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REVIEW

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Meningococcal serogroup B vaccines: Estimating breadth of coverage

Robert G. K. Donald[a], Julio Cesar Hawkins[a], Li Hao[a], Paul Liberator[a], Thomas R. Jones[a], Shannon L. Harris[a], John L. Perez[b], Joseph J. Eiden[a], Kathrin U. Jansen[a], and Annaliesa S. Anderson[a]

[a]Pfizer Vaccine Research and Development, Pearl River, NY, USA; [b]Pfizer Vaccine Research and Development, Collegeville, PA, USA

ABSTRACT

Neisseria meningitidis serogroup B (MenB) is an important cause of invasive meningococcal disease. The development of safe and effective vaccines with activity across the diversity of MenB strains has been challenging. While capsular polysaccharide conjugate vaccines have been highly successful in the prevention of disease due to meningococcal serogroups A, C, W, and Y, this approach has not been possible for MenB owing to the poor immunogenicity of the MenB capsular polysaccharide. Vaccines based on outer membrane vesicles have been successful in the prevention of invasive MenB disease caused by the single epidemic strain from which they were derived, but they do not confer broad protection against diverse MenB strains. Thus, alternative approaches to vaccine development have been pursued to identify vaccine antigens that can provide broad protection against the epidemiologic and antigenic diversity of invasive MenB strains. Human factor H binding protein (fHBP) was found to be such an antigen, as it is expressed on nearly all invasive disease strains of MenB and can induce bactericidal responses against diverse MenB strains. A bivalent vaccine (Trumenba®, MenB-FHbp, bivalent rLP2086) composed of equal amounts of 2 fHBP variants from each of the 2 immunologically diverse subfamilies of fHBP (subfamilies A and B) was the first MenB vaccine licensed in the United States under an accelerated approval pathway for prevention of invasive MenB disease. Due to the relatively low incidence of meningococcal disease, demonstration of vaccine efficacy for the purposes of licensure of bivalent rLP2086 was based on vaccine-elicited bactericidal activity as a surrogate marker of efficacy, as measured *in vitro* by the serum bactericidal assay using human complement. Because bacterial surface proteins such as fHBP are antigenically variable, an important component for evaluation and licensure of bivalent rLP2086 included stringent criteria for assessment of breadth of coverage across antigenically diverse and epidemiologically important MenB strains. This review describes the rigorous approach used to assess broad coverage of bivalent rLP2086. Alternative nonfunctional assays proposed for assessing vaccine coverage are also discussed.

Introduction

Neisseria meningitidis, the causative agent of invasive meningococcal disease (IMD), most often presents in clinical disease cases as meningitis and/or septicemia,[1,2] with peaks in disease incidence observed in infants, adolescents, and young adults.[2] Approximately 20% of survivors experience permanent sequelae including neurologic impairment, hearing loss, or limb amputation.[3,4]

Six serogroups defined by their capsular polysaccharides (A, B, C, W, X, and Y) cause almost all meningococcal disease globally.[5] During recent years, meningococcal serogroup B (MenB) has caused a large proportion of IMD cases in the United States,[6] Europe,[7] Canada,[8] Australia,[9] and New Zealand.[10] Moreover, in the past decade, MenB outbreaks have been observed at several university campuses in the United States[11] and in various settings within Europe, including family clusters, childcare settings, and schools.[12-16] These outbreaks may be prolonged and can result in significant morbidity and mortality.[17-19] While disease caused by meningococcal serogroups A, C, W, and Y can be prevented

effectively with capsular polysaccharide conjugate vaccines,[2] such vaccines are not possible for MenB due to the structural similarity of the MenB polysaccharide capsule with polysialic acid structures on human neuronal cells and the associated low immunogenicity of the MenB polysaccharide.[20]

Outer membrane vesicle (OMV) vaccines have been successfully used to control local MenB epidemics caused by single clones.[21,22] However, in contrast to capsular polysaccharide conjugate vaccines, which provide broad, serogroup-specific protection, the coverage provided by OMV vaccines is generally limited to the targeted epidemic meningococcal strain due to the sequence heterogeneity of the dominant OMV vaccine antigen, porin A (PorA).[23-26] This limitation of OMV vaccines and the predominance of MenB in several large geographic regions emphasized the need for development of an efficacious vaccine to confer broad protection against diverse invasive disease strains of MenB.

To address broad coverage against IMD, researchers sought to identify immunogenic surface proteins present in a large

proportion of circulating MenB disease strains and also capable of stimulating protective immune responses against the diversity of MenB isolates.[27,28] In this context, factor H binding protein (fHBP) was identified as a surface lipoprotein present on nearly all disease-causing strains, inducing bactericidal responses against MenB strains expressing antigenically diverse fHBP variants.[29,30] Importantly, fHBP amino acid sequence variants segregate into 2 antigenic and immunologically diverse subfamilies, designated A and B.[30,31] Each strain of MenB expresses a single variant of fHBP.

