NEISSERIA MENINGITIDIS SEROGROUP B VACCINE

Vaccine and Related Biological Products Advisory Committee
Pre-Meeting Background Document

04 March 2011

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LIST OF ABBREVIATIONS AND TERMS WITH DEFINITIONS

Abbreviation	Term
CI	confidence interval
EU	European Union
EU-IBIS	European Union Invasive Bacterial Infectious Surveillence Network
GMT	geometric mean titer
hSBA	serum bactericidal assay using human complement
IgG	immunoglobulin G
IMD	invasive meningococcal disease
IM	intramuscularly
LPS	lipopolysaccharide
LOS	lipo-oligosaccharide
mAb	monoclonal antibody
MFI	mean fluorescent intensity
MLST	multi locus sequence typing
MnB	*Neisseria meningiditis* serogroup B
MnC	*Neisseria meningiditis* serogroup C
PorA	porin A
rLP2086	recombinant lipoprotein 2086
rPorA	recombinant PorA
SAE	serious adverse event
SBA	serum bactericidal assay
USP	United States Pharmacopoeia
WCE	whole cell ELISA
WCB	working cell banks

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1.0 INTRODUCTION AND SUMMARY

Pfizer is developing a vaccine indicated for active immunization to prevent invasive meningococcal disease (IMD) caused by *Neisseria meningiditis* serogroup B (MnB) in adolescents and young adults 11-25 years of age. IMD is characterized by a high fatality rate and by permanent serious medical sequelae in survivors[1,2]. In the Unites States, the proportion of IMD caused by infection with *N. meningitidis* serogroup B represents approximately 30% of the IMD caused by all relevant meningococcal serogroups[1]. Accordingly, the medical need for a vaccine to prevent invasive MnB disease is clear. Given the epidemiological importance of IMD in adolescents, the Pfizer MnB vaccine program is focused on the development of a vaccine that will add protection against serogroup B disease to that already provided by the routine immunization of adolescents with licensed polysaccharide conjugate vaccines for *N. meningiditis* serogroups A, C, Y and W-135.

Unlike for the other serogroups, a capsular polysaccharide vaccine for MnB cannot be developed given that the MnB polysaccharide capsule is structurally identical to a human polysaccharide commonly expressed on human neuronal cells[3]. Pfizer research identified a MnB outer membrane protein, designated as LP2086 [4,5,6], which proved to be a bacterial virulence factor and a target for functional bactericidal antibodies. LP2086 was subsequently determined to be a bacterial factor H- binding protein (fHBP) that the bacterium uses to evade complement-mediated bacteriolysis[7,8]. The MnB fHBP/LP2086 sequence comprises a family of related sequence variants that is divided into two distinct subfamilies, A and B [4,5,6]; the sequence variants within each subfamily are highly conserved and are immunologically cross-reactive. Accordingly, the Pfizer MnB vaccine candidate is bivalent, composed of one representative variant from each of the subfamilies.

The fHBP gene is present in 100% of MnB strains in Pfizer's collection of more than 1200 MnB strains isolated from patients with invasive disease in the United States and Europe[9]. The two fHBP/LP2086 vaccine components are expressed as lipoproteins in *Escherichia coli,* and are

highly purified. The vaccine has been shown to be highly immunogenic in humans and to elicit broadly reactive anti-MnB bactericidal antibodies in phase 2 clinical trials.

While an important health concern, the incidence of the invasive MnB disease (~ 1 per 100,000 population) does not permit use of a clinical disease endpoint for hypothesis testing in phase 3 clinical trials. Nonetheless, while direct efficacy trials are not practical, the *in vitro* assay that measures serum bactericidal activity (SBA) is an established correlate of protection against IMD and well-accepted as a surrogate endpoint of vaccine efficacy. Pfizer proposes to use the SBA performed with human complement (hSBA) to establish the efficacy of the bivalent rLP2086 vaccine in support of the vaccine's licensure.

In order to demonstrate the breadth of serum bactericidal activity elicited by the bivalent rLP2086 vaccine, Pfizer believes that MnB SBA test strains used in the hSBA assays should be selected used a statistical approach from a representative pool of invasive MnB disease strains to avoid a potntial bias in the estimation of vaccine efficacy. Importantly, the selected SBA test strains should express fHBP/LP2086 protein sequences heterologous to the vaccine antigen sequences, thereby reflecting the fHBP/LP2086 sequence diversity and assuring that the SBA test strains will be representative of the MnB serogroup. In addition, the selected MnB SBA strains should be representative with regard to fHBP/LP2086 *in vitro* expression levels.

Accordingly, Pfizer used an unbiased, statistically-based approach to identify representative MnB SBA test strains from an extensive strain collection assembled with input from US and European public health laboratories to assess the vaccine's performance. The underlying conceptual logic for the use of four primary SBA test strains to estimate the breadth of coverage against the range of MnB strains is discussed in detail within this document. The hSBA data obtained with the four primary SBA test strains will be supplemented with data using appropriately selected additional exploratory MnB SBA test strains to further support vaccine coverage conclusions.

The ability of the bivalent rLP2086 vaccine to elicit broadly reactive anti-MnB hSBA activity, against MnB strains expressing fHBP/LP2086 proteins from both subfamilies, was demonstrated in a high proportion of adolescents in a phase 2 clinical trial conducted in Australia, Spain and Poland. The consistent nature of these observations across the SBA test strains supports the perspective that the four primary SBA strains are appropriately representative of the fHBP/LP2086 sequence diversity and are, therefore, suited for assessment of the overall efficacy of the bivalent rLP2086 vaccine candidate.

2.0 THE MEDICAL NEED FOR A MNB VACCINE

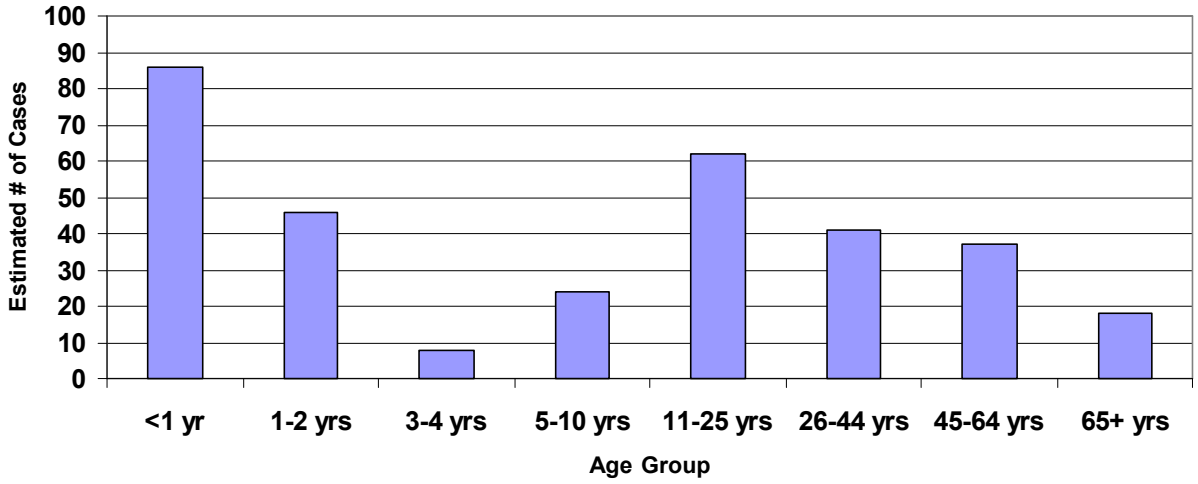
Pfizer is developing a vaccine indicated for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningiditis* serogroup B (MnB) in adolescents and young adults 11-25 years of age.

Neisseria meningitidis is a leading cause of bacterial sepsis and meningitis in infants, adolescents, and young adults. IMD is primarily due to serogroups A, B, C, W-135 and Y.

MnB invasive disease accounts for approximately one-third of IMD in North America and approximately two-thirds of IMD in Europe. The disease burden in US adolesecents is significant. In 2009, the incidence of meningococcal serogroup B disease in the US was 0.05 and 0.14 per 100,000 population for 11- to 17-year-olds and 18- to 24-year-olds, respectively (unpublished data, courtesy of Dr. Amanda Cohn, CDC). The incidence of IMD varies with age, with peak incidences in young infants and in adolescents/ young adults. The disease in adolescents is believed to be due to a number of factors, including social behavior and crowding of susceptible individuals in classrooms, dormitories, and military institutions.

While the incidence of MnB disease is somewhat greater among infants, the total burden of disease is similar for the group under one year of age and for the adolescent and young adult age group (Figure 2-1).

Figure 2-1: Age Stratified MnB Disease Burden in the US from 1999 – 2008 [10]



In the Unites States, the case fatality rate in <1 year olds and 11-19 year olds is similar (8.3% and 8.7% respectively). Slightly higher rates are seen in those 20 years of age and older (12.6%) [10].

Importantly, the rates of meningococcal nasopharyngeal carriage are highest in adolescents and young adults[11], and this age group may be an important source of transmission to younger age groups. Adolescents with meningococcal disease have a high risk of mortality, and among survivors, morbidity is substantial, including neurological disability, limb loss, and hearing loss, even when appropriate antibiotic therapy is initiated promptly.

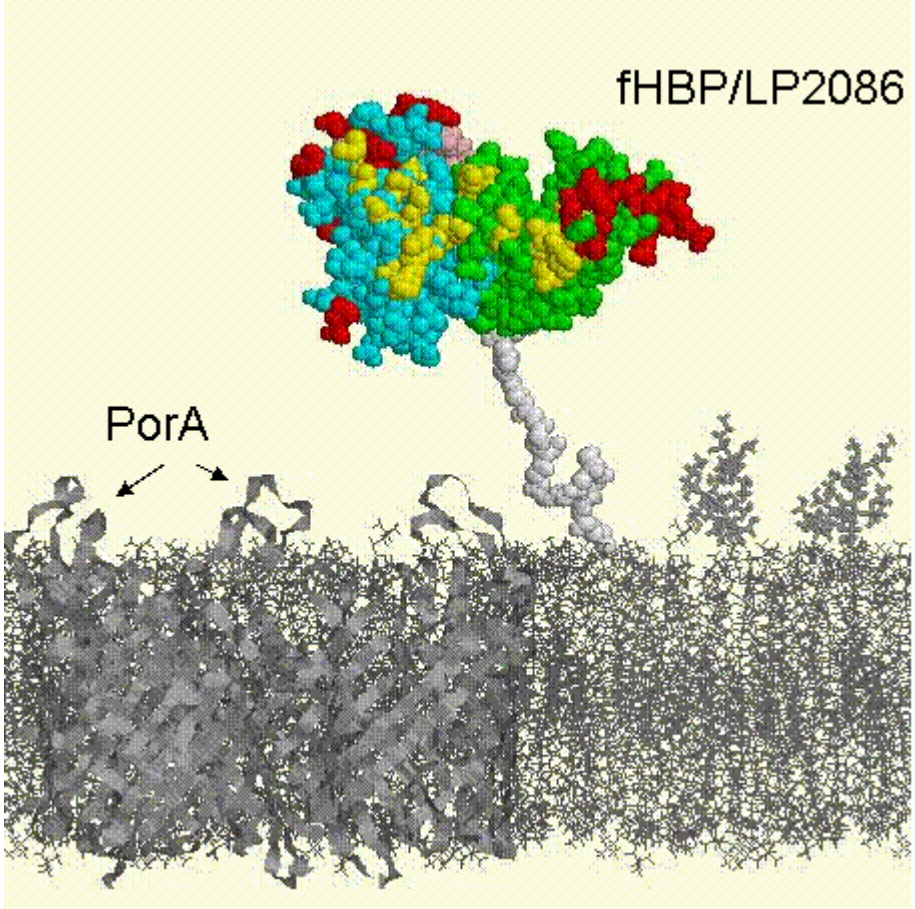
Efficacious capsular polysaccharide-protein conjugate vaccines for prevention of *N. meningitidis* serogroups A, C, Y and W-135 (MnACYW vaccine) have been licensed in the United States, and immunization of all adolescents is currently recommended with one of the two available vaccines. In contrast to these meningococcal serogroups, a capsular polysaccharide vaccine for MnB is not feasible, and prevention of invasive MnB disease therefore remains an important unmet medical need. The serogroup B capsular polysaccharide is composed of polysialic acid repeating units that are chemically similar to molecules found on human neuronal cells,

particularly during fetal development [3]. Therefore, various alternate approaches have been sought to develop vaccines to prevent MnB disease, including vaccines produced from outer membrane vesicles (OMVs) prepared from specific MnB strains. However, given the extensive sequence heterogeneity of the major immuno-protective component (PorA) in OMV vaccines, this approach is unlikely to result in a broadly protective vaccine against the MnB serogroup. Thus, efforts to develop a broadly effective anti-MnB vaccine have focused on the identification of non-polysaccharide bacterial surface antigen targets.

3.0 PFIZER'S MNB VACCINE COMPOSITION

The Pfizer MnB vaccine candidate is a bivalent vaccine composed of two variants (A05 and B01) of a family of bacterial outer membrane proteins designated LP2086 [4]. The proteins are lipoproteins that are anchored to the bacterial outer membrane through lipidated N- termini as shown in Figure 3-1 [12]. The main body of the protein extends above the bacterial surface via a flexible spacer domain. The two LP2086 components in the vaccine are expressed in their native, lipidated form in *E.coli*, and highly purified. Throughout this document, Pfizer's MnB vaccine candidate is referred to as bivalent rLP2086 vaccine.

Figure 3-1: Structure of the Lipoprotein LP2086



4.0 IMMUNOLOGICAL SURROGATE OF EFFICACY FOR MENINGOCOCCAL VACCINES

This document presents Pfizer's strategy for assessing the efficacy and breadth of coverage of the bivalent rLP2086 vaccine. Based on input from European and U.S. regulatory agencies, licensure will be based on an immunological surrogate efficacy endpoint, specifically the induction of serum bactericidal antibodies in human vaccines as assessed using an *in vitro* assay measuring bactericidal antibody with human complement (hSBA). The overall incidence of clinical disease is too low to permit vaccine development using efficacy trials with disease endpoints. The immunological surrogate is based on a long and well-validated history of epidemiological and clinical studies that have demonstrated the requirement for serum

bactericidal antibodies as measured by the hSBA for protection against invasive meningococcal disease[13,14,15].

The basis of the *in vitro* SBA for measuring serum bactericidal antibodies have only marginally changed since the time Goldschneider and colleagues demonstrated the correlation between serum bactericidal activity measured in SBA using human complement (hSBA) and protection against invasive meningococcal disease [13]. In the initial reports by Goldschneider et al., as well as in subsequent analyses, protection against invasive meningococcal disease was correlated with hSBA titers of ≥1:4 [13,14,15]. Subsequently, hSBA titers of ≥1:4 were also shown to correlate with protection afforded by licensed meningococcal polysaccharide and polysaccharide conjugate vaccines [15], as well as protein based vaccines (Section 5.0). Collectively, these observations provide the basis for the use of vaccine-elicited hSBA response rates (at titers ≥1:4) to predict meningococcal vaccine efficacy.

While there are currently no standardized hSBAs available for the assessment of MnB vaccines, Pfizer's hSBAs conform to the assay methods adopted by the World Health Organization[16] and Borrow and Carlone[17] and that have demonstrated a correlation between hSBA titers and efficacy afforded by existing meningococcal polysaccharide conjugate vaccines. The assay involves the mixture of viable meningococcal test strain bacteria, human serum complement and serially diluted test serum. Following a standard incubation, the surviving bacteria are enumerated. The assay titers are defined as the reciprocal of the test serum dilution that results in an at least 50% reduction of bacteria compared to the number of bacteria that survived in the absence of test serum. The lowest possible titer that can be determined in the SBA is a titer of 1:4 (lower limit of detection, LOD). Detailed descriptions and discussions of Pfizer's MnB hSBAs are presented in Appendix 1.

