The discovery and development of a novel vaccine to protect against *Neisseria meningitidis* serogroup B disease

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Vaccines have had a major impact on the reduction of many diseases globally. Vaccines targeted against invasive meningococcal disease (IMD) due to serogroups A, C, W, and Y are used to prevent these diseases. Until recently no vaccine had been identified that could confer broad protection against *Neisseria meningitidis* serogroup B (MnB). MnB causes IMD in the very young, adolescents and young adults and thus represents a significant unmet medical need. In this brief review, we describe the discovery and development of a vaccine that has the potential for broad protection against this devastating disease.

Introduction

Capsular polysaccharide based vaccines currently exist for prophylaxis of disease caused by meningococcal serogroups A, C, Y and W-135. However, similar approaches to develop a serogroup B *Neisseria meningitidis* (MnB) vaccine have failed. The serogroup B capsular polysaccharide is composed of polysialic acid repeating units that are similar to structures found on human neuronal cells (particularly during fetal development) and are not suitable as vaccine antigens.[1] An alternate vaccine strategy to prevent MnB disease has included vaccines produced from outer membrane vesicles (OMVs) prepared from regional epidemic MnB strains.[2] These OMVs have been successfully deployed to control epidemics in Norway, Cuba, New Zealand, and France.[3] The immune-dominant antigen in these vesicles is the outer membrane protein porin A (PorA).[4] For an OMV vaccine to be effective in an outbreak situation the PorA of the vaccine needs to match that of the epidemic strain. Since PorA proteins show

extensive sequence heterogeneity in their cell surface exposed immunogenic loops, many OMVs would be required to assure a broadly protective response against MnB invasive strains, thereby emphasizing the need for alternate approaches for vaccine development. To make a broadly effective vaccine candidate against MnB, vaccine antigens should be: (1) present in the majority of global clinical disease isolates; (2) surface exposed; (3) an important virulence factor; and (4) able to elicit a bactericidal immune response against a high proportion of diverse global invasive disease isolates. Serum bactericidal immune responses as measured in serum bactericidal antibody assays with human complement (hSBA) have been shown to correlate with protection against meningococcal disease.[5]

Several MnB surface proteins have been considered individually as vaccine antigens but have not satisfied all of the above mentioned criteria. Deficiencies have included; (1) an inability to demonstrate induction of functional immune responses measured in the hSBA (Transferrin Binding Protein, Neisserial Heparin Binding Protein);[6,7] (2) low surface expression on MnB invasive clinical isolates (Neisserial Surface Protein A);[8] (3) considerable sequence diversity (PorA, meningococcal enterobactin receptor FetA);[9,10] and (4) a lack of antigen expression in a significant subset of invasive isolates (Neisserial Adhesin A).[11] The development of a multi-antigen vaccine for the prevention of MnB IMD has been described by Giuliani and colleagues.[12] In this review, we outline the steps taken for the development of a vaccine candidate that targets a single protein on the meningococcal surface. The vaccine candidate contains two recombinantly expressed factor H binding protein variants and has been shown to elicit broad serum bactericidal activity against diverse MnB clinical isolates.

The Discovery of fHBP

The broadly protective vaccine potential of factor H binding protein (fHBP) was discovered using a combined biochemical

and immunological screening approach. The approach was developed to identify MnB surface expressed proteins with broad serogroup B distribution and sufficient amino acid sequence conservation to induce PorA independent hSBA responses against both endemic and epidemic strains. MnB strains were fractionated and the resulting outer membrane protein preparations were differentially solubilized with detergents and separated based on pI and surface charge into protein fractions which were used to immunize mice. The resulting immune sera were assessed in hSBA to identify fractions able to elicit robust bactericidal activity against diverse invasive MnB clinical isolates. The process was repeated until the most active fractions contained only a few proteins. The amino acid sequence of the proteins in the active fractions was determined and the corresponding genes were cloned, expressed in *E. coli* and purified. Immune sera raised to the recombinant proteins in preclinical species were tested to confirm that these gene products were able to elicit bactericidal activity in hSBA.[13] This screening approach, which relied heavily on the ability of the vaccine antigen to elicit broad serum bactericidal activity against diverse MnB strains, resulted in the identification of a single outer membrane lipoprotein, fHBP (also

known as Lipoprotein 2086, or LP2086), that had all the pre-requisite characteristics of a vaccine antigen as described above.

Human factor H is a negative regulator of the alternative complement pathway and the binding of factor H by fHBP expressed on the cell surface enables the bacteria to avoid attack by the complement system.[14] Consistent with a role for fHBP in meningococcal virulence, normal human serum has bactericidal activity against a MnB fHBP knock out strain while an isogenic wild type strain is much less susceptible.[15] In addition, the level of fHBP surface expression is inversely correlated with susceptibility to bacterial lysis by normal human serum or whole blood.[16] It is conceivable that a vaccine candidate based on fHBP induces protective antibodies mediating complement dependent bactericidal killing but possibly also may indirectly prevent factor H binding to the bacteria thereby limiting bacterial survival in vivo.[17,18]

Extensive molecular epidemiology of MnB clinical isolates demonstrated that meningococcal fHBP gene sequences segregate into two subfamilies, designated A and B.[13] The fHBP sequence diversity across the two subfamilies was an important contributor to the design of the recombinant LP2086 (rLP2086) vaccine. The lipoproteins of these two subfamilies are immunologically distinct.[13,19] Protein variants within subfamilies share ≥83% amino acid sequence identity, but only 60–75% identity between subfamilies (**Fig. 1**).[20] To obtain broad bactericidal immune responses against diverse MnB disease strains, a fHBP based vaccine containing one fHBP from each of the two fHBP subfamilies was required.