Early research efforts identified that a vaccine containing an fHBP variant from each of the 2 subfamilies (A and B) would greatly enhance the breadth of bactericidal antibody responses against MenB strains. Thus, bivalent rLP2086 (Trumenba®, MenB-FHbp; Pfizer Inc, Collegeville, PA), composed of equal amounts of recombinant, lipidated fHBP antigens from subfamily A (variant A05) and subfamily B (variant B01), was developed. Bivalent rLP2086 was subsequently the first MenB vaccine approved under an accelerated approval program by the US Food and Drug Administration in October 2014 for the prevention of MenB disease in adolescents and young adults 10 to 25 y of age.[32,33]

Similar to development programs for meningococcal serogroup C and meningococcal serogroups ACWY polysaccharide conjugate vaccines, large prelicensure clinical efficacy studies have not been feasible for MenB vaccines due to the relatively low overall incidence of disease.[34,35] Therefore, evaluations of vaccine efficacy for licensure are based instead on demonstration of vaccine-elicited serum bactericidal antibody responses as a surrogate marker of efficacy measured by *in vitro* serum bactericidal assays using human complement (hSBAs).[36] As an hSBA titer ≥ 1:4 correlates with protection against meningococcal disease,[34,36-38] hSBA responses have been accepted as surrogate measures of vaccine efficacy for licensure across meningococcal serogroups.[37,39] The approach also was supported by the experiences and data from postimplementation surveillance studies of polysaccharide conjugate vaccines and MenB OMV vaccines that were licensed or implemented on the basis of hSBA results.[36,40,41]

This review will describe the approach used to demonstrate broad coverage of bivalent rLP2086 with hSBAs using antigenically and epidemiologically relevant MenB strains. Additional discussion is provided regarding alternative nonfunctional assays that have been proposed for assessing vaccine coverage.

Serum bactericidal assay using human complement

Use of hSBAs to assess vaccine-elicited protection from meningococcal disease

hSBAs measure the functional ability of serum antibodies to kill meningococcal test strains in a complement-dependent fashion (Fig. 1).[36,42,43] An hSBA titer is defined as the highest serum dilution that kills at least 50% of the bacteria in the assay.[36] An hSBA titer ≥ 1:4 is the accepted correlate of protection, although a titer <1:4 is not always indicative of disease susceptibility.[34,36-38]

An hSBA titer ≥ 1:4 was initially identified as the correlate of protection using homologous human complement in the

assays (ie, the complement source was derived from the same individual whose serum was tested in the assay). The assays were later adapted to use exogenous human complement and test sera (ie, the human complement source was derived from someone other than the individual whose serum was tested in the assay).[37,44,45] The use of human complement has logistic difficulties, such as obtaining sera from human donors, prescreening the sera to ensure that the complement is active and compatible with the hSBA, and ensuring that the complement source does not result in nonspecific killing of the bacteria in the assay. Therefore, SBAs have also been performed with baby rabbit complement (rSBAs), as this reagent is readily available in high quantities and shows low nonspecific bactericidal activity.[34,42,46,47] However, only human complement is appropriate for testing bactericidal activity of human sera against MenB,[34] as the use of rabbit complement results in deceptively higher bactericidal titers to MenB strains than the use of human complement,[47,48] and rSBA responses do not correlate well with disease protection over time.[46]

Bactericidal activity as assessed in hSBAs with a single test strain can correlate well with protection against all disease strains of a given serogroup when the vaccine antigen is the serogroup-specific capsular polysaccharide, which is identical in, and expressed by, all strains within the targeted serogroup (eg, meningococcal serogroup C).[34,42] In these cases, vaccine efficacy can be inferred to apply to the whole serogroup, and thus, breadth of coverage can be demonstrated by hSBAs using a single representative serogroup-specific strain. However, when the vaccine antigen is not serogroup specific, as with many of the sequence-variable proteins expressed on the bacterial cell surface, assessment of breadth of coverage against disease strains is more complex and requires a different approach. Moreover, these proteins may not be expressed by all disease strains, and if they are expressed, expression levels can vary among different strains.[49] All of these factors are known to contribute to a lack of bactericidal activity in SBAs to a specific test strain.[49] The choice of test strains to determine both vaccine-elicited protection and breadth of coverage is therefore critical for protein antigen vaccines and is discussed in more detail below.

Assessing breadth of coverage of bivalent rLP2086

Fundamental to the use of hSBA responses as surrogates for vaccine efficacy is the selection of appropriate test strains and immunologic response endpoints. At the time of bivalent rLP2086 development, no standardized hSBAs were available to evaluate the breadth of protection of MenB vaccines based on surface protein antigens exhibiting sequence and expression variability.

For the purpose of evaluating the breadth of coverage of bivalent rLP2086, it was first necessary to conduct global surveillance and antigenic characterization of disease-causing strains to identify hSBA test strains appropriate for evaluation of protein-based vaccines for the prevention of MenB disease.