5.0 SERUM BACTERICIDAL ACTIVITY IS AN ACCEPTED SURROGATE OF PROTECTION FOR THE MENINGOCOCCAL SEROGROUP B

Early MnB vaccines were composed of purified bacterial outer membrane vesicles (OMVs) in which the major immunogenic component is the highly sequence divergent PorA protein. OMV vaccines have been used successfully to stem MnB disease outbreaks in regions where MnB

epidemics have been due predominantly to MnB strains that express a single PorA type [18,19]. Large-scale efficacy studies with OMV vaccines have been conducted in Cuba [20], Brazil [21,22] Chile[23], Norway[24] and New Zealand[25] and have confirmed the correlation between hSBA activity and protection from MnB disease (Table 5-1).

Table 5-1: Correlation of Observed Efficacy After Vaccination with PorA Containing OMV Vaccines and Percentage of Subjects Responding in hSBA [21,26,27,28,29]

Country (Years)	OMV/PorA Vaccine	Age	Observed Efficacy (95% CI)	SBA Responder Rate (% of Subjects with SBA Titer ≥1:4)
Norway (1988-1991)	B:15:P1.7,16	13-14 yrs	87% (62 – 100%)	97%
			57% (21- 87%)	42%
Brazil (1990-1991)	B:4:P1.15	3-23 mo	-37% (-100 – 73%)	13%
		24-47 mo	47% (-72 – 84%)	45%
		4-7 y	74% (16 – 92%)	52%
New Zealand (2004-2007)	B:4:P1.7b,4	0-3 y	85% (59 – 94%)	76-92%
		8-12 y	75% (52 – 85%)	93%

Since surface exposed PorA sequences are highly variable, the protection provided by each OMV vaccine in each region was predominantly directed against the epidemic strain that expressed the PorA type identical to the vaccine type. Relevant to the use of serum bactericidal activity as an immunological correlate, the hSBA responder rates for OMV vaccine recipients (percent of subjects with a hSBA titer of ≥ 1:4) reflected the observed clinical efficacy, and therefore served as an immunological predictive surrogate of efficacy for the respective MnB vaccine.

6.0 PFIZER'S IDENTIFICATION OF LP2086 AS A VACCINE ANTIGEN FOR ELICITATION OF BROAD SERUM BACTERICIDAL ACTIVITY AGAINST THE MENINGOCOCCAL SEROGROUP B

While the PorA/OMV vaccine experience validates both an outer membrane protein based approach for a MnB vaccine and the use of serum bactericidal activity as a surrogate of efficacy, PorA/OMV vaccines were only successful in epidemic settings where the majority of MnB disease causing strains expressed the PorA sequence identical to the vaccine antigen. Such an approach is not practical for broad, global protection against MnB disease, as the PorA surface accessible immunogenic loops (Figure 3-1) are highly variable and too many PorA serosubtypes would have to be included into a vaccine for broad coverage. In addition PorA/OMV vaccines have demonstrated clinical reactogenicity[30,18].

To overcome the limitations of OMV vaccines, Pfizer used a combined biochemical and immunological screening approach to identify bacterial proteins with sufficient amino acid sequence conservation to induce serum bactericidal antibodies broadly protective against the MnB serogroup. The approach was based on multiple sequential fractionations of the complex mixture of soluble outer membrane proteins. Fractions were used to immunize test animals and resulting immune sera were assessed for bactericidal activity against a number of diverse invasive MnB test strains. Only fractions that elicited broad and strong (high titers) bactericidal activity against these MnB strains were selected for further evaluation[3,4,5]. This screening approach, which relied heavily on the ability of the immunogen to elicit broad bactericidal activity against numerous MnB strains, resulted in the identification of only a single outer membrane lipoprotein, LP2086, which became the component of Pfizer's bivalent rLP2086 vaccine candidate[3,4,5].

7.0 LP2086 IS A FACTOR H BINDING PROTEIN (fHBP) AND AN ESSENTIAL BACTERIALVIRULENCE FACTOR

LP2086 was subsequently identified as a complement factor H binding protein (fHBP)[7] which enables the bacteria to bind human complement factor H, a key negative regulator of the alternative complement pathway[8]. Complement factor H in concert with several other proteins protects healthy human cells by preventing inappropriate activation of the complement system.

However, when factor H is bound to fHBP/LP2086 on the bacterial surface, it enables the bacteria to avoid attack by the complement system. This has been shown elegantly with MnB fHBP/LP2086 isogenic knock-out strains that in contrast to wild-type strains proved susceptible to killing in the presence of non-immune, normal human serum[7,31]. In addition, the surface expression levels of fHBP/LP2086 determined the amount of factor H that could be deposited on the bacterial surface. Low surface expression and consequently low factor H binding resulted in increased sensitivity to bacterial lysis by human serum/blood while higher fHBP/LP2086 expression and correspondingly higher amounts of factor H bound to the bacterial surface resulted in increased survival of the bacteria[7]. Substantial evidence exists that fHBP/LP2086 is expressed by the bacteria *in vivo* as well as *in vitro* [32,33,34]. Serological assessments of anti-LP2086 antibodies have been conducted in patients recovering from meningococcal disease that showed an increase in anti-LP2086 titers following the acute phase of the disease, thereby demonstrating that fHBP/LP2086 is expressed by the bacteria *in vivo* [32].

Given these observations, it is likely that antibodies targeting fHBP/LP2086 can attack the MnB bacterium by two mechanisms: (1) by direct complement dependent killing via the complement attack complex[7] and (2) by prevention of factor H binding[7] to the bacteria thereby limiting bacterial survival *in vivo*. Furthermore, these observations support the perspective that a MnB vaccine composed solely of fHBP/LP2086 proteins can provide substantial protection against MnB as bacteria that lack the expression of fHBP/LP2086 will likely survive poorly (if at all) *in vivo.*

8.0 fHBP/LP2086 SEQUENCE ANALYSIS AND CONSERVATION

The following terminology will be used in the subsequent sections to describe the classification of MnB invasive disease strains with regard to the amino acid sequence of the LP2086 vaccine antigen:

- Subfamily: The two major (A and B) phylogenetic groupings of the LP2086 amino acid sequences.
- Subgroup: Phylogenetic sequence groupings within each subfamily (e.g., N4/N5, N6, N1C1, N2C2, etc.)

- Variant: Specific LP2086 sequence within a subgroup (e.g. A05, B44, etc.)
- Strain: Individual MnB isolate expressing a specific LP2086 sequence variant.

To understand the sequence diversity and distribution of fHBP/LP2086 in the MnB serogroup and to estimate potential coverage by the bivalent rLP2086 vaccine, Pfizer collaborated with public health laboratories in Europe and the CDC (Active Bacterial Core [ABC] Surveillance Site network) to assemble a large collection of representative invasive MnB strains[9]. In collaboration with these laboratories, 1263 invasive MnB invasive disease isolates were systematically collected to establish a representative pool of MnB invasive disease strains (Table 8-1). This representative MnB strain pool was named SBA strain pool, as it was used by Pfizer also to select SBA test strains for clinical evaluation of Pfizer's bivalent rLP2086 vaccine candidate and to support the vaccine's licensure.

The United States strains in the SBA strain pool represented 85% of the available IMD strains from the CDC ABC surveillance sites for the years 2000 to 2005. The US ABC surveillance sites cover ~13% of the US population. MnB disease coverage for most of the European country collections is estimated to be >80% and , every 7th strain (Czech Republic) or 8th strain (UK, Norway, and France) received by each of the national surveillance centers during the years 2001 to 2006 was included in the SBA strain pool.

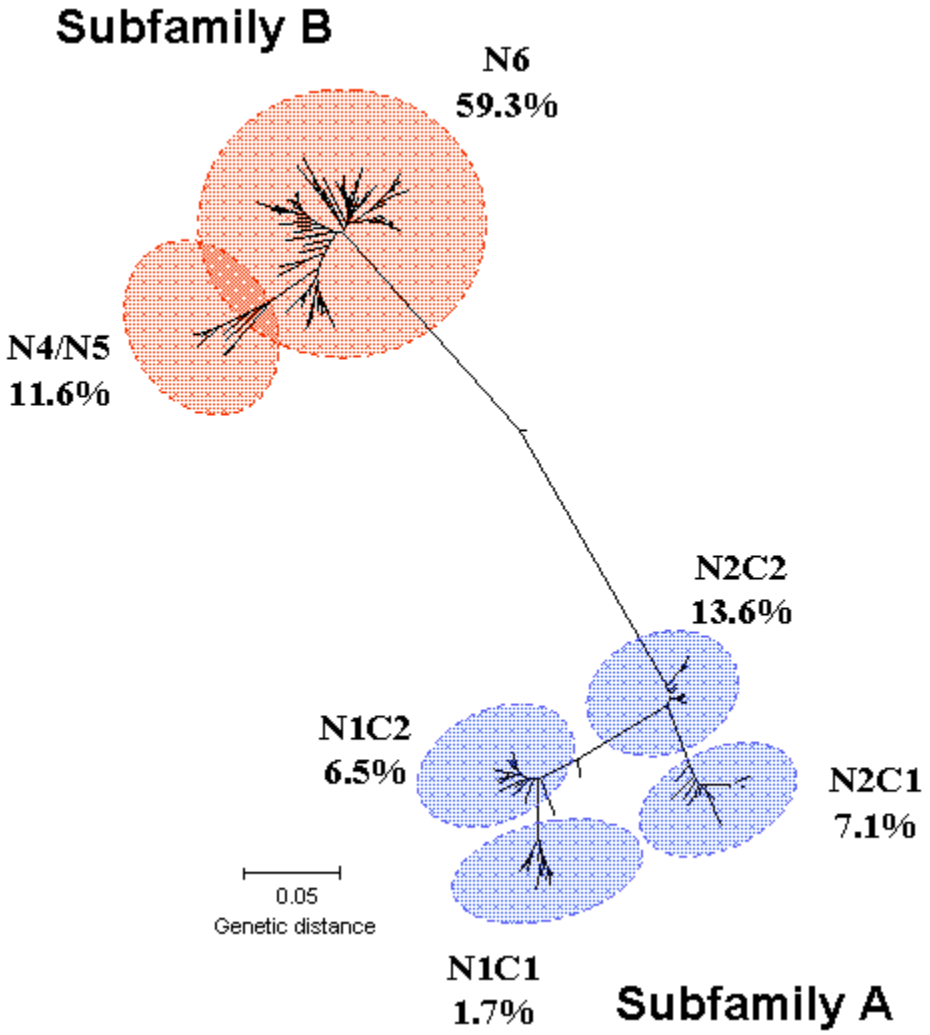
Table 8-1: Composition of Pfizer's Representative SBA Strain Pool

Country	**# of Isolates Included in Pfizer's Representative MnB SBA Strain Pool (% of Total)**
US (ABC sites)	432 (34.2)
UK	536 (42.4)
France	244 (19.3)
Norway	23 (1.8)
Czech Rep.	28 (2.2)
Total	1263 (100)

The fHBP/LP2086 gene was present in all 1263 strains and the intact sequence was found in all but one strain. 143 unique fHBP/LP2086 protein sequences (designated as variants) were identified. The fHBP/LP2086 amino acid sequences were used to construct a phylogenetic tree as described by Murphy et al[9] (Figure 8-1). The scale bar indicates genetic distance based on protein sequence. The end of each branch on the tree represents a variant. The 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 longer the line connecting two variants, the greater is the amino acid sequence diversity between them.

Variants segregated into two distinct subfamilies designated A and B. 29 % of strains in the SBA strain pool (Figure 8-1) are subfamily A strains, 71% of the strains in the SBA strain pool are subfamily B. Sequence conservation is substantial within each subfamily.

Figure 8-1: MnB LP2086 Protein Sequence Phylogenetic Tree



Phylogenetic tree of fHBP/LP2086 variants based on clustalW alignment and drawn with MEGA 4. Subgroups (N1C1, N4/N5 etc) contain closely related variants and are described in Murphy et al[9]. Percentages below each subgroup indicate the proportion of strains in the SBA strain pool (n=1263) that reside within each subgroup. The scale bar indicates genetic distance based on protein sequence, which is roughly equivalent to 1-(% amino acid identity/100). The 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 variant. The longer the line connecting the two variants, the greater is the amino acid sequence diversity between them.

Sequence identity of variants within each subfamily was >84% but was only 60 to 75% between subfamilies, which constituted an important observation for vaccine design (see Section 9.0).

fHBP/LP2086 protein variants could be further grouped within each subfamily into subgroups (N6, N4/N5, N1C1, N1C2, N2C1, and N2C2) that showed very high sequence identity within each subgroup (as indicated by the short lines between individual subgroup variants depicted in the tree). The average pairwise amino acid identity for all variants within each of the subfamily B subgroups N4/N5 and N6 was ~94% and within each of the subfamily A subgroups was ~97%. Because of the high sequence identity within each subgroup, any variant in a given subgroup can be considered representative for all other variants in the same subgroup. The proportions of MnB strains in the SBA strain pool that fall within each subgroup are shown in Figure 8-1.

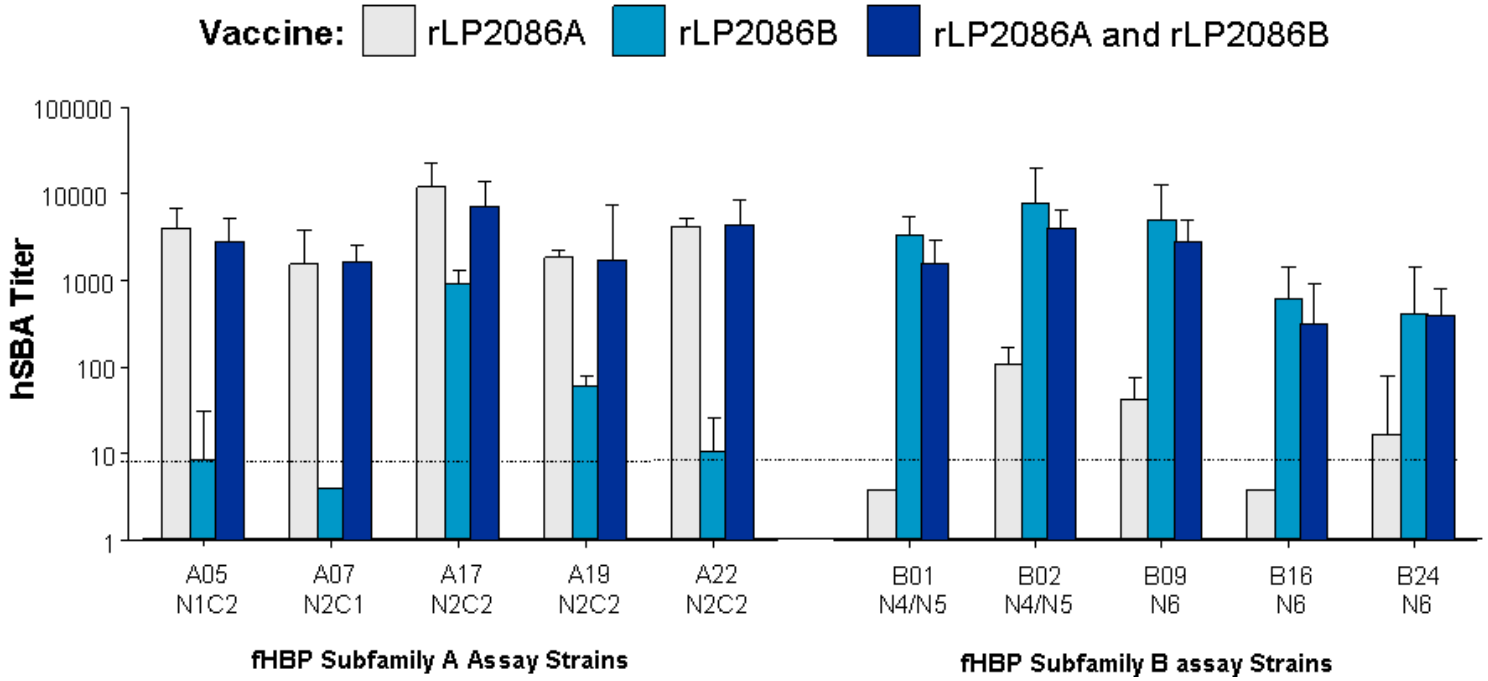
9.0 DEFINING PFIZER'S MNB VACCINE COMPOSITION

Pre-clinical studies were performed to determine whether the lipidated form of the LP2086 protein was required for optimal immunogenicity. Lipidated and non-lipidated forms of three LP2086 protein sequences were recombinantly produced and used to immunize mice. Corresponding immune sera were then tested in hSBA with MnB strains expressing heterologous LP2086 sequences (compared to vaccine sequences). In all cases, the lipidated forms induced at least 10-fold higher serum bactericidal antibody titers compared to the non-lipidated forms[4]. Based on these data, the lipidated forms of LP2086 were selected for further assessment and inclusion into the final vaccine.

