TABLE OF CONTENTS

Module 1	Vol. 1 , pg. 1 .
Table of Contents	Vol. 1 , pg. 1 .
Briefing Materials	Vol. 1 , pg. 3 .
Appendix 1	Vol. 1 , pg. 35 .
Appendix 2	Vol. 1 , pg. 41 .
Appendix 3	Vol. 1 , pg. 46 .
References	Vol. 1 , pg. 49 .
Bambini et al., 2009	Vol. 1 , pg. 49 .
Borrow et al., 2006	Vol. 1 , pg. 59 .
Brunelli et al., 2011	Vol. 1 , pg. 74 .
Donnelly et al., 2010	Vol. 1 , pg. 85 .
Feavers and Pizza, 2009	Vol. 1 , pg. 91 .
Findlow et al, 2010	Vol. 1 , pg. 100.
Frasch et al., 2009	Vol. 1 , pg. 111.
Giuliani et al., 2006	Vol. 1 , pg. 116.
Giuliani et al., 2010	Vol. 1 , pg. 122.
Goldschneider et al., 1969	Vol. 1 , pg. 130.
Kelley et al., 2007	Vol. 1 , pg. 150.
Lennon et al., 2009	Vol. 1 , pg. 157.
Litt et al., 2004	Vol. 1 , pg. 166.
Pizza et al., 2000	Vol. 1 , pg. 176.
Santos et al., 2009	Vol. 1 , pg. 181.

Serruto et al.,2010 Vol. 1 , pg. 184.

Tan et al ., 2010 Vol. 1 , pg. 190.

Vu et al., 2011 Vol. 1 , pg. 200.

Novartis Vaccines and Diagnostics, Inc

4CMenB

(Multicomponent Meningococcal B Vaccine (recombinant, adsorbed))

Briefing Materials for the Meeting of the

Vaccines and Related Biological Products Advisory Committee,

7th April, 2011

Property of Novartis Vaccines and Diagnostics, Inc.
350, Massachusetts Avenue,
Cambridge, MA 02139

Contents

LIST OF ABBREVIATIONS 4
LIST OF TABLES 4
LIST OF FIGURES 4
1.0 Executive Summary 5
2.0 Background 9
2.1 Current Meningococcal Serogroup B Vaccines and Obstacles to the Development of a Broadly Protective Vaccine 9
2.2 The Novartis 4CMenB Vaccine Contains Four Distinct Antigens Important for MenB Survival and Pathogenesis 10
2.3.0 The Recombinant Protein Antigens Contained in 4CMenB 10
2.3.1 NHBA 10
2.3.2 fHbp 11
2.3.3 NadA 11
2.3.4 Cross-protection by 4CMenB Protein Antigen Variants 12
2.4 Preservation of the Protective Potential of 4CMenB in the event of Antigenic Loss or Variation 12
3.0 Performance Measures for Protein based Meningococcal B Vaccines 13
3.1 Background 13
3.2 Step 1: Demonstrating that Each Major Component of the Vaccine Induces Bactericidal Antibodies 15
3.3 SBA Is a Valid Surrogate Marker of Protection for 4CMenB 17
3.4 Step 2. Relating Antigen Composition to Killing in the SBA: MATS 17
3.4.1 Overview of MATS Development 18
3.4.2 Positive Bactericidal Thresholds Provide a Link Between Functional Immunogenicity and Strain Coverage 20
3.4.3 Using MATS Relative Potency of NHBA, NadA and fHbp in a Model Population of Serogroup B Strains 21
3.4.4 Comparability of Coverage Estimated by MATS and by hSBA in a Model MenB Strain Population 22
3.4.5 Sera from Immunized Infants Are Able to Kill MenB Strains with Lower Levels of Antigen Expression 24

3.5 Multiple Antigens in 4CMenB Broaden Coverage and Provide a Defense Against Antigen Mutation or Loss 26

3.6 Use of MATS to Assess Region Specific Strain Coverage 28

4.0 Conclusion 28

5.0 References 30

LIST OF ABBREVIATIONS

Abbreviation	Definition
CC	Clonal Complex
ELISA	Enzyme-linked immunosorbent assay
fHbp	Factor H binding protein
HPA	Health Protection Agency
hSBA	Serum Bactericidal Assay using human complement
IMD	Invasive Meningococcal Disease
LLQ/LLOQ	Lower limit of quantitation
MATS	Meningococcal Antigen Typing System
MenB	Meningococcal serogroup B
MLST	Multi-locus sequence typing
NadA	Neisserial adhesin A
NHBA	Neisserial heparin binding antigen
NZ	New Zealand
OMV	Outer Membrane Vesicles
PBT	Positive Bactericidal Threshold
PCR	Polymerase Chain Reaction
RP	Relative Potency
SBA	Serum Bactericidal Assay
ST	Sequence type

LIST OF TABLES

LIST OF FIGURES

1.0 Executive Summary

1.1. Novartis' solution to the problem of antigenic variability of MenB

The diversity and antigenic variability of *Neisseria meningitidis* serogroup B (MenB) strains around the world has been a major challenge to developing a MenB vaccine with broad coverage. Polysaccharide or conjugate vaccines like those which have been successfully licensed for *N. meningitidis* serogroups A, C, Y, and W135 cannot be used for serogroup B because the structure of the B capsular polysaccharide is identical to a self antigen. Vaccines based on protein antigens, such as outer membrane vesicles (OMV), have been effective in controlling MenB outbreaks, proving the principle that bactericidal antibodies against protein antigens of MenB can provide protection. However, OMV vaccines alone lacked sufficient breadth of protection to be generally useful against diverse endemic strains of MenB.

Novartis' solution to this problem is to use four distinct antigen components, all of which are important for the ability of the bacteria to survive in a human host or to cause invasive disease. Three of the 4 major protein components of the Novartis vaccine were discovered by a genomic method termed "reverse vacccinology, " in which the total genome sequence of representative MenB bacteria was searched for proteins capable of inducing a serum bactericidal, and hence potentially protective, response (Pizza *et al.*, 2000). These proteins are: factor H binding protein (fHbp), which protects the bacteria from the action of complement by binding factor H, a negative regulator of the complement cascade (Welsch *et al*, 2003; Ram *et al*, 1999); Neisserial Heparin Binding Antigen (NHBA), which can protect MenB bacteria from the action of complement by binding heparin to the cell surface (Serruto, 2010), and Neisserial Adhesin A (NadA), which is important for attachment to, and invasion of, host cells by the bacteria (Capecchi *et al*, 2005). The fourth major component, OMV from the New Zealand 98/254 strain, was used in a mass vaccination campaign to control a meningitis outbreak in New Zealand (Kelly *et al*, 2007), and contains multiple antigens including PorA, which is an essential transport channel in the meningococcal outer membrane. The candidate vaccine is known as Multicomponent Meningococcal B Vaccine (4CMenB).

The 4 major protein components of the 4CMenB are found in the majority of MenB strains circulating worldwide (Pizza *et al*, 2000; Comanducci *et al,* 2002; Masignani *et al,* 2003; Serruto *et al,* 2010). The proteins themselves are survival factors, and, in addition, bactericidal antibodies against any one of these proteins are sufficient to kill MenB bacteria by complement-mediated lysis (Giuliani, 2006). Therefore, selective pressure that might result in changes to, or loss of, an antigen can be offset by the immune response to the other antigens. Knocking out any one antigen reduces the ability of the bacteria to survive in human blood or in the bloodstream of infant rats (Seib *et al*, 2010). In addition, applying selective pressure by means of specific antibodies and complement did not select for the emergence of mutants resistance to killing, even after repeated rounds of selection (Santos *et al*, 2009). Therefore, the use of multiple antigens, any one of which is capable of inducing a protective response, is an effective solution to the problem of providing broad and durable protection against diverse strains of MenB.

1.2. Assessing the public health impact of a MenB vaccine

To justify the use of a MenB vaccine it is necessary to assess its potential benefit to the public health. Because many thousands of different strains of MenB have already been defined, it is

not feasible to directly test the immune response of clinical trial subjects against every known strain variant. Therefore, Novartis broke the problem into three parts: first, determining whether a bactericidal immune response to the vaccine is induced in clinical trial subjects; second, determining what properties of MenB strains made them susceptible to being killed by the immune response to the vaccine; and third, determining the proportion of MenB strains in a region or country that could be expected to be covered by the immune response to the vaccine. A similar approach was used in pneumococcal conjugate vaccine efficacy trials (Black *et al*, 2000). Some pneumococcal serotypes, such as 4, 6B and 9V, did not cause sufficient numbers of cases of invasive disease to allow a direct demonstration of serotype specific efficacy in a Phase III trial (Black *et al*, 2000). In fact, there were no cases of serotype 4 in this trial. For those serotypes, efficacy was inferred based on the presence of an immune response and the use of capsule typing in epidemiologic studies to demonstrate the presence of the susceptible capsule type among invasive pneumococcal strains.

Novartis measures the presence of an immune response to 4CMenB using the serum bactericidal assay with human complement (hSBA) against a panel of invasive strains specifically selected to assess the functional immune response to each major component of the vaccine. The hSBA measures the ability of immune serum to kill bacteria in the presence of exogenous human complement, and therefore is a more direct measure of potential killing than the ELISA used in the pneumococcal efficacy trial cited above. Because bacteria express many antigens on their surface, and the Novartis vaccine contains multiple antigens, killing by immune serum in the hSBA is not necessarily specific as regards which individual antigen is responsible for killing.

To address this, Novartis developed a competitive hSBA in which the killing effects of antibody to one or more antigens could be specifically inhibited by the addition of soluble antigen to the bactericidal reaction, in order to create a condition of antigen excess and thereby assess the killing based on one antigen at a time (Giuliani *et al*, 2009). The hSBA itself imposes additional selection criteria on target strains for the measurement of immunogenicity. To achieve statistical significance, the competitive hSBA requires a combination of strain and test sera with initial bactericidal titers of 1:32 or greater. Furthermore, to be clinically useful, the target strain must be invasive, naturally occurring, and sufficiently rugged to be compatible with diverse sources of exogenous human complement and to give stable performance in the assay.

Using these criteria, we identified a subset of strains that were killed by immune serum, in which the killing effect resulted predominantly from one of the antigens in the vaccine, and that were suitable for clinical use. These strains were H44/76-SL for fHbp, 5-99 for NadA, M10713 for NHBA and NZ 98/254 for the OMV component. To establish a correlate of protection, we relied on the publications of Goldschneider *et al.* (1969), Holst *et al.* (2003), and Boslego *et al.* (1995), which defined a bactericidal titer of 1:4 with exogenous human complement as an indication of resistance to invasive disease both in case-control studies and in field trials of OMV vaccines against MenB. We further applied 95% confidence limits based on the intermediate precision of our validated hSBA to set our protective cutoff at 1:5, the measured titer that indicates with 95% certainty that the true titer is at least 1:4.

To establish a method that would predict accurately the ability of different strains of MenB bacteria to be killed by the human immune response to 4CMenB, we evaluated various methods of strain typing including genetic typing by Multilocus Sequence Typing (MLST), sequencing of the respective antigen genes, flow cytometric staining (FACS), Western Blot, and an antigen-specific ELISA. We evaluated each method on whether the presence of a

particular sequence type or level of signal would predict killing of MenB bacteria in the hSBA by immune serum from human subjects immunized with 4CMenB. For fHbp, NHBA and NadA, we found that neither genotype alone nor quantity of antigen alone was sufficient to predict killing of bacteria in the SBA, and that we needed a method that was sensitive to both parameters was necessary. Therefore, we selected a sandwich ELISA, using specific polyclonal antibodies against the individual vaccine components, as the typing method that was most relevant for these antigens. For PorA, we used conventional methods of typing the VR2 region of PorA. We combined these evaluations into a single system for characterizing MenB bacteria, which we termed the Meningococcal Antigen Typing System, or MATS.

1.3. The use of MATS to predict bactericidal killing of diverse MenB strains and estimation of strain coverage.

The development of vaccines based on capsular polysaccharides, such as *H. influenzae* type B, *S. pneumoniae*, and serogroups A, C, W135 and Y of *N. meningitidis* benefited from the previous establishment of capsule typing methods. For MenB, because three of the four major antigens in the vaccine were newly discovered and no typing method existed, we developed MATS. To determine whether MATS could be used to predict bactericidal killing in the hSBA, we sought to bridge the MATS typing results with killing of MenB strains in the hSBA.

Because of the large number of different MenB strains as well as the large specimen volume and effort required for the hSBA, initially we used pooled serum from adult, adolescent, and infant vaccinees to assess the ability of MATS to predict susceptibility of MenB bacteria to killing in the hSBA. First, we compared the hSBA titers of sera from individual adult subjects and pools of the same sera against a panel of 15 well-characterized MenB strains. We observed that, for pools comprised of equal volumes of serum from each subject, the hSBA titer of the pool generally equaled the arithmetic mean titer of the individual subject samples. We therefore defined positive killing for the pooled serum samples as a titer of 1:8 or greater, so that it would be more likely that the majority of individual subjects in the pool had hSBA titers of at least 1:4. Next, we evaluated 124 invasive MenB strains, obtained from many different countries and selected to represent a broad range of diversity of MenB, in hSBA with pooled sera from immunized adults. A subset of 57 of these strains then was tested with pooled serum from immunized infants. We defined strains as “killed” in SBA if the pooled serum samples gave a titer of 1:8 or greater, or if a fourfold difference in titer was observed between pre- and post- immunization serum pools.

By comparing MATS results from strains that were killed in the hSBA with MATS results from strains that were not killed, we found that strains were more likely to be killed if they had a MATS value above a minimum level for at least one of the three antigens: fHbp, NHBA, or NadA. We termed these levels the “Positive Bactericidal Threshold,” or PBT. To use MATS to predict whether or not strains would be killed in hSBA, we considered strains as predicted to be killed if they had any one of the antigens fHbp, NHBA, or NadA above the PBT, or had the PorA VR2 allele 1.4. Applying this model to 57 strains that were tested in the hSBA with pooled sera from infants immunized at 2, 4, 6 and 12 months of age, we observed that MATS had a positive predictive value of 89%, a negative predictive value of 79%, and an accuracy of 86%. Finally, we tested sera in the hSBA from 40-100 individual subjects immunized at 2, 4, 6, and 12 months of age with 4CMenB, against a panel of strains that MATS predicted would be killed. We found that the majority of individual subjects had hSBA titers $\geq$5 against these strains.

To simplify the analysis, we did not consider interactions of antigens on strains where more than one of the major antigen components of the vaccine was present. Recent data indicate that, where more than one antigen on the same MenB strain is targeted in the hSBA, antibodies to weakly crossreactive or sparse antigens, that do not kill independently, nonetheless may kill when acting together (Vu *et al*, 2010). In addition, the OMV component contains additional antigens, such as PorB, that are not typed in MATS but that can evoke bactericidal responses. Our method of analysis of MATS results does not consider these possibilities, and therefore is likely to underestimate the coverage potential of the vaccine. Using a larger panel of 124 strains tested in MATS and in hSBA with pooled serum from adults, we found that 85% of strains with a MATS result for a single antigen above PBT were killed in the hSBA. Among strains with two antigens above PBT, 94% were killed in hSBA, and among strains with 3 antigens above PBT, 100% were killed. Thus, targeting multiple antigens with 4CMenB can increase the efficiency of killing as well as providing a defense against loss or mutation of the target antigens. Among infants, where the breadth of crossreactivity of antibody responses is often significantly less than in adults, targeting multiple antigens is an effective way to provide coverage of diverse strains (Brunelli *et al*, 2011).

As the development and implementation of 4CMenB continues, health authorities in different countries or regions may desire to perform a local assessment of the potential public health impact of the vaccine. This can be accomplished in a two step process. First, researchers will determine the ability of the vaccine to induce bactericidal responses in clinical trial subjects in the country/countries of interest, using the standardized indicator strain panel which provides a link to the comparable data collected in other countries or regions. Second, strain typing by MATS can be used to estimate the proportion of MenB strains in a country or region that are likely to be killed by antibodies against 4CMenB. Together, these data can be used to assess the potential for public health impact of the vaccine. Novartis has transferred the MATS method to 7 national reference laboratories in the US and Europe. A survey of more than 1000 MenB strains isolated in 5 European countries in 2007-8 has been completed by the European collaborating laboratories. In a study that is in progress at the US Centers for Disease Control and Prevention, and is expected to finish within 2011, more than 800 MenB strains isolated from 2003-8 in the US are being evaluated for their potential coverage by 4CMenB.

2.0 Background

2.1 Current Meningococcal Serogroup B Vaccines and Obstacles to the Development of a Broadly Protective Vaccine

Currently, there are no broadly effective vaccines available in the US or Europe for prevention of disease caused by MenB. Although polysaccharide and glycocongugate vaccines have been proven efficacious against meningococcal serogroups A, C, W-135 and Y, the use of the serogroup B capsular polysaccharide is not possible because it is structurally identical to human fetal brain-cell adhesion molecules (Finne *et al.*, 1983). Although several vaccine candidates have undergone testing, most have met with limited success (reviewed in Tan *et al.*, 2010; Granoff, 2010).