Approximately 1800 invasive MenB disease strains from reference laboratories in Europe and the United States were collected, systematically assembled, and characterized.[27] All isolates were found to contain the gene encoding fHBP. Based
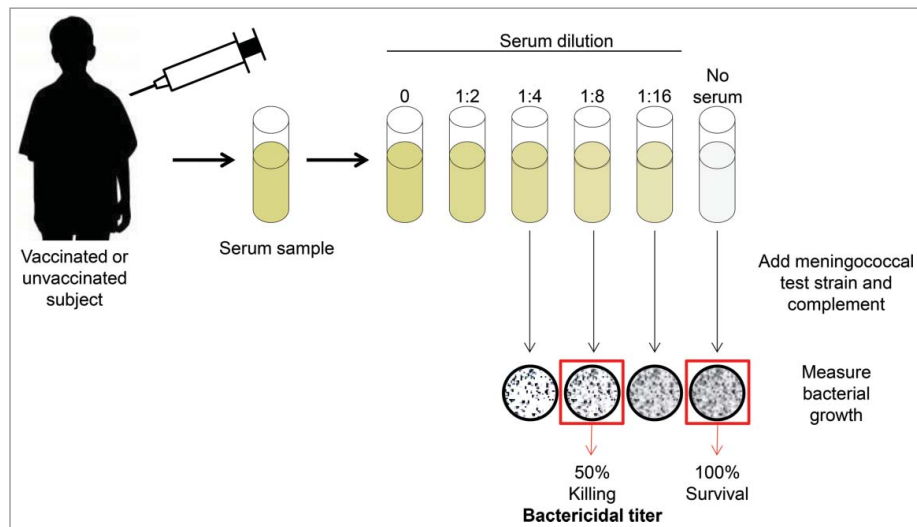
Figure 1. Serum bactericidal assay with human complement. Figure adapted from Ghandi, et al. *Postgraduate Medicine*, 2016.[82]

on amino acid sequences, fHBP variants segregated into 2 immunologically distinct subfamilies (designated A and B). The amino acid sequence identity of variants within each subfamily was found to be $\geq$ 83%, while variant sequence identity between subfamilies was just 60–75%.[27,29] Collectively, the 10 most prevalent fHBP variants in the strain pool were found to be expressed by nearly 80% of all strains.[27] Notably, the breadth of fHBP bactericidal activity is predominantly subfamily specific, with limited immunologic protection across subfamilies.[29,31]

Extensive exploratory hSBAs were then conducted using clinical isolates that represented the breadth of both the antigenic and epidemiologic diversity of the MnB serogroup. Results from these analyses revealed that bivalent rLP2086 induced bactericidal antibodies that killed MnB isolates, independent of the sequence diversity of the fHBP expressed by the bacteria strain (Table 1).[31,50] Pivotal licensure studies required the breadth of coverage to be assessed in a hypothesis-driven manner and necessitated an approach to select strains without bias. Therefore, a random approach was used to select the primary MenB test strains for use in hSBAs, while taking into account specific selection criteria to ensure that test strains appropriately represented the antigenic diversity of MenB isolates.[27] Selection criteria included: (i) expression of fHBP variants that differed from the vaccine antigens; (ii) expression of fHBP variants prevalent in contemporary IMD strains from the United States and Europe; (iii) representative *in vitro* surface expression levels of the fHBP vaccine antigens across all strains; and (iv) demonstration of low baseline bactericidal hSBA titers with selected strains. The last of these criteria is of particular note, as those most at risk for meningococcal disease have virtually nonexistent bactericidal activity against the majority of strains.

The bivalent rLP2086 clinical program rigorously validated hSBAs using 4 primary MenB test strains identified by the approach described above. Four test strains, each expressing fHBP variants different from (ie, heterologous to) the vaccine antigens were considered sufficient to provide indication of both efficacy and breadth of vaccine coverage. These 4 test strains [(PMB80 (A22/88.9%; fHBP variant/sequence identity to vaccine antigen of same subfamily), PMB2001 (A56/98.1%), PMB2948 (B24/86.2%) and PMB2707 (B44/91.6%)] were used in hSBAs in phase 2 and 3 studies designed to support licensure of bivalent rLP2086 in the United States and Europe (Table 1).[27,51-57] The relevance of these 4 test strains to be representative of all fHBP-expressing MenB strains was established

Table 1. MenB test strains used for the assessment of bivalent rLP2086.

Strain	fHBP Variant	fHBP Subgroup	Clonal Complex	Country of Origin
Primary strains				
PMB2001	A56	N1C2	CC213	France[83]
PMB2707	B44	N4/N5	CC269	United Kingdom
PMB80	A22	N2C2	CC41/44	United States
PMB2948	B24	N6	CC32	France[83]
Additional strains				
PMB3010	A06	N1C2	CC461	United Kingdom
PMB3040	A07	N2C1	CC162	Germany
PMB824	A12	N2C1	CC35	United States
PMB1672	A15	N2C1	CC103	France[83]
PMB1989	A19	N2C2	CC8	United Kingdom
PMB3175	A29	N1C1	CC32	United States
PMB1256	B03	N6	CC41/44	United Kingdom
PMB866	B09	N6	CC269	United Kingdom
PMB431	B15	N6	CC41/44	United States
PMB648	B16	N6	CC41/44	United Kingdom
Strains assessed in exploratory study[50]				
PMB3570	A22	N2C2	CC41/44	United Kingdom
PMB428	A22	N2C2	CC269	United Kingdom
PMB80	A22	N2C2	CC41/44	United States
PMB2001	A56	N1C2	CC213	France[83]
PMB17	B02	N4/N5	CC32	United States
PMB1256	B03	N6	CC41/44	United Kingdom
PMB1489	B09	N6	No complex	United Kingdom
PMB265	B09	N6	No complex	The Netherlands
PMB2932	B09	N6	CC41/44	United States
PMB1590	B16	N6	CC41/44	France[83]
PMB2882	B16	N6	CC41/44	United Kingdom
PMB2948	B24	N6	CC32	France[83]
PMB147	B44	N4/N5	CC269	United Kingdom
PMB2707	B44	N4/N5	CC269	United Kingdom
PMB5021*	B153	N6	CC41/44	US 2013 (University A)
PMB4479*	B24	N6	CC32	US 2013 (University B)

fHBP = factor H binding protein; MenB = meningococcal serogroup B.
*MenB test strain from US university outbreaks.

using a pairwise identity approach. The fHBP family can be divided into 6 subgroups; the relationship of each of the 4 test strains was assessed relative to the fHBP variants within its matched subfamily (ie, A and B) and its closest related subgroup (Fig. 2). As a result, the pairwise identity observed for a test strain and the fHBP sequences from the same subgroup that it represents ranges from 94.37% to 98.64%, indicating high sequence conservation between the test strains and other variants. The total minimun pairwise identity of variants to the vaccine antigens is 88.5% for A05 and 84.8% for B01, thus providing an opportunity to assess the breadth of coverage of the vaccine without the requirement of developing assays for every strain during clinical testing.

