human fH [70] and is the target of the monoclonal antibodies (mAbs) characterized to date (**Figure 3B**) [24, 68, 71-74]. Taken together, these observations may explain the variant-specific nature of the immune response induced by fHbp [8, 9] and why the identification of cross-reactive mAbs able to inhibit fH binding remained elusive for so long. Recently, three mAbs have been characterized that recognize a wide panel of variants and subvariants, and that target different surface-exposed epitopes on fHbp [75, 76]; two of these are able to inhibit binding to fH but have bactericidal activity only when tested in combination. On the other hand a murine IgG2b antibody has been reported to be non-bactericidal despite that fact that its epitope largely overlaps the fH-binding site [74]. These findings suggest that, contrary to previous hypotheses [49, 50], the ability of mAbs to efficiently promote complement-mediated

killing relies on the affinity and configuration of binding to the antigen, leading to efficient engagement of C1q, rather than on their ability to prevent fHbp-fH interactions. Mapping of the epitopes targeted by functional mAbs indicates that presentation of both domains is necessary to elicit an optimal immune response, despite the carboxyl-terminal domain having been identified as the immune-dominant portion of fHbp [77]. Understanding the conservation and configuration of functional epitopes is a valuable strategy to aid the design of broadly protective vaccine candidates, as previously shown by Scarselli and colleagues [78] who engineered a chimeric fHbp antigen that combined the antigenic repertoire of the three major fHbp variant groups into a single molecule.

fH:fHbp interactions involve both N- and C-terminal lobes of fHbp, but only two of the 20 domains of fH (short consensus repeats 6 and 7, also referred to as SCR67 or fH67) [70, 79]. The crystal structures of fH67:fHbp var1 and fH67:fHbp var3 show similar binding, although site-directed mutagenesis indicates that fH binding to fHbp variants is mediated by a distinct array of residues [67, 70]. Lack of conservation of the residues forming the area of fH:fHbp interaction in humans and lower vertebrates provides a structural explanation for human fH:fHbp binding selectivity [46].

The fHbp C-terminal domain is structurally similar to the lipocalin family, which includes siderophores that are organic chelators that have a strong affinity for ferric iron. All three variants of fHbp display *in vitro* binding to ferric enterobactin [$\text{FeIII}(\text{Ent})^{3-}$], a xenosiderophore produced by *Escherichia coli* and *Salmonella* spp. NMR mapping revealed that distinct zones of fHbp are

involved in interactions with enterobactin and fH [15]. The biological significance of binding of fHbp to $\text{FeIII}(\text{Ent})^{3-}$ has not yet been determined.

Key findings on the structural characterization of fHbp are summarized in **Table 1**.

fHbp as a vaccine component

Evaluation of fHbp as a vaccine candidate was conducted by two groups using different approaches. In the work by Maignani and colleagues [8], fHbp var1, var2 and var3 were purified as His-tagged recombinant proteins, formulated with Freund's adjuvant, and used to immunize CD1 mice. Surface expression of fHbp of different subvariants was detected by flow cytometry using mouse sera, but the level of recognition varied as a function of the level of fHbp expression in different strains. Of the 43 strains tested, approximately half were high expressing strains, whereas the remainders were medium or low fHbp expressing strains. Consistent with fluorescent-activated cell sorter data, the bactericidal response was mostly variant-specific, with only a minor level of cross-protection observed between var2 and var3 proteins. Of note, a recent analysis aimed at investigating the level of fHbp expression on a panel of more than 100 invasive meningococcal strains concluded that 24% of the strains had low or undetectable levels of fHbp, and that all but one of these strains low expressing strains carried *fHbp* var2 or 3 [80].

Fletcher and colleagues [9] expressed P2086 (*i.e.*, fHbp) proteins representative of subfamilies A and B as recombinant (rP2086) and lipidated (rLP2086) forms using the P4 lipoprotein signal sequence of *Haemophilus influenzae*. While rP2086 was soluble and localized in the cytoplasm, rLP2086 was associated with membrane fractions and was detergent-soluble. P2086 expression was confirmed in a panel of 95 *Neisserial* strains by Western blot. The antisera produced against

subfamily A and B rLP2086 molecules demonstrated strong surface reactivity to 13 of 15 strains tested using whole-cell ELISA, with higher titers and bactericidal activity against strains within the same family and negligible cross-protection between subfamilies. Antisera generated against rP2086 were less effective in the SBA assay, with titers approximately 10-fold lower than those obtained upon immunization with the lipidated form [9], likely as a result of the adjuvant effect of the lipid moiety [81] (**Table 2**).

Additional investigations of fHbp as a vaccine component have used native (non-detergent treated) OMVs generated from mutant strains with genetically attenuated endotoxin, which have been engineered to overexpress fHbp [82-85].

Studies to date on fHbp support the following conclusions: i) fHbp is expressed on the surface of almost all meningococcal strains tested, ii) fHbp is highly immunogenic and is a prominent target for bactericidal antibodies, iii) fHbp shows sequence variation and is classified into immunologically-distinct groups, which have limited cross-protection, and iv) fHbp expression levels vary significantly among different strains, and the level of expression affects strains susceptibility to killing. The following sections will detail the two vaccines containing fHbp.