Since fHBP is a lipoprotein, pre-clinical studies were performed to determine whether the lipidated form of the protein was needed for broad bactericidal activity against MnB invasive disease strains.[13] Lipidated and non-lipidated forms of fHBP variants were expressed as recombinant proteins and used to immunize mice.[13] In all cases, the lipidated protein antigens induced at least 10-fold higher hSBA titers compared with the non-lipidated forms. In addition to differences in relative potency, immune sera following vaccination with lipidated antigens had bactericidal activity against MnB isolates that expressed fHBP variants that were different from the vaccine antigen. One example, using fHBP variant B01 as antigen in mice and a hSBA strain expressing the heterologous fHBP variant B24, is provided in **Table 1**. The lipidated antigen rLP2086-B01 elicited a 10-fold higher hSBA titer compared with the non-lipidated rP2086-B01 against this heterologous hSBA strain. Similarly, vaccination of rhesus macaques with rLP2086-A05 elicited more robust hSBA responses than non-lipidated rP2086-A05. This was demonstrated using MnB strains that expressed fHBP variant A05, or the heterologous fHBP subfamily A variant A22 (**Table 2**). Based on these data, lipidated forms of fHBP were selected for further development of the final vaccine candidate.

Consistent with the sequence diversity between subfamily A and B, the breadth of bactericidal activity following immunization with lipidated fHBP antigens was mostly subfamily restricted.[13,19] Immunization with lipidated fHBP recombinant antigen from subfamily A elicited high serum bactericidal antibody activity as measured in hSBA against MnB invasive isolates
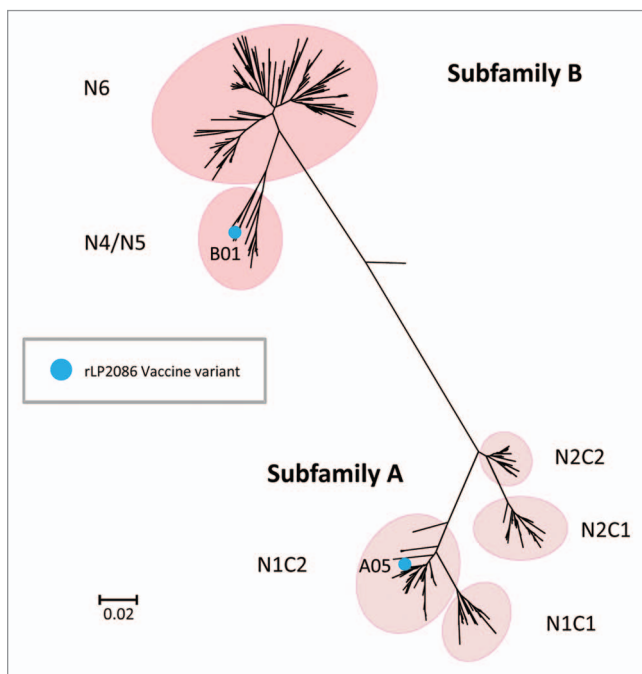

Figure 1. Phylogenetic relationship of meningococcal serogroup B fHBP variants. Figure adapted from Murphy et al.[20] Phylogenetic tree of fHBP/LP2086 variants based on clustalW alignment and drawn with MEGA 4. Highlighted are subfamily A and B, the subgroups within the respective subfamilies, and the components of the bivalent rLP2086 investigational vaccine, fHBP variants A05 and B01. During the development of bivalent rLP2086, alternative nomenclature systems were used for fHBP in the scientific literature.[21,38] The scale bar indicates phylogenetic distance based on protein sequence. The phylogenetic distance between any two variants is the length of the line traced from one branch back to the first common node on the tree and along the branch to the second variant. The end of each branch on the tree represents a specific fHBP variant. The longer the line connecting the two variants, the greater is the amino acid sequence diversity between them.

that expressed subfamily A protein variants, while showing much reduced or very low activity against isolates expressing subfamily B variants. Similarly monovalent lipidated fHBP antigen from subfamily B elicited high serum bactericidal antibody activity that was mostly restricted to MnB isolates that express subfamily B protein variants. In preclinical studies, immunization with a bivalent vaccine formulation composed of a lipidated fHBP antigen from both subfamily A and B was able to produce sera with broad spectrum bactericidal activity against most MnB strains tested.[19]

These preclinical immunogenicity observations together with preliminary epidemiological data supported the development of a vaccine candidate that contains two fully lipidated fHBP proteins, one from each of the two fHBP subfamilies, to assure protection against a broad spectrum of invasive MnB isolates from diverse geographical locations. Therefore, a bivalent vaccine composed of recombinantly expressed lipidated fHBP variants A05 and B01 (bivalent rLP2086) moved forward to clinical development (**Fig. 1**).