Given the fHBP/LP2086 protein sequence segregation into two distinct subfamilies (A and B), additional pre-clinical studies were performed to ascertain if more than one fHBP/LP2086 protein was needed for inclusion into the vaccine for induction of broad bactericidal activity. Lipidated LP2086 subfamily A and B proteins were produced in *E. coli*, purified and used as monovalent vaccine preparations or in combination as a bivalent vaccine formulation. Only the bivalent vaccine formulation induced serum bactericidal antibody responses in rabbits that were broadly bactericidal against MnB strains irrespective of whether they expressed fHBP/LP2086 protein sequences of the A or B subfamily, or expressed fHBP/LP2086 sequences in five subgroups. (Figure 9-1). In contrast, the monovalent vaccines predominantly induced serum

bactericidal antibodies against MnB strains expressing LP2086 proteins of either the A or B subfamily; generally the A subfamily monovalent vaccine induced A-subfamily specific responses while the B subfamily monovalent vaccine induced B subfamily specific antibodies.

Figure 9-1: Pfizer's Bivalent, Lipidated rLP2086 Vaccine that Contains One Subfamily A and One Subfamily B rLP2086 Protein Component Provides Broad Coverage Against Diverse MnB Stains Compared to Vaccines that Contains Only a Single rLP2086 Protein[35]



Individual rabbit immune sera were evaluated in hSBAs using diverse MnB strains. hSBA titers were interpolated as the reciprocal of the serum dilution that killed 50% of MnB bacteria in the assay. Each bar represents the Mean + SD of hSBA titers of 3 rabbits in each group. Most rabbit pre-immune sera showed undetectable SBA titers; a few sera had low hSBA titers between a titer of 9-55. The detection limit of the assay was an hSBA titer of 8 (lowest dilution tested in these experiments and indicated by the dotted line). Sera with hSBA titers ≤1:8 were assigned a value of 1:4. Refer to Figure 8-1 for description of subgroup designations.

These pre-clinical immunological observations supported that a bivalent vaccine composition containing fully lipidated LP2086 proteins from each of the two fHBP/LP2086 sequence subfamilies was required to provide broad serum bactericidal activity against the MnB serogroup.

10.0 BROAD SERUM BACTERICIDAL ACTIVITY IS AFFORDED BY PFIZER'S BIVALENT, RLP2086 VACCINE IRRESPECTIVE OF EPIDEMIOLOGICAL MARKERS OR fHBP/LP2086 PROTEIN SEQUENCE

To understand which factors could potentially influence the susceptibility of invasive MnB strains to serum bactericidal antibodies induced by Pfizer's bivalent, rLP2086 vaccine, 163 diverse MnB invasive disease strains were assessed in hSBAs using vaccine-elicited antisera. MnB SBA test strains were diverse with respect to country of origin, age of patient, disease outcome, epidemiological markers (particularly multi-locus sequence types [MLST] and porA serosubtypes), fHBP/LP2086 subfamily, subgroup and variant. Many strains were tested in the hSBAs using multiple human complement sources to reflect the biological diversity of this reagent and to document that the results were representative and reproducible. Complement sources were screened to have low intrinsic bactericidal activity (see Appendix 1). Early in the program, hSBA analyses were performed with 100 of the 163 MnB strains using rabbit rLP2086 immune serum pools. As the program progressed some of the earlier strains and additional strains (N = 109) were evaluated using human rLP2086 immune serum pools. hSBA data with bivalent rLP2086 rabbit immune sera demonstrated that over 87% of the 100 strains were susceptible to killing in the hSBA irrespective of MLST, PorA serosubtype, fHBP/LP2086 subgroup, fHBP/LP2086 variant or capsule expression[33,35]. Identical observations were obtained with human bivalent rLP2086 immune sera and 109 strains.

Figure 10-1 to Figure 10-4 demonstrate that the susceptibility of the 109 SBA test strains to the human bivalent rLP2086 immune sera was independent of MLST (Figure 10-1), PorA serosubtype (Figure 10-2), fHBP/LP2086 subgroup (Figure 10-3) and fHBP/LP2086 variant (Figure 10-4).

Figure 10-1: MnB Strains with Diverse MLST are Susceptible in hSBA to Bivalent rLP2086 Human Immune Sera

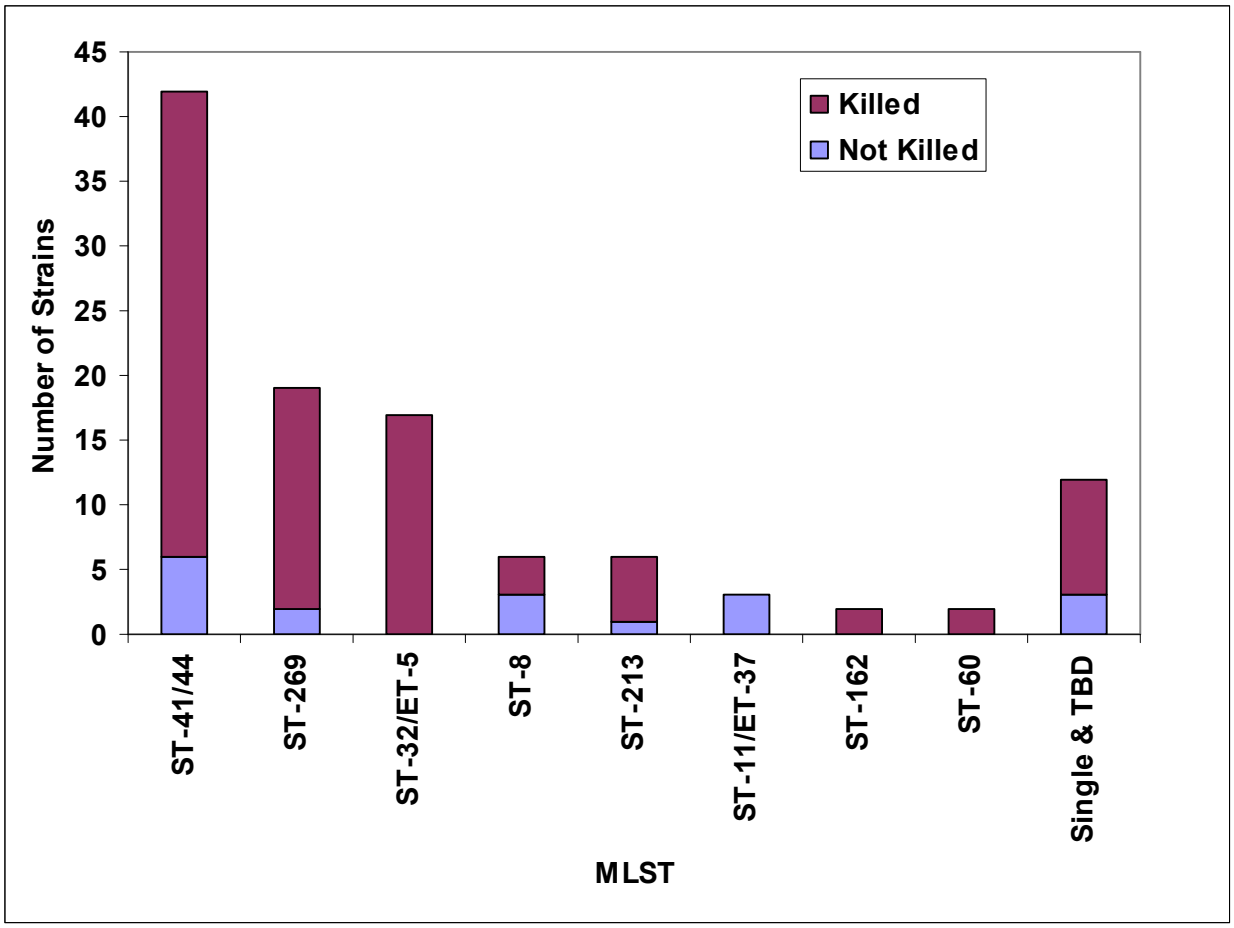


Figure 10-2: MnB Strains with Diverse PorA Serosubtypes are Susceptible in hSBA to Bivalent rLP2086 Human Immune Sera

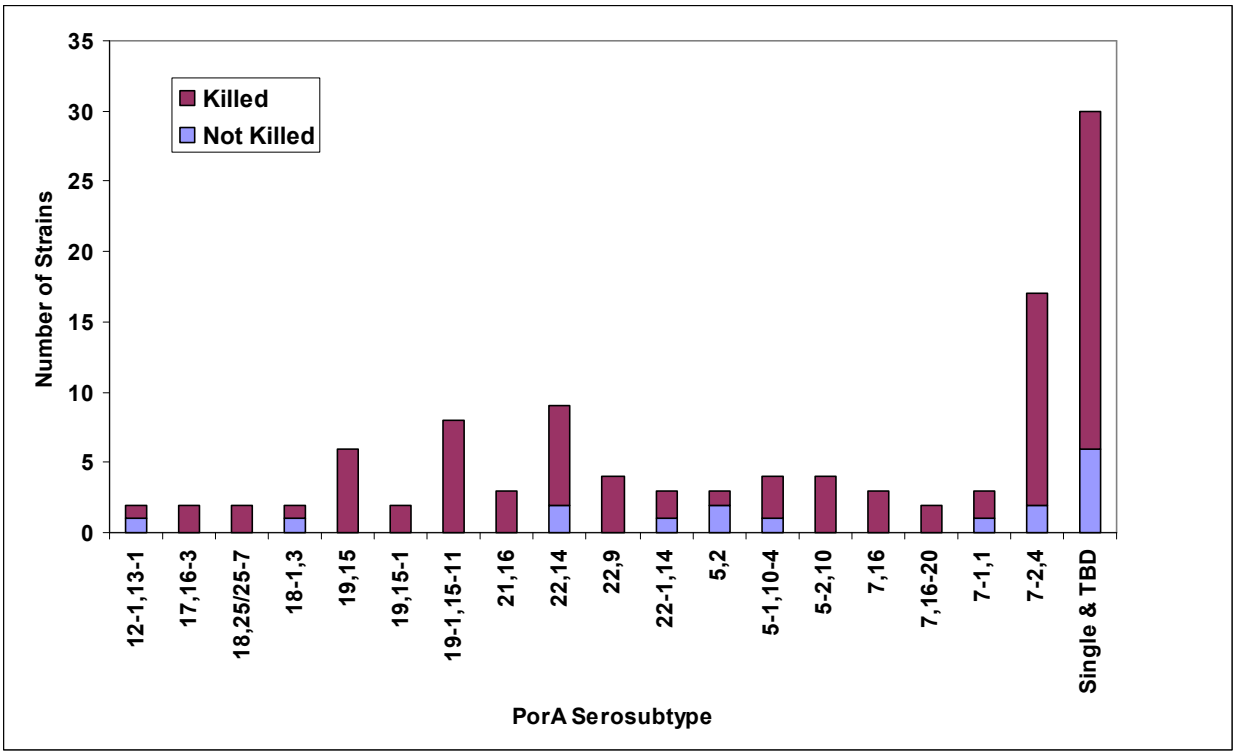


Figure 10-3: MnB Strains from all Six fHBP/LP2086 Subgroups are Susceptible in hSBA to Bivalent rLP2086 Human Immune Sera

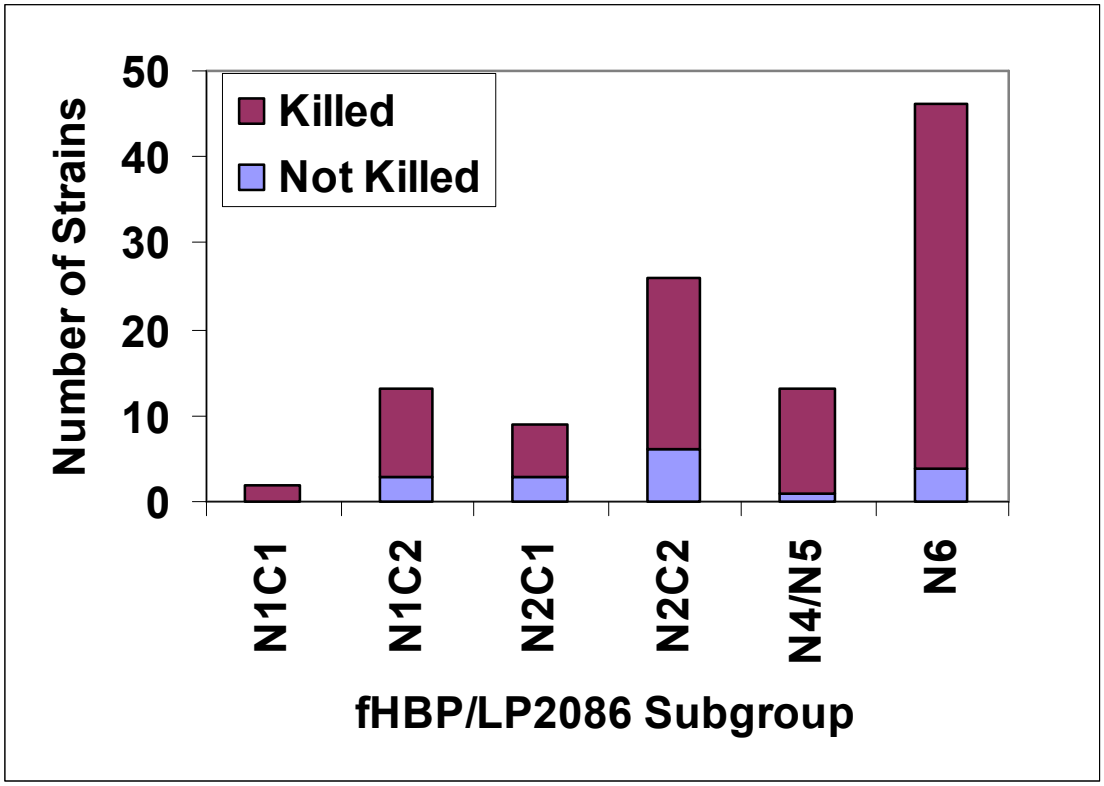


Figure 10-4: MnB Strains Expressing Prevalent fHBP/LP2086 Variants are Susceptible in hSBA to Bivalent rLP2086 Human Immune Sera