The Novartis Vaccines approach to MenB vaccine development

fHbp was one of the top candidates identified by reverse vaccinology, along with the *Neisseria* heparin binding antigen (NHBA, or GNA2132), and the *Neisseria* adhesin A (NadA, or GNA1994). Two other recombinant proteins GNA1030 and GNA2091 were also immunogenic in mouse models. Analysis of fusion proteins of these antigens revealed that fusion of fHbp and

NHBA to GNA2091 and GNA1030, respectively, enhanced protein stability and immunogenicity, and elicited higher serum bactericidal activity (SBA) titers [16]. The two fusion proteins fHbp-GNA2091 and NHBA-GNA1030 were combined with NadA (as a single protein antigen) in a multi-component vaccine, named rMenB [16]. The OMV component of the New Zealand outbreak strain NZ98/254 (containing PorA serosubtype P1.4) was also included in the final vaccine formulation, named 4CMenB, which progressed through clinical trials (**Table 2**). As shown by Findlow *et. al.* [86], the inclusion of the OMV component not only provided coverage of strains expressing the homologous PorA P1.4 serosubtype, but also greater immunogenicity was demonstrated against strains carrying mismatched PorA types. Although further investigations are required to fully elucidate the reasons for this effect, possible explanations include the presence of minor antigenic OMV components, possible synergy between antibodies against OMV and recombinant antigens, or a more general immunomodulatory effect of the OMV due to the presence of residual LPS and of other bacterial components [87].

4CMenB contains fHbp var1 (corresponding to subfamily B), which is the most commonly-represented variant in serogroup B meningococcal strains, with a frequency that ranges from 59% overall in the USA (reaching 83% and 84% in California and Oregon, respectively) [29, 31] to >65% in many European countries, including Norway, France, the Czech Republic, and reaching 76% in UK/Ireland [28]. Approximately 23–35% of MenB strains in Europe and the USA express fHbp var2/3 (subfamily A) [28]. More specifically, the fHbp-1.1 (corresponding to B24) genotype present in 4CMenB was identified in 129 of 1052 strains (12.3%) in a recent study, and was the third most frequent subvariant in the collection [88]. Work by Brunelli and colleagues

demonstrated that while mouse or adult sera obtained from immunization with fHbp-1.1 are bactericidal against strains expressing various subvariants of fHbp var1, less cross-protection was achieved by infant sera, thus supporting the rationale of having additional antigens included in the final vaccine formulation, especially for immunization of younger age groups [89].

NadA is present in approximately 50% of circulating MenB strains, is important for adhesion/invasion of human epithelial cells [90, 91] and its expression is up regulated *in vivo* by niche specific signals [92-94]. NHBA is present in virtually all MenB strains and is characterized as a heparin binding protein, a function that enhances bacterial serum resistance [95].

The Pfizer approach to MenB vaccine development

The group at Pfizer (previously Wyeth) used an approach based on the combination of two variants (subfamily A05/subvariant 3.45 and subfamily B01/subvariant 1.55) of lipidated fHbp (rLP2086) (**Table 2**) to protect against circulating meningococcal strains [9]. To date, very few strains carrying B01 have been identified, and A05 has a prevalence of 2%–11% [20, 28, 96]. Although the epidemiologic distribution of these subvariants in MenB strains is low both in Europe and the USA, preclinical data indicate a significant degree of intra-family protection, especially after immunization with the A05 subvariant. Jiang and coworkers [23] tested a panel of MenB strains expressing different fHbp subvariants in SBA assays using pooled rabbit and human sera obtained from immunization with the bivalent rLP2086 vaccine; 87 of 100 strains tested were killed in SBA assays by rabbit antisera, and 36 of 45 strains tested were also killed by pooled human sera. Although the level of killing did not correlate with the fHbp genotype, resistant strains had low fHbp expression [23]. Interestingly, mouse antibodies against

nonlipidated B01 were not very effective against other strains expressing subvariants from var1 group [97].

Prediction of coverage afforded by meningococcal vaccines

Clinical efficacy of MenB vaccines would provide the clearest demonstration of their benefit in disease prevention, but due to the low incidence of meningococcal disease, these studies are difficult and impractical. The use of a reliable method for determining coverage estimates is therefore a central issue in evaluating the effectiveness of meningococcal vaccines. Analysis of the distribution of fHbp, NadA, and NHBA variants in the meningococcal population indicated that vaccine coverage could not be predicted on the basis of multilocus sequence typing (MLST) [27], which is the gold standard for meningococcal classification [98]. Given the intrinsic diversity in their vaccine compositions, Novartis and Pfizer addressed this issue using different approaches.

Key findings on meningococcal vaccine coverage prediction are summarized in **Table 1**.