Although the serologic correlate for protection against IMD is an hSBA titer of ≥ 1:4, the lower limit of quantification (LLOQ) for hSBA using these 4 primary MenB test strains was determined to be a titer equal to 1:8 for strains expressing fHBP variants A56, B24, and B44, and a titer equal to 1:16 for the strain expressing fHBP variant A22. Because these LLOQ titers (rather than titers of ≥ 1:4) were employed for calculation of hSBA results during phase 2 and phase 3 clinical studies, the reported study results provide additional assurances for estimates of protection afforded by immunization with bivalent rLP2086.

The surrogate measure for licensure of bivalent rLP2086 required demonstration of bactericidal immune response by a high proportion of vaccine recipients using 5 different endpoints.[33] Study success was achieved only if predefined criteria were met for all 5 endpoints. Four of the 5 endpoints were based on vaccine-elicited 4-fold increases from baseline in hSBA responses to each primary test strain, thus providing evidence of vaccine benefit for recipients with low baseline titers

as well as those with higher hSBA titers prior to vaccination. The fifth endpoint measure was a composite response, demonstrating the proportions of vaccine recipients exhibiting hSBA titers ≥ LLOQ against not only 1, but all 4 primary test strains combined. These endpoints were included as prespecified exploratory endpoints in 3 of the phase 2 studies used for licensure[55,56,58] and as the primary immunogenicity endpoints in phase 3 studies that became post-marketing commitments under the accelerated approval process.[59,60] Successful achievement of the 5 endpoints described above supported accelerated licensure of bivalent rLP2086 in the United States and provided strong evidence of breadth of coverage across the diversity of invasive MnB strains.

Performance of hSBA with 10 additional test strains provided further evidence of breadth of vaccine coverage against disease strains circulating in the United States and Europe (Table 1). These results confirmed that the observed immune responses to the 4 primary strains were indicative of broad protection against MenB disease-causing strains that express diverse fHBP sequence variants.[27,61,62] As with the 4 primary strains, these 10 additional hSBA test strains all expressed fHBP variant sequences different from (ie, heterologous to) the vaccine antigens and were selected in a similar manner as the 4 primary test strains.[63] Strains with low to medium expression levels were chosen to mimic the normal distribution of fHBP expression among isolates.[27] Taken together, these 14 MnB test strains (4 primary and 10 additional) used in the clinical development of the bivalent rLP2086 vaccine represent both immunologic subfamilies of fHBP and address the potential diversity of fHBP in invasive disease isolates. Notably, the hSBA responses elicited by bivalent rLP2086 to these test strains all were assessed using sera from individual vaccine recipients
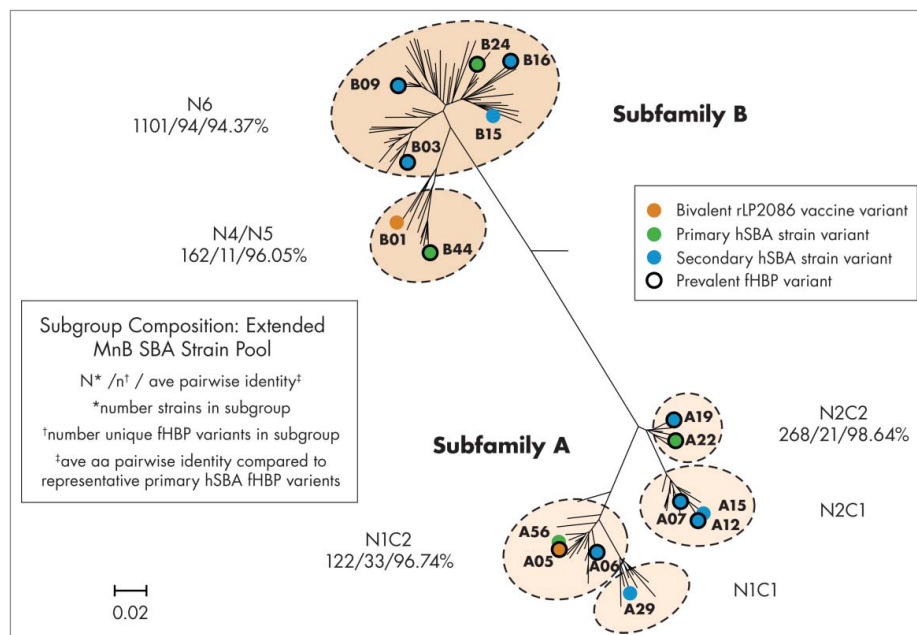