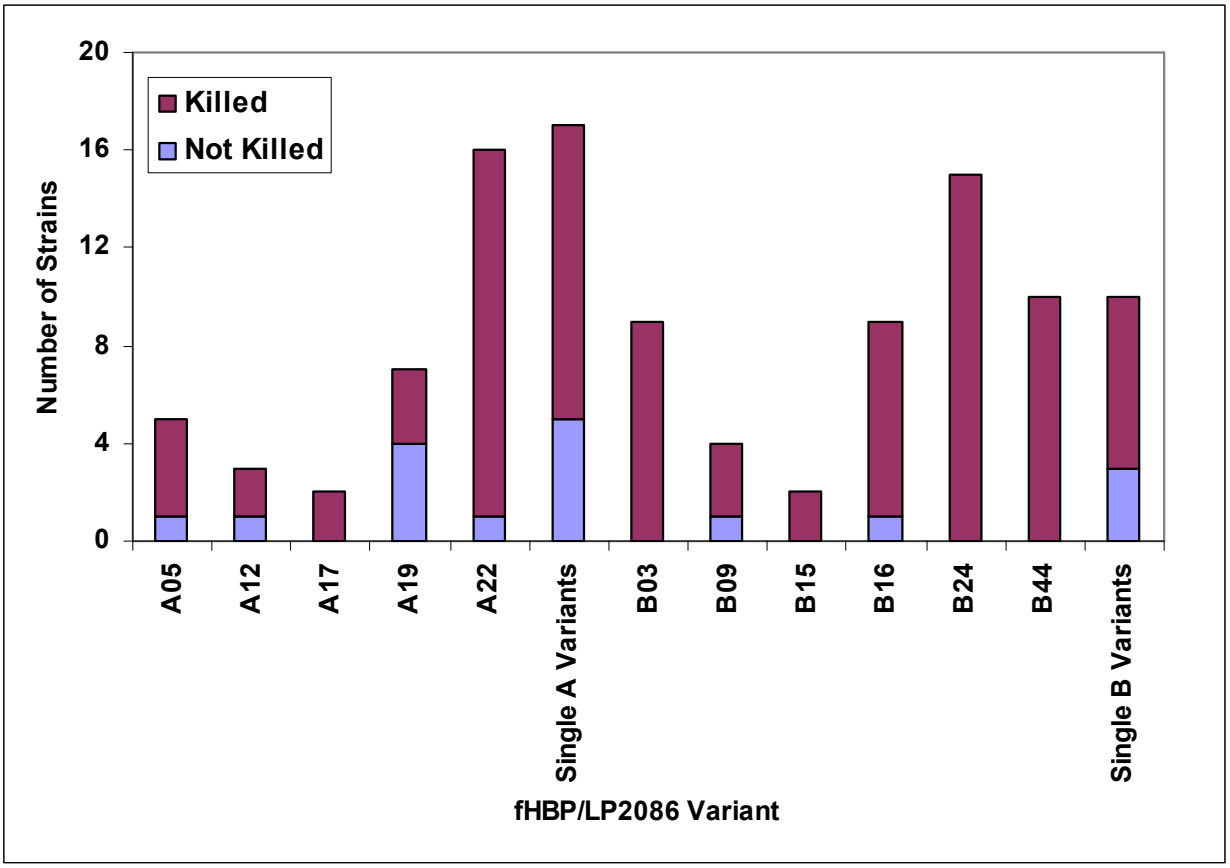
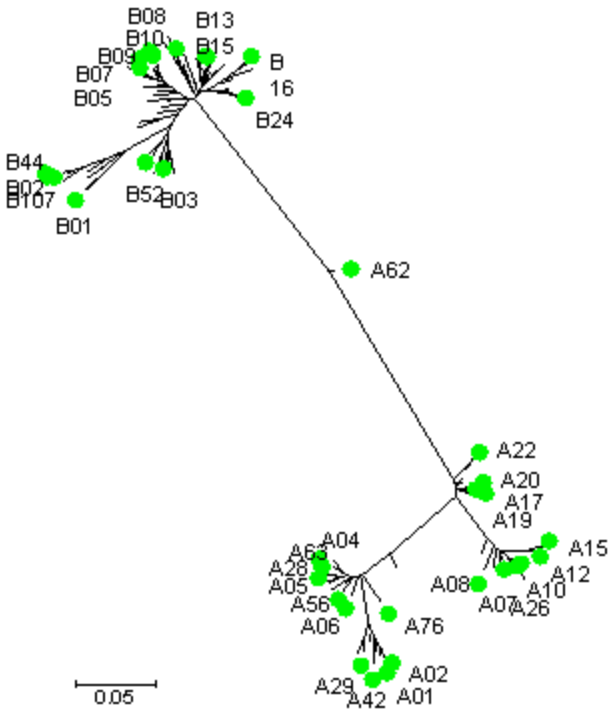


Figure 10-5 displays all fHBP/LP2086 variants expressed by MnB strains that were susceptible in hSBA using either rabbit or human immune sera elicited after immunization with the bivalent rLP2086 vaccine. As shown, the phylogenetic tree is well "decorated" supporting the conclusion that broad bactericidal coverage across the MnB serogroup is elicited by Pfizer's bivalent rLP2086 vaccine.

Figure 10-5: hSBA Coverage of MnB Strains Expressing Diverse fHBP/LP2086 Sequences by Immune Sera Elicited After Immunization of Rabbits or Humans with the Bivalent rLP2086 Vaccine



Each green dot on the tree represents a different variant that was killed in hSBA by immune sera elicited by the bivalant rLP2086 vaccine. Vaccine variants are B01 and A05.

11.0 ROLE OF FHBP/LP2086 *IN VITRO* SURFACE EXPRESSION FOR STRAIN SUSCEPTIBILITY IN THE HSBA

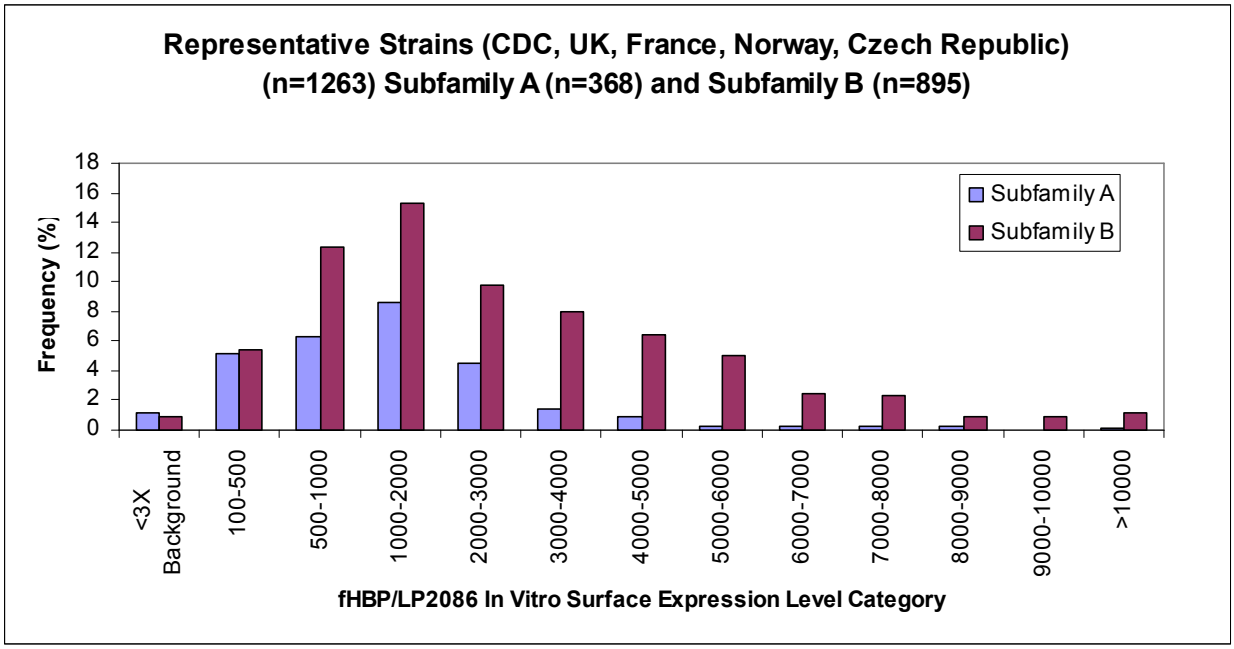
Even though the vast majority of diverse MnB strains evaluated in Section 10.0 were killed in hSBA, Pfizer sought to understand the reason why a small percentage of strains appeared to be not susceptible to serum bactericidal killing in the hSBA. To initiate the complement cascade, complement component C1q must bind to antibodies bound specifically to LP2086. Activation of the complement cascade only occurs when there is cross-linking between a single C1q and multiple antibodies bound to the bacterial surface[36]. Cross-linking can only occur when the vaccine target antigen is expressed in sufficient quantities on the surface of the bacteria to allow for sufficient antibody binding. Therefore, *in vitro* surface expression of LP2086 on bacteria

grown under the hSBA conditions was examined as a possible factor that could affect susceptibility of bacteria in the assay.

To determine relative surface expression/density of LP2086 on MnB invasive disease strains, a flow cytometry assay was developed (for details see Appendix 3) using a monoclonal antibody that recognized a functional epitope common to LP2086 subfamily A and B lipoproteins on the bacterial surface[33]. The assay was designed to quantify the amount of fHBP/LP2086 bacterial surface protein accessible to antibody, not the total bacterial cell-associated level of the protein, as only antibody accessible surface-expressed protein is biologically relevant.

When the relevant *in vitro* fHBP/LP2086 surface expression by each strain in the SBA strain pool (N= 1263) was examined, expression differences were noted (Figure 11-1). fHBP/LP2086 *in vitro* surface expression levels exhibited a normal distribution across the SBA strain collection, independent of whether strains expressed fHBP/LP2086 subfamily A or B variants.

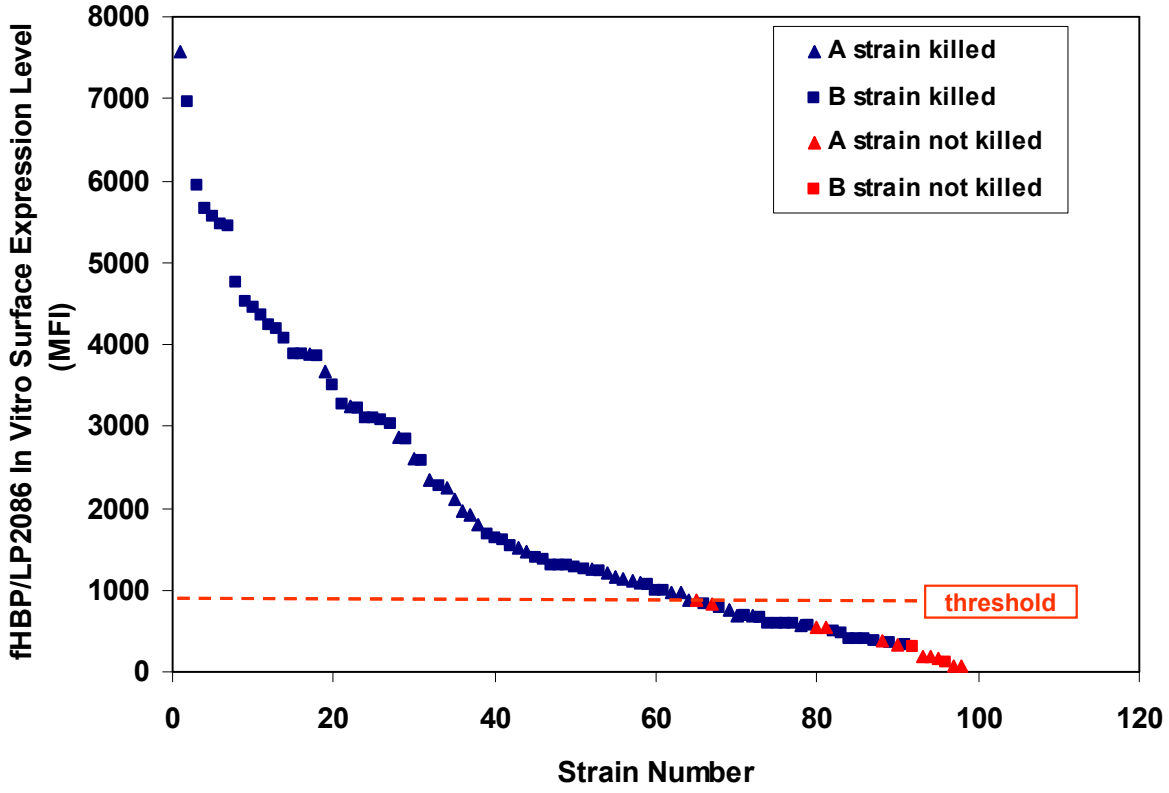
Figure 11-1: Distribution of fHBP/LP2086 *in Vitro* Surface Expression by MnB Strains in the SBA Strain Pool



The surface expression level of fHBP/LP2086 of the MnB strains was determined by flow cytometry as described in Appendix 3. Each *in vitro* surface expression level category is defined by a range of expression levels based on mean fluorescent intensities (MFI). Strains were sorted into the expression level categories as indicated based on their MFI.

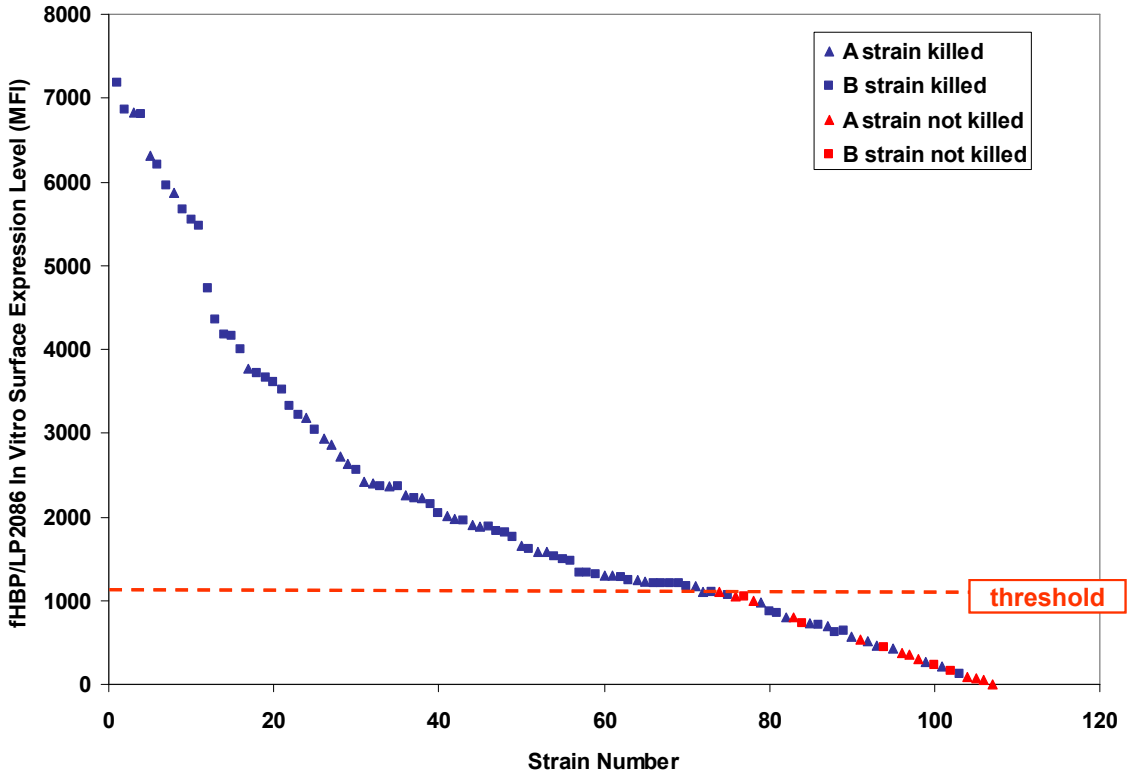
Next, the fHBP/LP2086 *in* vitro surface expression levels for each of the 163 strains used in the hSBA analyses discussed above were correlated with each individual strain's susceptibility in the hSBA (Figure 11-2 and Figure 11-3). The data demonstrated that, among the small proportion of MnB strains not susceptible in hSBA, all showed low fHBP/LP2086 *in vitro* surface expression levels irrespective of whether rabbit (Figure 11-2) or human (Figure 11-3) bivalent rLP2086 immune serum pools were used for the analyses.

Figure 11-2: Correlation of fHBP/LP2086 *in Vitro* Surface Expression with MnB Strain Susceptibility to Rabbit Immune Sera Following Immunization with the Bivalent rLP2086 Vaccine



Serum pools tested in hSBAs were from rabbits immunized three times with the bivalent rLP2086 vaccine. Preimmune serum pools from the same serum donors were negative in most hSBAs. A flow cytometry assay (described in Appendix 3) was used to determine fHBP/LP2086 *in vitro* surface expression. The readout of the flow cytometer is mean fluorescent intensity (MFI). Strains are ordered by MFI values from high to low and analyzed for susceptibility to serum bactericidal killing in hSBA. Strains killed in hSBA are represented by blue symbols and strains not killed in hSBA are represented by red symbols. Two highly expressing strains (both killed) were off the MFI scale and not plotted.

