

1   **Abstract**

2   **Background**

3   Use of a polysaccharide vaccine challenge to demonstrate immunologic memory after  
4   priming with capsular group C meningococcal conjugate vaccines (MenCC) risks  
5   induction of immunologic hyporesponsiveness. For this reason, MenCC vaccines are  
6   now used as probes of immunologic memory, however, no studies have demonstrated  
7   their ability to distinguish primed from unprimed children.

8

9   **Methods**

10   This study was part of a randomised controlled trial investigating the immunogenicity  
11   of a booster dose of the combined *Haemophilus influenzae* type b and MenC-tetanus  
12   toxoid vaccine (Hib-MenC-TT) in infants receiving reduced dose MenCC vaccine  
13   priming schedules (one MenC-CRM/MenC-TT or two MenC-CRM vaccine doses)  
14   compared with an unprimed group. Antibody kinetics were studied in a subset of 269  
15   children by measuring changes in the MenC serum bactericidal antibody, using rabbit  
16   complement, (MenC rSBA) titres and MenC specific IgG memory B-cells before and  
17   at 6 and 28 days following the 12 month booster vaccination.

18

19   **Results**

20   At 6 days after the 12 month MenCC vaccine, the rise in MenC rSBA titres and MenC  
21   specific IgG memory B-cells of the primed groups were significantly higher than the  
22   infant MenCC naïve group. Participants primed with one MenC-TT dose had the  
23   highest increase in MenC rSBA titres compared with all other groups. The MenC  
24   rSBA titres at the 28<sup>th</sup> compared with the 6<sup>th</sup> day after boosting was significantly  
25   higher in those primed with a single MenC-TT/MenC-CRM vaccine in infancy

compared with those who were not primed or who were primed with two doses of the MenC-CRM vaccine.

## **Conclusion**

Immunologic memory can be demonstrated by a MenCC booster vaccination but is affected by the type and number of MenCC doses used for infant priming. The MenC rSBA responses can be used to demonstrate successful immunologic priming.

**Trial registration number:** Eudract No: 2009-016579-31; NCT01129518; Study ID: 2008\_06 (<http://clinicaltrials.gov>)

## Introduction

Demonstration of immune memory is fundamental in defining a successful capsular group C meningococcal glycoconjugate (MenCC) vaccine in clinical trials [1]. Classically, immune memory was demonstrated in MenCC trials from the anamnestic response seen following a challenge with a fractional dose of MenC polysaccharide vaccine [2]. However, concerns have been raised about the induction of hyporesponsiveness following the administration of full as well as fractional doses of MenC pure polysaccharide vaccines after priming with a MenCC vaccine [3-5]. This is thought to be a result of terminal differentiation of B-cells to plasma cells without replenishment of the B-cell memory pool, resulting in a potentially increased susceptibility to invasive capsular group C meningococcal disease [6]. In addition, boosting with full doses of MenC pure polysaccharide vaccines after previous MenCC vaccination may result in severe systemic reactions [2,7]. It has therefore been proposed that the WHO guidelines on the clinical evaluation of MenCC vaccines be altered to recommend the use of booster doses of conjugate vaccines to assess immunologic memory [8]. One suggested means of doing this is to assess whether MenC serum bactericidal antibody (SBA) titres increase above baseline to a greater magnitude following a 'challenge' dose of a MenC vaccine in those who have previously been primed by prior immunisation with a MenCC vaccine, compared with those who are vaccine naïve. Furthermore, detection of MenC specific memory B-cells, that maintain circulating MenC bactericidal antibody above the threshold of protection by differentiating into plasma cells, may be another method for assessing immune memory [9]. Hyporesponsiveness is not known to occur following MenCC vaccines but no guidance exists on which methods should be used to demonstrate immunologic memory [1] and studies on the use of MenCC vaccines as probes of

immune memory are limited. This is the first study of the kinetics of the immune response to a MenCC challenge in MenCC primed and unprimed children, and in children receiving different MenCC vaccine priming regimens.

## **Methods**

### *Participants and recruitment*

This study was part of a phase 4 open label randomised control trial carried out in four centres in the UK and one centre in Malta from July 2010 until August 2013. The primary and booster stages of this trial as well as exclusion criteria and details of approvals from ethical and medicines regulatory agencies in both countries have been published elsewhere [10].

### *Visits and vaccines*

Briefly, 509 healthy infants (mean age: 8.5 weeks; Range: 6.9 – 10.6 weeks), were randomised in the primary vaccination phase in a 10:10:7:4 ratio to 4 different groups to receive one dose of the MenC-CRM vaccine (*Menjugate*, GlaxoSmithKline Vaccines, Siena, Italy) at 3 months of age (single infant dose MenC-CRM group), or two doses of the same MenC-CRM vaccine formulation at 2 and 3 months of age (two infant dose MenC-CRM group) or one dose of the MenC-TT vaccine (*NeisVac-C*, Pfizer Inc., New York, US) at 3 months of age (single infant dose MenC-TT group) or not to receive any MenCC doses (control group). All infants received the combined diphtheria-toxoid, tetanus-toxoid, acellular pertussis, inactivated polio and *Haemophilus influenzae* type b vaccine (DTaP-IPV-Hib, *Pediacel*, Sanofi Pasteur MSD, Lyon, France) at 2, 3 and 4 months of age and the 13-valent pneumococcal

conjugate vaccine (PCV13, *Prevenar 13*, Pfizer Inc., New York, US) at 2 and 4 months of age. The 478 participants who entered the booster phase (mean age: 12.5 months; Range: 11.9 – 13.6 months) received the Hib-MenC-TT vaccine (*Menitorix*, GlaxoSmithKline Biologicals, Rixensart, Belgium), together with the measles, mumps and rubella vaccine, at 12 months of age.