Coverage prediction studies of the 4CMenB vaccine should be able to simultaneously assess, in each strain under investigation, the presence, genetic variability and level of expression of each of the vaccine components. To this end, a high-throughput methodology, named the meningococcal antigen typing system (MATS) was developed. The MATS assay is an antigen-specific ELISA that measures immunologic cross-reactivity and quantity of fHbp, NHBA and NadA expressed by the particular meningococcal strain tested (and also assesses the PorA serosubtype by PCR sequencing) [99]. For each individual strain tested, the read out of the MATS ELISA is a Relative Potency (RP) calculated for each antigen with respect to a reference strain. By comparing the RP

with hSBA titers (*i.e.*, serum bactericidal assay antibody titres with human complement) from pooled post vaccination sera on a panel of 57 strains, a minimum RP level was determined, named the Positive Bactericidal Threshold (PBT), which predicts whether a given MenB isolate would be susceptible to killing in hSBA by antibodies induced by 4CMenB. A given strain is predicted to be covered if the RP is higher than the PBT for at least one antigen [99]. MATS analysis of large multi strain panels has predicted 86% and 77% protection from globally-circulating MenB strains by 4CMenB in adults and infants, respectively. The MATS assay has been transferred to and used in reference laboratories in different countries, to predict coverage afforded by the 4CMenB vaccine in geographically distinct strain collections [100]. A recent publication reported a predicted coverage by the 4CMenB vaccine of 78% of strains in a panel representative of the epidemiology in five European countries. Furthermore, approximately 65% of strains were predicted to be covered based on the presence of fHbp, either alone or in combination with one or more other antigens [88]. Of note, as a consequence of the well-known fluctuating nature of meningococcal epidemiology, MATS-predicted coverage estimates might change for the same country depending on the time of isolation of the strain panel being investigated [101]. Interestingly, a recent study conducted on a reference panel of 40 strains, representative of the epidemiology of MenB disease in England and Wales during 2007/08, predicted 70% coverage by MATS, while 88% of strains were killed in the hSBA assay using both infant and adolescent sera, indicating that MATS is a conservative predictor of strain coverage by the 4CMenB vaccine in infants and adolescents [102].

For rLP2086 vaccine, the Pfizer group followed a prediction-coverage approach based on the original finding that the bactericidal action of anti-fHbp antisera was directly dependent on fHbp

surface expression levels [23]. fHbp surface expression was measured using a monoclonal pan-fHbp antibody (MN86-994-11) that is claimed to recognize all fHbp variants and subvariants. Strains with fHbp surface expression values above a threshold of 1000 mean fluorescent intensity units were generally killed in SBA by both human and rabbit antisera, while strains with fHbp expression below this threshold survived. Furthermore, fHbp surface accessibility to the mAb was not affected by the level of strain encapsulation [24]. Unfortunately, information about the molecular aspects underlying mAb MN86-994-11 binding to fHbp, including the exact target epitope and the extent to which the binding affinity might be influenced by protein sequence divergence in the region surrounding the epitope, is not currently available. In conclusion, while this technique is optimal to monitor different levels of surface expression, it cannot address the antigenic diversity between the fHbp vaccine variants and the fHbp variant expressed by a given strain.

Clinical evaluation of MenB vaccines

The constituents of the 4CMenB and of the rLP2086 vaccines that have undergone clinical evaluation are listed in **Table 2**. The safety and immunogenicity of 4CMenB has been evaluated in preclinical, phase 1, and early phase 2 studies conducted in adults, adolescents and infants, and late-phase studies in infants, toddlers, children and adolescents have also been completed (**Table 3**). Several phase 1 and 2 clinical studies have also been reported for rLP2086 (**Table 3**). Reviews of 4CMenB [17] and rLP2086 [18] clinical studies have recently been published, and key data regarding these studies are outlined below and reported in **Supplemental Table 1**.

Correlates of clinical protection

When large-scale efficacy trials are unfeasible, correlates of clinical protection are needed for assessment of the potential benefits of a vaccine. Serum bactericidal antibodies detected with human complement (hSBA) are widely accepted as a correlate of protection against meningococcal disease; titers ≥ 4 are considered to be protective [103-105]. In current meningococcal vaccine trials, vaccine-induced antibodies are measured by hSBA assays that measure cell lysis using a combination of patient serum (containing antibodies), exogenous human complement, and bacterial reference strains matched to the vaccine antigens. For multicomponent vaccines like 4CMenB, a panel of “indicator” strains specifically matched to the antigen of interest (*i.e.*, lacking or mismatched to all other antigens) is used to evaluate the ability of each antigen to elicit antigen-specific bactericidal antibodies [106]. H44/76 is the commonly used indicator strain for fHbp [106] as it expresses fHbp var1, does not express NadA, has a PorA serosubtype different from P1.4 and is a weak antigenic match to NHBA.