Surveillance of Invasive MnB Isolates and the Epidemiology of fHBP

The discovery of the potential for fHBP to be used as a vaccine antigen to confer broad protection against MnB invasive disease isolates required a comprehensive understanding of the epidemiology of MnB isolates. In regards to fHBP gene presence, expression and variant distribution to determine the strategy for identifying MnB isolates for clinical hSBA testing in support of bivalent rLP2086. To support this, the fHBP epidemiology was determined for a collection of invasive MnB disease isolates obtained from the US, UK countries (England, Wales, and Northern Ireland), Norway, Czech Republic, and France was assembled to support this goal. A total of 1263 systematically collected isolates that represent approximately 13% of MnB disease isolates from these countries between 2001 and 2006 (2000 and 2005 from the US) were included, and the resulting collection was called the MnB SBA strain pool (**Table 3**). In addition to

characterization of fHBP, the strains were evaluated for other common epidemiological markers such as MLST and PorA subtype. Sequence analysis of the 1263 MnB invasive disease isolates in the MnB SBA strain pool demonstrated that the fHBP gene was present in all of the strains, and that intact sequences capable of coding for full-length proteins were found in all but one isolate. The analysis also revealed that fHBP subfamilies could be further divided into subgroups (**Fig. 1**).[20,21] Subfamily B fHBP variants were more common than subfamily A (**Table 3**), both in the total MnB SBA strain pool (71% compared to 29%) and in each of the component countries, including the US (65% compared to 35%). The subfamily distribution did change when fHBP variants were analyzed as a function of subject age. An increase in disease caused by strains expressing subfamily A variants was observed in the infant and the elderly population (**Fig. 2**)[22], which is consistent with findings from other regions.[23] Likewise MnB carriage strains also predominantly harbor fHBP subfamily A variants.[24] Despite the identification of 143 unique fHBP variants within the MnB SBA strain pool, a subset of ten fHBP variants is expressed in nearly 80% of all strains (**Fig. 3**). With one exception, there was little correlation observed between fHBP variants and other common epidemiological genetic markers (i.e., MLST, Clonal Complex, PorA subtype). The single exception noted was that the majority of strains expressing fHBP variant B24 were typed as CC32 (**Fig. 4**).

Table 1. Immunization of mice with rLP2086-B01 elicits greater bactericidal activity than rP2086-B01 using a MnB SBA test strain that expresses heterologous (to vaccine antigen) fHBP variant B24

Antigen	Lipidated	hSBA Titer Using MnB Test Strain Expressing fHBP Variant B24
rLP2086-B01	Yes	1:200
rP2086-B01	No	<1:25

Data adapted from Fletcher et al.[13] Mice (prevaccination titers <50) were immunized with 10 μg of rLP2086-B01 or rP2086-B01 (formulated with QS-21, 20 μg/0.2 mL dose) at weeks 0 and 4. Bactericidal titers in serum samples from week 6 are represented as the reciprocal of the dilution of antiserum that reduced the viable cell count by 50% using a MnB SBA strain that expresses heterologous fHBP variant B24.

Table 2. Immunization of rhesus macaques with rLP2086-A05 elicits greater hSBA responses than rP2086-A05 using MnB strains that express homologous or heterologous (to vaccine antigen) subfamily B fHBP variants

Antigen	Lipidated	Dose (μg)	MnB hSBA strains (% responders with a ≥4-fold rise in hSBA titer[a])	
			PMB1745 (A05) (n/N)	PMB663 (A22) (n/N)
rLP2086-A05	Yes	30	100 (10/10)	60 (6/10)
rP2086-A05	No	30	50 (5/10)	0 (0/10)
		60	50 (5/10)	20 (2/10)
		120	33 (3/9)	0 (0/9)

[a]The percentage of animals that responded to immunization with a ≥4-fold increase in hSBA titer from their pre-vaccination titers is presented. rLP2086-A05 and rP2086-A05 proteins were formulated with AlPO$_4$ and used to immunize Rhesus macaques (n ≥ 9/group). Animals received three intramuscular doses of vaccine antigen (weeks 0, 4, and 8), and the post dose 3 (week 10) serum samples were used to measure functional activity in the hSBA. hSBAs were performed using MnB strains that express fHBP variant A05 (PMB1745) or the heterologous (to vaccine antigen) fHBP variant A22 (PMB663). Data are reported as the percent of animals in the respective treatment groups that responded with at least a 4-fold increase in hSBA titer compared with pre-vaccination titers. Several animals had pre-vaccination serum samples with no baseline activity in the hSBA and were assigned a titer of 2 (by convention, one-half of the lowest serum dilution tested).

Table 3. The composition and fHBP subfamily distribution of the MnB SBA strain pool (2000–2006)

Region	Number of isolates	Approx. % in country disease coverage in the starting regional reference collection	fHBP subfamily (%)	
			Subfamily A	Subfamily B
US (ABCs)	432	13	35	65
United Kingdom	536	90	23	77
France	244	80–85	32	68
Norway	23	85–90	35	65
Czech Republic	28	50–70	25	75
Total	1263	-	29	71

Table adapted from Murphy et al.[20] The regional ABCs sites cover ~13% of US population, and all isolates are included in the MnB SBA strain pool. European collections survey the entire country, and every 8th isolate (every 7th from the Czech Republic) from the available collection of each country is included in the pool to represent approximately 12.5% of disease isolates.