As a result, an alternative approach to MenB vaccine development has focused on surface-exposed, outer membrane proteins presented as outer membrane vesicles (OMVs) as vaccine antigens. These OMV-based vaccines, one of which was developed by the Norwegian Institute of Public Health (NIPH) in Norway (MenBvac) and another by the Finlay Institute in Havana, Cuba, elicit bactericidal antibody responses, and clinical studies have demonstrated their efficacy against systemic MenB disease (Bjune *et al.*, 1991; Milagres *et al.*, 1994). Novartis Vaccines and Diagnostics has, in collaboration with NIPH, developed a MenB OMV-based vaccine (MeNZB™) prepared from the epidemic New Zealand strain NZ98/254 (B:4:P1.7-2,4). This vaccine was developed for and used in New Zealand in a national vaccination campaign to combat the ongoing MenB epidemic. Approximately 1 million individuals, aged 6 weeks to 19 years, were immunized with three doses of MeNZB. The initial effectiveness of MeNZB was estimated to be 73%; subsequent analyses have been consistent with this estimate (Kelly *et al.*, 2007).

The experience with MenB OMV-based vaccines has been instrumental in demonstrating the safety and effectiveness of meningococcal outer membrane proteins as vaccine antigens, and in demonstrating that *in vitro* serum bactericidal activity directed against MenB protein antigens correlates with protection (Holst *et al.*, 2003). However, the use of the OMV-based vaccines has been limited to epidemic settings because the protective, immunodominant protein antigens in the OMVs (mainly PorA) show sequence and antigenic variability and, as a result, induce protective antibodies that are primarily strain-specific especially in infants (Tappero et al, 1999; Holst *et al.*, 2009; Tondella et al., 2000).

Meningococci use a variety of mechanisms to evade natural immunity that are based on a highly fluid genome (Harrison, 2006). This striking feature of meningococcal biology is also one of the most significant obstacles to the development of broadly protective MenB vaccines. Antigenic variation by the meningococci can be achieved through acquisition of foreign DNA by horizontal gene transfer, or through autologous recombination (Howell-Adams and Seifert, 2000; Andrews and Gojobori, 2004). In addition, these bacteria are able to vary antigen expression via several mechanisms that include phase variation (Stephens et al., 1991; Saunders et al., 2000; Claus et al., 2004; Uria et al., 2008). As a consequence of antigenic variation, outer membrane proteins (OMPs) such as PorA, OpaC, and FetA, although effective as targets of bactericidal antibodies, generally provide only variant specific protection. Thus, the development of vaccines against such highly diverse and variable MenB strains required a paradigm shift from traditional vaccinology.

2.2 The Novartis 4CMenB Vaccine Contains Four Distinct Antigens Important for MenB Survival and Pathogenesis

Novartis' development of recombinant protein-based 4CMenB was intended to overcome these limitations by using the whole genome sequence of *N. meningitidis* serogroup B (MenB) to search for previously undiscovered novel vaccine candidates that were highly conserved across strains, and that could induce broadly protective bactericidal antibodies. This genomics-based approach, termed reverse vaccinology, identified a number of protein targets that were suitable for inclusion in a meningococcal serogroup B vaccine. These protein antigens included factor H binding protein (fHbp), Neisserial adhesin A (NadA) and Neisserial heparin-binding antigen (NHBA) (Giuliani *et al.*, 2006; Feavers and Pizza, 2009; Bai and Borrow, 2010). These vaccine antigens are important for both evasion of immune killing and pathogenesis, and are found in the majority of global MenB strains.

To increase the potency of the immune response and to facilitate large-scale vaccine manufacturing (Giuliani *et al.*, 2006), the fHbp protein was combined with the accessory protein GNA2091 and the NHBA protein was combined with GNA1030 to create two recombinant fusion proteins. NadA was included as single antigen as it did not perform well when fused to a partner, perhaps due to the disruption its native trimeric organization (Capecchi *et al*., 2005). The rationale behind combining three different antigens was to increase the spectrum of vaccine coverage, minimizing the possibility of bacterial immune evasion through mutation or loss. Pre-clinical investigations demonstrated that antibodies directed against each of these vaccine antigens are able to kill target MenB bacteria in SBA (Giuliani *et* al., 2006). The fHbp and NHBA fusion proteins, and NadA drug substances were formulated with OMVs derived from New Zealand strain NZ98/254 (OMV NZ) and then adsorbed to aluminum hydroxide as an adjuvant. The safety and immunogenicity of this final formulation, named 4CMenB, was subsequently evaluated in late Phase 2 and Phase 3 studies in infants, toddlers and adolescents. No significant safety concerns were identified in the clinical studies and recipients of 4CMenB from all age groups developed functional bactericidal antibodies to MenB strains expressing each of the homologous vaccine antigens as well as across heterologous MenB strains.

2.3 The Recombinant Protein Antigens Contained in 4CMenB

2.3.1 NHBA

NHBA (Neisserial Heparin Binding Antigen) is predicted to be a surface-exposed lipoprotein with a predicted molecular weight of 50 KDa. The protein is specific for *Neisseria* species and recent studies have demonstrated that the protein binds heparin *in vitro* through an arginine-rich region and this property correlates with increased survival of the unencapsulated bacterium in human serum (Serruto *et al.*, 2010). The *nhba* gene is detected in all neisserial strains examined thus far (Bambini S 2009; Jacobsson S 2006; Lucidarme 2009; Lucidarme 2010), and gene sequences from genetically diverse group B strains reveal variable segments of NHBA although the amino- and carboxyl-terminal regions are highly conserved. The vaccine-formulated NHBA derives from the NZ98/254 serogroup B strain and is present in 4CMenB as a fusion gene.

A recombinant NHBA elicited complement-mediated bactericidal activity against a variety of group B strains (Pizza *et al.*, 2000; Giuliani *et al.*, 2006). The resulting titers were of similar magnitude to that of control mice immunized with an outer membrane vesicle vaccine that, in humans, is known to confer protective immunity against developing meningococcal disease.

Previous studies reported that antibodies against NHBA may confer protection in humans based on opsonophagocytic immunity and passive protection in an *ex vivo* model of meningococcal bacteremia (Plested and Granoff, 2008; Plested *et al.*, 2009). Finally, NHBA is immunogenic in patients recovering from meningococcal disease and is able to induce antigen specific bactericidal antibodies in adult subjects immunized with the 4CMenB vaccine (Serruto *et al.*, 2010).

2.3.2 fHbp

fHbp (factor H binding protein) is a surface-exposed 29 kDa globular lipoprotein composed of two β barrels connected by a short linker and is bound to the outer membrane by an N-terminal lipid anchor (Cantini *et al.*, 2009; Schneider *et al.*, 2009). Analysis of the *fHbp* gene showed that it naturally occurs in three structurally distinct variants (variants 1 through 3). This protein is also known as rLP-2086, and has been classified in two subfamilies, A and B, which correspond to variants 2 and 3 combined and variant 1, respectively (Fletcher *et al.*, 2004; Zhu *et al.*, 2005). Amino acid identity is 74.1% between variants 1 and 2, 62.8% between variants 1 and 3, and 84.7% between variants 2 and 3. Sequences within each variant group are generally highly conserved (Masignani *et al.*, 2003). Variant 1 of fHbp is represented in the 4CMenB vaccine as a fusion protein and is derived from the MC58 MenB strain.

The 150 KDa alternative complement pathway regulatory factor H, has been identified as a ligand of all 3 variants of fHbp (Madico *et al.*, 2006; Schneider *et al.*, 2006). The amount of factor H-binding to bacterial cells correlates with the amount of fHbp expressed on the cell surface. Consistent with its protective role in invasive MenB strains, expression of fHbp was demonstrated be key for survival *ex vivo* in human blood and human serum, particularly in high-expressing strains (Seib *et al.*, 2009; Welsch *et al.*, 2008).

2.3.3 NadA

NadA (Neisserial adhesin A) is a surface-exposed oligomeric protein that binds epithelial cells *in vitro* and promotes adhesion and invasion into Chang epithelial cells when expressed ectopically in *E. coli* cells (Capecchi *et al.*, 2005). The *nadA* gene is known to be present in approximately 50% of meningococcal isolates globally and is more frequently associated with strains belonging to hypervirulent clusters (Comanducci *et al.*, 2002). It is ubiquitous in members of three of the four major non-serogroup A meningococcal hypervirulent clusters, namely, the ET-5 complex, the ET-37 complex, and the cluster A4, whereas it is absent in lineage III strains. NadA is well conserved, and five alleles have been identified (Comanducci *et al.*, 2004; Lucidarme, 2010). The gene product of *nadA* allele 3 is represented in the 4CMenB vaccine and derives from the 2996 serogroup B strain.

NadA induces strong bactericidal antibodies and is protective in the infant rat model (Comanducci *et al.*, 2004). Sera from young children convalescing from meningococcal disease, but not from healthy children, react strongly with the NadA protein (Litt *et al.*, 2004).

The level of NadA expression is highly variable because the *nadA* gene is subject to complex regulatory control and is highly dependent on various environmental cues (Comanducci et al., 2002; Martin et al., 2005; Metruccio et al., 2009; Schielke et al., 2009).

2.3.4 Cross-protection by 4CMenB Protein Antigen Variants

All of the major antigens of the Novartis 4CMenB vaccine (fHbp, NadA, NHBA and PorA P1.4) exhibit some natural sequence variation that may result in varying levels of cross-reactive potential (Bambini *et al.,* 2009).

The fHbp antigen is expressed by virtually all serogroup B meningococcal strains examined thus far. However, a small number of *N. meningitidis* serogroup C strains were recently identified that do not express fHbp (Harris *et al*., 2011). The level of fHbp expression varies considerably from strain to strain (Fletcher *et al.*, 2004; Masignani *et al.*, 2003) and is increased during oxygen limitation in a FNR-dependent manner (Oriente *et al.*, 2009). The *fHbp* gene naturally occurs in three structurally distinct variants (variants 1 through 3). Bactericidal activity is variant specific and antibodies against each of the three variants generally are not cross-protective (Masignani *et al*., 2003).

Of the five NadA sequence variants, NadA-1, NadA-2, and NadA-3 are highly cross-protective (Comanducci *et al*., 2002). A recombinant NadA-3 is represented in the 4CMenB vaccine. The NadA-4 is associated with carriage strains which cross-reacts poorly with variants 1, 2, and 3. NadA-5 is more closely related to NadA-4 than to the other forms, and is most associated with ST-213 clonal complex isolates. The NadA-5 cross-reacts weakly with the NadA1-3 variants.

Although the NHBA component of the 4CMenB naturally occurs in several genetically distinct forms (Bambini *et al*., 2009), antibodies directed against the different NHBA forms show cross-reactivity in SBA for most strains tested in pre-clinical studies (Giuliani *et al*., 2006), and cross-protection in the infant rat challenge model of bacteremia (Welsch *et al.,* 2003).

Several studies have demonstrated that immunity induced by OMV vaccines is predominantly mediated by bactericidal antibodies directed against variable region 2 (VR2) of the PorA outer membrane protein (OMP; Tappero *et al*., 1999; Martin *et al*., 2006), although minor OMV components may contribute to activity. Nonetheless, the degree of this serosubtype specific response appears to vary with age with the most cross-reactive SBA responses observed in adults (Tappero *et al.,* 1999; Holst *et al*., 2009). The OMV component in 4CMenB derives from the New Zealand epidemic NZ98/254 strain which harbors a P1.4 VR2 serosubtype.

2.4 Preservation of the Protective Potential of 4CMenB in the Event of Antigenic Loss or Variation

Novartis selected multiple antigens for inclusion in 4CMenB to strengthen the protective activity of the vaccine antigens, increase the breadth of MenB strains targeted by the vaccine, and limit the potential for selection of vaccine escape mutants.

To determine the importance of the antigens included in 4CMenB in the growth, survival and fitness of MenB, we generated a mutant lacking all three of the major recombinant protein antigens (fHbp, NHBA and NadA), as well as strains lacking the antigens individually (Seib *et al*., 2010). Our results show that abrogation of expression of these antigens in *Neisseria meningitidis* results in reduced growth *in vitro*, increased sensitivity of the bacterium to lysis by antibody and complement, and reduced fitness both in *ex vivo* models of infection and in an *in vivo* infant rat competitive index assay.

Because of the extensive diversity observed in MenB strains for antigens such as the porins and pili, it is possible that mass vaccination might create selective pressure that would lead to resistance to the vaccine. Therefore, we assessed the induction of resistance by immune selective pressure from bactericidal antibodies elicited in humans by the Novartis MenB vaccines.

MenB bacteria were exposed in vitro to bactericidal antibodies from immunized donors, and exogenous human complement, to a level that would result in killing of approximately 90% of the bacteria. The surviving bacteria were then plated, grown, and treated again with antibody and complement for a total of 5 cycles of selection. No development of resistance was seen, in that the ability of the immune serum to kill the bacteria was not significantly reduced (Santos *et al.*, 2009). While it is difficult to model *in vitro* the cumulative effects of the general use of a vaccine in a large human population over a sustained period, these studies show that resistance to bactericidal antibodies raised by the MenB vaccine was not easily induced in vitro.

3.0 Performance Measures for Protein-based Meningococcal B Vaccines

3.1 Background

Development of a MenB vaccine has lagged behind the other serogroups because of the need to target surface protein antigens instead of polysaccharide, since the serogroup B polysaccharide is not immunogenic in humans. In Appendix 1 and Section 2.2, we describe how surface proteins of MenB are targets for bactericidal antibodies and how antibodies against them can provide protective immune responses. We showed in Section 2.3 that MenB strains can be diverse in both the sequence and level of expression of surface proteins that are targets of bactericidal antibodies. Because of this diversity, without an efficient method to identify susceptible strains, it would be necessary to perform SBA on large panels of isolates in large numbers of human subjects. This is impractical, first, because the great diversity of MenB populations makes it unlikely that a sufficient number of different MenB strains could be tested in the SBA on human subjects to assess the extent of cross-reactivity among strains of the bactericidal response to this vaccine. Second, the SBA itself is labor-intensive and difficult to standardize (Borrow *et al.*, 2005, 2006). Since the use of human complement is essential to obtain accurate results in the meningococcal SBA (Appendix 2), and since most human complement sources have endogenous bactericidal activity against one or more MenB strains, finding suitable complement donors to support large panels of diverse MenB isolates is likewise labor-intensive and costly. Finally, the volume of sera available from the target infant population allows for the performance of only very limited numbers of bactericidal assays.

Therefore, Novartis broke the problem into parts: first, determining whether a bactericidal immune response was induced in clinical trial subjects; next, determining what properties of MenB strains made them susceptible to being killed by the immune response to the vaccine; and, finally, determining the proportion of MenB strains in a region or country that could be expected to be covered by the immune response to the vaccine. A parallel approach has been used for pneumococcal conjugate vaccines (Black et al., 2000). Pneumococcal serotypes, such as 4, 6B and 9V, did not cause sufficient numbers of cases of invasive disease to allow a direct demonstration of disease efficacy in a Phase III trial (Black et al., 2000). For those serotypes, efficacy was inferred based on the presence of a functional immune response and the use of capsule typing to demonstrate the presence of the susceptible capsule type among

invasive pneumococcal strains. Similarly, efficacy cannot be assessed directly against every class of MenB strains representative of each 4CMenB antigen. We therefore decided to use a similar approach based on demonstration of a functional immune response and using typing to identify susceptible strains.

Step 1. Measure immune response: We first needed to demonstrate that each major component of the vaccine was independently able to induce a bactericidal, and therefore potentially protective, antibody response (Step 1 in Figure 1). For the pneumococcal and meningococcal glycoconjugate vaccines, single strains representing each serotype or serogroup were used to assess functional immunogenicity. In a similar manner, we have identified four MenB strains that are predominantly killed by human antibodies that recognize specific protein components of 4CMenB.

Step 2. Perform strain typing: We next needed a method to categorize field strains of MenB into those that potentially could be killed by antibodies to the vaccine and those that could not (Step 2 in Figure 1). For this purpose we developed a phenotyping method (Meningococcal Antigen Typing System or MATS) that could detect both the quality of the immunological match between the vaccine and the target bacteria, and the quantity of antigen expressed by each strain. This method is being used to type large numbers of MenB isolates on a geographic basis.

To provide a bridge between the induction of bactericidal antibodies (Step 1) and the potential for protection against different strains of MenB (Step 2), we demonstrated that the phenotypes we assigned to different MenB strains could predict whether the strains could be killed by bactericidal antibodies elicited by the vaccine. Once we established the link between the phenotype and the ability of the bacteria to be killed by antibodies against the vaccine, we could then use the phenotyping method to survey different countries or regions to determine the likely proportion of covered strains among endemic MenB populations (Figure 1). This allows us to estimate the likely public health impact of implementing the vaccine.

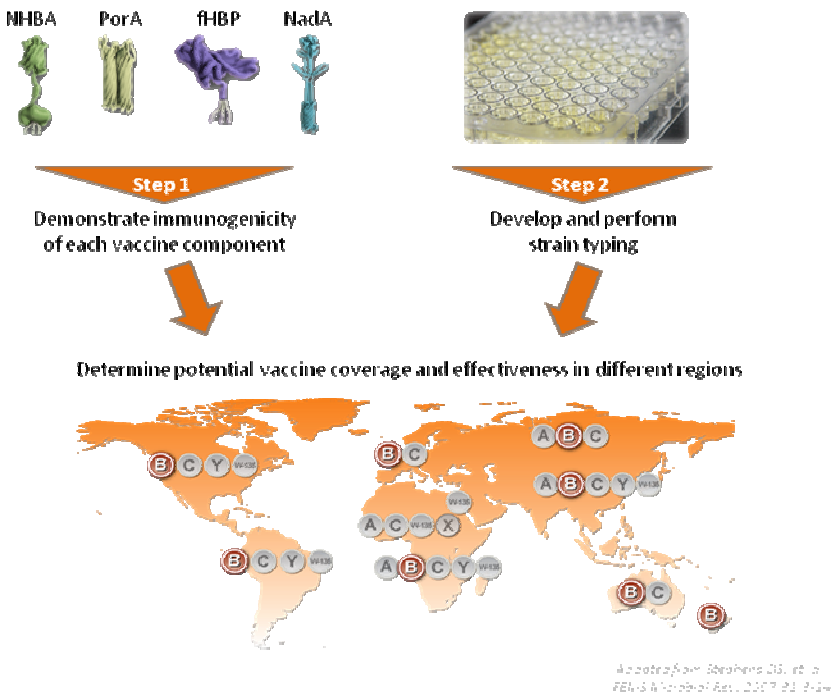


Figure 1. **Novartis strategy to evaluate 4CMenB performance.**

3.2 Step 1: Demonstrating that Each Major Component of the Vaccine Induces Bactericidal Antibodies

Our first task in evaluating the performance of the MenB vaccine was to measure functional immune responses in a cross-section of subjects. The SBA reflects the cumulative effect of all of the antibodies present in a sample which may interact with a target strain of bacteria. If a vaccine contains a mixture of different protein antigens, immune sera may contain antibodies against all of them, bacteria may express more than one of them, and therefore more than one antigen may participate in killing of the bacteria in the SBA. 4CMenB contains multiple, immunologically distinct antigens, each of which is capable of acting as a target for bactericidal antibodies. This feature of the SBA makes it very difficult to understand what each individual antigen is contributing to the function of the vaccine. Therefore, we have taken a subtractive approach to better understand the contribution of individual components of 4CMenB (Giuliani *et al.*, 2010). Using competitive inhibition of the SBA by individual antigens, we have identified four MenB strains that are predominantly killed by human antibodies that recognize specific protein components of our MenB vaccine (Figure 2). This has allowed us to demonstrate that each major component of the vaccine is able to induce bactericidal antibodies in human subjects. This is a necessary first step toward the registration of a multi-component, protein-based MenB vaccine with the potential for broad coverage of diverse MenB strains.

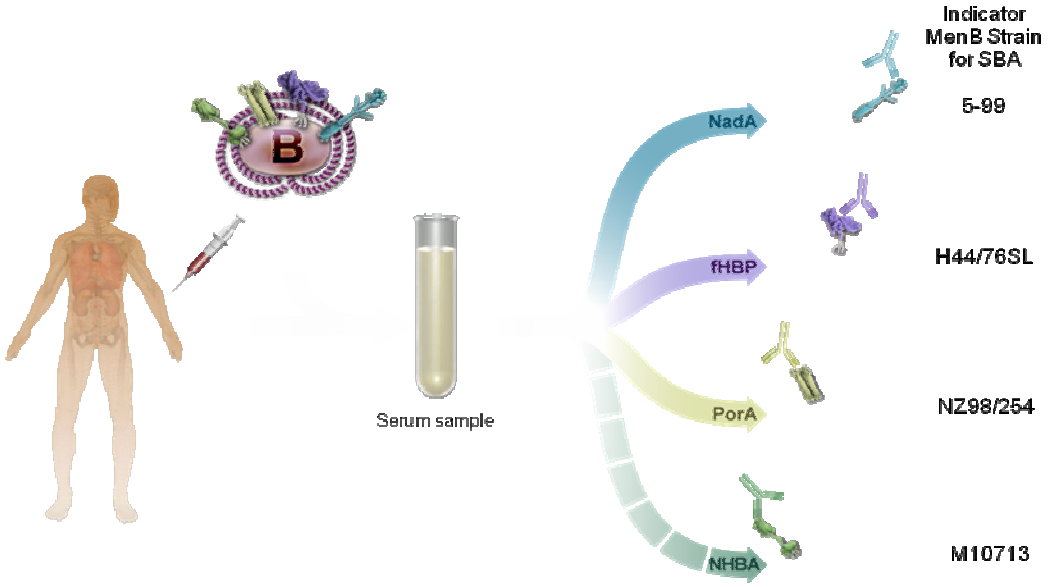


Figure 2. **Evaluating seroconversion after 4CMenB immunization.** The ability of each of the four major antigenic component of the vaccine to induce bactericidal antibodies (surrogate for protective immunity) is tested in SBA using human complement. Responses are tested against MenB "indicator" strains which were selected so that killing of each strain absolutely requires bactericidal antibodies directed against a different one of the major vaccine antigens.

The strains identified to assess functional immunogenicity elicited by the fHbp, NadA, NHBA and P1.4 PorA immunodominant antigen of OMV NZ (strain NZ98/254) are virulent strains of serogroup B meningococci that were isolated from cases of invasive disease (Table 1).

Table 1. **Indicator strains used to measure antigen-specific SBA responses to the four components in the vaccine (PorA, NadA, fHbp, NHBA).** The phenotype, MLST Sequence Type (ST) and Clonal Complex (CC) are provided for each indicator strain. The subvariants of each vaccine antigen and the PorA serosubtype present on each strain is also provided. The fHbp nomenclature used to describe the different variants and sub-variants of fHbp use a prefix (1, 2, or 3) that defines the variant and a suffix (e.g. 14) that defines the subvariant and distinguishes each by as little as a single amino acid change (Bambini *et al.*, 2009)..

Indicator Strain	Vaccine Antigen	Phenotype	ST	CC	Vaccine Antigen Genotype Profile			
					PorA	fHbp	NadA	NHBA
NZ98/254	PorA1.4	B:4:P1.7-2,4	42	41/44	P1.4	1.14	-	2
5/99	NadA	B:2b:P1.5,2	8	8	P1.2	2.23	+	20
44/76-SL	fHbp	B:15:P1.7,16	32	32	P1.16	1.1	-	3
M10713	NHBA	B:NT:P1.17,16-3	136	41/44	1.16-3	2.24	-	10

Antigen specific killing of these strains was demonstrated by competitive inhibition studies in which the binding of bactericidal antibodies to target antigens is inhibited by competitive binding of excess purified protein antigens. Using this assay, killing by bactericidal antibodies was shown to be directed against only one of the major vaccine antigen components (Giuliani *et al.*, 2010). Figure 3 shows that killing in the SBA can be inhibited by a single vaccine component in an antigen specific manner.

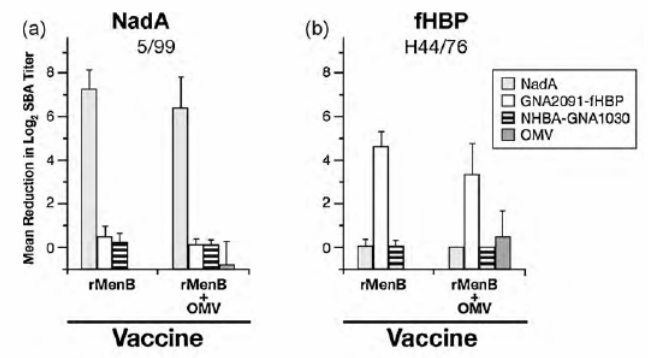


Figure 3. Specific inhibition of killing in the SBA by individual vaccine components is different for different strains, depending on their antigenic makeup. Competitive effect of vaccine components in SBA against a) 5/99 indicator strain for NadA b) H44/76 indicator strain for fHbp. The vaccines used were MenB recombinant proteins only (rMenB) or 4CMenB (rMenB+OMV). The mean difference in log2-transformed titers are shown, comparing SBA titer without competitors or with the indicated competitors added. Error bars denote 95% confidence limits of the mean. Confidence limits that include 0 indicate that on average there is not a statistically significant difference between the mean titers with and without competitor.

The basis for antigen specificity is thus related to the antigen "profile" of each strain. For example, inhibition of bactericidal activity against H44/76, a strain that harbors the same genetic subvariant of fHbp as that present in the vaccine (subvariant 1.1), but in which the other 3 major antigens are mismatched or absent (Table 1), was achieved only by pre-incubation of sera with excess purified fHbp antigen.

The inherent properties of the SBA put some important limitations on its usefulness for demonstrating these types of antigen specific effects. First, the low precision of the SBA means that in order for a reduction in killing to be significant, it normally should be in the

range of at least fourfold or greater. That means in turn that the response without inhibitor must be at least fourfold or more above the limit of detection. Not all combinations of subject/strain can meet these criteria. Second, the inherent lack of specificity of the SBA means that individual subjects may have naturally acquired antibodies that are able to kill the target strain alone or in combination with antibodies induced by the vaccine. Furthermore, individual subjects may make an immune response that reacts with more than one vaccine component (Weynants et al., 2007). In these situations, a single component may not be able to produce significant inhibition on its own. Finally, antibodies against different epitopes on the same antigen or against different antigens may cooperate, or synergize, in killing the strain in the SBA (Welsch et al., 2007). This may result in more than one competitor being required to reduce killing. Thus, there are some combinations of vaccine, strain, and subject for which it is possible to use the competition method to arrive at a clear demonstration of antigen specific killing in the SBA. However, for the reasons listed above, such a result is not guaranteed for every combination of strain and subject.

We have studied the immune responses elicited by immunization with 4CMenB and observed that both young and older infants mount a robust response against each of these indicator strains. This demonstrates that NadA, fHbp, NHBA and PorA P1.4 are capable of inducing bactericidal antibodies in these age groups. The details of these studies are presented in Appendix 3.

3.3 SBA Is a Valid Correlate of Protection for 4CMenB

Given the low incidence of meningococcal serogroup B disease, an efficacy trial for the Novartis 4CMenB vaccine is not considered practical or necessary and is therefore not included in the clinical development plan. In lieu of classical efficacy trials, appropriately validated correlates of protection may serve as valuable predictors of vaccine effectiveness. Immunity to meningococcal disease has been shown to be primarily mediated by bactericidal antibodies (Frasch *et al.*, 2009). The serum bactericidal assay with human complement (hSBA) measures the level of antibodies that are specific for bacterial surface determinants and are capable of directing complement-mediated bacterial lysis. The use of the hSBA as a correlate of protection for meningococcal vaccines including 4CMenB is discussed in further detail in Appendix 1.

The efficacy of the 4CMenB vaccine is inferred by the induction of bactericidal antibodies able to kill four “indicator” meningococcal serogroup B strains, each strain representing one of the major MenB vaccine antigens, in the SBA using human complement (hSBA). A description and standardization of the SBA assay, the preferential use of human complement in hSBA, details on complement selection and known mechanisms of serum resistance are provided in Appendix 2.

3.4 Step 2. Relating Antigen Composition to Killing in the SBA: MATS

Vaccine effectiveness results in part from the ability of a vaccine to provide coverage against circulating and potentially diverse isolates of the pathogens of interest. Because protein antigens may vary in their sequence and level of expression, the use in vaccines of novel proteins identified by whole-genome screening creates a challenge for determining which bacteria will be covered by the vaccine. In the case of meningococcal serogroup B, because of the known variability within this serogroup, with several thousands of distinct isolates

identified and variations over time, information is needed on recent case isolates to establish the coverage expected against diverse MenB strains.

The development of vaccines based on capsular polysaccharides, such as *H. influenzae* type B, *S. pneumoniae*, and serogroups A, C, W135 and Y of *N. meningitidis* benefited from the previous establishment of capsule typing methods. For MenB, three of the four major antigens in the vaccine were newly discovered and no typing method existed. The most widely-used typing method for meningococci, multi-locus sequence typing (MLST), relies on seven housekeeping genes, but these do not include any of the antigens in 4CMenB. We have continued to study the relationship between MLST and the immuno-phenotypes of the antigens in 4CMenB on multiple MenB strains. We have found significant heterogeneity of immuno-phenotypes within individual clonal complexes, which makes MLST less than optimal for estimating potential strain coverage by this vaccine. Therefore, a new typing method was needed that accounted for the specific protein antigens in 4CMenB in order to estimate coverage. More importantly, the results of such a typing method would need to be linked to killing of the strains in the SBA.

Recognition of target antigens and the subsequent activation of complement by bactericidal antibodies rely in large part to two major factors: the level of expression and extent of amino acid relatedness to the antigen represented in the vaccine. As the density of antigens on the bacterial surface increases, the distance between bound bactericidal antibodies decreases, increasing the likelihood for optimal complement engagement and activation (Michaelsen *et al.*, 1991; Weynants *et al.*, 2007). However, the quantity of antigen is not the only determining factor. The quality of the fit between antibody and antigen also is important. The significance of sequence variation of fHbp in immune recognition was recently highlighted by Brunelli *et al.* (2011). These investigators evaluated the contribution of fHbp sequence variability to vaccine coverage by constructing a recombinant MenB strain susceptible to bactericidal killing only by anti-fHbp antibodies and engineering it to express equal levels of 10 different fHbp sub-variants. Testing of these isogenic strains showed that sera from mice or adult volunteers vaccinated with fHbp variant 1.1 were bactericidal against all subvariant 1 sequences; however, the titer against the most distant subvariant 1 sequences were significantly lower. Importantly, sera from vaccinated infants were more susceptible to amino acid variations, and they displayed little or no bactericidal activity against the distant subvariant 1 sequences compared to sera from adults given the same vaccine.

Thus, the new typing system used to predict the susceptibility of a given strain to killing by vaccine-elicited immune sera would need to not only measure the levels of antigen expression, but also the extent of sequence relatedness to the antigen represented in 4CMenB.

3.4.1 Overview of MATS Development

The Meningococcal Antigen Typing System (MATS) was developed to link genotypic and phenotypic information about the major antigen components of 4CMenB in individual MenB strains to their ability to be killed in the hSBA by antibodies raised against the vaccine. The development of this method is described in a recent publication (Donnelly *et al.*, 2010.) PorA information is obtained from conventional PCR genotypic methods, because the presence of the PorA P1.4 marker is predictive of whether antibodies against the OMV NZ vaccine component are able to kill the strain in the SBA. For the antigens fHbp, NHBA and NadA, we have developed an ELISA that specifically measures both the level of immunological relatedness and expression levels of each of these three MenB vaccine antigens. There are therefore 3 separate antigen-specific MATS ELISA platforms. The typing system is

comprised of the three ELISAs and the presence or absence of the PorA 1.4 genotype or serotype. Figure 4 provides a schematic of the MATS ELISA test that is used to detect the three antigens NadA, fHbp and NHBA.

.

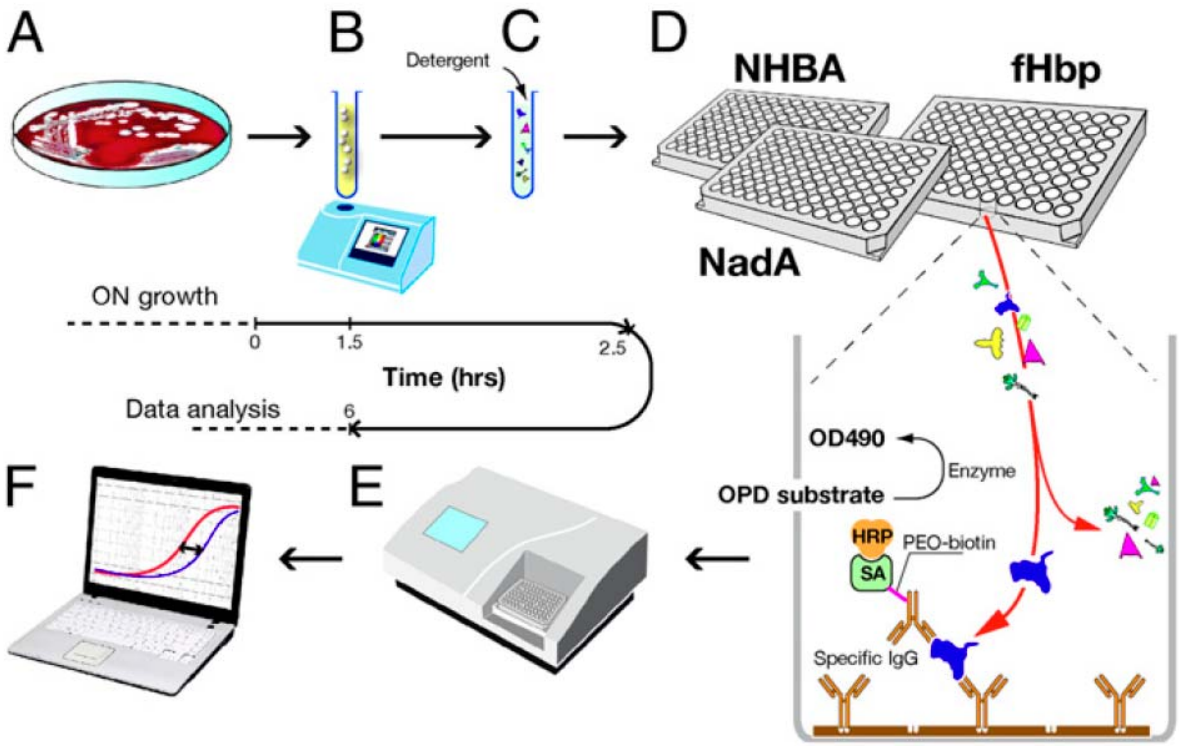


Figure 4. **Schematic of the MATS ELISA method.** IgG antibodies from sera derived from rabbits immunized with the recombinant vaccine antigen were purified on a Protein G column and plated onto 96-well microtiter plates.a) MenB bacteria are grown overnight on chocolate agar (at 37°C, 95% humidity, 5% CO2). b) A suspension of bacteria taken from the plate is prepared to a specified OD600. c) Detergent (0.5% Empigen) is added to the suspension to extract the capsule and expose the antigens. d) Two-fold serial dilutions of lysates are incubated in duplicate in microtiter plates coated with rabbit antibodies. A specific capture antibody binds one of the antigens from the extract (blue) which is then detected with a specific biotin-labeled antibody and a streptavidin-enzyme conjugate. Each plate contains reference meningococcal strain lysates as standards, and purified vaccine protein antigens as positive controls, and up to 6 unknown strains. After several washes (4 times with PBS, 0.05% Tween), antigen-specific binding is revealed by incubation with purified IgG from rabbits immunized with recombinant antigen. These secondary antibodies are conjugated with biotin and subsequently complexed with streptavidin-horse radish peroxidase (SA-HRP). e) Finally, signals are developed by incubating with the o-phenylene diamine (OPD) HRP substrate and monitoring the optical density (OD) at 490 nm in an ELISA reader. f) Results are calculated by comparing the curve of OD490 versus dilution obtained with the serially diluted unknown strain to a serially diluted reference strain tested in the same ELISA plate.

To demonstrate that MATS can be used to estimate the potential for strain coverage by a candidate MenB vaccine in different countries or geographical regions, Novartis performed an inter-laboratory standardization study (Plikaytis, B. *et al.*, manuscript in preparation). Independent laboratories in England and Wales (Health Protection Agency), Germany (University of Wurzburg), France (Institut Pasteur), Norway (Norway Institute of Public Health), Italy (Istituto Superiore di Sanità), and the United States (Centers for Disease Control and Prevention) received training to perform MATS and were supplied with the necessary

reagents. A common set of 17 different MenB strains was tested repeatedly in each laboratory. The reproducibility of relative potencies among laboratories and against consensus values derived from the present data was evaluated using a mixed-model analysis of variance (ANOVA). The results from this study showed good agreement[1] between the different laboratories.

3.4.2 Positive Bactericidal Thresholds Provide a Link Between Functional Immunogenicity and Strain Coverage

MATS was designed to predict whether a given strain would be killed in the hSBA by antibodies raised against the 4CMenB vaccine. To establish this correlation the MATS ELISA incorporates specific rabbit polyclonal antibodies raised against the fHbp, NHBA, and NadA components of 4CMenB. Therefore, the MATS ELISA is sensitive both to the level of antigen expressed by the bacteria and the extent of immunologic cross-reactivity between the antigen sequence variants that may be found in the target bacteria and the specific components of 4CMenB.

Conducting SBA testing across many strains requires a large volume of serum from vaccinees. This presents a practical problem in trials of vaccines in infants where only limited volume can be obtained. Thus, to identify PBTs for fHbp, NadA, and NHBA, we used pooled sera obtained from either 13-month-olds or from adults.

In order to assess the SBA results obtained with pooled serum in comparison with those of individual vaccinees, we initially demonstrated that titres from pooled adult sera were generally similar to the arithmetic mean SBAs of the individual vaccinees. Thus a pool titer of 1:8 would be expected to reflect an arithmetic mean titer of 1:8 in the individual members of the pool. To confirm that a pool titer of 1:8 or greater would reflect a positive (≥1:4) SBA response in the majority of individual subjects, we selected fHbp, NHBA and NadA antigen specific strains with RP values at or closer to the PBT. We then tested the ability of individual sera collected from 100 infants immunized with 3 or 4 doses of 4CMenB to kill these strains in the hSBA. The results of these studies are presented in Table 5, Section 3.4.5 of this document.

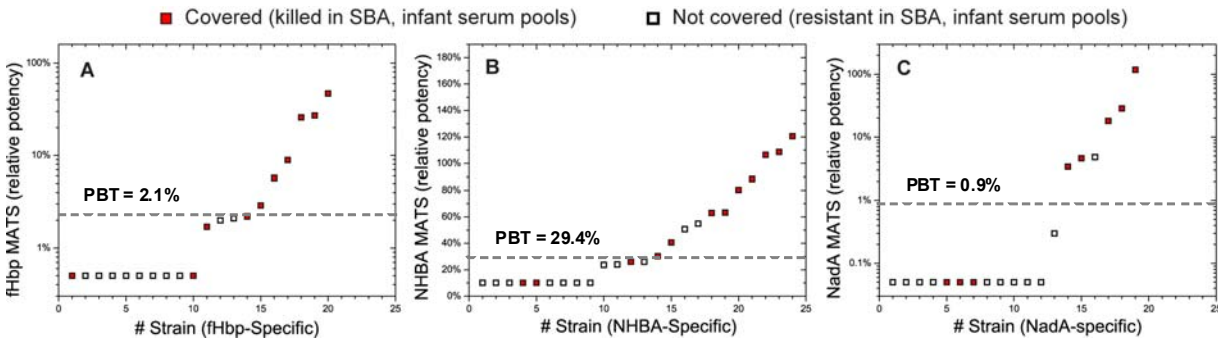
MATS ELISA results were compared with hSBA using pooled immune sera from different age groups, against MenB isolates ("Calibration" panel) taken from cases of invasive disease that were selected to represent a broad range of MLST and PorA types from various geographic regions. Pooled sera from adults were tested against a calibration panel of 124 MenB strains, while sera from children were tested against a 57-strain subgroup of the 124-strain calibration panel (Donnelly *et al*., 2010).

When the individual strains in each antigen-specific calibration panel were ranked according to their MATS relative potencies (MATS-RP) in the ELISA relative to a reference strain a score (MATS-RP "score") was observed above which at least 80% of strains were killed in the hSBA (also referred to as the "*positive bactericidal threshold*" or PBT; see Figure 5). The PBT can be used to make coverage estimates on an epidemiologically representative panel of invasive isolates from any region. Strain coverage is therefore estimated as the proportion of

[1] Pearson correlation coefficients (r), coefficients of accuracy (C_a), and concordance correlation coefficients (r_c) exceeding 99% in the large majority of comparisons. Summary measures of reproducibility (between-laboratory variance) expressed as CV's were 7.85% (fHbp), 16.51% (NadA), and 12.60% (NHBA), and the aggregate measure of variation over all strains, adjusted for laboratory, expressed as CV's were 19.8% (fHbp), 28.8% (NHBA) and 38.3% (NadA).

circulating strains that score above the PBT for at least one vaccine antigen (fHbp, NadA or NHBA) in MATS-RP analysis ("MATS positive" strains). Because the PBT is defined by the ability of clinical immune sera to kill a diverse set of MenB strains that express different levels of antigen of varying amino acid sequence, this threshold provides the necessary link between immunogenicity and strain coverage. MATS also includes a determination of the proportion of strains that carry the PorA P1.4 serosubtype, which would be susceptible to killing by antibodies elicited by the OMV component in 4CMenB.

Figure 5. **MATS Relative Potency (RP) of fHBP, NHBA and NadA determined by the MATS ELISA in antigen-specific sub-panels (the "Calibration panel" referred to in the text which contain strains mismatched for the other 3 antigens).** The strains are in ascending order of MATS RP. Strains covered (as determined by killing in the SBA with pooled post-immune infant sera) are shown with solid red circles, those not covered are shown with open circles. The dashed line represents the Positive Bactericidal Threshold (PBT) for each antigen, the relative potency above which at least 80% of MenB strains are killed in SBA with serum from vaccinated subjects (from Donnelly *et al.*, 2010).



3.4.3 Using MATS Relative Potency of NHBA, NadA and fHbp in a Model Population of Serogroup B Strains

To better understand how useful the MATS relative potency information might be in describing the properties of a population of MenB strains, we selected a model population of 124 serogroup B strains that represented a wide diversity of MLST and PorA types from varying geographic regions. The relative potency (the difference in ELISA reactivity as determined by comparison of serial dilution curves of a given MenB strain and a reference strain known to express the corresponding vaccine antigen), of fHbp, NadA, and NHBA was measured in these serogroup B strains. The strains were diverse in their RP values as a result of variations in antigen expression, cross-reactivity or both. Based on MATS, 33 of the 124 strains were at or below PBT for all of the antigens tested, 41 strains were above PBT for a single antigen, 34 for two antigens, and 16 for three antigens.

For fHbp, variations in amino acid sequence had a significant impact on RP. Strains with fHbp subvariant 1.1, which is matched to 4CMenB, had the highest RP (46% to 140% of that obtained for the reference strain). Strains classified within other fHbp variant 1 subvariants had RP values ranging from 1.6% to 38% of the reference strain value. For strains with fHbp variants 2 or 3 (40% of isolates tested), the RP was below the lower limit of quantitation (LLOQ).

For NHBA, 70% of isolates had values above the LLOQ, and RP ranged from 20% to 130% of that obtained for the reference strain. For NadA, up to 20% of strains gave RP results above the LLOQ and results across panel strains varied over a 1000-fold range.

3.4.4 Comparability of Coverage Estimated by MATS and by hSBA in a Model MenB Strain Population

We performed hSBA testing using pooled sera from 141 infants who had received 3 or 4 doses of 4CMenB against a subset of 57 serogroup B strains selected from the initial 124-strain panel. The strain panel was selected to evaluate strains with RP above the LLOQ but <50% of that of the reference strain for one antigen only. Fifty-four of the 57 strains were PorA mismatched to 4CMenB.

Strains from the panel of 57 were identified as "killed" if pooled sera from infants who received 4 doses of 4CMenB had an hSBA titer ≥8 (if the pre-immunization titers were <4) or achieved at least a four-fold rise in titer compared with baseline values. Other strains were considered "not killed." The point measurement of MATS RP for the majority of strains that were killed in the SBA was greater than the PBT for one or more antigens, calculated as described in Donnelly et al (2010)[2]. The PBT values were defined as relative potencies of **2.1%, 29.4%** and **0.9%** for **fHbp, NHBA** and **NadA**, respectively.

In the 57-strain panel, 89% of strains having a MATS relative potency above the PBT for one or more antigen were killed in the hSBA by pooled serum from 13-month-old children who received 4 doses of 4CMenB (Table 2). Among strains with MATS RP at or below the PBT for all antigens, 77% were not killed in the hSBA.

The overall accuracy of MATS in predicting whether or not strains were covered in this age group was 86%, with a statistically significant association ($p < 0.0001$) between MATS RP and the corresponding hSBA result (Table 2). When pooled serum from 7 month old infants given 3 doses of 4CMenB was analyzed, 83% of strains above the PBT were killed and 73% below the PBT were not killed, for an accuracy of 80%. Alternative methods of assigning PBTs, such as attempting to fix them to the relative potency of antigens on specific strains, significantly degraded the accuracy of the model, as described below in Section 3.4.5.

[2] To identify a MATS cut-off value for each antigen that best predicts SBA activity, we evaluated all combinations of MATS values for the 57-strain panel (n = 28, 35, and 15 for fHbp, NHBA, NadA, respectively, giving 14,700 combinations), using a stepwise optimization algorithm: (i) meeting overall and antigen-specific positive predictive value (PPV) ≥ 80% was fulfilled by 2,349 of the 14,700 possible combinations of fHbp/NHBA/NadA PBTs; (ii) maximizing overall and antigen-specific negative predictive value (NPV) was fulfilled by 3 of the 2,349 combinations; (iii) maximizing overall and antigen-specific accuracy was fulfilled by 2 of the 3 previously selected combinations; and (iv) selecting the most conservative combination (higher PBTs) gives the final PBT values: 2.1%, 29.4%, and 0.9% for fHbp, NHBA, and NadA, respectively.

Table 2. Positive and negative predictive values of MATS for vaccine antigens, based on killing in hSBA against a panel of 57 meningococcal serogroup B strains by pooled sera from 13-month-olds immunized with 4CMenB at 2, 4, 6 and 12 months of age.

Vaccine Antigen	Positive Predictive Value[a] (No. killed in hSBA/total[a])	Negative predictive value[b] (No. not killed / total[b])	Accuracy[c]	*p*-value[d]
NHBA*	82% (9/11)	77% (10/13)	79%	0.006
NadA**	83% (5/6)		79%	0.024
fHbp***	100% (7/7)		85%	0.002
Any antigen or combination	89% (39/44)		86%	<0.0001

[a] Proportion of strains with any 4CMenB antigen above the PBT that are killed in hSBA by immune serum

[b] Proportion of strains with all of the four antigens below the PBT that are not killed in hSBA by immune serum

[c] Accuracy, (number of strains predicted killed by MATS and actually killed in hSBA + number of strains predicted not killed by MATS and actually not killed in hSBA/total number of strains tested

[d] Fisher exact association test

*24 strains with fHbp, NadA, and PorA < PBT

**19 strains with fHbp, NHBA, and PorA<PBT

***20 strains with NHBA, NadA, and PorA < PBT

Strain coverage was defined as the proportion of circulating strains that demonstrate a RP above the PBT for at least one vaccine antigen (NadA, NHBA, or fHbp) in the MATS ELISA or are matched to the PorA 1.4 of the OMV component of 4CMenB.

We extended our observations to adults immunized with the 4CMenB vaccine. Using the entire panel of 124 strains, 85% were killed in the SBA by pooled serum from adults, and the proportion predicted by MATS to be covered was 72% (95% CL, 55%-81%). Thus MATS provides a robust and reasonably accurate means of estimating the proportion of MenB strains in a population that can be killed by pooled immune serum from 4CMenB vaccinees.

The antigens contained in 4CMenB also are found on bacteria from other serogroups (Pizza *et al.*, 2000; Comanducci *et al.*, 2002; Masignani *et al.*, 2003; Bambini *et al.*, 2009; Jacobsson *et al.*, 2009; Beernink *et al.*, 2009). Therefore, 4CMenB may also generate bactericidal responses against strains of serogroups A, C, W-135, and Y, although as for MenB, we would not expect all of the strains within a given serogroup to be covered (for example see Harris *et al.*, 2011). Because protein based vaccines such as 4CMenB are expected to provide less than full coverage within a serogroup, they are unlikely to be equivalent to the polysaccharide conjugate vaccines in their coverage of non-B serogroups.

3.4.5 Sera from Immunized Infants Are Able to Kill MenB Strains with Lower Levels of Antigen Expression

We previously described how Novartis defined the bactericidal thresholds as a tool to predict vaccine strain coverage by testing the ability to immune sera pooled from infants to kill 57 diverse MenB isolates (Section 3.4.2). To provide evidence that i) a significant proportion of these immune sera induced killing of multiple MenB strains, and ii) they were also able to kill strains with significantly less antigen than those present in the immunogenicity indicator strains, we also tested serum from individual infant vaccinees to kill strains at or closer to the PBT, in the hSBA.

Novartis identified 3 meningococcal serogroup B strains that, similar to the fHbp and NadA primary endpoint indicator strains used in Phase 3 studies, are predominantly killed by bactericidal antibodies directed against only one of the 4CMenB components. These strains, whose genotypic and phenotypic characterization are provided in Table 4, have significantly lower MATS-RP values than the primary indicator strains used in the Phase 3 studies for fHbp and NadA, and are closer to the associated PBT values of each antigen. Strain M10713 is considered a target for bactericidal killing by anti-NHBA antibodies because it expresses neither PorA P1.4 nor NadA, and it expresses levels of fHbp below the LLQ. The MATS values for the test strains for NHBA and NadA described in Table 2.3.2 are close to the PBTs associated with the 4CMenB antigen (29.4%, 0.9% and 2.1% for NHBA, NadA, and fHbp, respectively). The MATS value for the M14459 is more distant from the fHbp PBT, but nonetheless expresses a level of antigen significantly less than the H44/76 indicator.

Table 4. **Summary of Invasive MenB Strains Characterized.** The genetic classification of strains is given as MLST designations (ST: sequence type; na: not assigned). The absence (-) of the *nadA* gene is indicated for both strains. MATS Relative Potency (MATS-RP) values are expressed as a percentage relative to the reference strain for each antigen and are determined at 95% Confidence Level (the upper and lower confidence limits are given on either side of the relative potency value) using the within-lab precision associated with each antigen. LLQ: Lower Limit of Quantitation.

Strain	Major Vaccine Antigen	Clonal Complex (ST)	Typing	fHbp variant	NHBA variant	nadA gene	MATS Relative Potency (95% CI)		
							fHbp	NadA	NHBA
M10713	NHBA	41/44 (136)	B:P1. 17,16-3	2.24	10	–	<LLQ	<LLQ	40.7% (19.2-62.2)
M01-0240364	NadA	11 (ST11)	B:2a: P1.5,2	3.31	28	+	<LLQ	4.6% (1.5-7.7)	<LLQ
M14459	fHbp	na (ST2048)	B:NT: P1.22,9	1.180	19	–	27% (16.4-37.6)	<LLQ	(<LLQ)

Individual sera collected from infants immunized with 3 or 4 doses of vaccine were tested for their ability to kill these strains in hSBA. Serum samples from 100 infants (baseline and post-3rd dose) and a separate subset collected from another 100 infants (pre-boost and post-boost) were tested for the analysis presented in Table 5 below. These data show that immune sera from individual infants vaccinated with 3 or 4 doses of 4CMenB are able to kill strains at or close to the antigen PBTs**.** This provides proof of concept that the PBTs, determined with pooled sera, also are predictive of the ability of individual infant subjects to kill strains that

are diverse with respect to the antigenicity and quantity of the MenB vaccine antigens they express.

Table 5. Responses of Infants Against Strains Near the PBT

Strain	Major Vaccine Antigen	No. of Subjects	Percent subjects with a protective bactericidal titer (≥5) (95% CI)			
			Baseline*	Post-3rd*	Pre-boost**	Post-boost**
M01-0240364	NadA	100	2% (0-7)*	84% (75-91)*	20% (13-29)**	93% (86-97)**
M10713	NHBA	46	33% (24-43)*	84% (75-91)*	61% (51-71)**	98% (93-100)**
M14459	fHbp	100	9% (4-16)*	97% (91-99)8	24% (16-34)**	96% (90-99)**

*Titers at baseline (pre-dose 1) and post-dose 3 (2,4,6-month primary schedule) are from serum samples from 165 subjects from study V72P13, to have 100 valid results across all strains.

**Pre-boost and post-boost (at 12-months of age) samples were derived from 146 subjects from study V72P13E1, an extension study in which subjects from study V72P13 received a further immunization at 12 months of age, to have 100 valid results across strain. For strain M10713, 46 subjects were tested both in V72P13 and V72P13E1, for strain M14459, 9 subjects were tested for both studies and for strain M01-0240364, 8 subjects were tested for both studies.

We have defined PBTs for each antigen based on SBA with pooled infant immune sera on a panel of 57 invasive MenB strains, as described in Section 3.4.2 above. An alternative way of setting the PBTs that might be considered would be to define them as equal to the MATS relative potency of strains that are used to measure the specific immune responses in hSBA. However, as explained above, the many constraints on selection of strains for demonstrating immunogenicity (single antigen specificity, complement compatibility, etc. as described in Appendix 2), also would impose limitations on the MATS model, making it less predictive of the bactericidal ability of the immune serum pools. Table 6 below compares the two approaches for determining PBTs on the PPV, NPV and accuracy of the MATS model for two different example strain collections: the original 57 strain panel used in Donnelly (2010) and an expanded panel of 93 strains, representing the original 57 strains with 36 additional strains included. For purposes of illustration, we chose the three strains listed above as hypothetical candidates for use in the specific SBA to show immune responsiveness of the clinical trial subjects. For example, if the PBTs were set to correspond to the MATS levels of the three strains listed above, applying the method to a test panel of 93 strains, the PPV of the prediction relative to the SBA would now be 84%, the NPV would be 36%, and the Accuracy would be 53%. A model with an accuracy of 53% would be ineffective as a tool for predicting whether or not a given MenB strain was likely to be killed by the immune response to the vaccine.

Table 6: Coverage prediction accuracy of MATS using two alternative sets of PBTs

Sample Strain Panel	57 Strains		97 Strains	
fHbp PBT assigned	2.1*	27**	2.1*	27**
NHBA PBT assigned	29*	41**	29*	41**
NadA PBT assigned	0.9*	4.6**	0.9*	4.6**
Proportion of strains actually killed in SBA	74%	74%	71%	71%
Proportion of strains predicted killed by MATS	77%	47%	76%	34%
Positive Predictive Value[a]	89%	85%	83%	84%
Negative Predictive Value[b]	77%	37%	68%	36%
Accuracy[c]	86%	60%	80%	53%

[a] Proportion of strains with any 4CMenB antigen above the PBT that are killed in hSBA by immune serum

[b] Proportion of strains with all of the four antigens below the PBT that are not killed in hSBA by immune serum

[c] Accuracy, (number of strains predicted killed by MATS and actually killed in hSBA + number of strains predicted not killed by MATS and actually not killed in hSBA/total number of strains tested

* PBT based on the minimum MATS level required to achieve killing of 80% or more of MenB strains in SBA

**PBT based on an arbitrarily selected SBA target strain

3.5 Multiple Antigens in 4CMenB Broaden Coverage and Provide a Defense Against Antigen Mutation or Loss

It should be noted that the current MATS platform to estimate vaccine strain coverage relies on the reactivity and level of expression of single antigens on the target bacterium and does not consider the potential of additive or synergistic effects multiple antigens may provide. One of the major rationales behind the inclusion of multiple components in the 4CMenB vaccine was to increase the likelihood that strains expressing weakly cross-reactive or low levels of antigen are still killed by vaccine-elicited bactericidal antibodies that recognize more than a single vaccine antigen on target bacteria.

In our studies presented in Section 3.4.3, we showed that based on MATS, 33 of the 124 strains were at or below PBT for all of the antigens tested, while 91 strains were above PBT for at least one antigen. When tested in hSBA, using pooled sera from adults immunized with 4CMenB, against the 124-strain panel, 22 of 33 (67%) strains with no antigens above the PBT and with a mismatched PorA serosubtype, were killed. Among strains with MATS RP above the PBT for one antigen, 35 of 41 (85%) were killed, for strains with 2 or more antigens above the PBT, 48 of 50 (96%) were killed, while for strains with 3 or more antigens above the PBT, 16 of 16 (100%) were killed (Figure 6). Thus, targeting bacteria with antibodies to multiple antigens increased the proportion of strains killed. The negative predictive value of MATS for 4CMenB is less than 50% in adults, indicating a substantial underestimation of vaccine coverage. Further, MATS does not account for antibodies generated from non-PorA components of OMV or the synergistic effects of the multiple components of 4CMenB. Therefore, MATS is considered a conservative tool for predicting strain coverage in this age group.

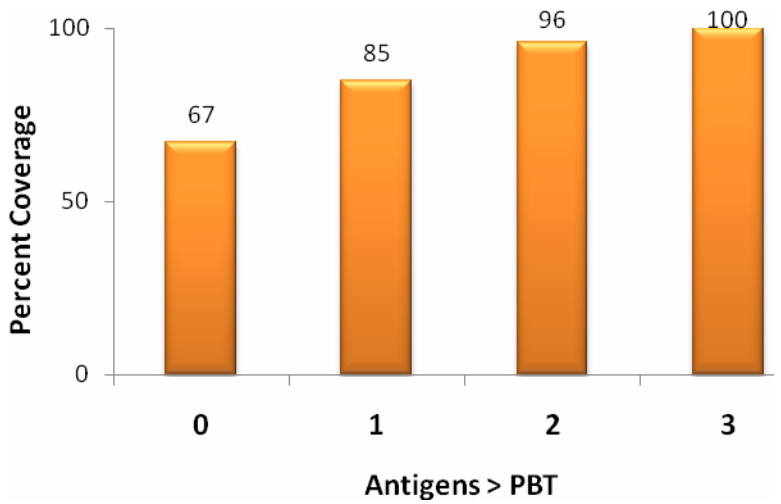


Figure 6. **Strains killed in the serum bactericidal assay using human complement (hSBA) by pooled sera from healthy adults who had received three doses of 4CMenB.** Strains that express PorA P1.4 subtype are considered covered and are listed as ">PBT". Among strains with their MATS relative potency above the positive bactericidal threshold (PBT): For strains with no antigens above the PBT and lacking PorA 1.4, 67% were nonetheless still killed in hSBA. For strains with one or more antigen above the PBT or with PorA 1.4, 85% were killed in hSBA. For strains with 2 or more antigens above the PBT or with PorA 1.4, 96% were killed in hSBA. For strains with 3 or more antigens above the PBT or with PorA 1.4, 100% were killed in hSBA.

In our studies described above, some strains with a MATS RP below the PBT for all antigens and mismatched PorA may still be killed in the hSBA, possibly by antibodies against additional immunogens in the OMV or by a combination effect of antigens below the PBT. Thus by introducing a necessary element of antigen specificity in our typing assay, our approach is inherently conservative as it does not take into account the additive or synergistic effects of bactericidal antibodies directed against more than a single vaccine antigen that potentially contribute to killing in hSBA.

The OMV component in 4CMenB contains multiple outer membrane proteins as well as lipo-oligosaccharides, and therefore many antigens in addition to PorA may play significant roles in the bactericidal antibody response. In a previous study, we observed a synergy between fHbp and OMV when used together as inhibitors of bactericidal killing by pooled adult immune sera (Giuliani *et al.*, 2010). This observation indicates that antibodies directed against antigens other than PorA, even though they may not be directly bactericidal on their own, may have cooperative bactericidal activity when combined with antibodies against fHbp. In addition, we demonstrated that NHBA can be a target of bactericidal antibodies on strain M4407, which lacks the *nadA* gene and is mismatched to the vaccine for PorA and fHbp. Moreover, the bactericidal anti-NHBA antibodies appeared to act in concert with antibodies to other antigens, which may or may not be bactericidal on their own (Giuliani *et al.*, 2010).

In a related study, investigators studied the ability of antibodies against individual antigens present in 4CMenB to interact and potentially augment protective immunity. Sera from six adults immunized with rMenB (vaccine containing only the 3 recombinant protein antigens) were tested in hSBA before and after depletion of antibodies to fHbp and/or NHBA (Vu *et al.*, 2011). All six subjects developed ≥4-fold increases in SBA titer against a test strain that expresses fHbp variant 1 matching that present in the vaccine (GMT <1:4 baseline, to 1:139 after 3 doses of vaccine). By adsorption, 88 to >95% of the SBA was directed against fHbp.

Four subjects developed ≥4-fold increases in SBA titer against a test strain with a heterologous fHbp variant 2 antigen and a homologous NHBA amino acid sequence that matched the vaccine antigen (GMT<1:4 baseline, to 1:45). SBA was directed primarily against NHBA in one subject, against fHbp in a second, while depletion of either anti-NHBA or anti-fHbp antibody removed the majority of SBA in sera from two subjects. In all four subjects, depletion of both anti-fHbp and anti-NHba antibodies removed more SBA than depletion of either antibody individually. Mixing a mouse non-bactericidal anti-fHbp variant 1 antiserum with a mouse anti-NHBA antiserum also augmented the anti-NHBA SBA titer against this test strain.

Collectively, these studies demonstrate that targeting bacteria with antibodies to multiple antigens increased the proportion of strains killed, and indicate that the potential effectiveness of 4CMenB could be expected to be greater if more than one vaccine component antigen is present on the target bacteria. For meningococcal vaccines that target relatively sparsely exposed antigens such as fHbp or NHBA, non-bactericidal antibodies against individual antigens can cooperate and elicit SBA. Finally, although we were unable to demonstrate induction of resistance by *in vitro* immune selective pressure (Section 2.4), the redundancy ensured by the strategy of targeting bacteria with antibodies to multiple antigens may nonetheless provide an additional measure of protection against antigen mutation or loss.

3.6 Use of MATS to Assess Region Specific Strain Coverage

During 2009-10, Novartis transferred the MATS method to 7 national reference laboratories to enable them to perform coverage assessments and monitor future changes in their endemic MenB isolates. Novartis Vaccines and the US Centers for Disease Control and Prevention (CDC, Atlanta) are working jointly to define the potential effectiveness of 4CMenB by typing isolates collected through the Active Bacterial Core surveillance (ABCs) program. This project is expected to finish before the end of 2011. In a parallel project in Europe, an analysis of potential 4CMenB vaccine strain coverage has already been conducted using a representative subset of the meningococcal group B collected by five European reference laboratories. This collection consists of over 1000 MenB invasive strains isolated during the 2007-2008 epidemiologic year (July 1, 2007 to June 30, 2008) in the following European countries: Norway, UK, Germany, France, and Italy. The MATS phenotype and the distribution of the major antigen components of the Novartis vaccine within these isolates have been assessed jointly with the respective national reference laboratories.

4.0 Conclusion

MenB is an extremely difficult vaccine target. The lack of a suitable capsular antigen and the consequent need to use protein antigens to immunize, the plasticity of the meningococcal genome, and the facility with which phase variation, mutation, and genetic exchange occur in this species pose formidable challenges to vaccine makers. The ability of the meningococcus to employ host regulatory pathways in a species-specific manner to inhibit effector functions such as complement gives it the ability to survive where other bacteria are killed, and complicates the life of the researcher by requiring the use of human proteins as reagents to study immune responses. Although devastating, invasive meningococcal disease is uncommon, except when it occurs in the form of case clusters or outbreaks, but these occur sporadically and without warning. Consequently, a field efficacy study of a MenB vaccine is impractical before licensure, would require a prohibitively large subject population to be

enrolled. The use of serologic markers is complicated by the need to use a functional assay, the hSBA, that is nonspecific, consumes large volumes of specimen, and requires human serum as one of the reagents. MenB disease occurs most frequently in infants and young children, the target population that yields the smallest quantity of specimen for analysis in this cumbersome test.

Against all odds, it is possible to make progress in the development of a MenB vaccine. Outer membrane vesicle vaccines provided protective immune responses, albeit of narrow breadth. The sequencing of the meningococcal genome provided a means to discover new antigens and to map their distribution in the species and potential for antigenic variation. From the more than 2000 genes in the meningococcal genome, a combination of four major antigens and two accessory antigens was found to give a bactericidal response much broader than ever was seen before with OMV.

To reduce such a vaccine to practice requires a means to measure bactericidal responses and a method of determining how effective those responses may be against the broad array of invasive meningococcal disease isolates. Since the bactericidal assay itself does not discriminate the effects of one antibody from those of another, and since the 4CMenB vaccine contains multiple distinct antigens, we used a subtractive method to identify appropriate strains to show the function of each of the vaccine's four major components. Since the number of different possible isolates against which it is necessary to show protection is prohibitively large, a means of classifying the different isolates was needed that would divide them according to their ability to be killed by bactericidal antibodies against the vaccine. For this we established MATS, a method of typing MenB bacteria by the quantity and antigenic relatedness of the vaccine antigens they express, and we identified phenotypes associated with susceptibility to killing by immune serum and complement. By typing large numbers of MenB bacteria, it is possible to assess the proportion of MenB strains within a country or region that can be killed by antibodies to the vaccine, and therefore against which protection would be expected. The project is now entering this last phase, large-scale typing of MenB strains in Europe and the US.

Novartis believes that a very high proportion of vaccinees, even in young age groups, will make protective bactericidal responses against the antigens in 4CMenB, and that the majority of MenB strains in both the US and Europe will have phenotypes that are susceptible to killing by the antibodies that 4CMenB induces. Consequently, the widespread implementation of immunization with 4CMenB will eliminate or substantially reduce this infectious threat.

5.0 References

Andrews TD and Gojobori T. (2004) Strong positive selection and recombination drive the antigenic variation of the PilE protein of the human pathogen *Neisseria meningitidis*, *Genetics* 166 (1):25–32.

Bai X and Borrow R. (2010) Genetic shifts of *Neisseria meningitidis* serogroup B antigens and the quest for a broadly cross-protective vaccine. *Expert Rev. Vaccines* 9(10):1203–1217.

Bambini S, Muzzi A, Olcen P, et al. (2009) Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus. *Vaccine*. 27(21):2794-803.

Beernink PT, Caugant DA, Welsch JA, Koeberling O, Granoff DM (2009) Meningococcal Factor H-Binding Protein Variants Expressed by Epidemic Capsular Group A, W-135 and X Strains from Africa. *J Infect Dis* 199:1360-8.

Bjune G., et al. (1991) Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. *Lancet* 338:1093-1096.

Black S, Shinefield H, Fireman B, *et al*. (2000) Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J* 19(3):187–95.

Borrow R, Aaberge IS, Santos GF, *et al*. (2005) Interlaboratory Standardization of the Measurement of Serum Bactericidal Activity by Using Human Complement against Meningococcal Serogroup B, Strain 44/76-SL, before and after Vaccination with the Norwegian MenBvac Outer Membrane Vesicle Vaccine. *Clin Diagn Lab Immunol.* 12(8): 970–976.

Borrow, R., et al. (2006) Neisseria meningitidis group B correlates of protection and assay standardization—International Meeting Report Emory University, Atlanta, Georgia, United States, 16–17 March 2005. *Vaccine* 24:5093-5107.

Boslego, J. et al. (1995) Efficacy, safety, and immunogenicity of a meningococcal group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile *Vaccine* 13:821-829.

Brunelli B, DelTordello E, PalumboE, et al. (2011) Influence of sequence variability on bactericidal activity sera induced by factor H binding protein variant 1.1. *Vaccine* xx:xxx-xxx.

Cantini F, Veggi D, Dragonetti S, *et al.* (2009) Solution structure of the factor H-binding protein, a survival factor and protective antigen of Neisseria meningitidis. *J Biol Chem*. 284(14):9022-6.

Capecchi B, Adu-Bobie J, Di Marcello F, et al. (2005) *Neisseria meningitidis* NadA is a new invasin which promotes bacterial adhesion to and penetration into human epithelial cells. *Mol Microbiol* 55:687–98.

Claus H, Borrow R, Achtman M *et al.*(2004) Genetics of capsule O-acetylation in serogroup C, W-135 and Y meningococci, *Mol Microbiol* 51 (1):227–239.

Comanducci M., Bambini S, Brunelli B, et al. (2002) NadA, a novel vaccine candidate of *Neisseria meningitidis*. *J. Exp. Med.* 195:1445–1454.

Comanducci M., Bambini S, Caugant DA, et al. (2004) NadA diversity and carriage in *Neisseria meningitidis*. *Infect. Immun.* 72:4217–4223.

Donnelly J, Medini D, Boccadifuoco G, et al. (2010) Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proc Natl Acad Sci U S A.* 107(45):19490-5.

Feavers IM and Pizza M. (2009) Meningococcal protein antigens and vaccines *Vaccine* 27(Suppl. 2):B42-B50.

Finne J, Leinonen M, and Mäkelä PH. (1983) Antigenic similarities between brain components and bacteria causing meningitis: implications for vaccine development and pathogenesis. *Lancet* 2(8346):355-357.

Fletcher LD, Bernfield L, Barniak V, et al. (2004). Vaccine potential of the Neisseria meningitidis 2086 lipoprotein. *Infect Immun* 72:2088–2100.

Frasch CE, Borrow R and Donnelly J (2009) Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease *Vaccine* 27 (Suppl. 2): B112-B116.

Giuliani MM, Biolchi A, Serruto D, et al. (2010) Measuring antigen-specific bactericidal responses to a multicomponent vaccine against serogroup B meningococcus. *Vaccine* 28:5023–5030.

Giuliani MM, et al. (2006) A universal vaccine for serogroup B meningococcus. *Proc Natl Acad Sci USA* 103:10834–10839.

Goldschneider I., et. al. (1969) Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* (6):1307-26.

Granoff DM (2010) Review of Meningococcal Group B Vaccines. *Clin Infect Dis* 50:S54–S65.

Harris SL, Zhu D, Murphy E, *et al.* (2011) Preclinical evidence for the potential of a bivalent fHbp vaccine to prevent *Neisseria meningitidis* serogroup C disease*. Human Vaccines* 7:1 Supplement, 1-2.

Harrison LH (2006) Prospects for vaccine prevention of meningococcal infection, *Clin Microbiol Rev* 19 (1):142–164.

Holst J, Martin D, Arnold R, et al. (2009) Properties and clinical performance of vaccines containing outer membrane vesicles from Neisseria meningitidis. *Vaccine* 27 Suppl 2:B3-12.

Holst, J., et al. (2003) Serum bactericidal activity correlates with the vaccine efficacy of outer membrane vesicle vaccines against *Neisseria meningitidis* serogroup B disease. *Vaccine* 21:734-37.

Howell-Adams B and Seifert HS. (2000) Molecular models accounting for the gene conversion reactions mediating gonococcal pilin antigenic variation, *Mol Microbiol* 37 (5):1146–1158.

Jacobsson S, Thulin S, Mölling P, *et al.* (2006) Sequence constancies and variations in genes encoding three new meningococcal vaccine candidate antigens. Vaccine. 15(12):2161-8.

Jacobsson S, Hedberg ST, Mölling P, *et al*. (2009) Prevalence and sequence variations of the genes encoding the five antigens included in the novel 5CVMB vaccine covering group B meningococcal disease. *Vaccine* 27(10):1579-84.

Kelly, C. et al. (2007) A Prospective Study of the Effectiveness of the New Zealand Meningococcal B. *Am J Epidemiol* 166:817-23.

Litt, D.J., Savino, S., Beddek, A., et al. (2004) Putative vaccine antigens from Neisseria meningitidis recognized by serum antibodies of young children convalescing after meningococcal disease. J Infect. Dis. 190:1488-1497.

Lucidarme J, Comanducci M, Findlow J, *et al*. (2009) Characterization of fHbp, nhba (gna2132), nadA, porA, sequence type (ST), and genomic presence of IS1301 in group B meningococcal ST269 clonal complex isolates from England and Wales. *J Clin Microbiol.* 47(11):3577-85.

Lucidarme J, Comanducci M, Findlow J, *et al.* (2010) Characterization of fHbp, nhba (gna2132), nadA, porA, and sequencetype in group b meningococcal case isolates collected in England and Wales during January 2008 and potential coverage of an investigational group B meningococcal vaccine. *Clin Vacc Immunol* 17(6):919–929.

Madico, J.A. Welsch, L.A. Lewis, A. McNaughton, D.H. Perlman and C.E. Costello *et al.* (2006) The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance, *J Immunol* 177(1):501–510.

Martin D, McCallum L, Glennie A, *et al.* (2005) Validation of the serum bactericidal assay for measurement of functional antibodies against group B meningococci associated with vaccine trials. *Vaccine*, 23:2218-21.

Masignani V, Comanducci M, Giuliani MM, et al. (2003) Vaccination against Neisseria meningitidis using three variants of the lipoprotein GNA1870. *J Exp Med* 197:789–799.

MetruccioMME,Pigozzi E, Roncarati D, Berlanda Scorza F, Norais N, Hill SA, et al. (2009) A novel phase variation mechanism in the meningococcus driven by a ligandresponsive repressor and differential spacing of distal promoter elements. *PLoS Pathog* 2009;5(12):e1000710.

Michaelsen TE, Garred P, Aase A et al. (1991) Human IgG subclass pattern of inducing complement-mediated cytolysis depends on antigen concentration and to a lesser extent on epitope patchiness, antibody affinity and complement concentratio. *Eur J Immunol* 21:11-6.

Milagres, L. G., et al. (1994) Immune response of Brazilian children to a Neisseria meningitidis serogroup B outer membrane protein vaccine: comparison with efficacy. *Infect Immun* 62(10):4419-24.

Oriente F, Scarlato V and Isabel Delany I (2010) Expression of Factor H Binding Protein of Meningococcus Responds to Oxygen Limitation through a Dedicated FNR-Regulated Promoter. *J Bacteriol* 192(3):691-701.

Pizza, M., Scarlato, V., Masignani, V., et al. (2000) Identification of vaccine candidates against serogroup b meningococcus by whole-genome sequencing. *Science* 287:1816-1820.

Plested JS and Granoff DM. (2008) Vaccine-induced opsonophagocytic immunity to Neisseria meningitidis group B. *Clin Vaccine Immunol.* 15(5):799-804.

Plested JS, Welsch JA and Granoff DM. (2009) Ex vivo model of meningococcal bacteremia using human blood for measuring vaccine-induced serum passive protective activity. *Clin Vaccine Immunol.* 16(6):785-91.

Ram S, Mackinnon FG, Gulati S, et al. (1999) The contrasting mechanisms of serum resistance of *Neisseria gonorrhoeae* and group B *Neisseria meningitidis Molec Immunol* 36:915-928.

Santos GF, Giuliani M, Santini L, et al. (2009) Investigation on the Effect of Immune Selection on Resistance toBactericidal Antibodies to Group B Meningococci In Vitro. *Clin Vaccine Immunol.* 16:1693–1695.

Saunders NJ, Jeffries AC, Peden JF, *et al*. (2000) Repeat-associated phase variable genes in the complete genome sequence of *Neisseria meningitidis* strain MC58, *Mol Microbiol* 37 (1):207–215

Schielke S, Huebner C, Spatz C, Nagele V, Ackermann N, et al. (2009) Expression of the meningococcal adhesin NadA is controlled by a transcriptional regulator of the MarR family. *Mol Microbiol* 72: 1054–1067.

Schnieder MC, Exley RM, Chan H, et al. (2006) Functional significance of factor H binding to *Neisseria meningitidis*. *J Immunol* 176:7566–75.

Schneider MC, Prosser BE, Caesar JJ, *et al.* (2009) *Neisseria meningitidis* recruits factor H using protein mimicry of host carbohydrates. *Nature*. 458(7240):890-3.

Seib KL, Oriente F, Adu-Bobie J, *et al.* (2010) Influence of serogroup B meningococcal vaccine antigens on growth and survival of the meningococcus in vitro and in ex vivo and in vivo models of infection. *Vaccine* 28:2416–2427.

Seib KL, Serruto D, Oriente F, et al. (2009) Factor H-binding protein is important for meningococcal survival in human whole blood and serum and in the presence of the antimicrobial peptide LL-37. *Infect Immun* 77:292–299.

Serruto, D., Spadafina, T., Ciucchi, L. et al. (2010) Neisseria meningitidis GNA2132, a heparin-bindingprotein that induces protective immunity in humans. *Proc. Natl. Acad. Sci (USA)* 107:3770–3775.

Stephens DS, Swartley JS, Kathariou S and Morse SA. (1991) Insertion of Tn916 in *Neisseria meningitidis* resulting in loss of group B capsular polysaccharide, *Infect Immun* 59 (11):4097–4102.

Tan LKK, Carlone GM and Borrow R (2010) Advances in the Development of Vaccines against *Neisseria meningitidis. N Engl J Med* 2010;362:1511-20.

Tappero JW, Lagos R, Ballesteros AM, et al. (1999) Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines: a randomized controlled trial in Chile. *JAMA* 281(16):1520–7.

Tondella ML, Popovic T, Rosenstein NE et al., (2000) Distribution of *Neisseria meningitidis* Serogroup B Serosubtypes and Serotypes Circulating in the United States. J Clin. Micro 38(9):3323-8.

Uria MJ, Zhang Q, Li Y, et al. (2008) A generic mechanism in Neisseria meningitidis for enhanced resistance against bactericidal antibodies. *J Exp Med.* 205(6):1423-34.

Vu DM, Wong TT, and Granoff DM (2011) Cooperative serum bactericidal activity between human antibodies to meningococcal factor H binding protein andNeisserial heparin binding antigen *Vaccine* xx:xxx-xxx.

Welsch JA and Granoff D. (2007) Immunity to Neisseria meningitidis group B in adults despite lack of serum bactericidal antibody. *Clin Vaccine Immunol.* 14(12):1596-602.

Welsch J, Ram S, Koeberling O, and Granoff D. (2008) Complement-Dependent Synergistic Bactericidal Activity of Antibodies against Factor H-Binding Protein, a Sparsely Distributed Meningococcal Vaccine Antigen. *J Infect Dis* 197:1053–1061.

Welsch JA., Moe GR, Rossi R, et al. (2003). Antibody to genome-derived neisserial antigen 2132, a *Neisseria meningitidis* candidate vaccine, confers protection against bacteremia in the absence of complement-mediated bactericidal activity. *J. Infect. Dis.* 188:1730–1740.

Weynants VE, Feron CM, Goraj KK, et al. (2007) Additive and synergistic bactericidal activity of antibodies directed against minor outer membrane proteins of *Neisseria meningitidi. Infect Immun* 75:5434-42.

Zhu D, Zhang Y, Barniak V, et al. (2005) Evaluation of recombinant lipidated P2086 protein as a vaccine candidate for group B *Neisseria meningitidis* in a murine nasal challenge model. *Infect Immun* 73:6838–45.

Appendix 1.

Serological Correlates of Protection for Meningococcal Vaccines and 4CMenB

A1.0 Summary

It is widely accepted that the primary immune mechanism responsible for protection against meningococcal disease is complement-mediated serum bactericidal activity (Frasch et al., 2009). Functional bactericidal antibodies induced by vaccination with the Novartis 4CMenB vaccine will be measured by the SBA, which uses human serum as the source of exogenous complement (hSBA). Human serum was used as the source of complement for the SBA in the original studies which established the correlation between serum bactericidal antibodies and protection against meningococcal disease. An hSBA titer ≥ 1:4 has been historically defined as the threshold titer that correlates to clinical protection. Although this level was established for antibodies directed against the capsular polysaccharides of meningococci, clinical efficacy trials of serogroup B OMV vaccines support a similar correlate for serum bactericidal antibodies to meningococcal outer membrane proteins. Several large trials using OMV vaccines were conducted to evaluate vaccine efficacy in response to meningococcal serogroup B outbreaks in Cuba, Brazil, and Norway (reviewed in Holst et al., 2009). The proportions of vaccinees with ≥4-fold rises in SBA pre- to post-vaccination or SBA titres ≥1:4 have been correlated with clinical efficacy in these large trials. In addition, the Novartis MenZB OMV vaccine has been administered to millions of subjects in response to a clonal seroroup B outbreak in New Zealand. It should be noted that the Novartis 4CMenB vaccine contains an OMV component identical to that used in the New Zealand immunization campaign.

At recent meetings conducted between Novartis Vaccines and the USFDA (Type B Meeting, June 2006) and the EMEA (Scientific Advice March 2007) regulatory agencies, a positive opinion was acquired on the Company proposal for use of the hSBA as a serological correlate for protection. It was accepted that hSBA titers ≥ 1:4 is an appropriate clinical end-point and that clinical efficacy trials would be not necessarily be required for licensure. In early Phase 1/2 studies prior to the validation of the SBA assay, the use of 1:8 as one endpoint was implemented by Novartis Vaccines, as a 1:4 titer provides only a two-fold difference from the 1:2 starting dilution and may be associated with significant error. Since this time the hSBA assay has been validated, and based on input from regulatory authorities, a titer of 1:5 has been identified as the dilution for which the lower 95% Confidence bound is 1:4.

As a result, an adequate proportion of pediatric subjects with hSBA titers ≥1:5 at one month after a primary course of three doses and a fourth dose at 12-months, against the reference ("indicator") meningococcal B strains was considered the primary immunological outcome for pivotal clinical studies. Novartis also plans to evaluate bactericidal antibody data using other endpoints as appropriate, including percentage of subjects achieving SBA titers ≥1:8, percentage of subjects with four-fold rise, GMTs, GMRs and Reverse Cumulative Distribution data.

A1.2 SBA titers > 1:4 are inversely correlated with age-related incidence of disease

The principal effector mechanism affording protection against meningococcal disease is the antibody-dependent activation of complement-mediated bacteriolysis, although antibody-mediated opsonization may also play a role in protection (reviewed in Granoff, 2009). Supportive evidence comes from the observation that rates of meningococcal disease are highest in infants between 6 and 12 months of age, an age group with the lowest bactericidal

antibody titers. In addition, individuals deficient in serum complement components C5, C6, C7, or C8 have a 5- to 10,000-fold greater risk of meningococcal disease than normal individuals, and are susceptible to repeated meningococcal infections (Figueroa and Densen, 1991). Based on these many observations, the serum bactericidal assay using human complement remains the method of choice to evaluate protection against MenB disease. Indeed, there was general agreement at the "*Neisseria meningitidis* Group B Correlates of Protection and Assay Standardization" international meeting that the SBA is an appropriate and robust surrogate for predicting the efficacy of a meningococcal group B vaccine (Borrow et al., 2005).

The original surrogate of natural protection against meningococcal serogroup C disease came from the pioneering work of Goldschneider and co-workers (Goldschneider *et al*. in 1969). In a study of military recruits commencing training, it was observed that only 3 out of 54 (6%) prospective meningococcal cases had naturally acquired, circulating SBA titers $\geq$ 4 prior to commencement of training, as compared with 444 of 540 (82%) controls who had titers $\geq$ 4 and who did not become infected. This correlate titer was then used to demonstrate the inverse relationship between the age-related incidence of disease and levels of bactericidal titers $\geq$ 4 against 3 strains of meningococci from serogroups A, B, and C (Figure A1-1). These studies revealed that the low incidence of disease correlated well with the high titers observed in very young infants. The peak incidence occurs at a window between approximately 6 and 24 months, the intermediate period separating maternal and acquired immunity. From age two onwards, the incidence of disease decreases as the percentage with SBA $\geq$ 4 rises, presumably through asymptomatic carriage of commensal meningococcal and/or related neisserial strains (particularly *N. lactamica*). It should be noted that more recent data suggest that the seroprevalence of group C bactericidal activity has generally decreased in the global population since the 1960s, when the Goldschneider et al. studies were done.

It is important to note that the Goldschneider studies were performed using serum collected in the field and containing only naturally acquired antibodies. The subjects were not immunized with any vaccine and no studies were performed to determine the specificity of the antibodies that killed the bacteria. Goldschneider *et al.* tested serum from the cases of meningococcal disease against their own isolates of *N. meningitidis* and showed that SBA activity was absent from pre-infection sera. Because the SBA reflects the summation of the killing activity of all of the antibodies present in the specimen, regardless of which meningococcal antigen(s) they may recognize, these studies were not a proof of concept of the effectiveness of anti-capsular antibodies against meningococcal disease. Rather, these studies showed a correlation between killing in the SBA, mediated by antibodies of unknown specificity, and resistance to meningococcal disease.

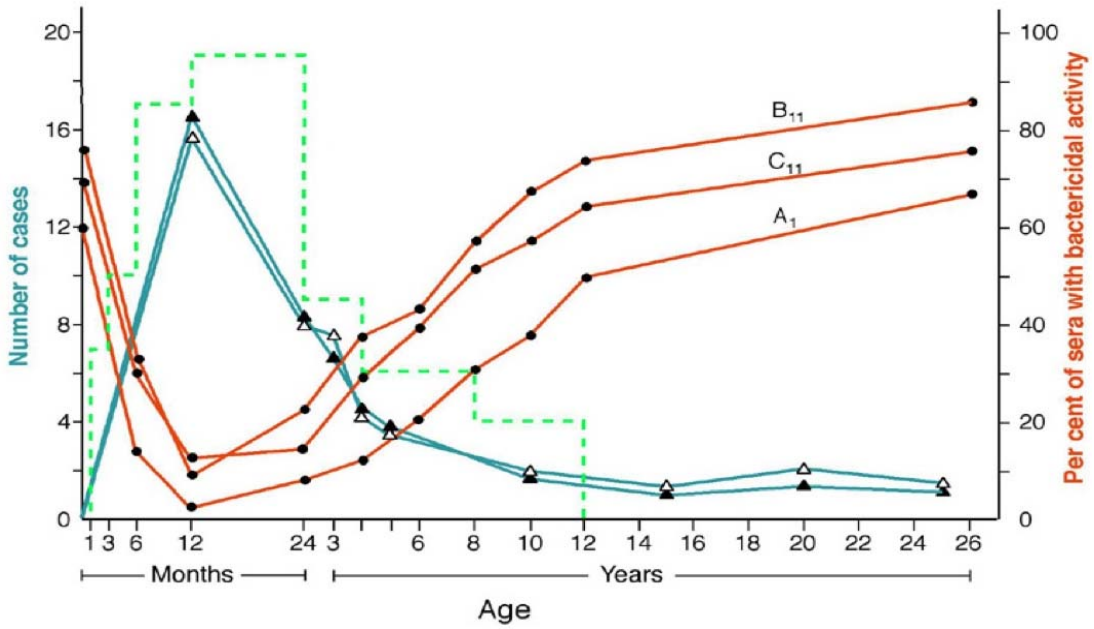


Figure A1-1. **The classical Goldschneider study**. Age-related incidence of meningococcal disease in the US and prevalence of serum bactericidal activity against 3 pathogenic strains of *N. meningitides.* ▲−▲, number of cases/100,000 age-specific population, 1965; Δ−Δ, number of cases/100,000 age-specific population, 1966;-------, age distribution of 72 cases admitted to L. A. Children's Hospital 1944-1953; ●−●, percent of sera in each age group having a bactericidal titer of 1:4 or greater against strains A1, B11 and C11. Sera from 282 children (at least 20 per age group) and 567 army recruits (ages 19-26) were tested in the presence of exogenous human complement. Each point in the figure represents the incidence of disease or prevalence of bactericidal activity among subjects in the age range encompassed by it and the previous point (e.g., 2.5% of children 6-12 months of age had serum bactericidal activity against meningococcal strain A1). Adapted from Goldschneider *et al.*, 1969.

Goldschneider also observed that not all of the military recruits who lacked an SBA response and who were colonized by the invasive strain went on to develop the disease. It is suggested then that the presence of SBA is associated with protection against meningococcal disease, but its absence does not necessarily imply susceptibility. Indeed, several studies have shown that SBA tends to underestimate the actual field efficacy of meningococcal vaccines (Balmer and Borrow, 2003; Borrow et al., 2006).

Goldschneider et al. also studied the age-related prevalence of bactericidal antibodies against *N. meningitidis* and compared it with the age related incidence of invasive meningococcal disease. In these studies, they chose an individual strain of serogroups A, B and C (A1, B11 and C11) as a representative of all of the meningococcal isolates within the serogroup. Again, the precise specificity of the antibodies responsible for the killing was not defined. It is likely that capsular polysaccharide was involved in killing of serogroup A and C strains although this is not directly demonstrated in this paper. Because of the inherent lack of immunogenicity of the serogroup B capsule (composed of α2-8–linked polysialic acid that is structurally identical to fetal brain-cell adhesion molecules), it is equally likely that the protective antibodies for group B were directed against non-capsular antigens. Yet, the same inverse relationship between SBA and age related disease incidence was seen also for serogroup B as it was for serogroups A and C.

A1.3 SBA correlates well with group B OMV vaccine efficacy

The great majority of the global meningococcal disease burden is associated with five serogroups: A, B, C, Y and W135. Currently polysaccharide and polysaccharide-conjugate vaccines are available for all but the serogroup B meningococci. Development of such a vaccine is hampered by the poor immunogenicity of the B polysaccharide in humans, perhaps relating to its similarity to a human neuronal carbohydrate. There is reluctance in industry to develop a group B polysaccharide-based vaccine due to the fears of the induction of autoantibodies. Instead, outer membrane vesicle vaccines (OMVs) have been developed to address group B epidemic outbreaks in Norway, Cuba, Chile, Brazil, and New Zealand (Bjune et al., 1991; Holst et al., 2003; Siera et al., 1991; Boslego et al., 1995 ; Milagres et al., 1994). These "tailor-made" vaccines are particularly well-suited for control of epidemic outbreaks that are dominated by a single serosubtype (defined by the highly variable PorA outer membrane protein).

The efficacy of these OMV vaccines is presented in Table A1-1. The Chile and Norway studies recapitulated the Goldschneider observation that there is an age-related efficacy that is reflected in the levels of bactericidal titers achieved. Importantly, these studies collectively support a correlation between serum bactericidal activity and efficacy of group B meningococcal OMV vaccines. Indeed, the proportions of vaccines with ≥4-fold rises in SBA pre- to post-vaccination or SBA titres ≥1:4 have been correlated with clinical efficacy in the Cuban, Brazilian and Norwegian efficacy trials (reviewed in Holst et al., 2009).

Table A1-1. Correlation between serum bactericidal activity and efficacy of group B meningococcal OMV vaccines.

Place (year; reference)	Vaccine OMV Serotype/ sero-subtype	Design	Efficacy [95% CI] (subject age)	% with ≥ 4-fold rise in SBA	% with SBA≥4 post-vaccination
Cuba (1987-89; Sierra et al., 1991)	4:P1.19,15	Randomized, double-blind, placebo-control	81% [44-93] (10-14 yrs.)	85%	nd
Sao Paolo, Brazil (1990-91; De Moraes et al., 1992, Milagres, et al. 1994)	4:P1.19,15	Case control	-37%[<100-83] (3-23 months)	22%	13%[a]
			47% [-79-88] (24-47 months)	45%	43%[a]
			74% [16-92] (>48 months)	52%	52%[a]
Iquique, Chile (1990-91; Boslego et al., 1995)	15:P1.7,3	Randomized, double-blind, placebo-control	No efficacy (1-4 yrs.)	12%	nd
			70% [3-93] (5-21 years)	65%	nd
Norway (1988-91; Bjune et al., 1991; Holst et al., 2003)	15:P1.7,16	Randomized, double-blind, placebo-control	87% [62-100] (14-16 years) 10 months post	80% (1.5 months post)	97% (1.5 months post)
			57% [21-87] (14-16 yrs.) 29 months post	26% (24 months post)	42% (24 months post)

[a] :% with SBA > 4 post-vaccination

A1.4 Conclusion

Novartis believes that the evidence suggesting that induction of bactericidal antibodies is the major immunological indicator of protection against all serogroups of meningococcus supports the use of the SBA regardless of antigen identity. Such evidence includes but is not limited to:

- The increased incidence of disease in individuals with deficiencies in terminal complement components,
- The inverse correlation between SBA and the age related incidence of meningococcal disease,
- The correlation between meningococcal group B OMV vaccine efficacy and the induction and persistence of serum bactericidal activity.

The proportions of vaccines with ≥4-fold rises in SBA pre- to post-vaccination or SBA titres ≥1:4 have been correlated with clinical efficacy in large OMV clinical trials. Novartis believes that these studies are strongly supportive of the use of a validated SBA titer of 1:5 (of which the lower bound 95% CI is 1:4) as an appropriate immune correlate of protection against serogroup B disease. Indeed, consensus support for this notion was achieved at the "*Neisseria meningitides* Group B Correlates of Protection and Assay Standardization" international meeting (Atlanta, 2005) attended by representatives from academia, health and regulatory agencies, and industry from the US and the European Union (Borrow et al., 2006)

A1.5 References

Balmer, P and Borrow, R. (2003) Serological correlates of protection for evaluating the response to meningococcal vaccines. *Expert Rev Vaccines* 3:89-99.

Bjune G., et al. (1991) Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. *Lancet* 338:1093-1096.

Borrow R, Aaberge IS, Santos GF, *et al*. (2005) Interlaboratory Standardization of the Measurement of Serum Bactericidal Activity by Using Human Complement against Meningococcal Serogroup B, Strain 44/76-SL, before and after Vaccination with the Norwegian MenBvac Outer Membrane Vesicle Vaccine. *Clin Diagn Lab Immunol.* 12(8): 970–976.

Borrow, R., et al. (2006) Neisseria meningitidis group B correlates of protection and assay standardization—International Meeting Report Emory University, Atlanta, Georgia, United States, 16–17 March 2005. *Vaccine* 24:5093-5107.

Boslego, J. et al. (1995) Efficacy, safety, and immunogenicity of a meningococcal group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile *Vaccine* 13:821-829.

Figueroa, JE and Densen P. (1991) Infectious diseases associated with complement deficiencies. *Clin Microbiol Rev.* 4(3):359-95.

Frasch CE, Borrow R and Donnelly J (2009) Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease *Vaccine* 27 (Suppl. 2): B112-B116.

Goldschneider I., et. al. (1969) Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* (6):1307-26.

Granoff DM (2009) Relative importance of complement-mediated bactericidal and opsonic activity for protection against meningococcal disease *Vaccine* 27 (Suppl. 2): B117-B125

Holst, J., et al. (2003) Serum bactericidal activity correlates with the vaccine efficacy of outer membrane vesicle vaccines against *Neisseria meningitidis* serogroup B disease. *Vaccine* 21:734-37.

Holst J, Martin D, Arnold R, et al. (2009) Properties and clinical performance of vaccines containing outer membrane vesicles from Neisseria meningitidis. *Vaccine* 27 Suppl 2:B3-12.

Milagres, L. G., et al. (1994) Immune response of Brazilian children to a Neisseria meningitidis serogroup B outer membrane protein vaccine: comparison with efficacy. *Infect Immun* 62(10):4419-24.

Sierra, G.V., et al. (1991) Vaccine against group B Neisseria meningitidis: protection trial and mass vaccination results in Cuba. *NIPH Ann* 14(2):195-207.

APPENDIX 2

The Use of the Serum Bactericidal Assay in MenB Vaccine Evaluation

A2.0 The SBA Assay, Complement Selection, and Standardization

N. meningitidis target strains are lysed in the presence of specific antibodies and complement ("antibody-mediated, complement-dependent" killing). To perform the SBA assay, serial dilutions of human sera (containing meningococcal-specific antibodies) are incubated with appropriate target strains and human complement. Specific antibody binds to the target cell surface, complement C1q binds the Fc portion of the surface bound immunoglobulin, activates the classical pathway, and leads to formation of the membrane attack complex (C5 through C9), and ultimately to target cell death. After incubation, the samples are plated onto solid media and viable bacteria are counted the following day. The SBA titer for each serum is expressed as the reciprocal serum dilution yielding > 50% killing as compared to the number of target cells present before incubation with serum and complement.

A2.1 Human vs. Rabbit Sources of Complement for Use in the SBA

The use of human complement for SBA (hSBA) has been a priority throughout Novartis meningococcal vaccine development programs. This includes the licensing of a serogroup C vaccine in Europe and elsewhere, a licensed OMV-based serogroup B meningococcal vaccine in New Zealand, and more recently, the quadrivalent glycoconjugate vaccine (Menveo®) licensed in the EU and the US. The bactericidal assay requires large volumes of specimen, and is often limited by the difficulty of finding human complement sources that are compatible with bacterial strains that may be desirable clinical endpoints.

SBA using baby rabbit complement (rSBA) has previously been used in assessing the performance of other glycoconjugate meningococcal vaccines. However, the choice of continued use of human rather than rabbit complement is based on several factors. It has been demonstrated that the rSBA and hSBA may not measure the same immune parameters (Santos *et al.*, 2001). Antibody titers measured with rabbit complement often are much higher than those measured using human complement (Zollinger and Mandrell, 1983; Santos *et al.*, 2001). The reason for this observation is that rabbit complement factor H (fH) does not bind to *N. meningitidis* (Granoff *et al.*, 2009). fHbp, one of the primary antigenic components of BEXSERO binds the human complement protein factor H (fH) in a species specific manner, with a strong preference for binding human fH (Madico *et al.*, 2006; Granoff *et al.*, 2009). fH was originally described as an element of the alternative pathway of complement activation, but later was found to inhibit a critical step in amplification of both the classical and anternative pathways. Binding of fH to the bacterial surface accelerates decay of C3/C5 convertase, which decreases the activation of complement on the surface of the target cell. Thus, by sequestering the fH-mediated down-regulating activity to the bacterial surface, the meningococci are able to avoid complement-mediated killing by non-immune human serum or blood.

The binding of fH to *N. meningitidis* is specific for human fH, explaining at least in part why *N. meningitidis* is strictly a human pathogen. The addition of human fH decreased rat and rabbit C3 deposition on the bacterial surface and decreased group C bactericidal titers measured with rabbit complement 10- to 60-fold in heat-inactivated sera from human vaccinees (Granoff *et al.*, 2009). Thus, in the absence of fH bound to the bacterial surface,

complement activation is not down-regulated, resulting in higher bactericidal titers when SBA analyses are conducted using rabbit complement. For this reason, Novartis uses the more physiologically relevant human complement in our SBA analyses.

A2.2 Selection and Qualification of Complement for Use in hSBA

It is clear that the complement source used in the SBA is of critical importance. Although the use of human complement is appropriate for evaluating the functional immunogenicity of serogroup B meningococcal vaccines, procurement of large volumes is difficult. Agammaglobulinemic sera is not available in large volumes as a complement source. Most normal human subjects acquire bactericidal antibodies during adolescence and adulthood as a result of oropharyngeal carriage of meningococci or other neisserial species. The need to find one or more complement donors who do not already have intrinsic bactericidal activity for a meningococcal strain of interest acts as a significant barrier to testing a large number of diverse MenB bacteria by a great number of clinical subjects in hSBA. Thus, the selection and qualification of potential donor sera is an important prerequisite to conducting hSBA in our clinical studies.

Various methods may be used for the screening of appropriate human complement sources. Donors may be prescreened for specific antibodies by immunoasssays other than SBA, e.g. by ELISA, if the antigen of interest is known. This allows donors with a high potential for bactericidal activity to be excluded from the screen. This practice is used for group C meningococci because anticapsular antibodies are the main cause of intrinsic bactericidal activity against this serogroup in normal human sera. For serogroup B, the intrinsic bactericidal activity may result from any combination of a plethora of protein and lipopolysaccharide antigens, some known and some unknown. Therefore, prospective complement donations usually are screened for the presence of intrinsic bactericidal antibodies able to kill a test MenB strain(Borrow and Carlone, 2001. Donations usually are tested at final concentrations of 25% and 50% for intrinsic bactericidal activity in the absence of exogenous antibody. Donations are selected if no significant reduction occurs in the number of colony forming units (cfu) added to the test at the beginning of the incubation period (usually 20-60 minutes). A threshold of 85% or 90% of the input cfu is often used to account for sampling error and avoid rejection of excessive numbers of complement donations. Complement donations also should be tested for proper function of the complement cascade. This is usually done by testing for hemolytic complement activity on pre-opsonized red blood cells (CH50), or by testing the ability of the complement donation to support killing of the MenB strain of interest using a known positive standard bactericidal serum. Sera lacking endogenous bactericidal activity against the target strain but with adequate complement activity in control experiments are then stored at –70°C. In cases where storage exceeds 6 months, it is generally considered to be good practice to re-qualify the complement in the same manner. Finally, if it should become necessary to pool complement donations from different individual human complement donors, the pooled product should be subjected to the same screening as is performed on the individual components, as described above.

Both serum (with clotting factors removed by coagulation) and plasma (with suitable anticoagulant) may be used as complement sources. Perkins *et al.* (1998) reported the use of hSBA with human plasma as complement source in a clinical trial of OMV vaccines in Iceland. Another study demonstrated that plasma complement can be used to obtain comparable bactericidal results in multiple laboratories (Borrow *et al.*, 2005). In addition, a validated hSBA assay with both plasma and serum complement was developed for efficacy

trials of the MenZB® vaccine (Martin *et al*., 2005). Use of either serum or plasma has its own strengths and weaknesses. Serum complement is straightforward to collect, but because the product is collected initially as whole blood, the amount that can be collected from a single donor is generally limited to one unit per 6 week period or as permitted by the hemoglobin status of the donor, as specified in the AABB Manual for Blood Banks. Plasma may be collected using an anticoagulant such as lepirudin, an anticoagulant obtained from vampire bat saliva, which has been found to have minimal ability to inhibit the action of complement. However, if applied to whole blood, the same volume restrictions also apply. Heparin is not recommended as a primary anticoagulant for whole blood for the purpose of collecting plasma complement because the concentrations normally used in anticoagulant tubes (around 25 U/mL) are sufficiently high to also have inhibitory activity on complement activation. Pheresis plasma is a ready source of complement and can be collected in much larger volumes, up to but not more than 1000 mL per two day period (CFR24 Part 640.65) for a typical donor. The anticoagulant in commercial pheresis sets generally is sodium citrate, which prevents coagulation by sequestering divalent cations. However divalent cations also are required for the activation of complement, and therefore pheresis plasma may require the replenishment of calcium and magnesium ions in order to have full complement activity. With the use of plasma complement at high concentrations, addition of divalent cations may result in some clotting of the specimen, which can make accurate colony counts difficult. Either lepirudin or a low concentration of sodium heparin (0.5-1 U/mL which is below the threshold that inhibits complement activation) generally is used to prevent clotting of the specimen and facilitate accurate colony counts.

A2.3 hSBA Standardization

The hSBA used by Novartis for analysis of clinical trials was originally established by Goldschneider et al (1969), but there are slight differences in every laboratory performing this assay. Therefore, a comparative study between four laboratories, the Health Protection Agency (HPA) in the UK, the New Zealand Institute of Environmental Science and Research Limited (NZ ESR), the Norwegian Institute of Public Health (NIPH), and Novartis (at the time Chiron) laboratory in Emeryville was performed (Borrow *et al.*, 2005). These laboratories measured SBA titers in the same panel of human sera from adults immunized with the Norwegian H44/76 OMV vaccine (MenBvac®). Initial results showed a number of discrepancies in results owing primarily to differences among laboratories in the maintenance of the Men B test strain. In a repeat study using the same, freshly distributed frozen isolate to all four laboratories, correlation coefficients of SBA results the HPA as comparator were as follows for NZ ESR, NIPH, and Novartis, respectively, using $\log_{10}$ titers: (r): 0.966, 0.967, and 0.936; intercepts were 0.08, 0.15, and 0.17; and slopes were 0.930, 0.851, and 0.891. After harmonization of the assay at the different labs, good assay comparability was demonstrated in terms of seroconversion rates, n-fold changes in titers, and percentages of subjects with SBA titers of ≥ 4.

In order to maintain the reproducibility of an hSBA it is necessary to use a series of assay controls. The assay controls should cover the critical titer range of the assay and ensure equal conditions over the entire titer range. They should be included in each assay to ensure that during the testing of a clinical trial no shifts in results will be introduced. Acceptance criteria should be established for each control based on repetitive tests by multiple operators, and each of the control sera should meet its defined acceptance criteria in order for the assay to be valid.

Control sera may be prepared from non-immunized donors or from clinical trial subjects immunized with the vaccine of interest, depending on the desired titer level. Individual

donations of high, medium, or low control should be kept separately and in order to avoid instability during storage, control sera from different donors should not be pooled.

The negative controls are serum donations from unvaccinated subjects. Negative controls are tested in the same way as described above for the high and low control and must always have a titer of below the lower limit of detection (LLD). The negative control in each assay must be <LLDin order for the assay to be valid. Should the negative control have a titer of ≥LLD, the assay is should be repeated.

The SBA assay using human complement has been appropriately validated by Novartis for each immunogenicity indicator strain. Although early clinical studies evaluated the percentages of subjects achieving hSBA ≥ 1:4 and 1:8 as outcomes, the hSBA titer cut-off was eventually changed from 1:4 to 1:5. Using the 1:5 cut-off ensures with 95% confidence that subjects with hSBA titers of 1:5 or greater will have achieved a titer of at least 1:4, which is considered to be indicative of protective efficacy. This change was based on the validation studies that have shown that the lower limit of the two-sided 95% confidence interval for a titer of 1:5 is a titer of 1:4, using linearly interpolated hSBA titers.

A2.4 Mechanisms of Serum Resistance in Meningococci

Serum resistance and mechanisms by which bacteria evade immune attack have been studied and reviewed extensively (Lambris et al., 2008). The principal mechanisms by which meningococci acquire resistance to the action of complement are thought to be by binding of regulatory proteins and by sialylation of capsular polysaccharide and of LOS (Ram et al., 1999). Binding of factor H, which converts active C3b into inactive iC3b, occurs on meningococci by the action of the species-specific factor H binding protein (fHbp) and also by neisserial surface protein A (NspA) (Granoff et al., 2009; Lewis et al., 2010). Sialylation of the bacterial surface introduces strong negative charges at physiological pH, which can interfere with the formation of the Membrane Attack Complex (MAC) and decrease the effectiveness of complement mediated lysis (Vogel et al. 1997, reviewed by Ram, et al. 1999). More recently, increased resistance by serogroup C isolates was shown to be due to the presence of an insertion sequence (IS*1301*) between operons responsible for capsule biosynthesis and export (Uria et al., 2008). This lesion resulted in an increased expression of capsule and the down-regulation of the alternative pathway of complement activation. Although the IS*1301* mutation is found in virtually all ST269 strains from the United Kingdom (Lucidarme et al., 2009), British ST269 strains can be killed by serum from human subjects immunized with 4CMenB (Donnelly *et al.,* 2010), indicating that the protective effect of this mutation on complement-mediated bacteriolysis is not absolute. Carriage strains, which often do not express a capsule, and capsule null strains, which lack the necessary genes to express a capsule (Claus *et al*., 2002; Dolan-Livengood *et al.*, 2003; Weber *et al.,* 2006) are very rarely invasive (Caugant e*et al.*, 2007). Growth of meningococci in culture may decrease their sialic acid content and increase their serum susceptibility. For this reason most SBA protocols use either rich media such as chocolate or Columbia agar or, if liquid media are used, supplement them with a carbon source such as glucose or CMP-NANA (Borrow, et al., 2005; reviewed in Ram, 1999).

A2.5 References

Borrow R and Carlone GM (2001) Serogroup B and C Serum Bactericidal Assays In: Pollard AJ and Maiden MCJ, eds. Methods in Molecular Medicine, vol. 66: Meningococcal Vaccines: Methods and Protocols. Humana Press Inc., Totowa, NJ. pp. 289-304.

Borrow R, Aaberge IS, Santos GF, *et al*. (2005) Interlaboratory Standardization of the Measurement of Serum Bactericidal Activity by Using Human Complement against Meningococcal Serogroup B, Strain 44/76-SL, before and after Vaccination with the Norwegian MenBvac Outer Membrane Vesicle Vaccine. *Clin Diagn Lab Immunol.* 12(8): 970–976.

Caugant DA, Tzanakaki G and Kriz P. (2007) Lessons from meningococcal carriage studies, *FEMS Microbiol Rev* 31(1):52–63.

Claus H, Maiden MC, Maag R, *et al.* (2002) Many carried meningococci lack the genes required for capsule synthesis and transport, *Microbiology* 148(6):1813–1819.

Dolan-Livengood JM, Miller YK, Martin LE, *et al.* (2003) Genetic basis for nongroupable *Neisseria meningitidis*, *J Infect Dis* 187(10):1616–1628.

Donnelly J, Medini D, Boccadifuoco G, et al. (2010) Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proc Natl Acad Sci U S A.* 107(45):19490-5.

Goldschneider I., et. al. (1969) Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* (6):1307-26.

Granoff DM (2009) Relative importance of complement-mediated bactericidal and opsonic activity for protection against meningococcal disease *Vaccine* 27 (Suppl. 2): B117-B125

Lambris JD, Ricklin D and Geisbrecht BV (2008) Complement evasion by human pathogens. *Nature Rev Microbiol* 6:132-142.

Lewis LA, Ngampasutadol J, Wallace R, et al. (2010)The Meningococcal Vaccine Candidate Neisserial Surface Protein A (NspA) Binds to Factor H and Enhances Meningococcal Resistance to Complement. *PLoS Pathog* 6(7): e1001027

Lucidarme J, Comanducci M, Findlow J, *et al*. (2009) Characterization of fHbp, nhba (gna2132), nadA, porA, sequence type (ST), and genomic presence of IS1301 in group B meningococcal ST269 clonal complex isolates from England and Wales. *J Clin Microbiol.* 47(11):3577-85.

Madico, J.A. Welsch, L.A. Lewis, A. McNaughton, D.H. Perlman and C.E. Costello *et al.* (2006) The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance, *J Immunol* **177**(1):501–510.

Martin D, McCallum L, Glennie A, *et al.* (2005) Validation of the serum bactericidal assay for measurement of functional antibodies against group B meningococci associated with vaccine trials. *Vaccine*, 23:2218-21.

Perkins BA, Jonsdottir K, Briem H. *et al.* (1998) Immunogenicity of Two Efficacious Outer Membrane Protein–Based Serogroup B Meningococcal Vaccines among Young Adults in Iceland. *J. Infec. Dis.* 177:683-91.

Ram S, Mackinnon FG, Gulati S, et al. (1999) The contrasting mechanisms of serum resistance of *Neisseria gonorrhoeae* and group B *Neisseria meningitidis Molec Immunol* 36:915-928.

Santos GF, Deck RR, Donnelly J, et al. (2001) Importance of complement source in measuring meningococcal bactericidal titers, *Clin Diagn Lab Immunol* **8**(3):616–623.

Uria MJ, Zhang Q, Li Y, et al. (2008) A generic mechanism in Neisseria meningitidis for enhanced resistance against bactericidal antibodies. *J Exp Med.* 205(6):1423-34.

Vogel U, Weinberger A, Frank R, et al. (1997) Complement factor C3 deposition and serum resistance in isogenic capsule and LOS sialic acid mutants of serogroup B *Neisseria meningitidis*. *Infect Immun* 65(0):4022-29.

Weber MVR, Claus H, Maiden MC, *et al.* (2006) Genetic mechanisms for loss of encapsulation in polysialyltransferase-gene-positive meningococci isolated from healthy carriers, *Int J Med Microbiol* 296(7):475–484.

Zollinger, WD and Mandrell, RE (1983) Importance of complement source in bactericidal activity of human antibody and murine monoclonal antibody to meningococcal group B polysaccharide. *Infect. Immun.* 40:257–264.

APPENDIX 3

Evaluation of 4CMenB Immunogenicity in Infants

We have previously provided evidence that adults who receive 4CMenB vaccine are able to mount a significant response against 3 of the original indicator strains used to evaluate the immunogenicity of fHbp (H44/76), NadA (5/99) and PorA P1.4 (NZ98/254)(Kimura *et al.*, 2010). In order to assess the performance of the vaccine in the pediatric population, we conducted studies in young infants (aged 2 months at first dose) and older infants (aged 6-8 months at first dose).

A3.1 Clinical Studies in Young Infants

In a Phase 2, open label, multi-center, controlled, randomized study, the first to be conducted in healthy infants aged 2 months at time of enrollment. Subjects (n=147) received 4CMenB or rMenB (vaccine formulation containing only the 3 recombinant protein antigens) vaccinations intramuscularly either according to a 2, 4, 6 and 12 months of age immunization schedule, or as a single dose at 12 months of age. The details of this study have been recently published (Findlow *et al.*, 2010).

The immunogenicity results demonstrated that 4CMenB can effectively induce bactericidal antibodies in infants following three doses at 2, 4 and 6 months of age, as measured by hSBA against the three MenB strains H44/76, 5/99 and NZ98/254. Bactericidal antibody was also found to persist against the three vaccine antigens at 12 months of age, 6 months after the primary series, but the percentages of subjects with hSBA ≥1:4 had fallen from 87% at 1 month after the third vaccination to 63% at 12 months of age for strain 44/76, from 95% to 89% for strain 5/99, and from 85% to 34% for strain NZ98/254, demonstrating the need for a booster dose. The responses could be further boosted with a 4th dose of 4CMenB at 12 months of age, demonstrating that the infants had been primed with the three doses and immunological memory had been generated.

The control subjects in this study who received a single dose of 4CMenB vaccine at 12 months of age served as comparators for the 12-month booster response in the subjects receiving the vaccine at 2, 4 and 6 months . The proportion of subjects achieving hSBA ≥1:4 against the three indicator strains was consistently lower for the control subjects receiving a single dose at 12 months of age as compared to the fourth dose response in the 4CMenB subjects group at the same age (73% versus 100% for strain 44/76, 73% versus 96% for strain 5/99, and 18% versus 93% for strain NZ98/254).

In addition to testing the ability of infant immune sera to kill the 3 indicator strains, four additional UK strains were also tested. The strain characteristics are reported in Table A3-1. These four strains were selected because 1) they are strains which vary in their vaccine antigen composition and/or expression and 2) are representative of the major disease-causing types currently in the UK. Overall, the proportion of infants with hSBA titers ≥1:4 was lower against these 4 strains compared to the responses observed against the 3 indicator strains. As expected immune sera were unable to kill the M01 240355 strain, consistent with the lack of cross-reactive vaccine antigens and low levels of NHBA expression in this strain. After 3 doses of vaccine, the proportions ranged from lows of 0% (M01 240355) and 12%[1] (M01

[1] It is noteworthy that the proportion of infants who had serum hSBA titers ≥1:4 against this strain 6 months after the third dose was 21% (i.e. a significant *increase* in the proportion with titer ≥1:4), suggesting the serum sample for this time point may have been inappropriately handled.

240364) to highs of 47% (M01 24101) and 63% (M00 242922), consistent with their antigen reportoire. Similar to the indicator strains, 4CMenB elicited anamnestic responses against these strains after the fourth dose, with responses of 57% (M01 24101), 75% (M01 240364) and 78% (M00 242922).

Table A3-1. Phenotype and genotype of additional strains used in the studies of Findlow *et al.*, 2010 and Snape *et al.*, 2010.

Strain	Phenotype	Clonal Complex (ST)	fHbp variant	*nadA* gene	NHBA Variant[a]	Predicted Coverage By MATS (antigen)
M01 240101	B:NT:P1.19-1,15-11	269 (1049)	1.15	-	21	Yes (fHbp)
M00 242922	B:4:P1.7-2,4	41/44 (41)	1.4	-	2	Yes (PorA and fHbp)
M01 240364	B:2a:P1.5,2	11 (11)	3.31	+	28	Yes (NadA)
M01 240355	B:1:P1.22,14	213 (213)	3.31	+[b]	18	No

a: The nomenclature used here for NHBA variants represents an updated naming convention (manuscript in preparation) and differs from the original nomenclature (Bambini et al., 2009) used in the published reports of these clinical studies.
b: Although the M01-240355 harbors the *nadA* gene, it encodes the non-cross-reactive NadA5 variant.

This study demonstrated that the 4CMenB vaccine was immunogenic against a range of MenB strains when given to healthy infants at 2, 4, and 6 months of age. Furthermore, a booster dose administered at 12 months of age elicited an anamnestic response when compared with a single dose in naïve individuals of the same age. Results from this trial suggest that this vaccine has the potential to provide a broader protection against MenB disease than that provided by existing monovalent OMV vaccines in infants.

A3.2 Clinical Studies in Older Infants

We have also evaluated the immunogenicity and reactogenicity of a 3-dose course of either 4CMenB or rMenB administered to healthy, older infants (Snape *et al.*, 2010). This study was a randomized, single-blind, comparative study of 60 healthy infants enrolled at 6 to 8 months of age and immunized with 4CMenB or rMenB at day 0, day 60, and at age 12 months. Blood samples obtained at baseline and 1 month following the second and third doses of vaccine were analyzed for serum hSBA against the same 7 MenB strains used in the study on younger infants discussed above.

At baseline, 29% (7/24) of infant subjects had a bactericidal titer ≥1:4 when tested against the H44/76 strain, while no baseline titers were detected against the 5/99 and NZ98/254 strains. One month after the second vaccination with 4CMenB, hSBAs ≥1:4 were achieved by 100% of subjects against strains H44/76 and 5/99. These percentages were unchanged one month after the third vaccination, suggesting that two vaccinations with 4CMenB were sufficient to produce the required response against these indicator strains in this age group. For the NZ98/254 strain, 95% to 96% of subjects in the 4CMenB group achieved a bactericidal titer ≥1:4 after the second and third injections, respectively. Almost all subjects achieved a four-fold increase in titers above baseline against all three strains after the second and third injection, except in the rMenB group against the NZ98/254 strain.

Titers of ≥1:4 were also seen for ≥88% participants for strains M00 242922 (homologous PorA) and M01 240364 (cross-reactive NadA) after two doses of 4CMenB. For the remaining 2 strains, this figure was 67% (M01 240101) and 7% (M00 240355). Similar variations were seen in hSBA GMTs and after 3 doses of vaccine. In addition, all recipients of 2 or 3 doses of 4CMenB demonstrated a 4-fold or greater rise in NHBA-GNA1030 fusion protein-specific IgG concentrations over pre-immunization values.

These observations suggest that three doses of 4CMenB in the second half of infancy induces bactericidal antibodies against strains expressing cross-reactive vaccine antigens. It appears that responses in older infants are generally more substantial than those observed in younger infants and suggests that the repertoire of antigen specific bactericidal antibodies is significantly expanded in infants with a more developed immune system.

A3.3 References

Bambini S, Muzzi A, Olcen P, et al. (2009) Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus. *Vaccine*. 27(21):2794-803.

Findlow J, Borrow R, Snape MD, Dawson M, Holland A, John MT, et al. (2010) Multicentre, open-label, randomised phase II controlled trial of an investigational recombinant meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy. *Clin Infect Dis.* 51(10):1127-37.

Kimura A, Toneatto D, Kleinschmidt A, *et al*. (2010) Immunogenicity and safety of a multicomponent meningococcal serogroup B vaccine and a quadrivalent meningococcal CRM197 conjugate vaccine against serogroups A, C, W-135, and Y in adults who are at increased risk for occupational exposure to meningococcal isolates. *Clin Vaccine Immunol.* Dec 22. [Epub ahead of print]

Snape MD, Dawson T, Oster P, Evans A, John MT, Ohene-Kena B, et al. (2010) Immunogenicity of Two Investigational Serogroup B Meningococcal Vaccines in the First Year of Life : A Randomized Comparative Trial. *Pediatr Infect Dis J.* 29(11):e71-9..