Clinical evaluation of 4CMenB

All the clinical data described for both vaccines is from published work, as highlighted in Table 3. Across 12 published phase 1 to 3 clinical studies, more than 8300 healthy subjects (infants, toddlers, children, adolescents, and adults) have received 4CMenB (**Table 3**) with acceptable tolerability. In all these studies local and systemic reactions were transient and mostly of mild to moderate severity, with fever (body temperature $\geq 38^{\circ}\text{C}$) and injection site pain being the most notable reactions in infants and adolescents, respectively [86, 107, 108]. Various clinical trials that investigated rMenB (protein antigens only, no OMV) alongside 4CMenB (rMenB+ OMV) indicated that both vaccines induce similar reactogenicity rates, although 4CMenB was associated with greater proportions of vaccinees with local reactions and fever [86, 107, 109, 110]. This is

likely due to the presence of OMV, which is known to be moderately reactogenic, because of the presence of lipopolysaccharide (LPS), which is a very potent activator of the TLR4 receptor [111]. This was further confirmed by a recent clinical study comparing immunogenicity and safety profile of 4CMenB formulations with different OMV doses; although all formulations were generally well-tolerated, groups with no or low-dose OMV displayed slightly lower reactogenicity profiles [112, 113].

Overall, 4CMenB produced robust hSBA responses, and most subjects achieved hSBA titers ≥ 4 after the vaccination courses (**Table 3**). In an early phase 2 study conducted in infants 2 months of age at enrollment, 87% of infants had hSBA titers ≥ 4 against fHbp after 3 doses of 4CMenB [86, 107, 109, 110], while in large late phase 2 and phase 3 studies 99% to 100% of infants had hSBA titers ≥ 4 against fHbp strain after 3 doses of 4CMenB [107, 109, 110]; all infants (100%) had hSBA titers ≥ 4 after receiving 4 doses [86, 107]. The immune response against fHbp strain was not affected by different dosing schedules (2, 3 and 4 months or 2, 4 and 6 months), nor by concomitant administration of routine childhood vaccinations or prophylactic paracetamol [107, 110, 113]. In adolescents (11–17 years of age), hSBA titers ≥ 4 were detected in 92% of recipients after 1 injection, and in 100% after 2–3 injections spaced 1–6 months apart [108]. Phase 2 studies in adults demonstrated similar immunogenicity [108, 114]. Among adults receiving 2–3 doses of vaccine, seroprotection (>90% of participants with hSBA titer ≥ 4) was generally maintained at 4 months [114].

Recent studies have evaluated antibody persistence and booster efficacy in children following early infant immunization with 4CMenB [115, 116]. Long-term antibody persistence after

vaccination with 4CMenB was investigated in adolescent populations and one study has shown that two doses of 4CMenB resulted in seroprotective activity that was sustained over at least 18-23 months in 81% to 84% of adolescents against fHbp [117]. The carriage rate after vaccination with 4CMenB was also evaluated in a phase 3 study performed in UK university students, indicating a potential effect of this vaccine on carriage (27% carriage reduction of BCWY capsular groups compared to the MenACWY-CRM control vaccine) [118]. Although this study was not designed to provide precise estimates of herd protection, these results suggest a possible impact of 4CMenB on transmission when widely implemented in a campaign targeting a population (e.g., adolescents) in which high transmission occurs [118]. Larger studies are required to confirm these preliminary findings, thus ultimately providing valuable information about the full impact of vaccination, as an unexpected outcome of meningococcal serogroup C vaccination in the United Kingdom was indirect herd immunity, with disease reduction in unvaccinated individuals and carriage reduced by as much as 66% [119].

The 4CMenB vaccine has been approved by the European Medicines Agency (EMA) and European Commission and the Australian Therapeutic Goods Administration (TGA) for use in individuals aged >2 months, and by Health Canada for use in individuals aged from 2 months through 17 years, under the commercial name of Bexsero[®] [120-122]. More recently, 4CMenB has also been approved in Chile, Uruguay and Colombia. In late 2013 the Food and Drug Administration (FDA) authorized the use of the 4CMenB vaccine to control two MenB outbreaks on US college campuses, namely Princeton and Santa Barbara. Between December 2013 and May 2014, 15,346 participants were vaccinated and 28,229 doses of the vaccine were administered [123, 124]. Safety monitoring demonstrated no concerning or unexpected patterns

of serious adverse events (SAEs) and no cases of MenB disease occurred among vaccinated subjects [125].

On January 23rd 2015, Bexsero[®] has been formally approved by FDA for extensive use in adolescent population.

Clinical evaluation of rLP2086

Results from 7 clinical studies, including 2 dose-ranging studies, with rLP2086 have been published, comprising 794 toddlers, adolescents, and adults (**Table 3**) [126-129]. While a set of other studies involving more than 4,000 subjects are either ongoing or concluded, these results are not yet published (www.clinicaltrials.gov). hSBA responses were assessed against MenB test strains with 86%–100% sequence identity with the vaccine antigens. In phase 1 studies, seroprotection rates (% of subjects with hSBA titer ≥ 4) and seroconversion rates (% of subjects showing >4 -fold increase in titer) after 3 doses varied by target strain but generally increased in a dose-dependent fashion [126-128]. In a phase 2 immunogenicity and safety study of rLP2086 in 539 adolescents, 68–100% of participants who received three 120- or 200- μ g doses exhibited hSBA titers greater than or equal to the lower limit of strain-specific quantitation (reciprocal titer from 7–18) [129]. Seroconversion rates were similar for both doses and varied by strain. The most commonly reported vaccine-related adverse effects were pain, headache, syncope, swelling, and pallor [129]. A recent Phase 1/2 clinical study conducted in infants was discontinued due to cases of fever observed also with the lowest antigen dosage [130]. It was suggested that this reactogenicity in infants is caused by an increased innate immune response to the lipidated protein, even though the same lipidated vaccine is acceptable in older age groups [130]. On October 29th 2014 the rLP2086 vaccine was approved by the USA Food and Drug Administration