Assessment of the Potential Breadth of Coverage of Bivalent rLP2086

hSBA responses have been correlated with protection from meningococcal disease, and therefore hSBA is the accepted surrogate serological assay for MnB vaccine efficacy.[25] To appropriately evaluate the breadth of coverage elicited by vaccination with rLP2086, Pfizer developed robust hSBAs to measure the ability of rLP2086 immune serum samples from nonclinical and clinical studies to kill MnB isolates expressing fHBP variants that are different (heterologous variants) from the vaccine antigen variants (homologous variants). Initial studies evaluated the ability of MnB isolates to be killed in the hSBA using pooled bivalent rLP2086 immune serum.[19] Isolates (n = 100) were selected that represented the breadth of fHBP diversity in addition to having diverse geographical and genetic backgrounds. Over 75% of strains were killed in the hSBAs. To explore why a small percentage of strains were resistant in the hSBAs, a large number of genetic factors were analyzed, including fHBP subgroup, fHBP variant and MLST. No correlation was identified between s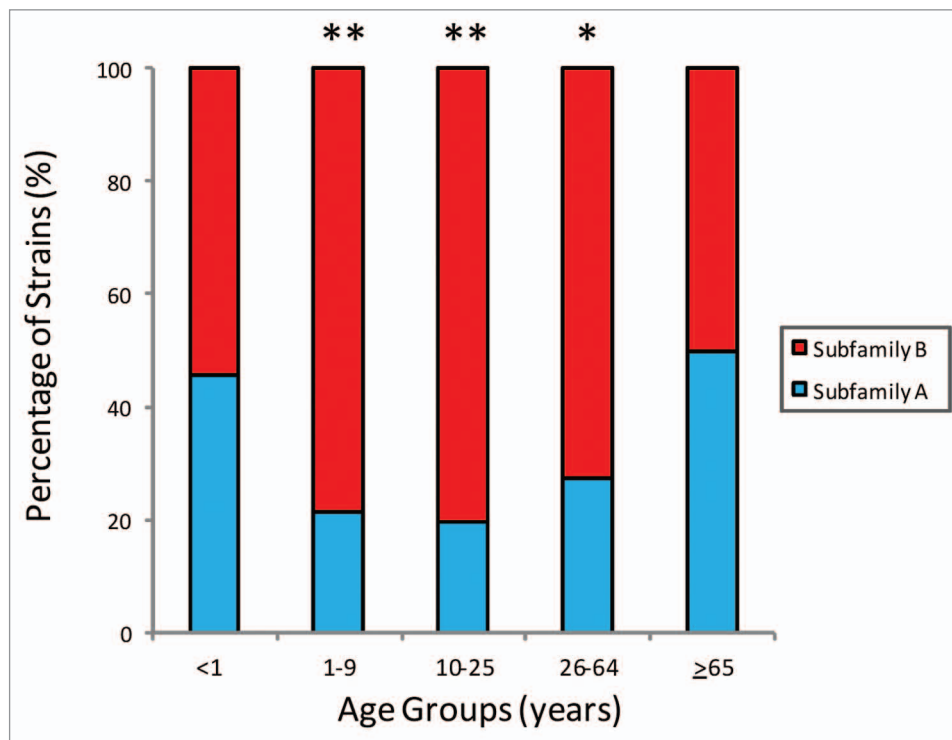train susceptibility and any of the markers studied. The hSBA measures antibody mediated, complement dependent bactericidal activity. It is dependent upon the formation of a membrane attack complex (MAC).[26] To initiate the complement cascade and MAC formation, complement factor C1q must bind to antibodies bound specifically to fHBP. Activation of the complement cascade is triggered by cross-linking between C1q and multiple antibodies bound to the bacterial surface. Such cross-linking can only occur when the fHBP protein is expressed in sufficient quantities on the surface of bacteria. Therefore, fHBP surface expression of MnB strains grown under hSBA conditions was examined as a variable that could impact susceptibility to hSBA killing. To determine relative surface expression/density of fHBP among MnB invasive disease strains, a flow cytometry assay was developed using a monoclonal antibody that recognized an epitope common to fHBP variants from both subfamily A and B (**Fig. 5**).[27] As only antibody-accessible

Figure 2. Distribution of fHBP subfamily A or b expressing strains stratified by patient age in the MnB SBA strain pool. Figure adapted from Hoiseth et al.[22] The distribution of fHBP subfamily A and subfamily B expressing strains within different age groups in the MnB SBA strain pool (age data available for 1215 subjects). Age groups with significant differences (*P < 0.0005) and with highly significant differences (**P < 0.0001) in subfamily distribution compared with the <1 y age group are marked (Fisher Exact Test).

cell surface antigen (not total cell associated antigen) is relevant to elicit the bactericidal response, the assay was designed to selectively quantify levels of surface expressed fHBP. The distribution of expression levels was independent of fHBP subfamily or subgroup, but relative differences in expression levels from strains expressing one variant compared with another variant were notable. A similar distribution was also observed when comparing the US subset of strains (n = 432 strains) to the entire MnB SBA strain pool (n = 1263 strains).

Comparative analysis of in vitro fHBP expression levels and hSBA susceptibility to bivalent rLP2086 immune serum using the 100 isolates tested revealed that there was a threshold level of fHBP expression above which MnB isolates were predictably killed with pooled rabbit sera (**Fig. 6**). While approximately 38% of strains with expression levels below the threshold level were susceptible to killing in the hSBA, no prediction could be made as to which of these strains with lower expression levels would be susceptible. Since fHBP surface expression level is an important factor in hSBA susceptibility, surface expression was included as a criterion in the unbiased selection of hSBA strains selected for clinical testing.