Figure 2. Phylogenetic relationship of different fHBP subgroups in the extended MenB SBA strain pool. Representation of the phylogeny of fHBP is based on a clustalW alignment and drawn with MEGA 4.2. The relative phylogenetic position of subfamily A and B fHBP variants, as well as the 6 major subgroups of fHBP, are outlined. The phylogenetic position of fHBP variants expressed by the 4 primary and 10 secondary SBA test strains, as well as 2 vaccine variants, is highlighted. The numbers beneath each of the fHBP subgroups represent the number of isolates, number of unique fHBP variants, and average amino acid percent identity from the respective subgroups. The scale bar represents phylogenetic distance based on the deduced fHBP protein sequence. Phylogenetic relationship of MenB fHBP variants first described by Murphy, et al. *J Infect Dis.* 2009.[30]

rather than pooled serum from multiple subjects, thus permitting an estimate of the breadth of vaccine coverage at the population level by reporting on the proportion of vaccinated subjects with functional bactericidal antibodies.

Further evidence of the breadth of coverage for bivalent rLP2086 is available by performance of hSBA using MnB isolates collected during recent disease outbreaks. Several documented clusters or outbreaks of MenB disease have occurred during the last several years at US university campuses.[64] Additional analyses, including previously unpublished data, examined MenB strains from 5 of these clusters or outbreaks in exploratory hSBAs using sera from individuals who participated in an earlier clinical study and who received bivalent rLP2086 according to a 3-dose regimen of 0, 2, and 6 months (Table 2). Three of these outbreaks were caused by strains expressing the prevalent B24 fHBP variant (Universities B, C, and D). Two of the strains expressing B24 fHBP belonged to the common clonal complex that is associated with a prolonged outbreak in Oregon, and the third represented a less prevalent clonal complex (unpublished data and[65]). The other 2 isolates were strains expressing rare fHBP variants B153 (University A) and B133 (University E), both of which were identified for the first time among MenB isolates in the United States. A strain expressing the B153 variant has recently been observed in a collection of invasive MenB isolates from England and Wales, whereas the B133 variant has been noted in isolates from Germany, England, Wales, and the Netherlands (unpublished data and[66,67]).

The proportion of bivalent rLP2086 vaccine recipients with hSBA titers of $\geq 1:4$ to the B24 outbreak strains ranged from 20–78% after the second vaccination and from 40–100% after the third vaccination (Universities B, C, and D; Table 2). For the B153 variant (University A), the proportions of hSBA responders were 67% and 78% after the second and third vaccine doses, respectively, and for the B133 variant (University E), corresponding values were 80% and 80%, respectively (Table 2). The B133 variant had a higher proportion of subjects with prevaccination hSBA titers $\geq 1:4$ (27%) than had been observed for the other outbreak strains, while hSBA titers of $>1:4$ were observed for 80% of vaccinees after both doses 2 and 3 (Table 2; Fig. 3). As observed with outbreak strains from Universities B, C, and D, which all express the B24 fHBP variant, differences in hSBA responses still occur. These observations further highlight the need for an unbiased selection of MenB test strains for hSBAs as well as the need for more extensive hSBA evaluations to accurately discern population response rates to MenB vaccination.

As the B133 variant was newly identified in the United States, additional hSBA testing was conducted on other B133-expressing isolates (Fig. 3). A second isolate [PMB5543 (B133)] from the outbreak at University E yielded post-dose 2 and 3 responder rates of 93%, although prevaccination titers were observed to be higher (60%). Baseline hSBA titers determined using a third strain expressing fHBP variant B133 [PMB5507 (B133)], which was obtained from the Netherlands during routine surveillance in Europe, were low (7%) but increased to 93% and 100% following 2 and 3 vaccine doses, respectively. Together, these hSBA results using recent outbreak strains provide assurance of the breadth of bivalent rLP2086 coverage predicted by the hSBA responses obtained with the 4 primary strains used in phase 2 and 3 clinical studies.[51-57]

Alternative nonfunctional assays to infer vaccine breadth of coverage

Although the benefits of hSBA titers as an immunologic correlate of vaccine efficacy are well established, some limitations do exist. For instance, hSBAs are labor intensive and logistically challenging when a large number of strains have to be tested due to the need for considerable amounts of sera and sources of assay compatible complement.[68] Differences among laboratories in performing hSBAs can also hinder the ability to compare responses and assessments of breadth of coverage between meningococcal vaccines.[69] Therefore, alternative assays have been proposed to predict vaccine

Table 2. Bactericidal activity of bivalent rLP2086 immune sera against university outbreak strains in the United States.[*]

Strain	fHBP Variant	Clonal Complex	Timing of Serum Sampling	Subjects With hSBA $\geq$ 1:4, %[£]
PMB5021[†] [50] University A	B153	CC41/44	Prevaccination	0
			1 month after dose 2	67
			1 month after dose 3	78
PMB4479[†] University B	B24	CC32/ET5	Prevaccination	0
			1 month after dose 2	78
			1 month after dose 3	100
PMB5519[‡] University C	B24	CC32/ET5	Prevaccination	20
			1 month after dose 2	53
			1 month after dose 3	60
PMB5522[‡] University D	B24	TBA[§]	Prevaccination	7
			1 month after dose 2	20
			1 month after dose 3	40
PMB5544[‡] University E	B133	CC32/ET5	Prevaccination	27
			1 month after dose 2	80
			1 month after dose 3	80

fHBP = factor H binding protein; hSBA = serum bactericidal assay using human complement.