(FDA) (based on 7 clinical studies with 4335 subjects, see www.clinicaltrials.gov) for use in individuals of 10 through 25 years of age under the commercial name of Trumenba[®].

Summary

Independent approaches to developing a MenB vaccine have identified the surface-exposed protein fHbp as an important target for bactericidal antibodies. Preclinical studies directed at understanding the structure, function, and expression of fHbp and its distribution in MenB strains have confirmed its potential as a vaccine candidate, and have also revealed its role as one of several key meningococcal virulence factors that aid bacterial evasion of the host complement system. This work has led to the development of two MenB vaccines that include the fHbp antigen: 4CMenB, which contains fHbp and three other main antigens, and rLP2086, which contains two subvariants of recombinant lipidated fHbp.

As a result of more than 20 years of pioneering vaccine research, 4CMenB has been recently approved in Europe, Australia, Canada, Chile, Colombia and Uruguay, for use in all age groups, including infants over 2 months, which are the most susceptible to meningococcal disease, and in USA for use in adolescents. Similarly, the rLP2086 vaccine was also approved by the FDA in late 2014 for use in adolescents. Both vaccines hold great promise for protection from the devastating consequences of MenB infection.

Expert commentary

After more than four decades of searching for potential MenB vaccine targets, fHbp was identified as a result of novel genomic and proteomic based approaches and is now present in two

licensed MenB vaccines. The ongoing interest surrounding fHbp as a meningococcal vaccine antigen, and also a virulence factor, was clear at the recent XIXth International Pathogenic Neisseria Conference (IPNC) held in Asheville North Carolina (Oct 12-17 2014) where an entire session was dedicated to fHbp. Presentations covered the characteristics of currently licensed vaccines, fHbp epidemiology and expression profiling, epitope mapping, the impact of fHbp-human factor H binding on immunogenicity, the inclusion of fHbp in OMV based vaccines, the redundancy in complement escape mechanisms and the basis of resistance to anti-fHbp bactericidal activity seen in some meningococcal strains.

From an epidemiologic standpoint, the presence of the *fHbp* gene in almost all meningococcal strains suggests that a combination of the main fHbp variants could elicit immunity against the entire MenB population. Such a vaccine would be relatively easy to develop and manufacture. However, the variable expression of fHbp by different strains, which in some cases is almost undetectable, and the high sequence diversity limit the efficacy of anti-fHbp-specific antibodies in low expressing and genetically distant strains. It has long been claimed that there is a strong selective pressure to maintain a functional fHbp protein, as this is required for immune evasion and survival of meningococci in the bloodstream. However, it has now been proved that, beside fHbp, several additional protein factors contribute to regulation of the alternative pathway of complement, including other fH-binding proteins like NspA and PorB, and the NalP protease through its ability to cleave C3. In principle, all these factors could complement the activity of fHbp in fHbp-low expressing or fHbp-deficient strains.

In light of these observations, a multicomponent vaccine may be ideal, as it would enable targeting of multiple meningococcal proteins and therefore be effective also against strains that express very low amounts of one antigen. The use of combined antigens could ultimately limit

selection of escape mutants (*i.e.*, bacteria with a mutation in an antigen-encoding gene that would allow them to evade vaccine-induced antibodies) and be more effective in protection against meningococcal infection in the long term.

Five-year view

In November 2012, the European Medicines Agency (EMA) approved the 4CMenB vaccine (Bexsero[®]) and European Commission licensure followed in January 2013. Unexpectedly, in July 2013 the Joint Committee on Vaccination and Immunisation (JCVI), the independent committee that advises the UK Government on vaccine policy, released an interim statement advising against the introduction of routine infant or adolescent immunization, concluding that Bexsero[®] implementation was highly unlikely to be cost effective at any vaccine price. The announcement led to immediate responses from charities, clinicians, academics, stakeholders and politicians challenging the statement and calling for vaccine introduction. As a result of new cost-effectiveness analyses, and prompted by the reaction of vaccines advocates, in March 2014 the JCVI reverted its original statement and recommended the inclusion of Bexsero[®] in the National Immunisation Programme in the UK, a country with a high burden of meningococcal disease. Currently, Bexsero[®] is approved in 37 countries, including the European Union, Australia, Canada, Colombia, Chile and Uruguay. Following the recent outbreaks of MenB cases in college campuses in the USA, the FDA licensed the rLP2086 bivalent vaccine (Trumenba[®]) (October 29th 2014) and the 4CMenB multicomponent vaccine (Bexsero[®]) (January 23rd 2015) for prevention of invasive meningococcal disease in adolescents.