Figure 11-3: Correlation of fHBP/LP2086 *in Vitro* Surface Expression with MnB Strain Susceptibility to Human Immune Sera Following Immunization with the Bivalent rLP2086 Vaccine



Serum pools tested in hSBA were from humans immunized three times with the bivalent rLP2086 vaccine. Preimmune serum pools from the same serum donors were negative in most hSBAs. A flow cytometry assay (described in Appendix 3) was used to determine fHBP/LP2086 *in vitro* surface expression. The readout of the flow cytometer is mean fluorescent intensity (MFI). Strains are ordered by MFI values from high to low and analyzed for susceptibility to serum bactericidal killing in hSBA. Strains killed in hSBA are represented by blue symbols and strains not killed in hSBA are represented by red symbols. Two highly expressing strains (both killed) were off the MFI scale and not plotted.

MnB strain characteristics such as MLST, PorA sersubgroup, fHBP/LP2086 subgroups or variant sequences did not influence susceptibility of strains in the hSBAs (Section 10.0) . An *in*

vitro surface expression level of approximately 1100 MFI (as defined by the flow cytometry assay) was identified above which all MnB strains were susceptible to killing by vaccine antisera in the hSBA. Below this threshold, about 50% of MnB strains were still susceptible but prediction of which of these strains were susceptible was not possible.

It is uncertain how or whether the observed correlation *in vitro* between fHBP/LP2086 surface expression and susceptibility to killing in the hSBA translates to the potential of bacterial strain killing *in vivo*. Nonetheless, even if the correlation to *in vivo* susceptibility is direct, the data described above show that the bivalent rLP2086 vaccine candidate elicits serum bactericidal activity against >87% (rabbit immune sera) or >84% (human immune sera) of MnB invasive disease strains irrespective of the *in vitro* surface expression levels of fHBP/LP2086.

12.0 SELECTION OF REPRESENTATIVE MNB SBA STRAINS TO ASSESS THE BIVALENT RLP2086 VACCINE EFFICACY

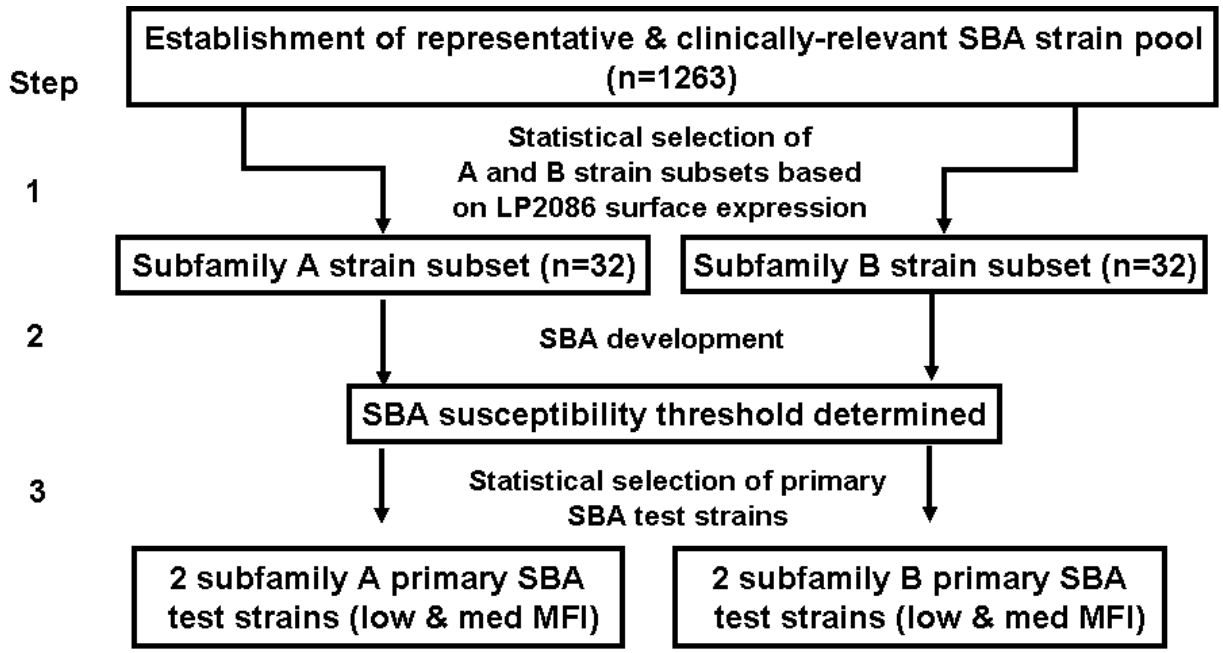
In the context of phase 3 efficacy trials, it is not feasible to assess the vaccine-elicited immune response against a large number of diverse MnB strains in a statistically meaningful way using fully validated assays. Therefore, Pfizer proposes to use four primary SBA test strains (two each from the A and B subfamilies) to assess the overall immunogenicity and efficacy of the bivalent rLP2086 vaccine candidate, and to supplement these hSBA data with additional assessments using a larger pool of "exploratory" strains.

The method of selection for the four "primary" SBA strains is critical as it is essential that bias not be introduced into the selection process. This section describes the unbiased statistically driven approach used by Pfizer to select the four primary SBA test strains.

Pfizer considered the fHBP/LP2086 sequence segregation into two subfamilies and the role of bacterial surface expression in determining *in vitro* MnB strain susceptibility to killing by vaccine-elicited antiserum in hSBAs, and therefore took both of these factors into consideration in the selection process.

Rigorous assessment of vaccine efficacy requires that the MnB SBA test strains should be selected using a statistical approach from a representative pool of invasive MnB disease strains to avoid a potential bias in the estimation of vaccine efficacy. Importantly, it is essential that the selected SBA test strains express fHBP/LP2086 protein sequences heterologous to the vaccine antigen sequences, thereby reflecting the fHBP/LP2086 sequence diversity and assuring that the SBA test strains will be representative of the MnB serogroup. In addition, the selected MnB SBA strains should be representative with regard to fHBP/LP2086 *in vitro* expression levels. Since complement lots can also affect serum bactericidal activity (see Appendix 2), the use of complement lots from multiple individuals rather than single sources to reflect the natural human biological diversity of this reagent was also taken into consideration. Pfizer designed the MnB SBA strain selection strategy depicted in Figure 12-1 so that all these critical factors would be addressed. In the following sections a brief description is provided of how the strategy was implemented.

Figure 12-1: Outline of SBA Strain Selection Strategy



12.1 Generation of Representative MnB Strain Pool for Selection of SBA Strains

Generation of Pfizer's representative MnB SBA strain pool (N =1263 invasive disease strains) was described earlier (Section 8.0, [9].) Participating countries and contribution of strains from these countries are shown in Table 8-1. The SBA strain pool is extensive and represents strains recently circulating in Europe and the United States. The pool only contains MnB clinical strains that have caused invasive disease. Pfizer has demonstrated that analyses of additional isolates did not change any conclusions obtained with the representative strain pool [9,37].

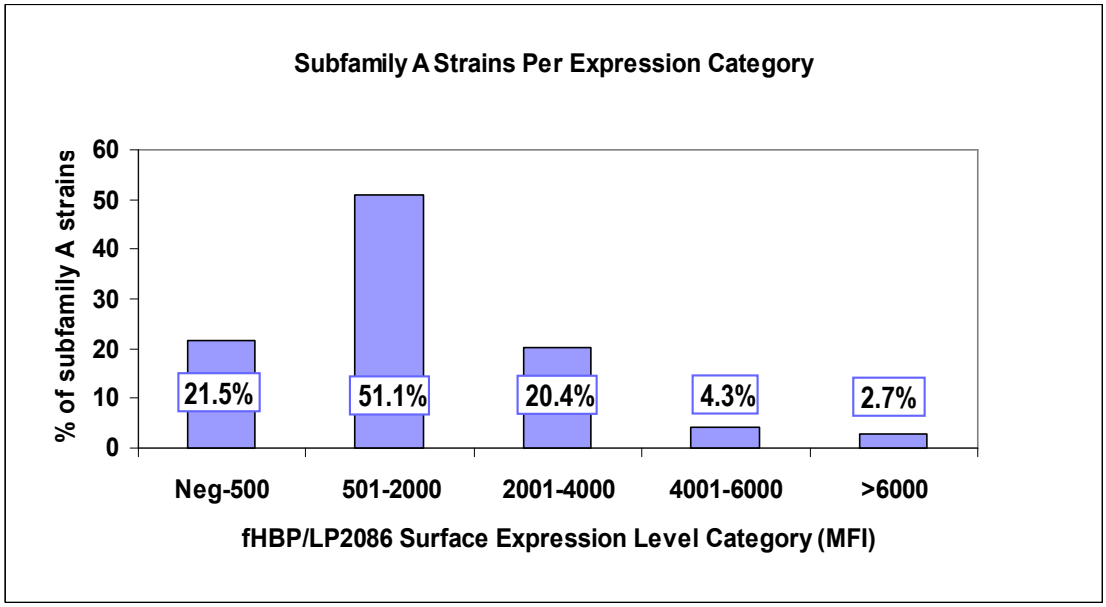
12.2 Statistical Selection of Primary SBA Test Strains Representing Each Subfamily

12.2.1 Statistical Selection of A and B Subfamily Strains Taking into Account the *in Vitro* fHBP/LP2086 Surface Expression Distribution within the SBA Strain Pool (Step 1)

As depicted in Figure 12-1, initial statistical selection of subfamily A and B strain subsets was performed. Based on Pfizer's pre-clinical findings, 32 strain subsets per subfamily were considered sufficient to address the MnB strain epidemiological (major MLSTs) and fHBP/LP2086 sequence diversity (prevalent variants and subgroups) and to establish the *in vitro* expression threshold. Furthermore, the development of individual hSBAs and the assessment of each strain with 5 different complement sources (to establish robustness and reproducibility with this biological reagent), was considered technically feasible using a total of 64 strains (32 A subfamily and 32 B subfamily strains). Establishment of the LP2086 expression threshold was important since low LP2086 expression levels (below the threshold) under the *in vitro* hSBA conditions could hinder the development of robust hSBAs needed to support high-throughput clinical sample testing. Strains with expression levels below the threshold were therefore excluded from the selection process of the primary strains, but as discussed in Section 12.3, these strains were considered for exploratory hSBA analyses.

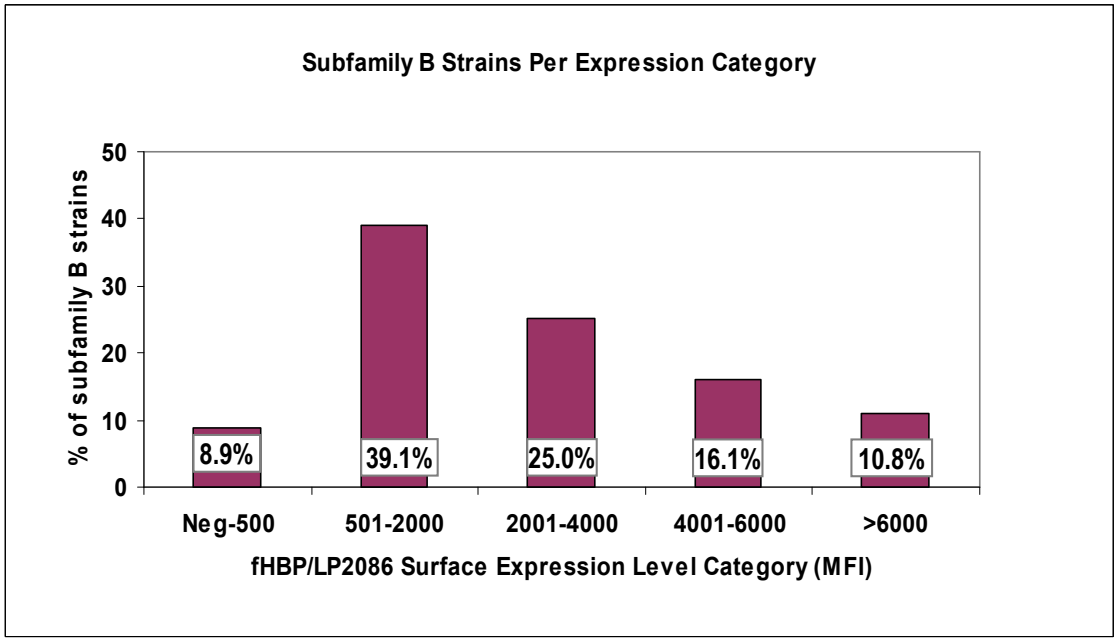
To select the 32 strains per subfamily, the MnB SBA strain pool was separated by subfamily into 368 and 895 subfamily A and B strains, respectively. Strains within each subfamily were then grouped into five categories based on the *in vitro* LP2086 surface expression as shown in Figure 12-2 and Figure 12-3.

Figure 12-2: Subfamily A strain (N=368) Distribution Based on fHBP/LP2086 *in vitro* Surface Expression Level



 # strains selected: 7 16 7 1 1

Figure 12-3: Subfamily B Strain (N=895) Distribution Based on fHBP/LP2086 *in vitro* Expression Level



 # strains selected: 3 13 8 5 3

By subfamily, the percentage of total strains in each *in vitro* fHBP/LP2086 surface expression level category was then determined. The percentage in each expression category was multiplied by the total number of strains to be selected for the subfamily (n=32) to yield the number of strains to be selected from each category. For example, 39.1% of all subfamily B strains have *in vitro* surface expression levels of 501-2000 MFI (Figure 12-3). Since 32 subfamily B strains were to be selected, then (0.391 x 32) or 13 subfamily B strains would be selected from this surface expression category. The calculation was performed for all other subfamily A and B expression categories to yield the number of strains per expression category to be selected. The numbers calculated are depicted in Figure 12-2 and Figure 12-3.

Statistical selection involved assigning all strains in the SBA strain pool a random number using a random number generator; the strains were then ordered numerically in ascending order. The

calculated number of strains required for each expression category in each subfamily was selected based on the strain order.

An example of how this was done is shown in Figure 12-4 for a hypothetical expression group. Ten strains were assigned a random number (left panel), then ordered numerically (right panel). This process applied to surface expression category of 501-2000 MFI for subfamily B strains in Figure 12-3 (containing ~350 subfamily B strains with LP2086 expression levels between 500 and 2000 MFI) would select the first 13 strains after ordering the strains by random number. This process was used for all expression groups in both subfamilies, yielding the strains comprising the subfamily-specific subsets of 32 strains each.

Figure 12-4: Example of Using a Random Number Approach to Order Strains

strain	random number
1	0.287
2	0.150
3	0.052
4	0.828
5	0.694
6	0.940
7	0.419
8	0.082
9	0.863
10	0.414

order →

strain	random number
3	0.052
8	0.082
2	0.150
1	0.287
10	0.414
7	0.419
5	0.694
4	0.828
9	0.863
6	0.940

After applying the statistical selection approach, the LP2086 variant distribution in the SBA strain pool (N =1263 isolates) was compared to the statistically selected subset of 64 strains to demonstrate that the selected subset of 64 strains exhibited a similar distribution of fHBP/LP2086 variants. This comparison (Table 12-1) supports the perspective that the selection process was appropriately representative, i.e. the statistically selected 64 SBA strains represent a similar distribution of fHBP/LP2086 variants to the larger SBA strain pool. In addition, the selected 64 strains represented the most common MLST and PorA types, as well as all six fHBP/LP2086 subgroups, and the range of fHBP/LP2086 surface expression levels (data not shown).