[*]Exploratory hSBAs using prevaccination (baseline titers) and serum samples 1 month after dose 2 and dose 3 of bivalent rLP2086 from randomly selected adolescent subjects aged 11–<19 y of age. Bivalent rLP2086 was administered at months 0, 2, and 6.

[†]Serum samples from 9 subjects tested in hSBAs using outbreak strains from 2013.

[‡]Serum samples from 15 subjects tested in hSBAs using outbreak strains from 2015/2016.

[§]To be assigned.

[£]Responder rates are based on 2-fold step titers and so differ from rates based on GMTs >1:8 reported for PMB4479 and PMB5021 in 'Harris et al. *Pediatr Infect Dis J.* 2016'.[50]
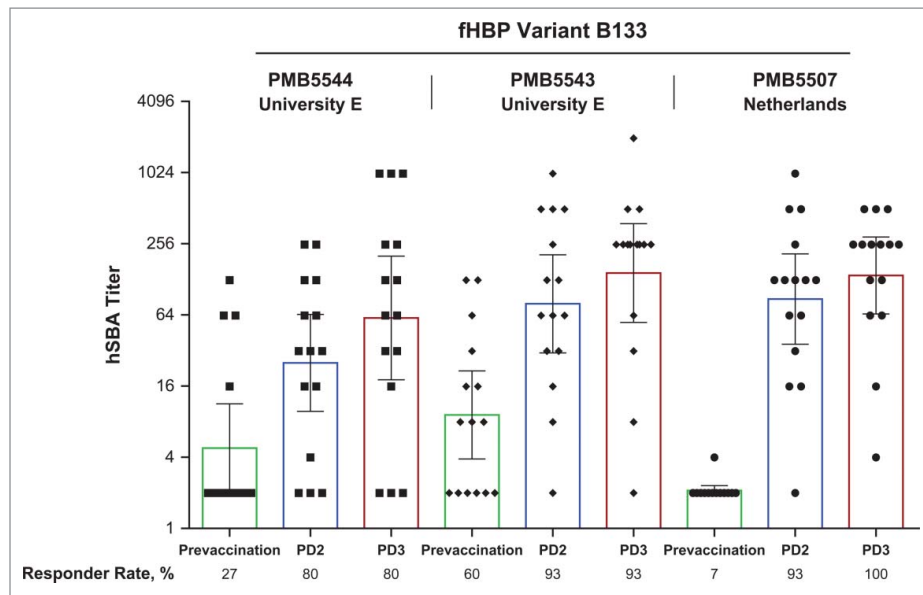
Figure 3. Activity of rLP2086 immune sera against fHBP B133 isolates in exploratory hSBAs. Isolates tested were from the University E outbreak (PMB5543 and PMB5544) and from the Netherlands (PMB5507). Sera are from 15 subjects vaccinated with bivalent rLP2086. Sera were collected before vaccination, after the first vaccination (PD1), after the second vaccination (PD2), and after the third vaccination (PD3). Responder rates based on individual titers ≥1:4 are indicated. Open rectangles represent geometric mean titers with 95% CIs (error bars). fHBP = factor H binding protein; hSBA = serum bactericidal assay using human complement; PD = postdose.

coverage (not efficacy) that are technically easier to perform than SBAs; these approaches are summarized in the following section.

Meningococcal antigen typing system

4CMenB (Bexsero®, MenB-4C; Novartis Inc, Cambridge, MA) is the only other MenB vaccine licensed in the United States for use in adolescents and young adults 10 to 25 y of age;[32] 4CMenB is also approved in other regions.[70] 4CMenB is comprised of 4 main components: a subfamily B fHBP (variant B24), 2 meningococcal proteins (Neisserial adhesin A [NadA]; Neisserial heparin binding antigen [NHBA]) expressed as recombinant fusion proteins, and OMVs (PorA serosubtype P1.4).[32] fHBP appears

to be the major target of vaccine-induced serum bactericidal activity for both 4CMenB and bivalent rLP2086.[71]

Clinical studies of 4CMenB have predominantly used the Meningococcal Antigen Typing System (MATS) assay to assess vaccine coverage (Fig. 4). This method consists of 3 antigen-specific sandwich enzyme-linked immunosorbent assays (ELISAs) that measure the reactivity of lysed bacterial extracts prepared from the test strain of interest with rabbit polyclonal antibodies against fHBP, NHBA, or NadA; PorA is evaluated using genotyping.[72,73] Reference strains (ie, H44/76, NGH38, 5/99) against which the activity of test strains are compared express the same fHBP, NHBA, and NadA antigens present in 4CMenB.[71,72] The relative ELISA potency for each antigen is then compared with a corresponding bactericidal
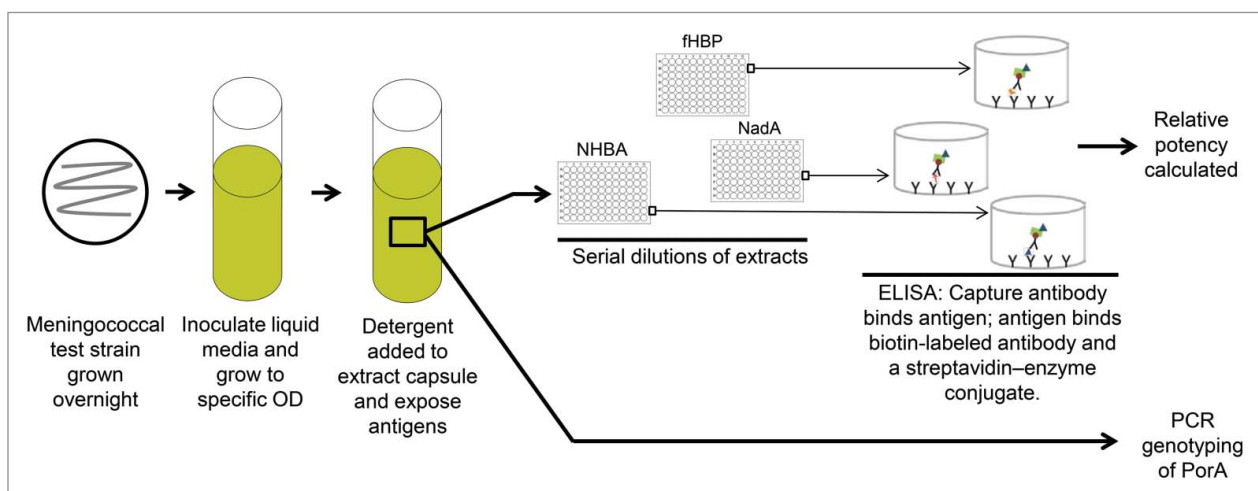