In the booster phase blood samples were obtained at 12 months of age, prior to administration of the Hib-MenC-TT vaccine, and 28 days later. At least 64 participants from each of the two infant dose MenC-CRM, single infant dose MenC-CRM and single infant dose MenC-TT groups, as well as all participants in the control group, were randomly selected to have a blood sample 6 days after the 12 month Hib-MenC-TT vaccine (Figure 1). Selection of the subset of participants in the MenC groups who were to have an additional blood sample 6 days after the Hib-MenC-TT vaccine at 12 months of age was part of the original randomisation process as described elsewhere [10]. The parents of enrolled participants as well as the study staff were not masked to allocation to the subset who needed blood sampling 6 days after the Hib-MenC-TT vaccine.

#### *Serologic assays*

Group C serum bactericidal antibody was measured using a SBA assay targeting the *N. meningitidis* C11 (C:16:P1.7-1,1) strain (MenC SBA) and baby rabbit serum (Pel-Freeze Incorporated, Rodgerson, AZ) as the complement source (MenC rSBA). MenC rSBA assays were performed at the Vaccine Evaluation Unit, Public Health England, Manchester Laboratory, Manchester Royal Infirmary, Manchester, UK.

*MenC specific IgG memory B-cells*

Heparinised whole blood samples were taken from participants who could provide a sufficient volume of blood and whose blood could be processed within 6 hours from sampling at the laboratory facilities in Oxford. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation (*Lymphoprep*; Alere, UK), stimulated for differentiation of MenC memory B-cells into MenC IgG secreting cells as described by Blanchard et al and Kelly et al [9, 11], and then measured using an enzyme linked immunospot (EliSpot) assay [12].

*Statistical analysis*

In order to provide a power of 88%, a sample size of 56 (increased to 64 to allow for a 12.5% drop out) was required in each group to detect a 10% difference in the mean rSBA response between each MenCC groups and the control group at 6 days after the 12 month Hib-MenC-TT vaccination, with a two sided significance level of 5%. Analysis of the outcome variables was performed on those participants within each group who had both pre- and post-boost MenC rSBA titre results. MenC rSBA titres below the lower limit of detection of the SBA assay ( $<4$ ) were given an arbitrary value of 2 in order to be able to perform the analysis. Since it was expected that some primed participants would have detectable MenC rSBA titres before the 12 month Hib-MenC-TT vaccine dose an analysis of the immune kinetics of the pre-boost and the 6-day post boost antibody titres was carried out by fitting a regression analysis model to the log-transformed rSBA titres that adjusted for the pre-boost titres (i.e. the analysis of the 6-day post boost antibody titres included an adjustment for detectable pre-boost titres in order to have a comparable pre-boost antibody baseline between the groups). Since we were assessing differences in antibody titres, analysis of unadjusted

data would have resulted in a falsely higher fold increase in the groups with the smaller pre-vaccination titres and would not have reflected the true differences between the groups. Geometric means and their 95% confidence intervals (95% CI) were calculated from the antilog of the mean of the log transformed rSBA titres and the geometric mean ratios (GMR) comparing randomised groups, and their 95% CI from the parameter estimates in the regression model. An analysis of the antibody kinetics at the 28<sup>th</sup> compared with the 6<sup>th</sup> day after the Hib-MenC-TT vaccine was performed in participants having results available from these two time points by computing the adjusted GMRs from a similar regression analysis model that adjusted for the 6 day post-boost antibody titres. The analysis was performed using STATA 13.

Analysis of the MenC specific IgG memory B-cells was exploratory and the sample size had been predetermined by the MenC rSBA analysis. Blood was taken only from those participants who lived in the vicinity of the Oxford research laboratory facilities where blood could be processed within 6 hours of sampling. The statistical methods used to analyse the pre-boost and the 6<sup>th</sup> and 28<sup>th</sup> day post boost results were similar to the rSBA analysis, with calculation of the geometric means, and their 95% CI as well as the GMRs using a regression analysis model that adjusted for pre-boost numbers. When no cells were identified on the EliSpot assay, a value of 0.31 cells/million peripheral blood mononuclear cells (PBMC), equivalent to half the lowest level of detection of the assay, was assigned in order to be able to log<sub>10</sub> transform the results.