Table 12-1: Comparison of Statistically Selected Strains (N= 64) to SBA Strain Pool (N=1263) by Variant Frequency

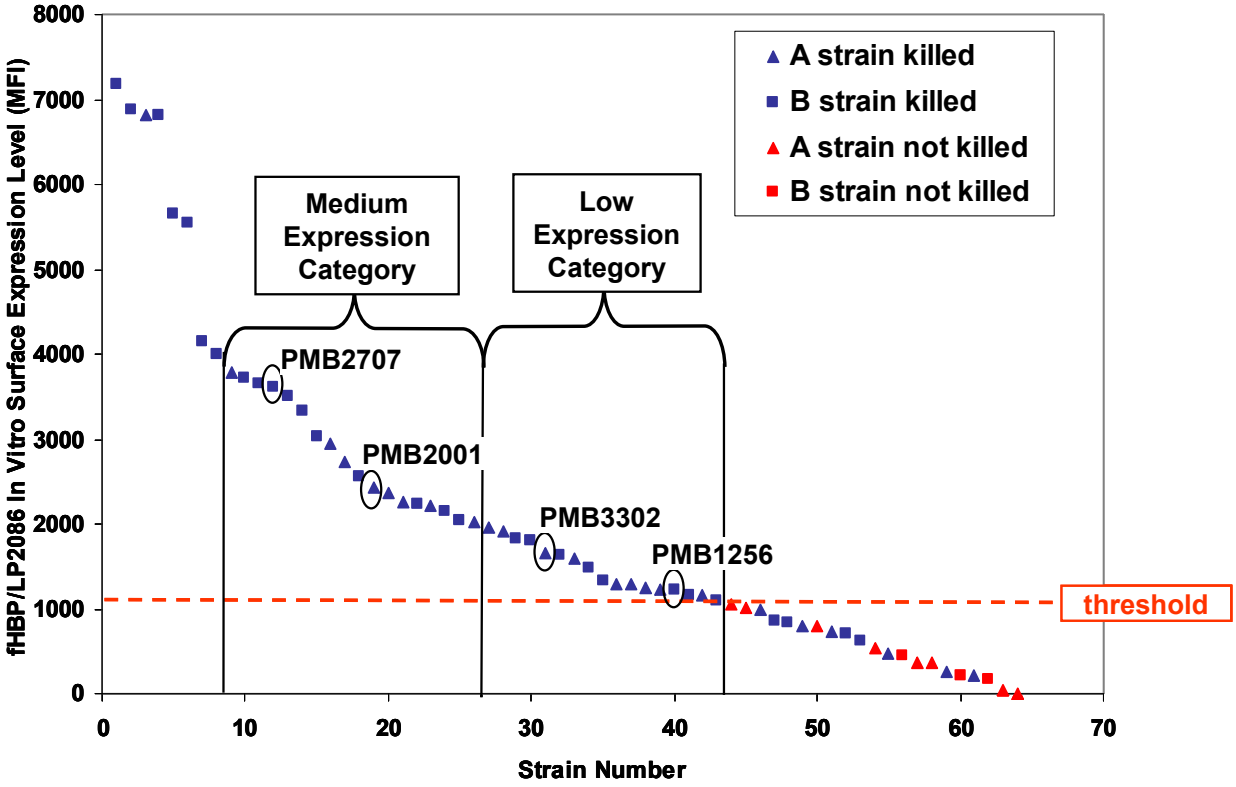
LP2086 Sequence	Total	Expected Frequency	Actual Frequency Selected Strains
A22	125	10.9	9
A12	38	3.3	2
A19	33	2.9	2
A05	29	2.5	4
A07	21	1.8	2
A06	18	1.6	1
A15	12	1	1
A10	3	0.3	0
B24	266	9.5	5
B16	167	6	5
B44	137	4.9	9
B03	111	4	6
B09	73	2.6	2
B82	15	0.5	0
B15	10	0.4	1
B23	10	0.4	0

12.2.2 Evaluation of the Subfamily A and B Strain Subsets to confirm the LP2086 *in vitro* Surface Expression Threshold (Step 2)

For each of the 64 statistically selected MnB strains, human complement sources were identified with low non-specific serum bactericidal activity to determine the strain's susceptibility in the hSBA. (See the Appendix 2 for an in-depth discussion of hSBA complement selection.) The

susceptibility threshold of the statistically selected SBA strain pool, tested with the human immune serum pool, is shown in Figure 12-5.

Figure 12-5: hSBA *in vitro* Susceptibility Threshold for the 64 Statistically Selected MnB SBA Test Strains



12.2.3 Selection of Primary SBA Test Strains (Step 3)

Step 2 permitted the determination of two surface expression categories for the selection of the four primary SBA strains: A low expression category (low MFI) and a medium expression category (medium MFI).

One primary SBA strain for each subfamily was selected conservatively from the low surface expression category so as to reflect effectiveness of the vaccine against strains with lower *in vitro* LP2086 expression levels (low MFI). A second primary SBA strain for each subfamily was statistically selected from a surface expression category that contained strains with higher LP2086 surface expression levels (i.e. medium MFI). The random number selection process, as described in Section 12.0, was then used to select the final four primary SBA strains.

After random number-based ordering, the first strain in each list of subfamily A and B strains, in the low or medium expression categories, was selected as a primary SBA strain if appropriate complement sources could be identified that (i) demonstrated low intrinsic bactericidal activity and (ii) were found in high enough frequency in the human complement donor population to assure the continuous supply for high-throughput hSBA testing required to support clinical phase 3 vaccine development.. In three cases, the first statistically selected strain could not be developed for hSBA testing due to complement constraints as discussed. In these cases, the next statistically selected strain was used instead (Figure 12-6).

Figure 12-6: Characteristics of the First Three Statistically Selected SBA Strains for Each Subfamily and Expression Category

Surface Expression Level

Subfamily A

	Rank Order	Strain	Subgroup	Variant	MFI
Low	*1*	*PMB1321*	*N2C2*	*A22*	*1292*
	2	**PMB3302**	**N1C2**	**A04**	**1658**
	3	PMB876	N1C2	A28	1908
Medium	*1*	*PMB1168*	*N1C2*	*A05*	*2724*
	2	**PMB2001**	**N1C2**	**A56**	**2420**
	3	PMB1675	N2C1	A12	2257

Subfamily B

	Rank Order	Strain	Subgroup	Variant	MFI
Low	**1**	**PMB1256**	**N6**	**B03**	**1217**
	2	PMB1610	N6	B08	1806
	3	PMB2948	N6	B24	1479
Medium	*1*	*PMB147*	*N4/N5*	*B44*	*3333*
	2	**PMB2707**	**N4/N5**	**B44**	**3610**
	3	PMB3036	N6	B24	3512

Bolded strains represent the final selection of primary SBA strains. Strains in italics were rejected based on difficulty identifying sufficient human complement lots with low intrinsic bactericidal activity required to support the large number of phase 3 clinical hSBAs.

hSBAs for the four primary strains shown in bolded type in Figure 12-6 were developed. Development included identification of suitable human complement lots, assay optimization, and qualification. All hSBAs will be fully validated before phase 3 clinical evaluations are performed. The four primary SBA strains represent both subfamilies and three of six sequence subgroups, thereby representing over 77% of MnB invasive disease isolates (Figure 8-1).

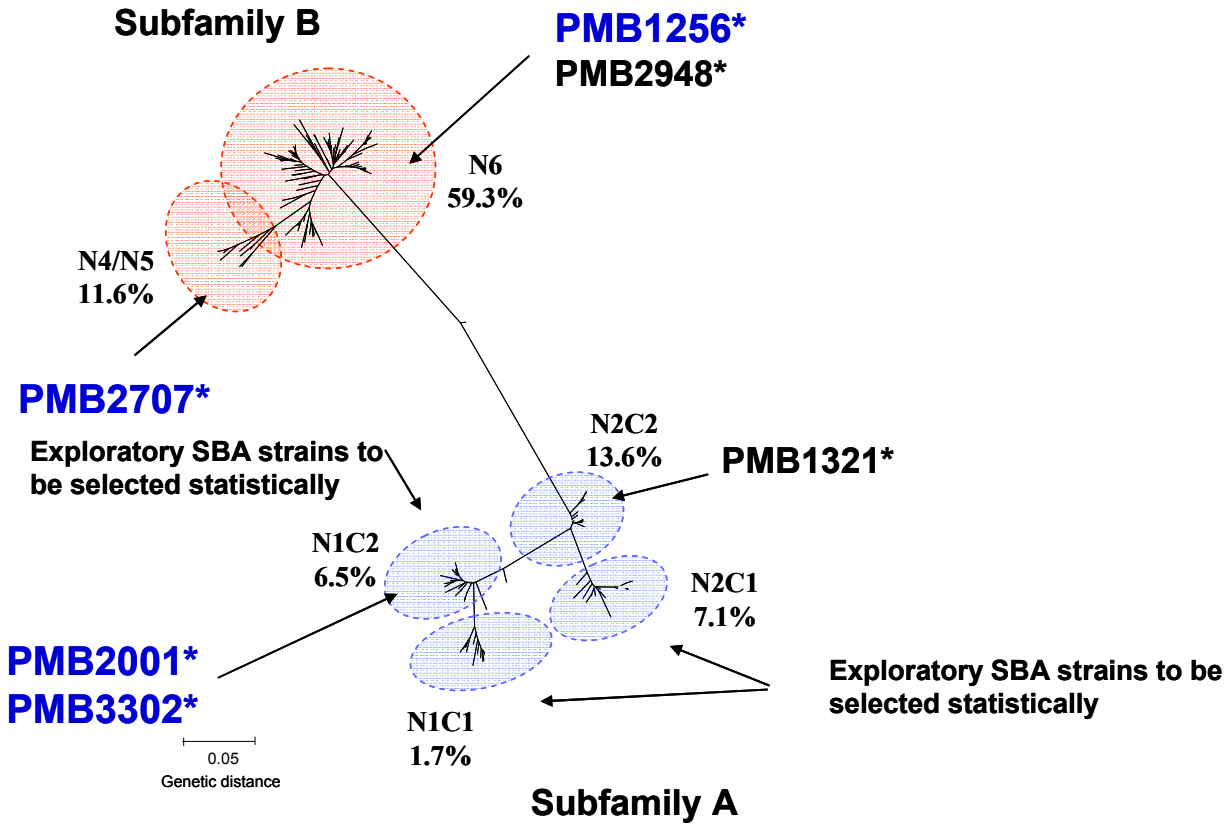
The statistical selection of the primary SBA test strains as described represents an approach that avoided bias and selected strains that represent the "natural" diversity of MnB strains circulating in the United States and Europe. Furthermore, the selection of two primary SBA test strains each from subfamilies A and B will enrich the assessment of the vaccine clinical responses (efficacy). For example, evaluation of two MnB test strains per subfamily decreases the potential of having

chosen an outlier strain by chance that might not be broadly representative. If the hSBA responses measured with the selected SBA test strains are truly representative of the MnB serogroup as a whole, then similar hSBA responder rates would not just be expected between the two SBA strains within a given subfamily, but responses would be expected to be similar across all four primary SBA strains, as was indeed observed in phase 2 studies of the vaccine (see Section 13.0).

12.3 Exploratory hSBA Analyses With Additional Statistically Selected MnB Strains to Provide Supporting Evidence that The Primary MnB SBA Test Strains Adequately Describe the Efficacy of Pfizer's Vaccine Against the MnB Serogroup

The four primary SBA test strains cover 3 fHBP/LP2086 sequence subgroups representing over 77% of all MnB strains in the SBA strain pool. To provide additional evidence in support of the primary clinical efficacy endpoints using these four primary SBA test strains, Pfizer will perform exploratory hSBA analyses with additional "exploratory" strains (N~10). These strains will represent fHBP/LP2086 variants in subgroups not represented by the four primary strains (i.e. N1C1; N2C2; N2C1) and will also represent strains with *in vitro* fHBP/LP2086 surface expression levels below the hSBA susceptibility threshold. These supportive analyses will complement the clinical evaluations with the primary SBA test strains and will be conducted with qualified hSBAs on prospectively chosen subsets of vaccine recipients. It is expected, based on Pfizer's clinical analyses shown in Section 13.0 and discussed in Section 14.0 , that these exploratory analyses are expected to confirm the vaccine efficacy estimates generated with the four primary SBA test strains. Figure 12-7 shows the phylogenetic tree of fHBP/LP2086 and annotated on the tree, the primary SBA strains (bold blue font) and anticipated exploratory SBA strains (black font). As discussed in the next section, clinical phase 2 hSBA data support that this strategy can adequately describe the efficacy afforded by the bivalent rLP2086 vaccine candidate.

Figure 12-7: The Statistically Selected Primary SBA Test Strains in Conjunction with the Exploratory SBA Strains Will Cover All Six fHBP/LP2086 Sequence Subgroups



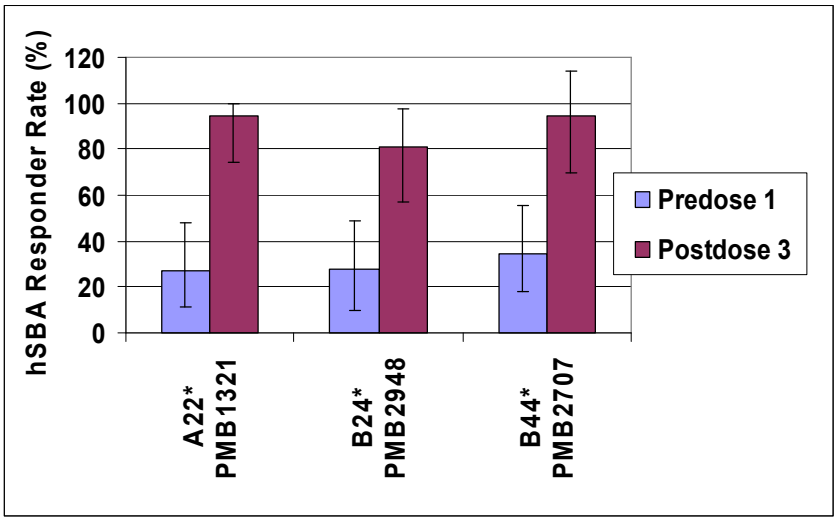
Asterisk indicates that SBA test strains express fHBP/LP2086 heterologous to vaccine components. Percentages under subgroups indicate proportion of strains in SBA strain pool that express variants of the subgroup.

13.0 SUPPORTING DATA FROM THE ADOLESCENT PHASE 2 AND ADULT PHASE 1 CLINICAL TRIALS OF THE BIVALENT RLP2086 CANDIDATE VACCINE

Safety and immunogenicity data are available from a completed phase 1/2 study in adults with a 0-, 2-, and 6 month vaccination schedule (B1971003) and from an adolescent phase 2 dose-ranging study utilizing a 0-, 2-, and 6-month vaccination schedule (B1971005). Immunogenicity evaluations using hSBA were performed with serum samples obtained prior to the first dose (predose 1) of the bivalent rLP2086 vaccine and then 1 month after the third vaccine dose (postdose 3) in studies B1971003 and B1971005.

Study B1971003 was a phase 1 / 2 open-label study of safety, reactogenicity and immunogenicity study in healthy adults. Sixty (60) adult subjects, 18 to 40 years of age (mean age 28.6 years), were enrolled in this study and received three doses of 120 mcg (final dose level) of the bivalent rLP2086 MnB vaccine. Serum samples obtained predose 1 and one month after third study dose (postdose 3) were evaluated in hSBA performed with MnB test strains called phase 1 strains expressing fHBP/LP2086 variants homologous to vaccine antigen (PMB1745, fHBP/LP2086 variant A05) and heterologous to vaccine antigen (PMB17, fHBP/LP2086 variant B02) . The hSBA response rates were expressed as percentage of subjects with hSBA titers ≥ 1: 4 to each of the SBA test strains. Most of the adult study subjects did not have measurable hSBA titers before their first dose of vaccine. Postdose 3, approximately 94 % of the subjects had hSBA titers ≥1:4. An exploratory analysis with hSBA using MnB SBA test strains expressing heterologous fHBP/LP2086 variants (PMB1321, variant A22, and PMB2948, variant B24, and PMB2707, variant B44) also was performed on a subset of subjects ages 18 to 25 (N ~19-25). The percent of subjects with hSBA titers ≥ 1:4 predose 1 were 27%, 28%, and 35% for SBA test strains PMB1321, PMB2948 and PMB2707, respectively (Figure 13-1). The percent of subjects with hSBA titers ≥ 1:4 postdose 3 were 95%, 81% and 95% for SBA test strains PMB1321, PMB2948 and PMB2707, respectively (Figure 13-1).