While a surface expression level threshold can predict susceptibility of a MnB isolate in hSBA, it cannot predict the percentage of human subjects that will mount protective antibody responses to a vaccine as measured in hSBA and it is the hSBA response rate that correlates with protection.[5,25] To determine response rate and assessment of the potential breadth of vaccine coverage of the investigational bivalent rLP2086 vaccine, serum samples from individual human subjects need to be taken prior to and following immunization with bivalent rLP2086 and evaluated in the hSBA. Therefore great care was needed to identify the MnB strains that would be used for clinical testing to confirm that the vaccine can confer broad protection against circulating MnB strains.
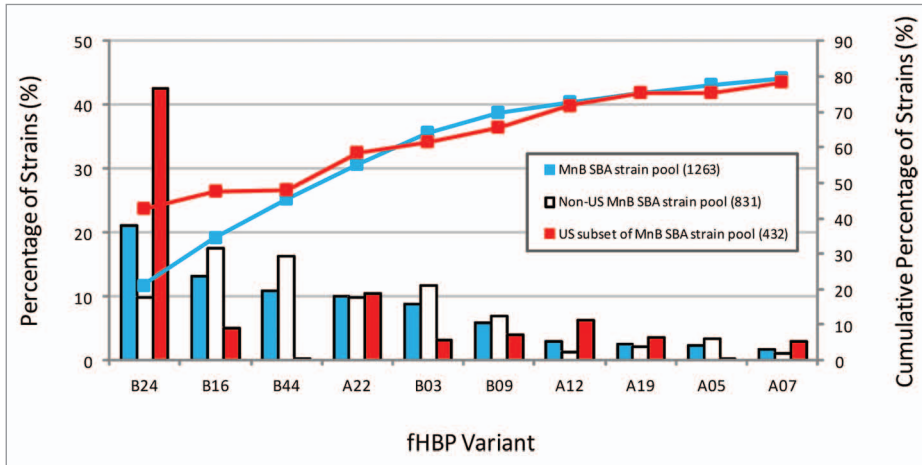

Figure 3. Prevalence of fHBP subfamily A and B variants in the MnB SBA strain pool and the US subset of the MnB SBA strain pool. Data taken from Murphy et al.[20] The ten most prevalent fHBP variants (each with ≥20 representative strains) and the cumulative percentage of strains in the MnB SBA strain pool (n = 1263) are pictured. The prevalence of these ten fHBP variants in the US subset of the MnB SBA strain pool (n = 432) and the non-US subset of the MnB SBA strain pool (n = 831) are shown.
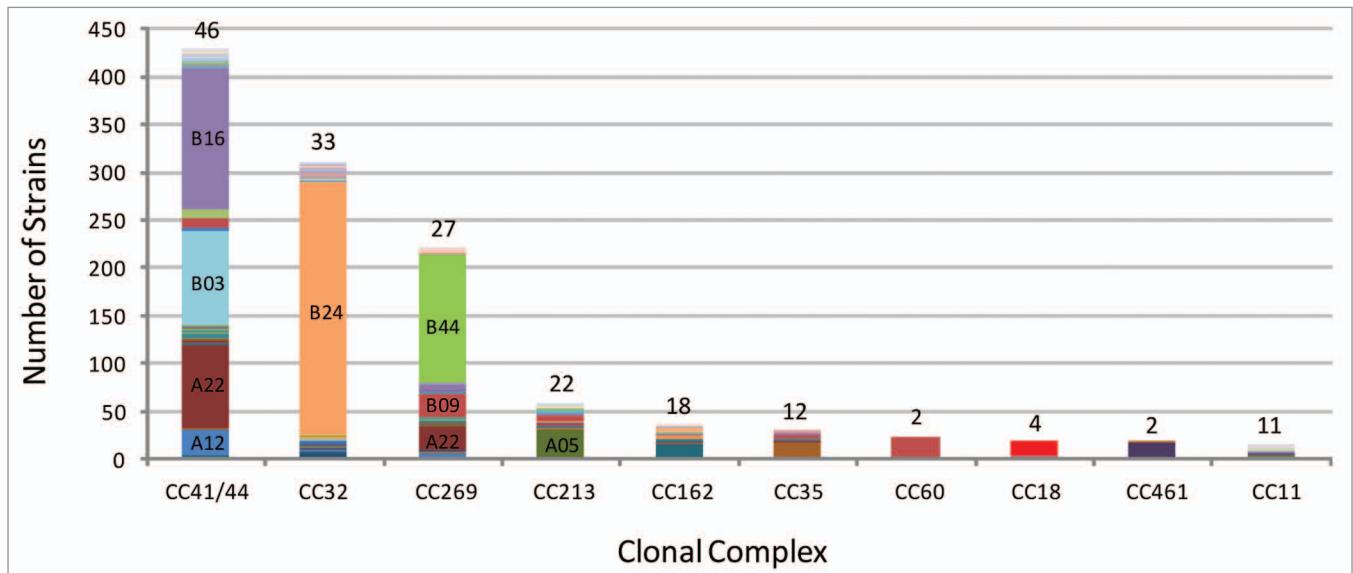

Figure 4. The fHBP variant distribution within the major clonal complexes of the MnB SBA strain pool. Data taken from Murphy et al.[20] The relative abundance and fHBP variant distribution within the ten most prevalent CCs in the MnB SBA Strain Pool (n = 1263) is shown. Each color in the respective columns corresponds to an individual fHBP variant and the prominent variants are labeled. Above each of the columns is the number of different fHBP variants that were found associated with the respective CCs.