Figure 4. MATS assay. Bacteria are grown overnight. A bacterial suspension is grown to a specific OD and detergent is added to the suspension to extract the capsule and expose the antigens. Serial dilutions of the extract are tested by ELISA. fHBP, NHBA, and NadA coverage is assessed by defining a relative potency of the tested strain versus a reference strain for each antigen and then extrapolating a relative potency to a positive bactericidal threshold. PCR genotyping is used to identify PorA. ELISA = enzyme-linked immunosorbent assay; fHBP = factor H binding protein; MATS = Meningococcal Antigen Typing System; NadA = Neisserial adhesin A; NHBA = Neisserial heparin binding antigen; OD = optical density; PCR = polymerase chain reaction; PorA = porin A.

threshold that is the minimum value predictive of killing in the hSBA. The positive bactericidal thresholds for each antigen are determined separately in hSBAs with vaccine immune sera.

The main advantage of MATS is that it is comparably faster and less resource intensive to perform than hSBAs.[68] However, because the MATS system was designed specifically to measure coverage pertaining to 4CMenB antigens, ELISAs used to assess coverage of NHBA or NadA antigens and PorA genotyping cannot be used to measure strain coverage of other MenB vaccines with different antigen compositions.[72] In addition, MATS results are assessed against hSBAs performed using pooled serum,[73] which is less informative than using individual samples as population responses to vaccination cannot be inferred. Further limitations of MATS include uncertainties with linking MATS results to specific age groups or administration schedules, as well as the recognized challenges for estimation of potential vaccine benefit in the absence of direct testing of individual serum samples by hSBA; these challenges were highlighted in a recent study by Basta and colleagues that used hSBAs to examine 4CMenB's response to the University A outbreak strain.[74]

MEASURE flow cytometry assay

The Meningococcal Antigen Surface Expression (MEASURE) assay has been used to assess whether surface expression levels of vaccine antigen on MenB strains predict whether a MenB strain can be killed in hSBA by bivalent rLP2086 immune sera (Fig. 5). MEASURE is a flow cytometry method that uses a monoclonal antibody that is specific to an epitope common to fHBP variants from both subfamilies, thereby allowing a phenotypic assessment of fHBP expression and selective quantification of surface-expressed fHBP concentrations on intact bacteria that are prepared using standard hSBA procedures.[27,75,76] Because only the cell surface exposed portion of the antigen is accessible to serum bactericidal antibodies, MEASURE selectively quantifies the concentration of fHBP expressed on the surface.[27] Qualification of the MEASURE assay found that the distribution of fHBP expression levels is independent of subfamily or subgroup, but differences in expression levels between fHBP variant groups and MenB strains were found.[27] The MEASURE assay was found to be specific, robust, precise, and transferable to other laboratories.[76] Unlike some other meningococcal epidemiologic markers, fHBP surface expression levels determined by the flow cytometric-based

MEASURE assay were predictive of strain susceptibility in the hSBA.[31] In addition, the applicability of MEASURE to predict bactericidal activity and breadth of coverage of bivalent rLP2086 to globally collected disease strains has been suggested.[76] MEASURE shares some of the shortcomings of the MATS assay because it does not provide information on the proportion of the population that responds to vaccination or that is protected against a given strain. In addition, vaccine-elicited benefit in a population above baseline coverage cannot be inferred.

Selected reaction monitoring mass spectrometry

Selected reaction monitoring mass spectrometry (SRM-MS) is a technique for the specific and accurate quantification of a selected protein in a complex biological mixture. SRM-MS has been used for the absolute quantification of total cellular fHBP protein in a large panel of MenB disease strains representative of the genetic diversity of *N meningitidis* isolates.[77] This method attempts to correlate fHBP detected by SRM-MS with SBA activity. In principal, SRM-MS is analogous to the flow cytometry-based MEASURE assay but is less direct, because it detects levels of total antigen in a bacterial lysate rather than levels exposed on the bacterial surface. Moreover, attempts to correlate SBA activity with this approach are based on bactericidal assays with rabbit complement and mouse sera, rather than SBAs with human sera, thereby making it irrelevant for human vaccine studies.