The real cost-effectiveness and impact of the new MenB vaccines on invasive meningococcal disease will only be fully understood post implementation. Hopefully, over the

next five years there will be widespread implementation of Bexsero[®] and Trumenba[®] in national immunization programs worldwide. The decline in the number of IMD cases, deaths, and IMD-related long-term sequelae, will ultimately inform us about the real, tangible value that these preventive measures can have on public health. In parallel, continued investigation on fHbp's function, distribution, diversity and immunogenicity will provide greater understanding of its potential as a vaccine antigen as well as its role in pathogenesis.

Key issues

- Factor H binding protein (fHbp, previously called GNA1870 or LP2086) is a *Neisseria* specific lipoprotein and an important virulence determinant that is a component of two recently licensed vaccines – Bexsero[®] (4CMenB, Novartis Vaccines) and Trumenba[®] (rLP2086, Pfizer).
- Bexsero[®], a multicomponent vaccine that contains fHbp variant 1.1, NadA, NHBA and outer membrane vesicles (OMVs) of the New Zealand outbreak strain NZ98/254, is currently licensed in several countries for use in individuals >2 months of age and in USA for use in adolescents. Trumenba[®], a bivalent vaccine containing two subvariants of recombinant lipidated fHbp (subfamily A05/subvariant 3.45 and subfamily B01/subvariant 1.55), is licensed in the USA for use in adolescents.
- Several vaccine trials have shown both vaccines to be immunogenic and able to induce bactericidal antibodies in all age groups. Although some indications are available about the potential of Bexsero on carriage, more data are needed to demonstrate a clear herd immunity effect of both vaccines on MenB carriers.

- Although Bexsero[®] has acceptable safety and tolerability profiles, increased reactogenicity has been observed in infants upon concomitant administration of routine vaccines. In the case of Trumenba, clinical studies in infants have shown an unacceptable reactogenicity profile in this age group.
- Although fHbp is present in most meningococcal strains, a few invasive isolates have been identified which either have a frame-shifted gene or express fHbp at minimal levels, suggesting that this factor is not essential for virulence and therefore the emergence of escape mutants could be possible in principle.

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Reference annotations

* Of interest

** Of considerable interest

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Vega Massignani, Maria Scarselli and Daniela Toneatto are currently employed by Novartis Vaccines. During the writing this manuscript, Maurizio Comanducci and Kate Seib were employees of Novartis Vaccines. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Figure 1. fHbp binding to fH enables immune evasion and is a mechanism of meningococcal survival in the bloodstream. A) During bacterial invasion, bacteria not expressing a fH binding molecule, are recognized as non-self by the alternative pathway of complement, C3 binds and the complement pathway readily kills them. B) In the case of fHbp expressing *N. meningitidis*, the bacteria are coated by factor H (in blue) and therefore they are not recognized as non-self; they survive and multiply in human blood causing sepsis.

Figure 2. The three-dimensional structures of fHbp currently available from the RCSB Protein data bank. (A) NMR structure of the variant 1.1 protein in aqueous solution (PDB code 2KC0). (B) NMR structure of the variant 1.55 or B01 allele in micellar solution (PDB 2KDY). (C) Crystal structure of the fHbp variant 3 (PDB 4AYI). (D) Crystal structure of the C-terminal beta barrel domain of fHbp variant 2 (PDB 4AYN). (E) Crystal structure of fHbp variant 1.1 in complex with CCP6-7 of human factor H (in purple) (PDB 1WH0). (F) Crystal structure of fHbp variant 3 in complex with CCP6-7 of human factor H (in purple). fHbp, factor H binding protein; NMR, nuclear magnetic resonance. Pictures were created with the CHIMERA software.

Figure 3. fHbp variability and functional epitopes. (A) Visual representation of fHbp sequence variability on the structure, calculated with Consurf (<http://consurf.tau.ac.il/>). (B, C) Epitope mapping of fHbp. Epitopes recognized by monoclonal antibodies mapped so far are reported: (B) MAb502 (red) [73], 12C1 (blue) [74], 17C1/12C1 (beige) [75], JAR4 (green) [72]. (C) JAR5 (dark cyan) [71] MN86-1075-6 (purple) [24], MN86-440-18 (pale blue) [24] and MN86-1042-2 (gold) [68].