Figure 13-1: The Bivalent rLP2086 Vaccine Demonstrates High hSBA Responder Rates in Young Adults ages 18- 25 Against MnB Strains Expressing Vaccine Heterologous fHBP/LP2086 Variants A22, B24 and B44. All Three Strains were Statistically Selected. PMB2707 is a Primary SBA strain, PMB3632 and PMB2948 are Exploratory SBA Strains



*, indicates SBA strains expressing heterologous fHBP/LP2086 variants to vaccine antigens

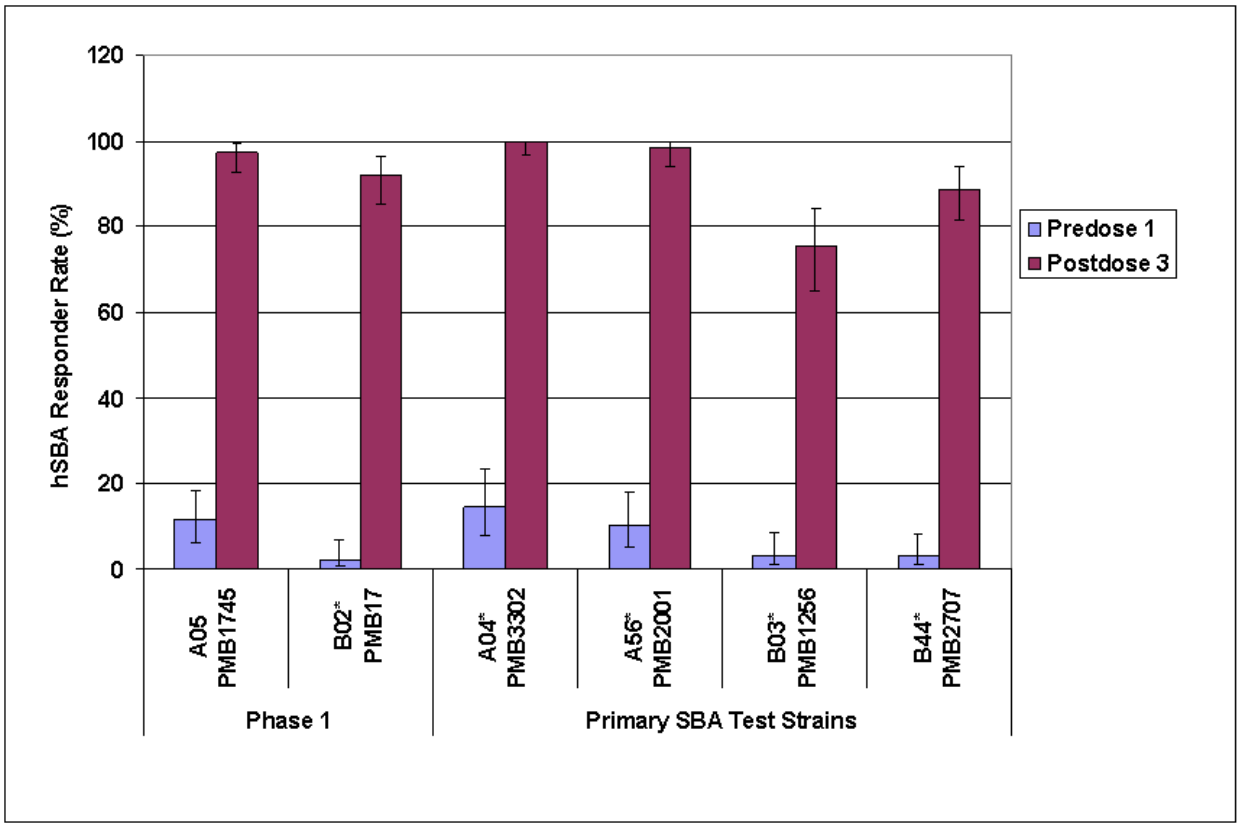
The hSBA data with the bivalent rLP2086 vaccine in adults demonstrated high hSBA responder rates against diverse SBA test strains including strains that expressed fHBP/LP2086 variants heterologous to vaccine antigens.

Study B1971005 is an on-going randomized, single-blind, placebo-controlled trial to assess the safety and tolerability of the bivalent rLP2086 MnB vaccine in healthy adolescents, aged 11 to 18 years. A total of 539 adolescents were enrolled. The 120 µg dose level was chosen to complete the clinical development program and therefore the immunogenicity data described here are from recipients of this dose level only.

Most subjects had no circulating anti-MnB antibody before their first dose of vaccine (Figure 13-2). Following the third dose, the percent of subjects with hSBA titers ≥1:4 to the two phase 1 strains PMB1745 and PMB17 (variants A05 and B02, respectively) were 97.4% and 92%. hSBA responses to the four primary SBA test strains, PMB3302 (A04 variant), PMB2001

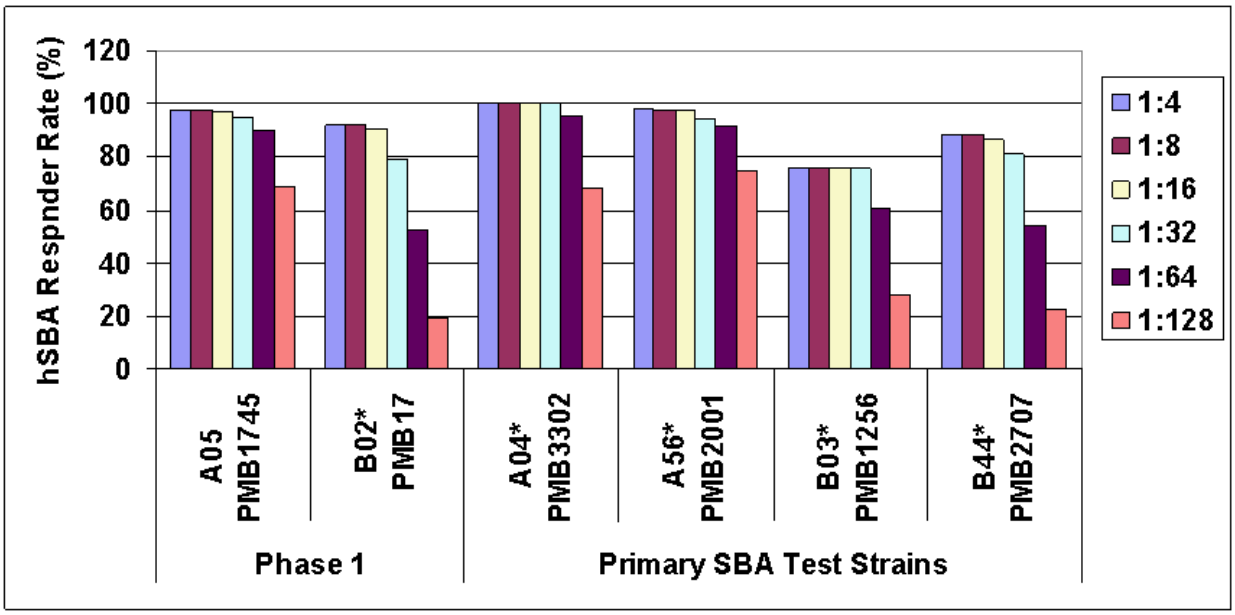
(A56 variant), PMB1256 (B03 variant) and PMB2707 (B44 variant), were also assessed. All four primary SBA test strains and phase 1 strain PMB17 express fHBP/LP2086 variants heterologous to the vaccine variants. The percent of subjects with post-immunization hSBA titers ≥1:4 to strains PMB3302, PMB2001, PMB1256 and PMB2707 strains was 100% , 98%, 76% and 89% , respectively (Figure 13-2).

Figure 13-2: Percentage of hSBA Responders After Immunization with 3 Doses of the Bivalent rLP2086 Vaccine



When responder rates were calculated using different hSBA titer cut-offs (i.e. 1:4, 1:8, 1:16, etc), the robustness of the hSBA response was demonstrated (Figure 13-3).

Figure 13-3: Post-Dose 3 Bivalent rLP2086 Vaccine Responder Proportions at Different hSBA Titer Levels



The MnB SBA test strains used in these vaccine immunogenicity assessments represent both fHBP/LP2086 subfamilies, and four of the six fHBP/LP2086 subgroups (harboring over 90% of all invasive MnB strains). Therefore, the hSBA data presented in Figure 13-1 to Figure 13-3 suggest a vaccine efficacy between 76 to 100% for adolescents and young adults using serum bactericidal antibody activity as the efficacy surrogate. The bivalent rLP2086 vaccine candidate was well-tolerated in the immunized population (data not shown).

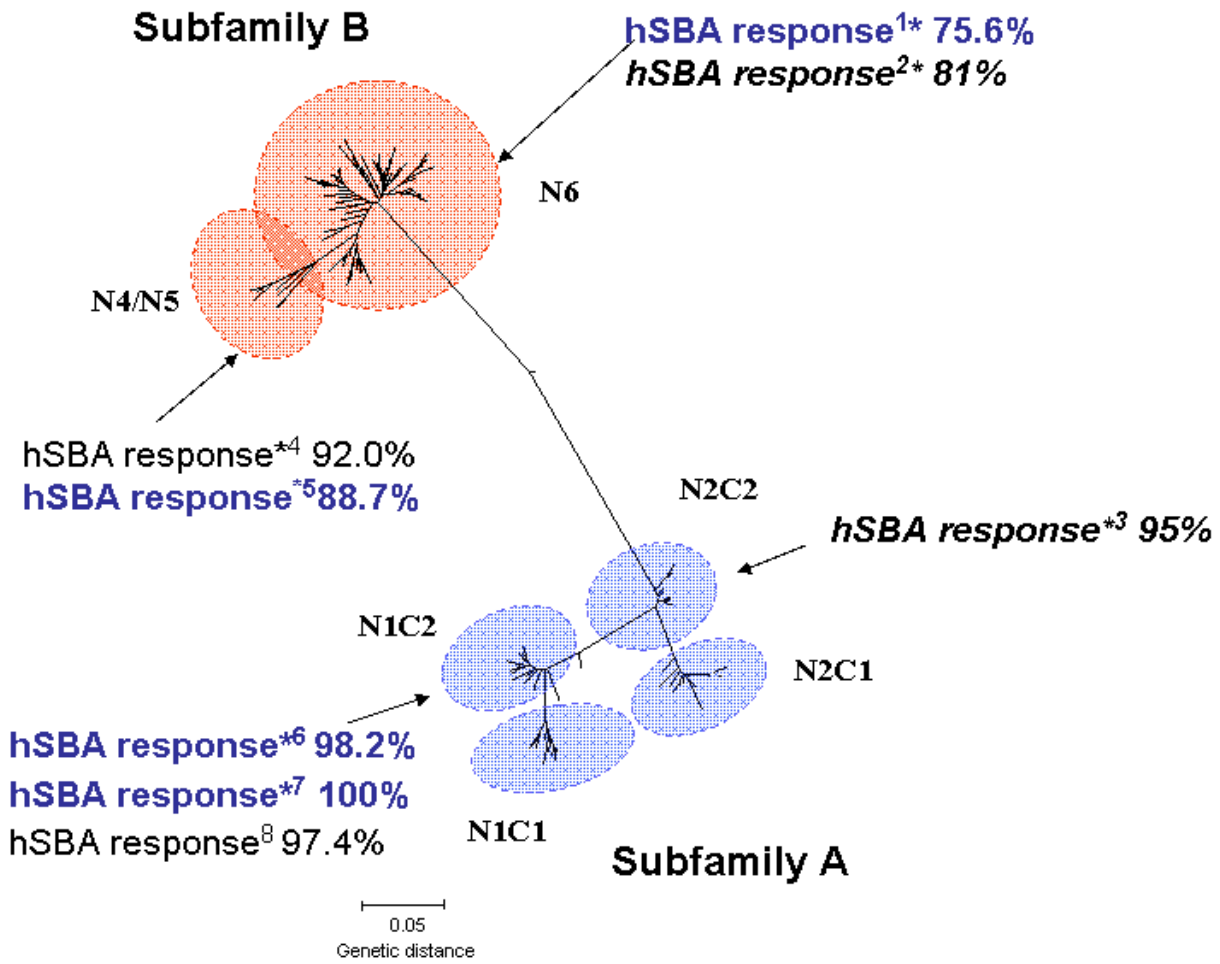
14.0 BRIDGING OF CLINICAL HSBA RESPONDER PROPORTIONS AGAINST PRIMARY AND EXPLORATORY SBA STRAINS TO ASSESSMENT OF VACCINE EFFICACY

Figure 14-1 presents the hSBA responder rates against the four statistically selected primary SBA test strains, two exploratory strains and two phase 1 strains annotated on the protein sequence phylogenetic tree of fHBP/LP2086. As described in Section 12.0, the primary SBA strains PMB3301, PMB2001, PMB1256 and PMB 2707 represent MnB strain expressing variants from the fHBP/LP2086 subgroups N1C2, N4/N5 and N6. The exploratory strain,

PMB1321 expresses an fHBP/LP2086 variant (A22) of the N2C2 subgroup and therefore allows an estimate of vaccine efficacy against MnB strains in this subgroup. The exploratory strain PMB2948 expresses an fHBP/LP2086 variant (B24) of the N6 subgroup and provides confirmation of vaccine responses provided by the primary SBA test strain, PMB1256 (variant B03) which is also of the N6 subgroup.

Robust serum bactericidal antibody responses were observed (76 to 100%) in phase 1 and 2 clinical assessments of the bivalent LP2086 vaccine irrespective of SBA test strain. The robustness of the post-immunization responder rates is particularly noteworthy since the four primary SBA test strains, the two exploratory strains and one of the phase 1 strains all expressed fHBP/LP2086 variant sequences heterologous to the vaccine antigens. Results of hSBA performed with the statistically selected primary SBA test strains showed consistent high responder rates at hSBA titers of 1:4 (correlate of protection) and up to hSBA titers of 1:32. The consistent nature of these observations across the SBA test strains supports the perspective that the four primary SBA strains are appropriately representative of the fHBP/LP2086 sequence diversity and are, therefore, suited for the assessment of the overall efficacy with the bivalent rLP2086 vaccine candidate.

Figure 14-1: Phase 2 hSBA Responder Proportions Using Primary and Exploratory SBA Strains in Relation to fHBP/LP2086 Sequence Diversity



[1,5,6,7]Primary SBA strains PMB1256, PMB2707, PMB2001 and PMB3302, respectively[2,3]. Exploratory SBA strains PMB2948 and PMB1321, respectively. [5,8]Phase 2 indicator strains PMB17 and PMB1745, respectively. The asterisk denotes SBA strains expressing a heterologous fHBP/LP2086 variant compared to vaccine variants. hSBA response is defined as percentage of subjects with an hSBA titer ≥1:4.

In conclusion, Pfizer has demonstrated through pre-clinical and clinical analyses described in this document that the four primary SBA test strains selected by a scientifically rigorous and unbiased approach are appropriately representative of MnB invasive disease strains and will allow demonstration of the benefit of the bivalent rLP2086 vaccine in prevention of MnB

disease. Pfizer has selected four primary MnB SBA test strains using a statistical and unbiased approach that incorporated consideration of both the sequence diversity of Pfizer's LP2086 vaccine antigen and the *in vitro* surface expression levels of fHBP/LP2086. Pfizer has demonstrated that hSBAs with these four primary SBA strains can be used for clinical hypothesis testing in support of phase 3 development and licensure of Pfizer's bivalent rLP2086 vaccine and that the hSBA data generated with the four primary SBA strains are sufficient to provide proof of overall vaccine efficacy. The range of hSBA responses with the four primary SBA test strains (76 to 100%) that was observed during phase 2 clinical development was further supported by preliminary analyses using additional exploratory SBA strains. The robustness and similarity of the clinical hSBA responses observed with the SBA strains, which express fHBP/LP2086 sequence variants heterologous to the vaccine antigens and which cover four of six fHBP/LP2086 sequence subgroups (representing > 90% of all MnB strains), support the perspective that the vaccine will demonstrate efficacy against the large majority of MnB invasive disease strains.