The Strategy for Identifying MnB Strains for Clinical Testing

While clinically devastating, the incidence and sporadic occurrence of IMD do not permit the conduct of phase 3 studies based on clinical disease endpoints, and therefore hSBA responses have to be used as a surrogate of efficacy. Nonclinical and exploratory clinical studies established that bivalent rLP2086
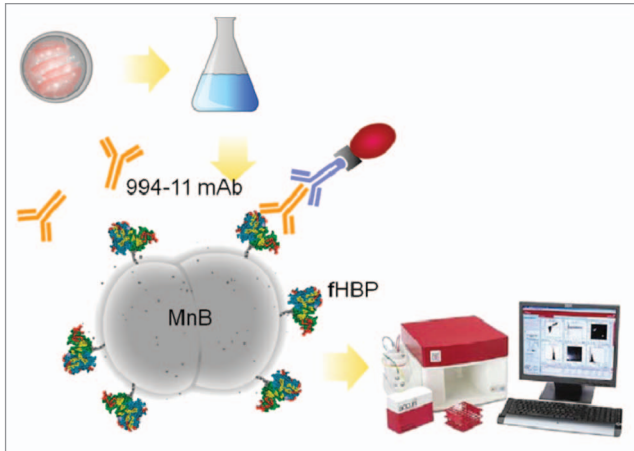

Figure 5. Overview of the MEASURE assay. Bacteria are grown following standard hSBA procedures. Briefly, MnB bacteria are streaked onto plates and grown overnight before inoculating liquid media with colonies and growing to a specific OD. Bacteria are then stained using a 3-step antibody staining method. Bacteria were stained with the primary anti-LP2086 antibody, MN86–994–11–1, followed by a biotinylated mIgG secondary antibody and tertiary PE-Streptavidin to amplify the fluorescence signal and increase the dynamic range.
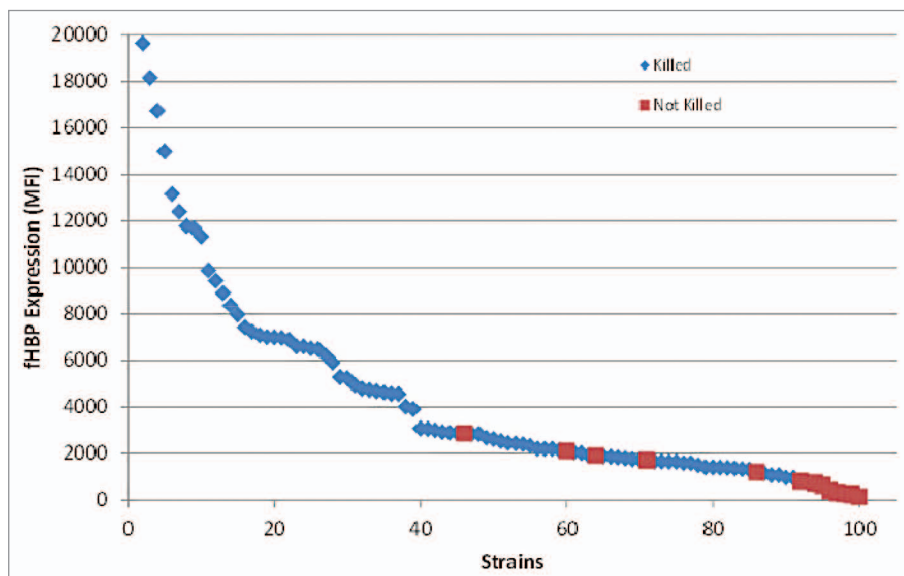

Figure 6. fHBP surface expression correlates with strain susceptibility in the SBA. Data adapted from Jiang et al.[19] Median fluorescence intensity (MFI) was determined using the validated MEASURE assay (McNeil et al., manuscript in preparation). Invasive MnB strains (n = 100) were tested using the Measure assay and the MFI was plotted. Each strain was also run in the hSBA assay. Red squares represent MnB strains that were not killed in the hSBA and blue triangles represent MnB strains that were killed.

elicits a dose-dependent immune response that is capable of killing MnB isolates in hSBA irrespective of fHBP variant sequence or any other epidemiological marker studied.[19,28] Considering the diversity of potential MnB strains and the practical limitations for the number of strains that could be tested in hSBA, an unbiased approach was used to select MnB hSBA test strains that represented either fHBP subfamily A or B expressing strains from the MnB SBA strain pool.[20] Differences in fHBP surface expression were taken into consideration by randomly selecting strains with low to medium rather than high surface expression levels to reflect more closely the normal distribution of fHBP expression among disease isolates. Furthermore, low baseline positivity rate was an important consideration for test strain identification for use in hSBA, as the populations at risk for meningococcal disease are characterized by non-existing or low baseline bactericidal activity to most strains. In addition, to demonstrate that vaccine induced responses provide broad coverage against IMD strains, the hSBA test strains had to express fHBP variants different (heterologous) from the vaccine antigens and strains that reflected the fHBP diversity of meningococcal disease isolates. Based on these considerations, the 4 primary hSBA test strains selected were PMB2948 (fHBP variant B24), PMB2707 (fHBP variant B44), PMB80 (fHBP variant A22), and PMB2001 (fHBP variant A56).[29] These four primary hSBA test strains include representatives of both subfamily A and B and four of the six major fHBP subgroups, representing at least 84% of strains circulating in the US (**Fig. 7**). These strains were used to estimate vaccine efficacy using hSBA immunogenicity endpoints.