Tables

Table 1. Key Features of fHbp

Citation (Author/Year)	Design/primary aims	Outcomes	Implications
Discovery, Classification, and Distribution			
Masignani et al, 2003 [8]	Characterization of GNA1870	GNA1870 exists in 3 variants. Each variant elicits bactericidal activity against strains expressing fHbp from the same variant group	GNA1870 is a potential vaccine candidate
Fletcher et al, 2004 [9]	Identification and characterization of LP2086	LP2086 exists in two subfamilies and elicits bactericidal response	A vaccine including representatives of the two subfamilies can have broad coverage
Bambini et al, 2009 [27]	Analysis of the prevalence and distribution of the fHbp variants in 85 MenB strains isolated worldwide	Overall prevalence of fHbp var1 is 51%, var2 is 41% and var3 is 8% in MenB strains. No correlation with MLST genotype	MLST is not a useful predictor of fHbp variant
Murphy et al, 2009 [28]	Analysis of the prevalence and distribution of the fHbp variants in 1837 MenB strains isolated worldwide	Overall prevalence of fHbp subfamily A was 30% and B was 70% in MenB strains. No correlation with MLST genotype	As above
Wang et al, 2011 [31]	Analysis of the prevalence and distribution of the fHbp variants in 896 invasive MenB, MenC and MenY US strains	Subfamily B fHbp has a prevalence of 59%, 39% and 3% respectively in MenB, MenC and MenY strains, respectively.	Distribution of genotypic variants of fHbp varies among different serogroups

Citation (Author/Year)	Design/primary aims	Outcomes	Implications
Expression and Regulation			
Oriente et al, 2010 [38]	Investigation on the mechanism of fHbp regulation	Expression of fHbp is induced upon oxygen limitation	fHbp might play an important role in microenvironments lacking oxygen, such as the submucosa or intracellularly
Litt et al, 2004 [41]	Analysis of serum from children convalescing after invasive meningococcal disease	Antibodies to fHbp were detected in patient serum	The presence of antibodies to fHbp after natural meningococcal disease indicates that fHbp is expressed during infection
Ala'Aldeen et al, 2010 [43]	Determine the host response to fHbp during carriage and disease	fHbp-specific antibody response was detected in both carriers and subjects with IMD	fHbp is expressed in vivo during carriage and invasive disease
Virulence			
Madico et al, 2006 [10]	Investigation of fHbp function	fHbp binds fH, a key regulatory protein of the human complement system, enabling the meningococcus to down-regulate the activity of the immune system and host evade killing	fHbp is an important survival factor of the meningococcus
Granoff et al, 2009 [46]	Investigation of the blocking role of fHbp antibodies Assessment of the ability of fHbp to bind fH of different species	Antibodies to fHbp decrease binding of fH to the bacteria fHbp binding to fH is specific for human fH	Vaccine induced antibodies against fHbp may decrease meningococcal evasion of the host immune system Scientific explanation for species specificity of meningococcal infection

Citation (Author/Year)	Design/primary aims	Outcomes	Implications
Structural Characterization			
Cantini et al, 2009 [65]	Structural determination of vaccine antigen GNA1870	Determination of the NMR solution structure of the C-terminal portion of GNA1870	GNA1870 is a two-domain protein. The C-terminal domain is an independently folded beta barrel
Mascioni et al, 2009 [24]	Investigation of the topology of fHbp on the biologic membrane	Compare the structure and topology of fHbp in its lipidated and nonlipidated forms	Overall structure of lipidated and nonlipidated forms of fHbp is maintained
Schneider et al, 2009 [70]	Unravelling of the molecular mechanism of fHbp binding to human fH	Crystal structure of the complex between fHbp variant 1 and CCP6 and CCP7 domains of fH	Understanding the fHbp:fH complex may lead to fHbp vaccines that can elicit responses against an array of protective epitopes
Johnson et al, 2012 [67]	Provide explanation on the different modes of human fH interaction by var2 and var3 fHbp respect to var1	Crystal structure of var3 fHbp in complex with fH and of the C-terminal beta barrel of var2 fHbp. By site directed mutagenesis provide a catalogue of non-fH binding fHbp from all variant groups.	Despite remarkable conservation of var1, 2 and 3 atomic structures, there are differences in key amino acids necessary for interactions with fH. Non-functional fHbp could be included in next generation vaccines

Citation (Author/Year)	Design/primary aims	Outcomes	Implications
Prediction of Coverage of Meningococcal Vaccines			
Giuliani et al, 2006 [16]	Development of a universal vaccine against MenB	Identification of antigens to be included in the combination vaccine and evaluation of their potential strain coverage	A combination vaccine can be effective to protect against the majority of circulating MenB strains
Jiang et al, 2010 [23]	Evaluation of the vaccine potential of the bivalent rLP2086 vaccine	fHbp surface expression was determined using the cross-reactive mAb on a panel of MenB strains. Rabbit and human sera from immunization with rLP2086 killed the majority of MenB strains tested	Bactericidal activity of anti-fHbp sera correlates with fHbp surface expression in vitro
Donnelly et al, 2011 [99]	Development of an assay able to predict 4CMenB vaccine coverage	Definition of the MATS assay	Possibility to reliably predict coverage afforded by 4CMenB vaccine
Vogel et al, 2013 [88]	Assessment of the 4CMenB predicted strain coverage in Europe by MATS	MATS predicted that 78% of >1000 MenB strains from 5 European countries would be killed by anti-4CMenB sera	The 4CMenB multi component vaccine has the potential to protect against a substantial proportion of invasive MenB strains isolated in Europe
Frosi et al, 2013 [102]	Assess the accuracy of MATS-based predictions of vaccine strain coverage	MATS predicted 70% coverage of 40 representative strains from England/Wales, while 88% if these strains were killed in hSBA assays	MATS is a conservative predictor of strain coverage by the 4CMenB vaccine in infants and adolescents

fH=factor H; fHbp=factor H binding protein; IMD=invasive meningococcal disease; MenB, C, Y=Meningococcus serogroups B, C, Y; MLST=multi locus sequence typing; NMR=nuclear magnetic resonance; US=United States of America.