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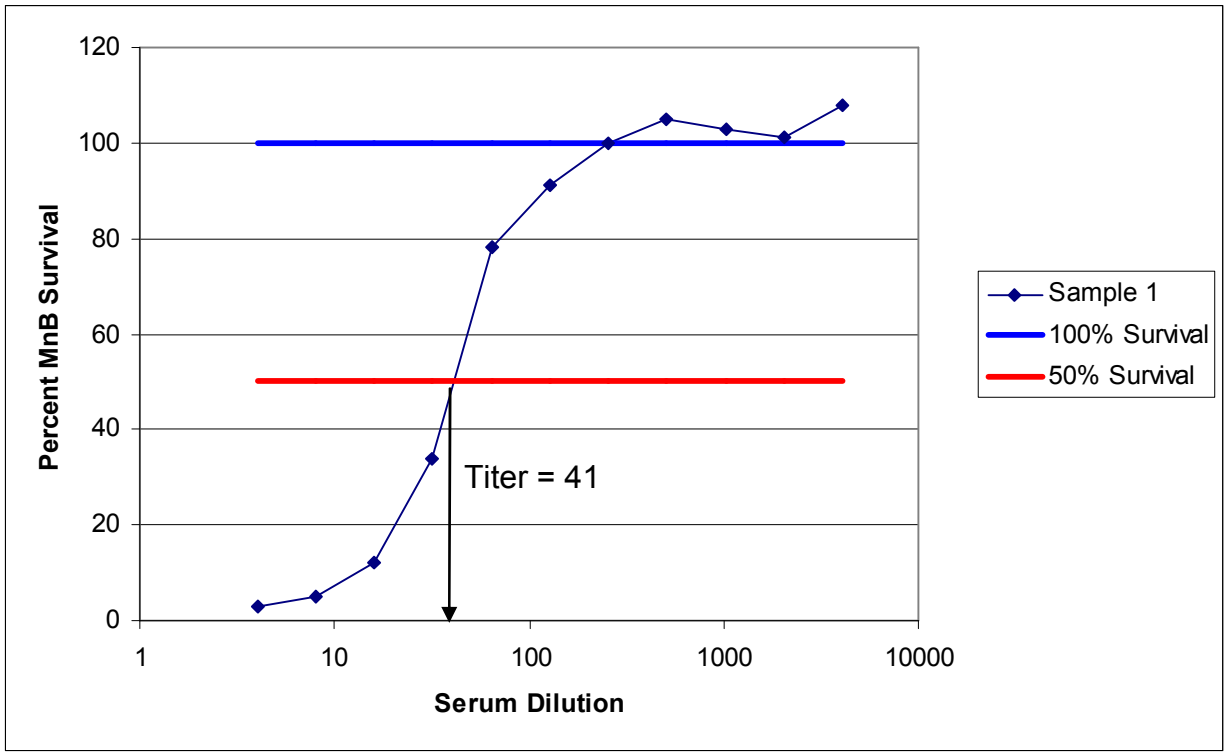
APPENDIX 1: DESCRIPTION OF PFIZER'S HSBAS

In the following section, Pfizer will describe in more details the hSBAs. In particular, emphasis will be placed on how Pfizer controls critical assay reagents, i.e. human complement lots.

The serum bactericidal assay using human complement (hSBA) measures the amount of anti-meningococcal serogroup B (MnB) antibody in serum capable of initiating complement-mediated bactericidal activity. Pfizer's hSBAs are based on the assay described previously by the World Health Organization[16] and Borrow and Carlone[17]. Briefly, test serum is serially-diluted in 2-fold steps and added to 96-well assay plates. MnB SBA test strains and human serum complement are added, initiating the bactericidal reaction. Then, the assay plates are incubated at 37°C for 30-60 minutes (depending on SBA test strain). Bacteria surviving this incubation are diluted and transferred to microfilter plates. Following overnight incubation, the colony-forming units (CFU) are quantified using an Immunospot Analyzer. The raw CFU data are recorded electronically and transferred to a SAS-based data analysis application that calculates the hSBA titer. The hSBA titer is the reciprocal of the interpolated dilution of a test serum that results in a 50% reduction of MnB bacteria (50% baterial survival) compared to a control which represents bacteria not subjected to test serum (100% bacterial survival). It is important to note, that GMTs cannot be compared across different hSBAs as there are no international reference standards to compare titers across different assays. Each hSBA has been optimized to give accurate and reproducible results and titers are dependent on the SBA test strain and complement source. However, the lower limit of detection (LOD) is a titer of 1:4 (the lowest titer that can be determined in SBA) and is the same for all SBAs.

An example of an average hSBA "kill" curve is exemplified in Figure 1.

Figure 1: Typical hSBA Kill Curve



Performance of the hSBAs are controlled using Quality control Sera (QCS) included on every assay plate. The data from the QCS are used for the assay suitability assessment and assay trending over time.

Clinical hSBA Detail and Plate Layout

The hSBA is conducted over a two day period: on day 1 assay plates are prepared for the opsonization and bactericidal phases of the assay, and on day 2 the surviving bacteria (CFU) in samples and controls are enumerated to calculate serum bactericidal antibody titers. A typical assay plate contains 4 serum samples and one quality control serum, QCS (Table 1). Test sera are in rows A-G, and a QCS is in row H of each assay plate. For each assay run, there are two different QCS, one QCS yields titers in the low part of the assay range and the other QCS yields titers in the medium-high part of the assay range. The two QCS are alternated on the assay

plates in the run. Unlike the test sera which are tested in replicate, each QCS is tested as a single determination on each assay plate. Replicate evaluation of test sera is a quality measure to prevent the reporting of sample titers that are extravariable. QCS, on the other hand, are tested individually on each plate for plate suitability purposes but are tested multiple times during an assay run and each QCS titer is compared to pre-determined specification limits. Column 12 of each titer-determining assay plate contains target bacteria and active complement, but no test serum. The average CFU from the column 12 wells is used to calculate the 100% bacterial survival control used in the calculation of the hSBA titer for samples on that assay plate as depicted in Table 1.

Table 1: Example the hSBA Plate Layout

Dilution^{-1}:	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**4096**	**100% Bacterial. Survival**
Well No.:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12 (T_{30})[a]**
A	Clinical Sample 4[b]											Bacteria + Complement
B	Clinical Sample 3											
C	Clinical Sample 3											
D	Clinical Sample 2											
E	Clinical Sample 2											
F	Clinical Sample 1											
G	Clinical Sample 1											
H	Quality Control Serum											

a. Bacteria + human complement, control wells used to determine 100% survival (0% bactericidal killing).
b. The replicate of the sample run in row A is run on another assay plate

APPENDIX 2: COMPLEMENT SOURCES AND SBA TEST STRAIN ARE CRITICAL REAGENTS FOR THE MENINGOCOCCAL SEROGROUP B HSBA

Control of reagents critical to the performance of the MnB SBA is necessary to maintain consistent assay performance over time. The most critical reagents identified for hSBA that affect the outcome of the assay are the MnB SBA test strain banks and the human complement lots. In some cases a MnB strain cannot be developed for high-throughput testing as sufficient human donors with acceptable complement performance cannot be identified (i.e. the strain is non-specifically killed by complement from most donors) or the strain becomes susceptible to non-specific killing after pooling of multiple lots. Pfizer believes that rejection of strains that are too vulnerable to intrinsic bactericidal killing by complement is a conservative approach to assure that the % responder rates truly reflect a representative estimate of the vaccine efficacy against the MnB serogroup.

MnB SBA Test Strain Banks

In order to maintain control of the bacterial strains used in the hSBA, well-characterized, colony purified master bank lots are created for each SBA test strain in sufficient quantity to last the lifetime of the project. When the SBA test strain is initially received, the strain origin is documented. The strains are inoculated on solid media plates and incubated overnight to determine the purity. Several individual colonies from the purified source of bacteria are selected. This initial culture is grown to late-log phase and single use aliquots containing glycerol are stored frozen at -80°C. The master banks are tested for purity and characterized based on WHO recommendations (1999 Laboratory methods for the diagnosis of meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Hamemophilus influenzae*) to confirm species and serogroup. This testing includes biochemical characterization, oxidase testing, agglutination based on capsular serogroup, and Gram staining. The fHBP/LP2086 gene is sequenced and translated to deduce its amino acid sequence, thereby confirming the LP2086 variant designation.

To establish sufficient aliquots for each bacterial strain for routine testing, master bank aliquots were expanded in a two step process by growing bacteria in liquid culture to late log phase,

harvesting the bacteria and cryo-preserving them in single-use aliquots at -80ºC. These final experimental banks are qualified for used in the assay by confirming their species, serogroup and LP2086 sequence.

Human Complement

Human complement is the recommended and accepted complement source for MnB SBA[a]. However, hSBA titers can vary depending on the human complement lot used. As an example, the hSBA titers obtained with multiple complement lots from individual donors and for each of four test sera could vary as much as 5- to 20-fold for a given test serum (Table 2). Consequently, human complement must be tightly controlled through extensive screening and rigorous qualification to ensure consistent assay performance.

Table 2: SBA Titers Can Vary with Complement Lot from Individual Donors

	Titer			
Individual C Lot[a]	**Test Serum 1[b]**	**Test Serum 2[b]**	**Test Serum 3[b]**	**Test Serum 4[b]**
A	54	6	222	20
B	64	16	146	26
C	72	23	129	47
D	76	15	232	27
E	78	14	351	38
F	82	24	199	56
G	89	11	256	27
H	92	20	236	70
I	93	26	166	50
J	101	11	431	34
K	105	37	303	130
L	106	27	126	55
M	106	43	181	175
N	108	30	263	81
O	120	9	384	31
P	122	45	265	87
Q	127	9	287	Not tested
R	146	10	259	42
S	164	10	370	97
T	167	31	343	54
U	178	28	182	48

[a] Borrow, R, et al. 2006. *Neisseria meningitidis* group B correlates of protection and assay standardization. Vaccine 24: 5093-5107.

Table 2: SBA Titers Can Vary with Complement Lot from Individual Donors

	Titer			
Individual C Lot[a]	**Test Serum 1**[b]	**Test Serum 2**[b]	**Test Serum 3**[b]	**Test Serum 4**[b]
V	190	69	425	169
W	191	43	273	60
X	195	26	355	88
Y	195	49	388	183
Z	197	26	203	161
AA	219	20	348	56
BB	234	121	489	182
CC	344	38	668	109
DD	256	52	449	6

a. Individual complement lots in bold have average reactivity against at least 3 of the 4 test sera.
b. Titers in bold are within 1.5 stndard deviations of the geometric mean titer of the test serum.

To generate enough human complement for at least one clinical study, Pfizer pools human serum complement lots from multiple normal donors. Individual human complement lots are obtained from healthy adults aged 18 to 64 years with hemoglobin levels ≥ 12.5 g/dL and hematocrit values ≥ 38%. The complement donors provide a unit volume of blood (approximately 500 mL per bleed) collected without anticoagulants. After clotting, serum is separated by centrifugation at 4°C and immediately removed and frozen at -70 to -80°C in order to maintain complement activity.

The individual human complement lots are screened and selected for pooling as described below:

1. Individual human complement lots are screened initially for intrinsic bactericidal activity. Only those human complement lots lacking intrinsic bactericidal activity (i.e. ≤30% reduction of CFU after 30-min incubation with the complement lot alone) are retained.
2. hSBA titers for human immune reference sera are then determined with the individual human complement lots lacking intrinsic bactericidal activity. A statistical algorithm is applied to identify "average" complement lots as follows: individual complement lots that yield titers within 1.5 standard deviations of a previously established GMT (specification range) for at least 75% of the reference serum samples are candidates for pooling. The previously established GMT specification range for each of the reference sera was determined by testing each serum with approximately 50 human complement lots. hSBA titers for each reference serum were ordered from high to low and then the top

and bottom 10% of hSBA titers were excluded from calculating the GMT specification range (GMT+/- 1.5 SD). Individual complement lots were accepted for further consideration only if their hSBA titers for at least 75% of the reference sera were within the GMT specification range. Individual complement lots passing this screen are candidates for pooling.

3. The pooled complement lots are qualified for use in the hSBA by testing a proficiency panel of immune sera (whose titers cover the breadth of the assay range) in multiple runs and comparing the resulting titers to those obtained from a qualified reference complement lot.
4. A new complement lot is qualified for use in the hSBA based on the hSBA titer bias ratio. To generate data for the bias ratio analysis, a proficiency panel of immune serum is tested in the hSBA at least three times each with the test complement lot and the qualified reference lot. The bias is calculated as the difference in log-titers from the same sample serum, as follows:
   $\text{Bias} = \log_{10}(\text{titer}_{\text{Test}}) - \log_{10}(\text{titer}_{\text{Reference}}) = \log_{10}(\text{titer}_{\text{Test}}/\text{titer}_{\text{Reference}})$

The mean bias is the average of all bias determinations. The equivalence-90% confidence interval on the mean bias should be completely contained within 0.67-1.50, and ideally encompass 1.0, for the test complement lot to be considered equivalent to the reference lot.

The same reference lot of complement is used to qualify new complements lots as they become available. In the example shown in Table 3, complement lot D is the reference lot. After proficiency panel testing over multiple runs, three pooled complement lots , B, C and E, were qualified for use in the hSBA for a given SBA test strain as they had an acceptable SBA titer bias ratio while pooled complement lot A, was not acceptable since the bias equivalence interval exceeded the limits.

Table 3: Equivalence Intervals for the Comparison to Reference Lot D

		90% Confidence Interval	
Pooled Complement Lot	**SBA titer ratio**	**Lower**	**Upper**
A	1.61	1.34	1.92
B	0.86	0.72	1.03
C	1.04	0.87	1.24
E	0.87	0.72	1.04

In summary, the extensive screening, and pooling to yield "average" working pooled complement lots, and rigorous complement lot qualification facilitate (i) the generation of accurate and reproducible titers for individual test sera; and (ii) the reliable comparison of percent responder rates and geometric mean titers (GMTs) between in clinical studies.

Validation of Meningococcal Serogroup B hSBAs

Pfizer will validate the MnB hSBA for the four primary SBA test strains according to ICH guidelines and assess relative accuracy/sample linearity, precision, lower limit of quantitation and assay range. For all hSBAs the lower limit of detection is a titer of 1:4 which is the lowest hSBA titer that can be determined in the assays. Validation experiments will determine an hSBA lower limit of quantitation (LLOQ) that will exhibit adequate relative accuracy and precision per ICH guidelines. The validated LLOQ (which in some cases may be higher than the assay LOD, titer of 1:4) will be used to determine the % hSBA responder rates.

APPENDIX 3: DESCRIPTION OF PFIZER'S FLOW CYTOMETRY ASSAY TO DETERMINE THE *IN VITRO* SURFACE EXPRESSION OF FHBP/LP2086

To determine relative surface expression/density of LP2086 on MnB clinical isolates, we screened murine monoclonal antibodies for their ability to detect LP2086 on the surface of MnB isolates using flow cytometry. One murine monoclonal antibody, mAb 994-11, was identified that recognized a functional epitope common to most LP2086 subfamily A and B lipoproteins. A monoclonal antibody was preferred for the analyses, since sequences of LP2086 differ and a polyclonal serum against one LP2086 variant would not be appropriate to compare LP2086 expression levels across other variants because the serum would recognize different number of epitopes on each strain. MnB isolates were grown under the *in vitro* conditions used to prepare the bacteria for the hSBAs and then exposed to the mAb 994-11. Bound mAb 994-11 was detected with biotinylated anti-mouse IgG, then streptavidin-phycoerythrin and analyzed by flow cytometry. Mean fluoresence intensity (MFI) values were then used to compare and rank order strains by LP2086 surface expression level. The fHBP/LP2086 *in vitro* surface expression level as assessed by the flow cytometry assay correlated with the total fHBP/LP2086 amount in outer membrane extracts determined by an orthogonal method (data not shown). Inter-assay variability of the flow cytometry assay was less than 20%.