The Clinical Performance of Bivalent rLP2086

The clinical development of bivalent rLP2086 began in 2006 with 3 early Phase 1 studies in subjects aged 18 to 25 y,[30] 8 to 14 y,[31] and 18 mo to 36 mo.[32] These studies were conducted using an initial formulation of bivalent rLP2086, at dose levels of 20 μg, 60 μg, or 200 μg administered on a 0, 1, 6-mo schedule. Overall, bivalent rLP2086 was shown to be well tolerated and immunogenic in these Phase 1 trials. Subsequently, the drug substance manufacturing process and the drug product formulation were enhanced to increase scalability of manufacture and to ensure long-term stability of the vaccine. The resulting final formulation was used for all subsequent clinical studies including a Phase 1 Study (B1971004) and a Phase 1/2 Study (B1971003) conducted in subjects 18 to 40 y of age, where the final selected dose level of 120 μg (60 μg of rLP2086 variant A05 and 60 μg of rLP2086 variant B01) was confirmed.[33,34] The potential for bivalent rLP2086 to generate broadly

bactericidal antibodies was demonstrated in the Phase 2 proof-of-concept and dose ranging study (B1971005) conducted in subjects 11 to 18 y who received vaccine administered on a 0, 2, 6-mo schedule (**Table 4**).[30] Additional Phase 2 and 3 studies with the 120 μg dose level were then initiated to collect safety, immunogenicity, lot consistency and concomitant vaccine use data (**Table 5**). The functional immunogenicity for most of these studies was assessed using the four primary MnB strains that were randomly selected to demonstrate breadth of coverage. Completed phase 1 and 2 studies have demonstrated a robust hSBA response to MnB test strains expressing either subfamily A or B fHBP variants after 2 or 3 vaccine administrations of rLP2086 at a dose level of 120 μg.[30] Across the phase 1 and phase 2 studies, bivalent rLP2086 showed consistent and robust immune responses to a panel of MnB test strains expressing fHBP variants that are heterologous to the vaccine, including the four primary MnB test strains that are currently being used to evaluate the immune responses to bivalent rLP2086 in phase 3 clinical studies.

Conclusion

A new bivalent vaccine for protection against MnB disease has been designed based on the discovery of a vaccine antigen (fHBP) that meets all the prerequisites of a preferred vaccine antigen. Specifically, fHBP is: (1) present in the vast majority of global clinical disease isolates; (2) surface exposed; (3) an important meningococcal virulence factor; and (4) able to elicit a bactericidal protective immune response against
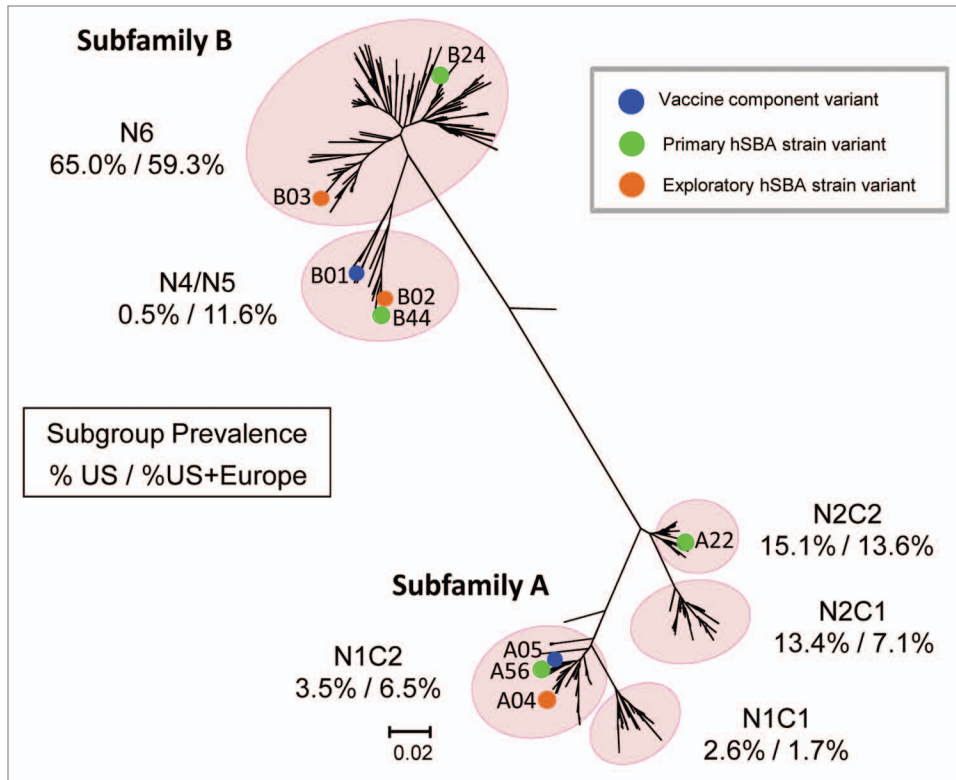

Figure 7. fHBP Phlyogenetic Tree Illustrating MnB hSBA Strain and Vaccine Component Variants. Phylogenetic tree of fHBP/LP2086 variants in the MnB SBA strain pool based on clustalW alignment, and drawn with MEGA 4. Highlighted are the fHBP variant components of bivalent rLP2086 (A05 and B01), the fHBP variants expressed by the primary hSBA strains, and the fHBP variants expressed by the exploratory hSBA strains listed in **Table 4**. The numbers beneath each fHBP subgroup in the figure represent the percentage of isolates in the US subset of the MnB SBA strain pool (on the left) or the MnB SBA strain pool (on the right) that reside in each subgroup. The scale bar indicates phylogenetic distance based on protein sequence.

Table 4. The bivalent rLP2086 vaccine induces a broad immune response in adolescents against MnB isolates that express diverse fHBP variants

Strain (fHBP variant)	Predose 1				1 mo postdose 3			
	N[a]	n[b]	%[c]	95% CI	N[a]	n[b]	%[c]	95% CI
PMB1321 (A22)	34	3	8.8	1.9, 23.7	31	28	90.3	74.2, 98.0
PMB3302 (A04)	90	12	13.3	7.7, 22.0	108	108	100.0	93.1, 100.0
PMB1745 (A05)	122	14	11.5	6.9, 18.4	114	111	97.4	92.2, 99.1
PMB2001 (A56)	119	10	8.4	4.6, 14.9	114	111	97.4	92.2, 99.1
PMB2948 (B24)	38	6	15.8	6.0, 31.3	33	30	90.9	75.7, 98.1
PMB17 (B02)	124	3	2.4	0.8, 7.2	113	104	92.0	85.4, 95.8
PMB1256 (B03)	118	3	2.5	0.8, 7.6	86	65	75.6	65.4, 83.5
PMB2707 (B44)	120	4	3.3	1.3, 8.5	115	102	88.7	81.5, 93.3

The proportion of subjects vaccinated from Study B1971005 with hSBA titers ³1:8 adapted from[30] [a]Number of subjects with valid and determinate hSBA titers to the given strain. [b]Number of subjects with observed hSBA titer for the given strain ³1:8. [c]Percentage of subjects with valid and determinate hSBA titers that had an observed hSBA titer for the given strain ≥ 1:8.

Table 5. Phase 1, 2, and 3 studies using the final formulation of bivalent rLP2086

Study number (Country/Region[a])	Phase	Study description	Study population
B1971004 (US)	1	Safety and Immunogenicity	Adults 18 to 40 y.o.
B1971003 (Aus)	1/2	Safety and Immunogenicity	Adults 18 to 40 y.o.
B1971005 (Aus/EU)	2	Proof of Concept, Safety and Immunogenicity	Adolescents 11 to < 18 y.o.
B1971010 (EU)	2	Safety and Immunogenicity, concomitant with dTap/IPV vaccines	Adolescents 11 to < 19 y.o.
B1971011 (US)	2	Safety and Immunogenicity, concomitant with Gardasil® (HPV) vaccine	Adolescents 11 to < 18 y.o.
B1971012 (EU)	2	Safety and Immunogenicity, various dose schedules	Adolescents 11 to < 19 y.o.
B1971042 (US)	2	Safety and Immunogenicity in laboratory workers	Adults 18 to ≤ 65 y.o.
B1971015 (US)	2	Safety and Immunogenicity, concomitant with Menactra® (MCV4) and dTap vaccines	Adolescents 10 to < 13 y.o.
B1971014 (WW)	3	Large Scale Safety	Adolescents and Young Adults 10 to < 26 y.o.
B1971009 (WW)	3	Safety, Immunogenicity and Lot Consistency	Adolescents 10 to < 19 y.o.
B1971016 (WW)	3	Safety and Immunogenicity	Young Adults 18 to < 26 y.o.

[a]Key to Country/Region: United States (US), Australia (Aus), Europe (EU), worldwide (WW). HPV, human papilloma virus; IPV, inactivated poliomyelitis virus; dTap, tetanus, low-dose diphtheria, and low-dose acellular pertussis; MCV4, quadrivalent meningococcal polysaccharide conjugate vaccine.

the majority of diverse invasive disease isolates globally. Bivalent rLP2086 hSBA responses which represent and are being used as surrogates of efficacy are robust and have been demonstrated to kill MnB strains that are representative of endemic and epidemic disease causing isolates.

Taken together, the non-clinical and clinical studies with bivalent rLP2086 using hSBA as the critical immunological/efficacy assessment tool, demonstrated the potential for this vaccine to broadly protect against MnB disease. The potential of this vaccine to meet the unmet medical need for prevention of MnB invasive disease has been recognized by having received breakthrough therapy designation from the FDA, with the possibility of accelerated regulatory approval.[35,36] Introduction of this vaccine will make it possible to answer additional questions that were not the subject of this review including whether the vaccine has the potential to protect against other meningococcal serogroups that also express fHBP[37] and whether the vaccine can impact meningococcal carriage in the nasopharynx. By targeting adolescents and young adults for vaccination, not only does bivalent rLP2086 have the potential to prevent MnB invasive disease in this susceptible population, but it may also limit transmission of MnB in other age populations. Thus, demonstration of prevention of carriage will have the potential to further reduce the burden of disease, especially in the very young infant populations through herd protection.[37]

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