IgG ELISA

Immunoglobulin G (IgG) ELISAs have been employed for assessment of population-based immunity, but the results are not generally accepted as a correlate of individual protection and are not used as surrogates of efficacy for licensure.[47] This approach most recently has been assessed with respect to meningococcal A vaccines.[78] An IgG meningococcal serogroup A polysaccharide antibody concentration of > 2 μg/mL was considered indicative of protection against MenA disease based on the results of a Finnish clinical study of a meningococcal polysaccharide vaccine conducted nearly 40 y ago, but differences were noted among some populations in the African meningitis belt, where antibody concentrations were generally higher.[69] It has been suggested that much of the antibody detected by this ELISA technique is nonfunctional, potentially because it is induced by cross-reacting bacteria or due to low avidity of the antigen-antibody complex. Limitations
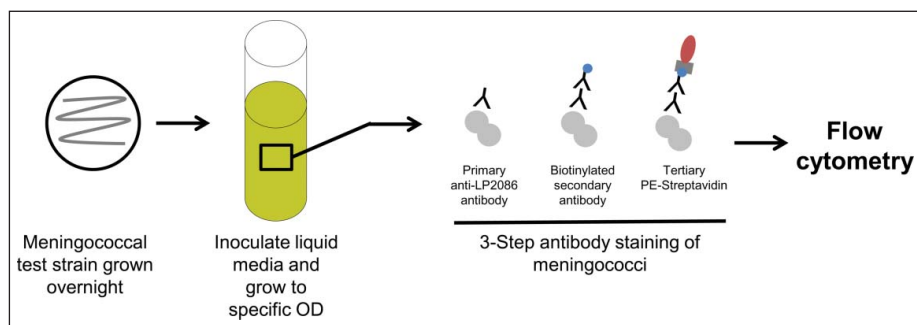

Figure 5. MEASURE assay. Bacteria are grown overnight using standard hSBA procedures. Bacteria are then stained using a 3-step antibody staining method. Flow cytometry is used to selectively quantify the concentration of surface-expressed fHBP. fHBP = factor H binding protein; hSBA = serum bactericidal assay using human complement; MEASURE = Meningococcal Antigen Surface Expression; OD = optical density.

regarding the use of ELISA for meningococcal-specific IgG rather than SBA for serogroup C disease have also been discussed in other published studies.[79,80]

Conclusions

Bivalent rLP2086, composed of recombinant, lipidated fHBP protein variants representing each fHBP subfamily, was designed to provide broad protection against antigenically and epidemiologically diverse MenB strains that cause invasive disease. fHBP is a conserved meningococcal virulence factor that is expressed in nearly all invasive MenB strains. In completed clinical studies, bivalent rLP2086 has demonstrated an acceptable safety profile and has elicited hSBA titers above the accepted correlate of protection in a high percentage of individuals when given alone or with concomitant vaccines.[51-57]

Disease caused by MenB is generally rare, making assessment of MenB vaccine efficacy with clinical disease endpoints challenging and requiring measurement of hSBA responses as surrogate markers of efficacy.[27,34,35] The individual immune responses elicited by vaccination with bivalent rLP2086, and measured in hSBAs performed with a panel of MenB strains representative of antigenically and epidemiologically diverse invasive disease isolates, support the notion of broad protection afforded by bivalent rLP2086 against MenB disease.[50] The totality of hSBA data from completed, randomized, controlled studies met rigorously and prospectively established primary endpoints and success criteria, including a composite hSBA response to primary test strains.[51-54,56,57,59,60,81] Supported by additional hSBA analyses with 10 additional epidemiologically diverse MenB disease strains,[61,62] these vaccine-elicited responses provide assurance of the broad protection afforded by bivalent rLP2086 against IMD.

hSBA titers of ≥ 1:4 are recognized as the serological correlate of protection against meningococcal disease and the assay results are accepted as surrogate measures of efficacy for vaccine license. The assay also remains the most comprehensive, informative, and biologically relevant means to assess breadth of vaccine coverage. While the logistical challenges to perform quality hSBAs in a consistent manner have led to the development of alternative methods to assess immunogenicity and breadth of coverage, these methods do not measure vaccine-elicited functional antibodies, are not used as surrogates for vaccine licensure, and to date have limited utility in assessing breadth of coverage against diverse disease strains. These limitations of alternative assays highlight the importance for pre-censure studies using hSBAs with rigorously selected, unbiased test strains that express antigen sequences different from the vaccine antigens.

Abbreviations

4CMenB	Bexsero®, MenB-4C
Bivalent rLP2086	Trumenba®, MenB-FHbp
ELISA	enzyme-linked immunosorbent assay
fHBP	factor H binding protein
hSBA	serum bactericidal assay using human complement
IMD	invasive meningococcal disease
IgG	immunoglobulin G
LLOQ	lower limit of quantification
LP2086	lipoprotein 2086
LOD	limit of detection
MATS	Meningococcal Antigen Typing System
MEASURE	Meningococcal Antigen Surface Expression assay
MenB	meningococcal serogroup B
NadA	Neisserial adhesin A
NHBA	Neisserial heparin binding antigen
OD	optical density
OMV	outer membrane vesicle
PCR	polymerase chain reaction
PD	postdose
PorA	porin A
rSBA	serum bactericidal assay using rabbit complement
SBA	serum bactericidal assay
SRM-MS	selected reaction monitoring mass spectrometry

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