Table 2. Composition of the rLP2086 and 4CMenB vaccines

rLP2086 [131]				4CMenB [132]			Considerations
Components	Variant	Form	Prevalence	Variant	Form	Prevalence	
fHbp	A05 (var3.45)	lipidated	2-11%	fHbp	Recombinant, fused to accessory antigen GNA2091		fHbp binds fH and has an important role in pathogenesis by increasing serum resistance. Very immunogenic and excellent target for bactericidal antibodies. fHbp expression is variable between strains. Some level of intra-family cross-protection observed (mainly in adult population).
	B01 (var1.55)	lipidated	n.a.	NHBA	Recombinant, fused to accessory antigen GNA1030		NHBA binds heparin and has an important role in meningococcal pathogenesis by increasing serum resistance. It is present in all meningococcal strains. Cross-protection observed.
				NadA	Recombinant	<i>nadA</i> gene present in 50% of MenB strains. Cross-protection observed	NadA has a crucial role in adhesion/invasion of human epithelial cells. Cross-protection observed when gene is present. NadA expression is repressed by the regulator NadR under <i>in vitro</i> growth conditions, and induced <i>in vivo</i> by niche-specific signals
Adjuvant	Aluminum phosphate			OMV	Deoxycholate detergent		Main component of the MeNZB™ vaccine. Induced excellent protection during the New Zealand vaccination campaign.
				Aluminum hydroxide			

fHbp=factor H binding protein; n.a.=not applicable; NadA=neisserial adhesin A; NHBA=neisserial heparin binding antigen; OMV=outer membrane vesicles; var=variant.

Table 3. Clinical Meningococcal B vaccine studies

Study*	Age at Enrollment	Reference
4CMenB		
<i>Studies in Adults</i>		
Phase 1		
V72P5 [#] (Switzerland)	18–40 y	Toneatto et al., 2011 [133]
<i>Studies in Adolescents and Adults</i>		
Phase 2/3		
NCT00560313 (V72P4 [#]) (Italy, Germany)	18–50 y	Kimura et al., 2011 [114]
NCT00661713 (V72P10 [#]) (Chile)	11–17 y	Santolaya et al., 2012 [108]
NCT01148524 (V72P10 [#]) (Chile)	13–19 y	Santolaya et al., 2013 [117]
NCT01214850 (V72_29 [#]) (UK)	18–24 y	Read et al., 2014 [118] [134]
<i>Studies in Infants, Toddlers and Children</i>		
Phase 2		
NCT00381615 (V72P6 [#]) (UK)	2 mo	Findlow et al., 2010 [86]
NCT00433914 (V72P9 [#]) (UK)	6–8 mo	Snape et al., 2010 [109]
NCT01027351 (V72P6E1 [#]) (UK)	40 mo	Snape et al., 2013 [115]
NCT00937521 (V72P16 [#]) (Europe)	2 mo	Prymula et al., 2014 [113]; Esposito et al, 2014 [112]
Phase 2/3		
NCT00721396 (V72P12 [#]) (Europe)	2 mo	Gossger et al., 2012 [110]
NCT00657709 (V72P13 [#]) (Europe)	2 mo	Vesikari et al., 2013 [107]
NCT00847145 (V72P13E1 [#]) (Europe; extension)	2 mo	Vesikari et al., 2013 [107]
rLP2086		
<i>Studies in Adults</i>		
Phase 1		
NCT00879814 (B1971004 [#]) (USA)	18–40 y	Sheldon et al., 2012 [126]
NCT00297687 (Australia)	18–25 y	Richmond et al., 2012 [127]

Phase 2		
NCT00808028 (B1971005 [#]) (Australia/Europe)	11–18 y	Richmond et al., 2012 [129]
NCT00780806 (B1971003 [#]) (Australia)	18–40	Marshall et al., 2013 [135]
<i>Studies in Adolescents and Adults</i>		
Phase 1/2		
NCT00387725 (Australia)	8–14 y	Nissen et al., 2012 [136]
<i>Studies in Children</i>		
Phase 1		
NCT00387569 (Australia)	18–36 mo	Marshall et al., 2012 [137]
Phase 1/2		
NCT00798304 (Spain)	2 mo	Martinon-Torres et al., 2014 [130]

UK=United Kingdom

* Clinicaltrials.gov registry identifier unless otherwise noted

[#] Manufacturer's trial identifier