## Results

There were 181 participants who had paired sera before and 6 days after the Hib-MenC-TT vaccination at 12 months of age (Figure 1). Before Hib-MenC-TT immunisation only around 30% of the participants primed with a MenCC vaccine in infancy had MenC rSBA titres  $\geq 8$ , whilst none of those in the control group had detectable MenC SBA (Table 1). At 6 days after the Hib-MenC-TT vaccination a significantly higher proportion of participants who were primed with a MenCC vaccine in infancy had MenC rSBA titres  $\geq 8$  compared with those in the control group, who had not been primed (100% in the MenC primed groups vs 82.6% in the unprimed group; all  $p \leq 0.006$ ; Fishers Exact tests) (Table 1). MenC rSBA titres and circulating MenC specific IgG memory B-cells after the booster vaccine were significantly higher in participants primed in infancy compared with those who were not primed (Figures 2, 3 and Tables 1, 2). Participants primed with MenC-CRM vaccines had an increase in rSBA titres (as measured by post-immunisation rSBA GMTs adjusted for pre-immunisation levels) almost 4 times more than control participants in both single dose CRM recipients (GMR compared with controls 3.82, 95% CI: 1.88 to 7.77,  $p < 0.0001$ ) and two dose MenC-CRM recipients (3.99, 95% CI: 1.93 to 8.23,  $p < 0.0001$ ) (Figure 2, Table 1). MenC IgG memory B-cells, after adjusting for pre-boost levels, were similarly significantly higher in the single and two dose MenC-CRM groups when compared with the control group (GMR 6.23, 95% CI: 2.04 to 18.96,  $p = 0.002$  and GMR 3.99, 95% CI: 1.23 to 12.92,  $p = 0.022$ ; respectively) (Table 2). Participants primed with a single dose of MenC-TT had an increase in rSBA titres and memory B-cells 13.5 times and 8 times more than control participants, respectively (MenC rSBA GMR 13.49, 95% CI: 6.68 to 27.25,  $p < 0.0001$  and MenC memory B-cells GMR 8.12, 95% CI: 2.33 to 28.35,  $p = 0.0015$ ). The



response to the Hib-MenC-TT booster in the single infant dose MenC-TT group was also significantly higher than in those primed with MenC-CRM (MenC rSBA GMR 3.53, 95% CI: 1.79 to 6.97;  $p < 0.0001$  and higher than in the two infant dose MenC-CRM group (MenC rSBA GMR 3.38, 95% CI: 1.69 to 6.77;  $p = 0.001$ ), although the change in the number of circulating MenC IgG memory B-cells was not significantly different. In contrast, no significant differences were seen in the MenC rSBA rise six days after the Hib-MenC-TT boost between the single and the two infant dose MenC-CRM groups (Table 1). Similarly, the change in the number of circulating memory B-cells was not significantly different between these MenC-CRM primed groups (Table 2).

Differences observed in the GMRs were not reflected in the proportion of participants with protective titres above accepted thresholds. Priming with one MenC-TT dose at 3 months of age did not induce significant differences in the percentage of participants with MenC rSBA titres  $\geq 8$  or  $\geq 128$  six days after the Hib-MenC-TT 12 month booster dose when compared with the MenC-CRM primed groups.

A separate analysis of the 6 day post-boost MenC bactericidal antibody and memory B-cell kinetics of primed participants who had undetectable pre-boost MenC rSBA titres ( $< 4$ ) showed a significantly higher increase in MenC rSBA titres, but not circulating MenC IgG memory B-cells, in the primed compared with the control group (Supplementary figures 1a, 2a and Supplementary table 1). However, no significant differences in the post boost MenC rSBA GMTs and memory IgG B-cells were noted in participants with pre-boost MenC rSBA titres  $< 4$  compared with those with titres  $\geq 4$  within the same primed group (Supplementary figures 1, 2 and

Supplementary table 1). No significant differences were noted in the rise in post-boost MenC rSBA titres and memory IgG B-cells of participants with pre-boost titres >4 between the single and two infant dose MenC-CRM groups. Although the change in the number of MenC IgG memory B-cells was not significantly different, the rise in the MenC rSBA titres was significantly higher in the MenC-TT when compared with the MenC-CRM groups (Supplementary figures 1b, 2b and Supplementary table 1).

In order to assess the MenC rSBA kinetics at the 28<sup>th</sup> compared to the 6<sup>th</sup> day after the Hib-MenC-TT boost, an exploratory analysis of changes in the MenC rSBA titres and MenC IgG memory B-cells was performed after the Hib-MenC-TT vaccine given at 12 months of age (Figure 2, 3). Although there was a decline in the MenC rSBA GMTs in all groups at the 28<sup>th</sup> compared with the 6<sup>th</sup> day after the Hib-MenC-TT vaccine, which was only statistically significant for the two infant dose MenC-CRM group, the MenC rSBA titres of participants primed in infancy with a single MenC-CRM dose at day 28 (after adjusting for the 6<sup>th</sup> day levels) remained 4 times higher than those in the control group (GMR 4.04, 95% CI: 1.96 to 8.31;  $p<0.0001$ ). