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3 **Serum Bactericidal Antibody Responses of Adults Immunized**  
4 **with the MenB-4C Vaccine Against Genetically Diverse Serogroup B**  
5 **Meningococci**

6

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35 **Conflict of interest:** DMG is an inventor on patent applications or on issued patents, in  
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53

54 **Abstract**

55 **Background.** MenB-4C is a meningococcal vaccine for prevention of serogroup B  
56 disease. The vaccine contains Factor H binding protein (FHbp) and three other  
57 antigens that can elicit serum bactericidal activity (SBA). For vaccine licensure, efficacy  
58 was inferred from SBA responses against three indicator strains. The relation between  
59 the results and broad protection against circulating genetically diverse strains is not  
60 known.

61 **Methods.** 20 adults were immunized with two doses of MenB-4C given 1 to 2 months  
62 apart. SBA was measured against 3 reference strains and 15 serogroup B test strains  
63 (6 from college outbreaks).

64 **Results.** Compared to pre-immunization titers, 70%, 95% and 95% of subjects had  $\geq 4$ -  
65 fold increases in SBA against reference strains for anti-PorA P1.4, anti-NadA, and anti-  
66 FHbp antibody, respectively. In contrast, only 25 to 45% of the subjects had  $\geq 4$ -fold  
67 responses to ten of the 15 test strains, including eight that expressed one to three  
68 antigens in the vaccine. The majority of subjects at 1 month with  $< 4$ -fold titer increases  
69 had serum titers  $\geq 1:4$ , which are considered sufficient for protection. However, for four  
70 strains, titers declined to  $< 1:4$  by 4 to 6 months in greater than 50% of subjects tested.

71 **Conclusions.** Clinically relevant isolates often are more resistant to SBA than indicator  
72 strains used to measure antigen-specific SBA. A working model is that the percentage  
73 of subjects with titers  $\geq 1:4$  at 1 month post-immunization correlates with short term  
74 protection against that strain, whereas the percentage of subjects with  $\geq 4$ -fold titer  
75 increases represent more robust responses.

## 76 Introduction

77 A multicomponent meningococcal vaccine (Bexsero®, GlaxoSmithKline) for  
78 prevention of serogroup B disease was licensed in Europe in January 2013 for infants  
79 beginning at two months of age, as well as for older children and adults. The vaccine,  
80 referred to either as “4C-MenB” (1, 2) or “MenB-4C” (3, 4), contains four components  
81 that can elicit serum bactericidal antibodies (SBA). Three components are recombinant  
82 proteins containing Factor H binding protein (FHbp), *Neisserial* heparin binding antigen  
83 (NHba), or *Neisserial* adhesin A (NadA) (5). The fourth is outer membrane vesicles  
84 (OMV) from a serogroup B strain responsible for an outbreak in New Zealand (6, 7).  
85 The OMV elicits SBA largely directed at an accessible loop on PorA defined by the  
86 serosubtype P1.4 (8) MenB-4C subsequently was approved in the United States for  
87 adolescents and young adults, ages 10 to 25 years. The vaccine also is recommended  
88 for individuals older than age 25 who are at increased risk of developing meningococcal  
89 disease, such as persons with complement deficiencies or laboratory workers with  
90 occupational exposure to serogroup B meningococci (3, 4). In September 2015 MenB-  
91 4C was introduced for routine vaccination in the United Kingdom for infants beginning at  
92 two months of age.

93 For inferring vaccine efficacy, the Committee for Medicinal Products for Human  
94 Use (CHMP) in Europe, and the U.S. Food and Drug Administration (FDA), approved  
95 MenB-4C based on SBA measured against three reference strains, each of which  
96 expresses one MenB-4C antigen (FHbp, NadA, PorA P1.4). A suitable reference strain  
97 for measuring SBA to the fourth antigen, NHba, was not available. In contrast to the  
98 reference strains, circulating disease-causing serogroup B strains are genetically

99 diverse (9-11) and exhibit a wide range of expression of the surface proteins that are  
100 target antigens in MenB-4C (12, 13). These properties can affect strain susceptibility to  
101 SBA (12, 14-18). While indirect methods such as antigen typing have been proposed to  
102 predict strain coverage (12), the relationship between SBA measured against the  
103 reference strains and circulating strains is not known. For example, MenB-4C  
104 vaccination recently failed to elicit protective SBA titers in a third of students exposed to  
105 an a University campus outbreak despite predictions based on antigen typing that the  
106 strain was susceptible (19).

107 The purpose of the present study was to investigate SBA responses of adults  
108 immunized with MenB-4C against 18 genetically diverse meningococcal serogroup B  
109 strains.

110

## 111 **Material and methods**

112 *Study design.* The sera were obtained from 20 adults, ages 21 to 44 years,  
113 immunized with MenB-4C in post-licensure immunogenicity studies. Fifteen were  
114 immunized with two doses separated by two months at the Oxford Vaccine group,  
115 University of Oxford, U.K., and five were immunized with two doses separated by one  
116 month at the UCSF Benioff Children's Hospital Oakland, California, U.S. The 1 month  
117 interval in Oakland was used to accelerate protection since the subjects were  
118 microbiologists with occupational exposure to *N. meningitidis*.

119 The median age at immunization was 29.5 years. Five subjects (25%) were  
120 males; 13 (65%) were white European ancestry, 6 (30%) were Asian (2 from India and 4  
121 from other countries), and one was of black African ancestry. Twelve (60%) were

122 healthcare or laboratory workers. Serum samples were taken at the time of the first  
123 dose (pre-immunization sample), and one month after the second dose. Midway in the  
124 study, the protocol was amended to obtain additional follow-up serum samples 4- to 6  
125 months after the second dose of vaccine to determine antibody persistence: Nine  
126 Oxford subjects and two Oakland subjects provided this additional sample. The studies  
127 were approved by the Institutional Review Board of UCSF Benioff Children's Hospital  
128 Oakland (protocols 2015-055 and 2016-24), and by the National Research Authority  
129 Ethical Committee, Bristol UK (15/SC/0172, protocol 2014-12). The Oxford protocol was  
130 registered at clinicaltrials.gov (protocol NCT02398396).

131 *Neisseria meningitidis* test strains. FHbp can be classified into two sub-families,  
132 A and B (20, 21), or three variant groups, 1, 2 or 3 (22). Sub-family B corresponds to  
133 variant group 1, and sub-family A includes variant groups 2 and 3. We tested SBA  
134 against 18 capsular group B isolates (Table 1). Two isolates, H44/76-SL (referred to  
135 herein as H44/76 WT) and 5/99, were reference strains provided by the manufacturer  
136 for testing anti-FHbp and anti-NadA SBA, respectively (23-25). A third strain, SK016,  
137 was used in a previous study to measure anti-PorA P1.4 responses (25). SK016  
138 expresses a sub-family A FHbp antigen (Table 1), which is mismatched for the sub-  
139 family B FHbp in MenB-4C. The SK016 strain was used a reference isolate instead of  
140 the anti-PorA P1.4 reference strain NZ98/254 previously used by the manufacturer, as  
141 NZ98/354 has sub-family B FHbp that could contribute to vaccine-induced SBA. The  
142 remaining 15 test isolates included 7 with FHbp subfamily A amino acid sequence  
143 variants, which were mismatched with the FHbp sub-family B antigen in MenB-4C, and  
144 8 with FHbp subfamily B sequence variants. Two FHbp sub-family B isolates were from

145 patients hospitalized in 2009 and 2013 in Quebec during a period of hyperendemic  
146 capsular group B disease (26). Five of the 6 remaining FHbp subfamily B strains, and  
147 one of the sub-family A isolates, were from outbreaks on U.S. college campuses (Table  
148 1). The isolate from the Princeton University outbreak (CDC strain designation,  
149 M26312), was from blood and is ST 409 [cc 41/44/], PorA P1.5-1, 2-2, FHbp ID 276,  
150 NHba ID 2 and NadA-negative. This phenotype is identical to the CSF isolate from the  
151 same patient used in the study by Basta et. Al. (19) (CDC strain designation M26313,  
152 written personal communication, Ray Borrow and Xilian Bai, Vaccine Evaluation Unit,  
153 Public Health England, Manchester Royal Infirmary, Manchester, United Kingdom).  
154 Three sub-family A FHbp strains (labeled B2, B9 and B11, Table 1) were selected  
155 because of absence of a gene encoding NadA, PorA serosubtypes other than P1.4, and  
156 varying levels of NHba expression to assess SBA that may be directed at NHba. The  
157 designations and antigenic phenotypes of the 18 strains with respect to MenB-4C  
158 antigens, are summarized in Table 1, and genetic characterization of the strain antigens  
159 is provided in Supplemental Table S1.

160 *Human complement source.* Pooled serum from three healthy adults was used  
161 as a complement source to measure bactericidal activity. The human serum pool was  
162 depleted of IgG using a protein G column (HiTrap Protein G; GE Life Sciences,  
163 Piscataway, NJ), which was performed as previously described (27). The procedure  
164 results in some dilution of the sample and loss of complement activity (28). To  
165 compensate, we used 35% IgG-depleted serum (equivalent in classical and alternative  
166 pathway activity and hemolytic activity to 20 to 25% serum that had not been IgG-  
167 depleted).

168       *Serum bactericidal assays.* Bacteria were grown to early log phase in liquid  
169   Frantz media supplemented with 4 mM D,L-lactate (Sigma-Aldrich), and 2 mM cytidine  
170   5'-monophospho-N-acetyl-neuraminic acid (CMP-NANA; Carbosynth) to enhance  
171   sialylation of lipooligosaccharide (29). Test sera were heated for 30 minutes at 56°C to  
172   inactivate endogenous complement. The 40 µl bactericidal reaction contained two-fold  
173   serial dilutions of test sera, 300 to 400 CFU of bacteria, and 35% IgG-depleted human  
174   complement. SBA titers were the interpolated dilution resulting in 50% survival of the  
175   bacteria, compared to CFU/ml in negative control sera and complement.

176       *Antigen expression.* We used flow cytometry to measure surface-accessible  
177   NadA, FHbp and NHba on live *N. meningitidis* bacteria, which was performed as  
178   previously described (30). Polyclonal antisera from mice immunized with recombinant  
179   NadA, NHba or FHbp ID 22 (sub-family A) or FHbp ID 1 (sub-family B) were used for  
180   antigen detection. After washing the bacteria, the cells were incubated with Alexa Fluor  
181   488 goat anti-mouse IgG (H+L) (Invitrogen) for 1 h at room temperature. Strain  
182   expression of FHbp, NadA and NHba were compared in parallel to control strains with  
183   high expression of the respective antigens.

184       *Statistical analyses.* For calculation of geometric mean titers, titers below the limit  
185   of the detection were assigned half the value of the lowest dilution tested (i.e., 1:2 for  
186   titers <1:4). For defining 4-fold or greater increases in SBA titers 1 month post-dose 2,  
187   subjects with pre-immunization serum titers <1:4 were required to have titers of ≥1:16 in  
188   post-immunization serum. Confidence intervals (95%) of proportions were determined  
189   from the binomial distribution. All statistical tests were two-tailed; probability (*p*) values  
190   of less than or equal to 0.05 were considered statistically significant.



191 **Results**

192       *Serum bactericidal responses.* Figure 1 summarizes the percentages of subjects  
193 with  $\geq 4$ -fold increases in SBA titers at one month post-dose 2. For the three reference  
194 indicator strains (Panel A), 95%, 95% and 70% of subjects had  $\geq 4$ -fold responses to  
195 FHbp, NadA and PorA P1.4, respectively. These results are nearly identical to those  
196 reported by the manufacturer from two studies supporting MenB-4C licensure in the U.S  
197 (historical data from package insert shown as open and hatched bars, Panel A). In  
198 contrast to the reference strains, only 25 to 45% of the subjects in the present study had  
199  $\geq 4$ -fold increases in SBA titers against the 7 test strains with FHbp sub-family A  
200 sequence variants (Panel C), or against 3 of the 8 FHbp sub-family B test strains (Panel  
201 D). All three resistant FHbp sub-family B strains had FHbp ID 15, which as shown in the  
202 dendrogram (Panel B) is relatively divergent from ID 1 in the vaccine. The relative  
203 resistance of the Princeton University outbreak isolate with FHbp ID 276 is similar to  
204 that reported by Basta et al (19).

205       Figure 2, Panel A, illustrates SBA titers pre- and 1 and 4 to 6 months post-  
206 immunization measured against the three reference strains (each symbol represents the  
207 titer of an individual subject). The data are stratified based on pre-immunization  
208 bactericidal titers  $\leq 1:8$  (persons likely to benefit most from immunization), and those  
209 with pre- titers  $> 1:8$  (persons with natural immunity who likely were protected before  
210 vaccination). For the PorA and FHbp reference strains, subjects with pre-immunization  
211 titers  $> 1:8$  had 4- to 10-fold higher bactericidal titers at 1 month post-dose 2 than those  
212 with pre-immunization titers  $\leq 1:8$ . By 4 to 6 months, titers decreased against all three

213 reference strains but remained  $\geq 1:4$  with the exception of one subject against strain  
214 SK016 (1:4 is considered the minimal titer for protection (31)).

215 Figure 2, Panel B, shows SBA titers against three representative strains with  
216 FHbp sub-family A amino acid sequence variants (i.e., “mismatched” with respect to the  
217 sub-family B FHbp vaccine antigen). The highest responses were against strain 03s-  
218 0673, which expressed NadA and NHba. The lowest responses were against strain  
219 B11, which expressed NHba, and the Rutgers University isolate, which had no known  
220 antigens matched to MenB-4C (Table 1). Panel C shows the corresponding data for  
221 three representative strains with FHbp sub-family B. Each had high expression of two  
222 other MenB-4C antigens, NadA and NHba. Yet there was a 7-fold range in geometric  
223 mean serum titers 1 month post-dose 2, being highest against the UC Santa Barbara  
224 isolate with an exact FHbp sequence match to FHbp ID 1 in the vaccine, intermediate  
225 against the Santa Clara University isolate (FHbp ID 510), and lowest against the Ohio  
226 University isolate (FHbp ID 15). Similar respective results were observed against the  
227 remaining test strains (supplemental Figures S1 and S2).

228 Figure 3 summarizes the reciprocal geometric mean titers (GMT) against the  
229 three reference strains and 15 test strains for subjects with pre-immunization titers  $\leq 1:8$ .  
230 The high GMTs 1 month post-dose 2 were against the three reference strains (Panel A;  
231 note, the Y axis extends to 1:1000, whereas the Y axes in panels B and C showing the  
232 data for the 15 test strains extend only to 1:100). Among the 7 strains with FHbp sub-  
233 family A (Panel B), the lowest GMT 1 month post-dose 2 was to strain “B9”, which, with  
234 respect to MenB-4C, only expressed NHba (Table 1). Among the 8 FHbp sub-family B  
235 strains (Panel C), the lowest GMT 1 month post-dose 2 was to the Ohio University

236 isolate, which as described above, also expressed NadA and NHba. The H44/76 mutant  
237 with 50% lower expression of sub-family B FHbp ID 1 than the parent WT strain had a  
238 GMT at 1 month post-dose 2 of 24.7 compared to 47.8 for the parental wildtype strain.  
239 Similarly, the Quebec 2013 isolate had a GMT of 12.7 compared to 21.0 for the 2009  
240 Quebec isolate. Neither of the respective differences, however, was significant ( $p \geq 0.10$ ).

241 Figure 4 summarizes the percentages of subjects with protective SBA titers of  
242  $\geq 1:4$  at different time points. Data are shown for subjects with pre-immunization titers  
243  $\leq 1:8$ . At one-month post-dose, 100% of subjects had protective titers against the three  
244 reference strains (Panel A), and 85% to 100% had protective titers against 13 of 15 test  
245 strains (Panels B and C). The proportion of subjects with protective titers against the  
246 Princeton University isolate (100%) was higher than in a student population (66%)  
247 reported by Basta et al (19). The difference could reflect different study populations  
248 (Princeton students vs. older laboratory or hospital workers), small sample size in the  
249 present study, or possible strain factors (CSF in the Basta et al study, and blood isolate  
250 in the present study, see methods). Although the number of subjects with follow-up sera  
251 at 4 to 6 months is small (shown in Figure 4 above the respective bars), 70% or greater  
252 of the subjects at this time point had bactericidal titers  $\geq 1:4$  against four of the six sub-  
253 family A FHbp strains (Panel B) and six of eight of the sub-family B FHbp B strains  
254 (Panel C). The four exceptions were the mutant H44/76 and Rutgers University isolates  
255 with sub-family A FHbp, and the B2 and Ohio University isolates with sub-family B  
256 FHbp, where 38 to 55 percent of the subjects tested at 4 to 6 months had titers  $< 1:4$ .

257

## 258 Discussion

259 In this study, we measured SBA responses of 20 adults immunized with two  
260 doses of MenB-4C against 18 serogroup B strains. Previous studies measured SBA  
261 primarily against three indicator strains selected to provide information on antigen-  
262 specific bactericidal activity (32-36). However, the relationship between SBA measured  
263 against the reference strains and broader protection against circulating serogroup B  
264 strains is unknown. Important limitations of the present study are the small sample size  
265 of immunized adults, use of two study sites in the UK and the U.S. with different natural  
266 exposures to meningococcal strains, and different recommended vaccinations  
267 schedules (two injections separated by one or two months). The study was not  
268 designed to compare the responses at the two study sites. Nevertheless, for nearly all  
269 of the strains, the respective proportions of subjects with  $\geq 4$ -fold increases in SBA titers  
270 1 month post-dose 2 were similar (data not shown). The two possible exceptions were  
271 strains B9 and 03s-0451 (13% and 22% in the UK and 60 and 80% in the U.S. ( $P=0.08$   
272 and 0.13, respectively).

273 An important strength was testing bactericidal activity in a panel of sera from  
274 vaccinated subjects against 18 genetically and antigenically diverse meningococcal  
275 strains, including three reference strains. A second strength was the use of an identical  
276 complement source for testing all of the isolates (pooled serum from three adults that  
277 had been depleted of IgG - see methods). Comparing serum bactericidal data across  
278 strains is potentially confounded by use of different complement sources for different  
279 isolates. The sera may contain naturally acquired antibodies that by themselves lack  
280 bactericidal activity but can contribute to bactericidal activity in the presence of vaccine-

281 induced antibody) (37). Our most important finding was that the clinical isolates were  
282 generally more resistant to MenB-4C-induced SBA than the reference strains. Also, by 4  
283 to 6 months after 2 doses of vaccine, SBA titers declined and, for three of the test  
284 strains, were below protection ( $<1:4$ ) in a third or more of the subjects.

285 Serum anti-FHbp antibodies are thought mainly to confer sub-family-specific  
286 protection (22). Because MenB-4C does not have a sub-family A FHbp antigen, SBA  
287 against strains with sub-family A FHbp have been thought to depend largely on  
288 antibodies to the three other MenB-4C antigens, NHba, NadA or PorA P1.4 (38). It is  
289 noteworthy that only 25 to 45% percent of the subjects had  $\geq 4$ -fold SBA titer increases 1  
290 month post-dose 2 against all seven test isolates with sub-family A FHbp, and five of  
291 these resistant strains expressed one or two other MenB-4C antigens. Similarly, all  
292 three resistant strains with sub-family B FHbp had two other MenB-4C antigens. These  
293 data underscore the complexity of defining antigen-specific SBA induced by MenB-4C,  
294 particularly for the NadA and NHba antigens.

295 For measuring anti-NadA bactericidal activity, the manufacturer used strain 5/99,  
296 which is mismatched for all of the MenB-4C antigens except NadA. This strain is a high  
297 expresser of NadA and is highly susceptible to MenB-4C antibodies (geometric mean  
298 titer 5- to  $>10$ -fold higher than to the other 17 strains we investigated (Figure 3). Our  
299 panel of test strains included five with relatively high NadA expression as measured by  
300 flow cytometry (summarized in Table 1). These isolates showed much lower  
301 susceptibility to vaccine-induced SBA than the NadA reference strain. Possibly anti-  
302 NadA resistance of the test isolates was related to previous findings that, in vitro,  
303 expression of NadA can be repressed by the regulator NadR (39). Irrespective of the

304 mechanism, titers against the NadA reference strain overestimate strain coverage by  
305 anti-NadA antibodies.

306       The role of antibodies to NHba in eliciting MenB-4C bactericidal activity is not  
307 well understood. We investigated SBA responses against three test strains selected to  
308 be mismatched with all of the vaccine antigens except NHba. Strain B11 (M4407) has  
309 NHba with 100% amino acid identity to the NHba variant in the vaccine, and strains B2  
310 and B9 have NHba with 87% and 88% amino acid identity, respectively. All three  
311 isolates were relatively resistant to MenB-4C bactericidal antibodies (Figures 1 and 3).  
312 Conceivably, the ability of endogenous proteases in some strains to cleave NHba and  
313 decrease the amount of accessible NHba on the bacterial surface may have contributed  
314 to anti-NHba SBA resistance (40). Note also that the Rutgers University outbreak isolate  
315 had similar vaccine-induced SBA titers as those of the three strains expressing NHba.  
316 The target antigen(s) for the Rutgers University isolate is unknown since this strain was  
317 mismatched for all four MenB-4C antigens (low expression of a sub-family A FHbp, low  
318 expression of NHba, absence of a functional NadA gene, and a PorA serosubtype other  
319 than P1.4, Table 1).

320       Few data are available from humans on the extent of anti-FHbp protection within  
321 a sub-family. It is of interest, therefore, that SBA responses were high against the  
322 H44/76 reference isolate with FHbp ID 1 (100% amino acid identity to the FHbp variant  
323 in MenB-4C), and against three other test isolates with FHbp ID 1. In contrast, SBA  
324 responses were much lower against the three test isolates with relatively high  
325 expression of sub-family B FHbp ID 15 (89% amino acid identity) (Table 1). These  
326 results were consistent with our previous findings in mice immunized with a recombinant

327 FHbp ID 1 vaccine: high serum bactericidal activity against strain H44/76 with FHbp ID  
328 1, and much lower activity against a H44/76 mutant with FHbp ID 15 (14). Collectively,  
329 the data underscore that MenB-4C coverage can be affected by FHbp amino acid  
330 sequence diversity within a sub-family.

331 A  $\geq 4$ -fold bactericidal response requires a minimum post-immunization serum  
332 titer of 1:16 (i.e.,  $< 1:4$  to 1:16). However, a titer of  $\geq 1:4$  is considered sufficient to confer  
333 protection (31, 41). Therefore, immunized persons who fail to achieve  $\geq 4$ -fold titer  
334 increases at 1 month can be protected from disease (see for example, **Figure 5**,  
335 showing bactericidal responses of subjects with pre-immunization titers  $< 1:4$  when  
336 tested against two strains for which the majority of subjects did not have  $\geq 4$ -fold  
337 bactericidal responses at 1 month). By 4- to 6 months, serum titers declined to  $< 1:4$  in  
338 50% or more of the subjects with available follow-up sera. Similar declines in protective  
339 titers at 4 to 6 months were seen with two other resistant test strains: Ohio University  
340 and strain B9 from Georgia. Thus persons with underlying diseases who are at  
341 increased risk of meningococcal disease may benefit from an off-label third booster  
342 dose MenB-4C dose. In a previous study, three doses of MenB-4C were given to  
343 adolescents in Chile and appeared to be safe but had no major advantage with respect  
344 to higher or longer duration of SBA responses than two doses (35, 42) when measured  
345 against reference strains. Conceivably, had titers been measured against more  
346 antigenically diverse resistant strains, the authors might have observed higher  
347 responses to three doses. Whether SBA titers after a third dose will be of longer  
348 duration than after two doses also is not known.

349

350 **Author contributions:**

351           DMG and AJP designed study; analyzed and interpreted results; MMG, CR and  
352 CD designed and conducted the clinical trial, SG and EL performed the assays and  
353 analyzed results; all authors contributed to writing the manuscript. Primary data and  
354 materials are available from the authors.

355

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363 **References**

- 364 1. **Law DK, Zhou J, Deng S, Hoang L, Tyrrell G, Horsman G, Wylie J, and**  
365 **Tsang RS.** 2014. Determination of serotyping antigens, clonal analysis and  
366 genetic characterization of the 4CMenB vaccine antigen genes in invasive  
367 *Neisseria meningitidis* from Western Canada, 2009 to 2013. *J Med Microbiol*  
368 **63**:1490-9.
- 369 2. **Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, Caugant**  
370 **DA, Kriz P, Abad R, Bambini S, Carannante A, Deghmane AE, Fazio C,**  
371 **Frosch M, Frosi G, Gilchrist S, Giuliani MM, Hong E, Ledroit M, Lovaglio**  
372 **PG, Lucidarme J, Musilek M, Muzzi A, Oksnes J, Rigat F, Orlandi L, Stella**  
373 **M, Thompson D, Pizza M, Rappuoli R, Serruto D, Comanducci M,**  
374 **Boccadifuoco G, Donnelly JJ, Medini D, and Borrow R.** 2013. Predicted strain  
375 coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a  
376 qualitative and quantitative assessment. *Lancet Infect Dis* **13**:416-25.
- 377 3. **Macneil JR, Rubin L, Folaranmi T, Ortega-Sanchez IR, Patel M, and Martin**  
378 **SW.** 2015. Use of Serogroup B Meningococcal Vaccines in Adolescents and  
379 Young Adults: Recommendations of the Advisory Committee on Immunization  
380 Practices, 2015. *MMWR Morb Mortal Wkly Rep* **64**:1171-6.
- 381 4. **Folaranmi T, Rubin L, Martin SW, Patel M, Macneil JR, and Centers for**  
382 **Disease C.** 2015. Use of serogroup B meningococcal vaccines in persons aged  
383  $\geq 10$  years at increased risk for serogroup B meningococcal disease:  
384 Recommendations of the Advisory Committee on Immunization Practices, 2015.  
385 *MMWR Morb Mortal Wkly Rep* **64**:608-12.

- 386 5. **Giuliani MM, Adu-Bobie J, Comanducci M, Arico B, Savino S, Santini L,**  
387 **Brunelli B, Bambini S, Biolchi A, Capecchi B, Cartocci E, Ciocchi L, Di**  
388 **Marcello F, Ferlicca F, Galli B, Luzzi E, Massignani V, Serruto D, Veggi D,**  
389 **Contorni M, Morandi M, Bartalesi A, Cinotti V, Mannucci D, Titta F, Ovidi E,**  
390 **Welsch JA, Granoff D, Rappuoli R, and Pizza M.** 2006. A universal vaccine for  
391 serogroup B meningococcus. *Proc Natl Acad Sci U S A* **103**:10834-9.
- 392 6. **Oster P, Lennon D, O'hallahan J, Mulholland K, Reid S, and Martin D.** 2005.  
393 MeNZB: a safe and highly immunogenic tailor-made vaccine against the New  
394 Zealand *Neisseria meningitidis* serogroup B disease epidemic strain. *Vaccine*  
395 **23**:2191-6.
- 396 7. **O'hallahan J, Lennon D, Oster P, Lane R, Reid S, Mulholland K, Stewart J,**  
397 **Penney L, Percival T, and Martin D.** 2005. From secondary prevention to  
398 primary prevention: a unique strategy that gives hope to a country ravaged by  
399 meningococcal disease. *Vaccine* **23**:2197-201.
- 400 8. **Martin DR, Ruijne N, McCallum L, O'hallahan J, and Oster P.** 2006. The VR2  
401 epitope on the PorA P1.7-2,4 protein is the major target for the immune response  
402 elicited by the strain-specific group B meningococcal vaccine MeNZB. *Clin*  
403 *Vaccine Immunol* **13**:486-91.
- 404 9. **Hoiseth SK, Murphy E, Andrew L, Vogel U, Frosch M, Hellenbrand W, Abad**  
405 **R, Vazquez JA, Borrow R, Findlow J, Taha MK, Deghmane AE, Caugant DA,**  
406 **Kriz P, Musilek M, Mayer LW, Wang X, Macneil JR, York L, Tan CY, Jansen**  
407 **KU, and Anderson AS.** 2013. A multi-country evaluation of *Neisseria*

- meningitidis serogroup B factor H-binding proteins and implications for vaccine coverage in different age groups. *Pediatr Infect Dis J* **32**:1096-101.
10. **Hill DM, Lucidarme J, Gray SJ, Newbold LS, Ure R, Brehony C, Harrison OB, Bray JE, Jolley KA, Bratcher HB, Parkhill J, Tang CM, Borrow R, and Maiden MC.** 2015. Genomic epidemiology of age-associated meningococcal lineages in national surveillance: an observational cohort study. *Lancet Infect Dis* **15**:1420-8.
11. **Wang X, Cohn A, Comanducci M, Andrew L, Zhao X, Macneil JR, Schmink S, Muzzi A, Bambini S, Rappuoli R, Pizza M, Murphy E, Hoiseth SK, Jansen KU, Anderson AS, Harrison LH, Clark TA, Messonnier NE, and Mayer LW.** 2011. Prevalence and genetic diversity of candidate vaccine antigens among invasive *Neisseria meningitidis* isolates in the United States. *Vaccine* **29**:4739-44.
12. **Donnelly J, Medini D, Boccadifuoco G, Biolchi A, Ward J, Frasch C, Moxon ER, Stella M, Comanducci M, Bambini S, Muzzi A, Andrews W, Chen J, Santos G, Santini L, Boucher P, Serruto D, Pizza M, Rappuoli R, and Giuliani MM.** 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**:19490-19495.
13. **Jiang HQ, Hoiseth SK, Harris SL, Mcneil LK, Zhu D, Tan C, Scott AA, Alexander K, Mason K, Miller L, Dasilva I, Mack M, Zhao XJ, Pride MW, Andrew L, Murphy E, Hagen M, French R, Arora A, Jones TR, Jansen KU, Zlotnick GW, and Anderson AS.** 2010. Broad vaccine coverage predicted for a

- 431 bivalent recombinant factor H binding protein based vaccine to prevent  
432 serogroup B meningococcal disease. *Vaccine* **28**:6086-93.
- 433 14. **Konar M, Granoff DM, and Beernink PT.** 2013. Importance of inhibition of  
434 binding of complement Factor H for serum bactericidal antibody responses to  
435 meningococcal Factor H-binding protein vaccines. *J Infect Dis* **208**:627-36.
- 436 15. **Pajon R, Lujan E, and Granoff DM.** 2016. A meningococcal NOMV-FHbp  
437 vaccine for Africa elicits broader serum bactericidal antibody responses against  
438 serogroup B and non-B strains than a licensed serogroup B vaccine. *Vaccine*  
439 **34**:643-9.
- 440 16. **Pajon R, Fergus AM, Koeberling O, Caugant DA, and Granoff DM.** 2011.  
441 Meningococcal factor H binding proteins in epidemic strains from Africa:  
442 Implications for vaccine development. *PLoS Negl Trop Dis* **5**:e1302.
- 443 17. **Pajon R, Beernink PT, Harrison LH, and Granoff DM.** 2010. Frequency of  
444 factor H-binding protein modular groups and susceptibility to cross-reactive  
445 bactericidal activity in invasive meningococcal isolates. *Vaccine* **28**:2122-9.
- 446 18. **Rossi R, Beernink PT, Giuntini S, and Granoff DM.** 2015. Susceptibility of  
447 meningococcal strains responsible for two serogroup B outbreaks on U.S.  
448 university campuses to serum bactericidal activity elicited by the MenB-4C  
449 vaccine. *Clin Vaccine Immunol* **22**:1227-34.
- 450 19. **Basta BE, Mahmoud AaF, Wolfson J, Ploss A, Heller BL, Hanna AB,**  
451 **Johnson PJ, Izzo R, Grenfell BT, Findlow H, Bai X, and Borrow R.** 2016.  
452 Immunogenicity of a meningococcal B vaccine during a university outbreak. *New*  
453 *England Journal of Medicine* **375**:220-8.

- 454 20. **Murphy E, Andrew L, Lee KL, Dilts DA, Nunez L, Fink PS, Ambrose K,**  
455 **Borrow R, Findlow J, Taha MK, Deghmane AE, Kriz P, Musilek M,**  
456 **Kalmusova J, Caugant DA, Alvestad T, Mayer LW, Sacchi CT, Wang X,**  
457 **Martin D, Von Gottberg A, Du Plessis M, Klugman KP, Anderson AS,**  
458 **Jansen KU, Zlotnick GW, and Hoiseth SK.** 2009. Sequence diversity of the  
459 factor H binding protein vaccine candidate in epidemiologically relevant strains of  
460 serogroup B *Neisseria meningitidis*. *J Infect Dis* **200**:379-89.
- 461 21. **Fletcher LD, Bernfield L, Barniak V, Farley JE, Howell A, Knauf M, Ooi P,**  
462 **Smith RP, Weise P, Wetherell M, Xie X, Zagursky R, Zhang Y, and Zlotnick**  
463 **GW.** 2004. Vaccine potential of the *Neisseria meningitidis* 2086 lipoprotein. *Infect*  
464 *Immun* **72**:2088-100.
- 465 22. **Masignani V, Comanducci M, Giuliani MM, Bambini S, Adu-Bobie J, Arico B,**  
466 **Brunelli B, Pieri A, Santini L, Savino S, Serruto D, Litt D, Kroll S, Welsch JA,**  
467 **Granoff DM, Rappuoli R, and Pizza M.** 2003. Vaccination against *Neisseria*  
468 *meningitidis* using three variants of the lipoprotein GNA1870. *J Exp Med*  
469 **197**:789-99.
- 470 23. **Snape MD, Dawson T, Oster P, Evans A, John TM, Ohene-Kena B, Findlow**  
471 **J, Yu LM, Borrow R, Ypma E, Toneatto D, and Pollard AJ.** 2010.  
472 Immunogenicity of two investigational serogroup B meningococcal vaccines in  
473 the first year of life: a randomized comparative trial. *Pediatr Infect Dis J* **29**:e71-9.
- 474 24. **Findlow J, Borrow R, Snape MD, Dawson T, Holland A, John TM, Evans A,**  
475 **Telford KL, Ypma E, Toneatto D, Oster P, Miller E, and Pollard AJ.** 2010.  
476 Multicenter, open-label, randomized phase II controlled trial of an investigational

- 477 recombinant meningococcal serogroup B vaccine with and without outer  
478 membrane vesicles, administered in infancy. Clin Infect Dis **51**:1127-37.
- 479 25. **Costa I, Pajon R, and Granoff DM.** 2014. Human factor H (FH) impairs  
480 protective meningococcal anti-FHbp antibody responses and the antibodies  
481 enhance FH binding. MBio **5**:e01625-14.
- 482 26. **Law DK, Lefebvre B, Gilca R, Deng S, Zhou J, De Wals P, and Tsang RS.**  
483 2015. Characterization of invasive *Neisseria meningitidis* strains from Quebec,  
484 Canada, during a period of increased serogroup B disease, 2009-2013:  
485 phenotyping and genotyping with special emphasis on the non-carbohydrate  
486 protein vaccine targets. BMC Microbiol **15**:143.
- 487 27. **Beernink PT, Shaughnessy J, Braga EM, Liu Q, Rice PA, Ram S, and**  
488 **Granoff DM.** 2011. A meningococcal factor H binding protein mutant that  
489 eliminates factor H binding enhances protective antibody responses to  
490 vaccination. J Immunol **186**:3606-14.
- 491 28. **Giuntini S, Granoff DM, Beernink PT, Ihle O, Bratlie D, and Michaelsen TE.**  
492 2016. Human IgG1, IgG3 and IgG3 hinge truncated mutants show different  
493 protection capability against meningococci depending on the target antigen and  
494 epitope specificity. Clin Vaccine Immunol **23**:698-706
- 495 29. **Mandrell RE, Kim JJ, John CM, Gibson BW, Sugai JV, Apicella MA, Griffiss**  
496 **JM, and Yamasaki R.** 1991. Endogenous sialylation of the lipooligosaccharides  
497 of *Neisseria meningitidis*. J Bacteriol **173**:2823-32.

- 498 30. **Giuntini S, Pajon R, Ram S, and Granoff DM.** 2015. Binding of complement  
499 Factor H to PorB3 and NspA enhances resistance of *Neisseria meningitidis* to  
500 anti-factor H binding protein bactericidal activity. *Infect Immun* **83**:1536-45.
- 501 31. **Borrow R, Carlone GM, Rosenstein N, Blake M, Feavers I, Martin D,**  
502 **Zollinger W, Robbins J, Aaberge I, Granoff DM, Miller E, Plikaytis B, Van**  
503 **Alphen L, Poolman J, Rappuoli R, Danzig L, Hackell J, Danve B, Caulfield**  
504 **M, Lambert S, and Stephens D.** 2006. *Neisseria meningitidis* group B correlates  
505 of protection and assay standardization--international meeting report Emory  
506 University, Atlanta, Georgia, United States, 16-17 March 2005. *Vaccine* **24**:5093-  
507 107.
- 508 32. **Lee HJ, Choe YJ, Hong YJ, Kim KH, Park SE, Kim YK, Oh CE, Lee H, Song**  
509 **H, Bock H, Casula D, Bhusal C, and Arora AK.** 2016. Immunogenicity and  
510 safety of a multicomponent meningococcal serogroup B vaccine in healthy  
511 adolescents in Korea-A randomised trial. *Vaccine* **34**:1180-6.
- 512 33. **Perrett KP, Mcvernon J, Richmond PC, Marshall H, Nissen M, August A,**  
513 **Percell S, Toneatto D, and Nolan T.** 2015. Immune responses to a  
514 recombinant, four-component, meningococcal serogroup B vaccine (4CMenB) in  
515 adolescents: A phase III, randomized, multicentre, lot-to-lot consistency study.  
516 *Vaccine* **33**:5217-24.
- 517 34. **Vesikari T, Esposito S, Prymula R, Ypma E, Kohl I, Toneatto D, Dull P,**  
518 **Kimura A, and Group EUMBIVS.** 2013. Immunogenicity and safety of an  
519 investigational multicomponent, recombinant, meningococcal serogroup B

- 520 vaccine (4CMenB) administered concomitantly with routine infant and child  
521 vaccinations: results of two randomised trials. *Lancet* **381**:825-35.
- 522 35. **Santolaya ME, O'ryan M, Valenzuela MT, Prado V, Vergara RF, Munoz A,**  
523 **Toneatto D, Grana G, Wang H, and Dull PM.** 2013. Persistence of antibodies in  
524 adolescents 18-24 months after immunization with one, two, or three doses of  
525 4CMenB meningococcal serogroup B vaccine. *Hum Vaccin Immunother* **9**:2304-  
526 10.
- 527 36. **Gossger N, Snape MD, Yu LM, Finn A, Bona G, Esposito S, Principi N, Diez-**  
528 **Domingo J, Sokal E, Becker B, Kieninger D, Prymula R, Dull P, Ypma E,**  
529 **Toneatto D, Kimura A, and Pollard AJ.** 2012. Immunogenicity and tolerability of  
530 recombinant serogroup B meningococcal vaccine administered with or without  
531 routine infant vaccinations according to different immunization schedules: a  
532 randomized controlled trial. *JAMA* **307**:573-82.
- 533 37. **Welsch JA, Ram S, Koeberling O, and Granoff DM.** 2008. Complement-  
534 dependent synergistic bactericidal activity of antibodies against factor H-binding  
535 protein, a sparsely distributed meningococcal vaccine antigen. *J Infect Dis*  
536 **197**:1053-61.
- 537 38. **Giuliani MM, Biolchi A, Serruto D, Ferlicca F, Vienken K, Oster P, Rappuoli**  
538 **R, Pizza M, and Donnelly J.** 2010. Measuring antigen-specific bactericidal  
539 responses to a multicomponent vaccine against serogroup B meningococcus.  
540 *Vaccine* **28**:5023-30.
- 541 39. **Fagnocchi L, Biolchi A, Ferlicca F, Boccadifuoco G, Brunelli B, Brier S,**  
542 **Norais N, Chiarot E, Bensi G, Kroll JS, Pizza M, Donnelly J, Giuliani MM,**



- 543 **and Delany I.** 2013. Transcriptional regulation of the *nadA* gene in *Neisseria*  
544 *meningitidis* impacts the prediction of coverage of a multicomponent  
545 meningococcal serogroup B vaccine. *Infect Immun* **81**:560-9.
- 546 40. **Serruto D, Spadafina T, Ciocchi L, Lewis LA, Ram S, Tontini M, Santini L,**  
547 **Biolchi A, Seib KL, Giuliani MM, Donnelly JJ, Berti F, Savino S, Scarselli M,**  
548 **Costantino P, Kroll JS, O'dwyer C, Qiu J, Plaut AG, Moxon R, Rappuoli R,**  
549 **Pizza M, and Arico B.** 2010. *Neisseria meningitidis* GNA2132, a heparin-binding  
550 protein that induces protective immunity in humans. *Proc Natl Acad Sci U S A*  
551 **107**:3770-5.
- 552 41. **Goldschneider I, Gotschlich EC, and Artenstein MS.** 1969. Human immunity  
553 to the meningococcus. I. The role of humoral antibodies. *J Exp Med* **129**:1307-  
554 26.
- 555 42. **Santolaya ME, O'ryan ML, Valenzuela MT, Prado V, Vergara R, Munoz A,**  
556 **Toneatto D, Grana G, Wang H, Clemens R, and Dull PM.** 2012.  
557 Immunogenicity and tolerability of a multicomponent meningococcal serogroup B  
558 (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised,  
559 observer-blind, placebo-controlled study. *Lancet* **379**:617-24.
- 560 43. **Vu DM, Wong TT, and Granoff DM.** 2011. Cooperative serum bactericidal  
561 activity between human antibodies to meningococcal factor H binding protein and  
562 *Neisseria* heparin binding antigen. *Vaccine* **29**:1968-73.
- 563 44. **Soeters HM, Mcnamara LA, Whaley M, Wang X, Alexander-Scott N,**  
564 **Kanadanian KV, Kelleher CM, Macneil J, Martin SW, Raines N, Sears S,**  
565 **Vanner C, Vuong J, Bandy U, Sicard K, Patel M, and Centers for Disease C.**

- 566 2015. Serogroup B meningococcal disease outbreak and carriage evaluation at a  
567 college - Rhode Island, 2015. *MMWR Morb Mortal Wkly Rep* **64**:606-7.
- 568 45. **Mandal S, Wu HM, Macneil JR, Machesky K, Garcia J, Plikaytis BD, Quinn**  
569 **K, King L, Schmink SE, Wang X, Mayer LW, Clark TA, Gaskell JR,**  
570 **Messonnier NE, Diorio M, and Cohn AC.** 2013. Prolonged university outbreak  
571 of meningococcal disease associated with a serogroup B strain rarely seen in the  
572 United States. *Clin Infect Dis* **57**:344-8.
- 573 46. **Mcnamara LA, Shumate AM, Johnsen P, Macneil JR, Patel M, Bhavsar T,**  
574 **Cohn AC, Dinitz-Sklar J, Duffy J, Finnie J, Garon D, Hary R, Hu F, Kamiya H,**  
575 **Kim HJ, Kolligian J, Jr., Neglia J, Oakley J, Wagner J, Wagner K, Wang X,**  
576 **Yu Y, Montana B, Tan C, Izzo R, and Clark TA.** 2015. First Use of a Serogroup  
577 B Meningococcal Vaccine in the US in Response to a University Outbreak.  
578 *Pediatrics* **135**:798-804.  
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Table 1. 18 meningococcal strains used to test serum bactericidal activity.						
Strain	Location (year)	MLST <sup>†</sup> (clonal complex)	Relative Strain Expression of MenB-4C Antigens*			OMV (PorA)
			FHbp (Pep ID)	NadA	NHba	P1.4
Reference strains						
H44/76 (FHbp strain) (23)	Norway (1976)	32 (32)	++ (1)	Absent	+/-	No
5/99 (CH245) (NadA) (23)	Norway (1999)	1349 (8)	+ (23)	++	+/-	No
SK016 (CH526) (PorA P1.4) (25))	North Carolina (2001)	103 (103)	+ (25)	Absent	+/-	Yes
FHbp Sub-family A isolates (N=7)						
CH865, college outbreak	Rutgers Univ, NJ (2016, 2 cases)	11 (11)	+/- (19)	Absent*	+/-	No
B11, M4407 (CH258) (43))	Minnesota (1996)	6160 (41/44)	+ (19)	Absent	++	No
B2 (CH740)	Georgia (2003)	13 (269)	++ (19)	Absent	+	No
H44/76 mutant (FHbp Sub-family A) (25)	Norway, 1976	32 (32)	++ (22)	Absent	++	No
03s-0673 (CH862)	California (2003)	32 (32)	++ (23)	+	++	No
B9 (CH687)	Georgia (1997)	136 (41/44)	++ (24)	Absent	++	No
03s-0451 (CH862)	California (2003)	1364 (32)	+ (76)	+	+	No
FHbp Sub-Family B isolates (N=8)						
H44/76 mutant (50% FHbp expression) (16)	Norway (see above)	32 (32)	+ (1)	Absent	+	No
CH840, college outbreak (18)	Univ. Calif Santa Barbara (2013, 5 cases)	32 (32)	++ (1)	+	++	No
CH852, college outbreak (44)	College in RI, (2015, 2 cases)	9069 (364)	++ (1)	Absent	++	Yes
CH860, hyperendemic (26)	Quebec, Canada, 2009	269 (269)	++ (15)	Absent	++	No

582

CH861, hyperendemic (26)	Quebec, Canada, 2013	269 (269)	++ (15)	Absent	++	No
CH855, college outbreak (45)	Ohio Univ. (2010, 13 cases)	269 (269)	+ (15)	++	++	No
CH819, college outbreak (18, 46)	Princeton Univ., (2013, 9 cases)	409 (41/44)	+ (276)	Absent	+	No
CH863, college outbreak	Santa Clara Univ., CA (2016, 3 cases)	New (32)	++ (510)	+	++	No

583 <sup>†</sup>Multilocus sequence typing.

584 \*Antigen expression was measured by flow cytometry using live bacteria and  
 585 monoclonal or polyclonal mouse sera, as previously described (25). For quantitation of  
 586 antigen expression, +/- is <20%; + is 20 to 80%; and ++ is >80% of expression  
 587 compared to a positive control strain tested in parallel. Expression of FHbp was  
 588 compared with strains H44/76 (sub-family B) or M44/07 (Sub-family A), depending on  
 589 the sub-family of the test isolate (Table 1). Expression of NadA was compared to strain  
 590 5/99 [4], and expression of NHba was compared to strain M4407. All of these strains  
 591 are naturally high expressers of the respective antigen being measured (23) (43). Absent  
 592 NadA indicates no detectable binding and absence of a NadA gene. For OMV, PorA P1.4  
 593 was detected with a specific mouse monoclonal antibody and the strain variable region  
 594 sequence type was inferred by gene sequencing (See Supplemental Table S1).

595 **Figure legends**

596

597 **Figure 1. Percentage of vaccinated adults with four-fold or greater increases in serum**  
598 **bactericidal antibody titer.** Four-fold responses, comparing titers 1 month post dose 2 to  
599 respective pre-immunization titers. ID, strain FHbp amino acid sequence variant as defined in a  
600 public database <<http://pubmlst.org/neisseria/fHbp/>>. Strain expression of other MenB-4C  
601 antigens are designated by \*(NHba), †(NadA) or #(PorA P1.4). **Panel A.** SBA, Reference  
602 indicator strains H44/76 WT (FHbp), 5/99 (NadA), and ant-PorA P1.4 (SK106 for current study;  
603 and strain NZ98/254, historical data; see text). Data for historical studies 1 and 2 are from the  
604 U.S. FDA MenB-4C package insert. **Panel B.** FHbp sequence variants. Unrooted maximum-  
605 likelihood phylogram of strain FHbp amino acid sequence variants computed with MEGA 7. ID  
606 1, sequence of the FHbp in MenB-4C. The scale bar indicates 5% amino acid sequence  
607 divergence. **Panel C.** SBA, seven FHbp sub-family A test strains. Rutgers University isolate  
608 and H44/76 mutant are mismatched for all four MenB-4C antigens reported to elicit serum  
609 bactericidal activity. **Panel D.** SBA, eight FHbp sub-family B test strains. H44/76 mutant has  
610 ~50% expression of FHbp ID 1 as its parent WT strain (see Panel A). Further details on strain  
611 antigens and clonal complexes are provided in Table 1 and Supplemental Table 1.

612

613 **Figure 2. Serum bactericidal antibody responses to 9 representative strains.** Each symbol  
614 represents the serum titer of an individual before and 1 and 4 to 6 months after two doses of  
615 MenB-4C. Numbers on top of the panels indicate GMT. Data are stratified based on titers  $\leq 1:8$   
616 or  $>1:8$  in pre-immunization sera. **Panel A.** Three reference indicator strains, H44/76 wildtype,  
617 5/99, and SK-016, each matched with only one MenB-4C antigen (FHbp, NadA and PorA P1.4,  
618 respectively). **Panel B.** Three representative strains with sub-family A FHbp (mismatched for  
619 the sub-family B FHbp antigen in vaccine). Strain 03s-0673 has two vaccine antigens, NadA  
620 and NHba; strain M4407 has one vaccine antigen (NHba); the Rutgers University isolate is

621 mismatched for all four antigens. **Panel C.** Three representative strains with sub-family B FHbp  
622 matched for the sub-family B of the FHbp antigen in vaccine. All three strains have NadA and  
623 NHba.

624

625 **Figure 3. Geometric mean serum bactericidal titers.** Pre-immunization, grey bars; 1 month  
626 post-dose 2, orange bars; and 4 to 6 months post dose 2, aqua-colored bars. **Panel A.** Three  
627 reference strains, H44/76 wildtype, 5/99 and SK-016, each matched with only one MenB-4C  
628 antigen (FHbp, NadA and PorA P1.4, respectively). **Panel B,** Seven test strains with sub-family  
629 A FHbp (mismatched for the sub-family B FHbp antigen in vaccine). **Panel C.** Eight test strains  
630 with sub-family B FHbp matched for sub-family B FHbp antigen in vaccine). Data shown are for  
631 subjects with pre-immunization titers  $\leq 1:8$ . Symbols above bars indicate FHbp sequence ID and  
632 expression of other MenB-4C antigens as described in legend to Figure 1. Note that Y axis for  
633 Panel A is from  $<4$  to 1000, and for Panels B and C, from  $<4$  to 100.

634

635 **Figure 4. Percent of subjects with serum bactericidal titers  $\geq 1:4$ .** Vaccine antigens  
636 expressed by each strain and symbols for the bars are described in legend to Figure 3. Data are  
637 for subjects with pre-immunization titers  $\leq 1:8$ . For each strain, the first number above the bars  
638 indicates the number of subjects included with pre-immunization titers  $\leq 1:8$  for analyses of the  
639 pre- and 1 month post-dose 2 titers; the second number refers to the number of subjects  
640 included for the analysis of the serum titers at 4 to 6 months post-dose 2.

641

642 **Figure 5.** Serum bactericidal titers of subjects with pre-titers  $< 1:4$  measured against  
643 representative of the strains for which less than 40% of subjects had 4-fold increases in  
644 SBA titer at 1 month post-immunization compared with respective pre-immunization  
645 titers. **Panel A.** Quebec 2013 Isolate, representative of strain causing hyperendemic

646 disease (26). **Panel B.** Case isolate from Rutgers University outbreak. Although the  $\geq 4$ -  
647 fold response rates were low, nearly all subjects had protective titer  $\geq 1:4$  one month  
648 post-dose 2, which were not sustained by 4 to 6 months in the greater than 50% of the  
649 subjects tested.  
650











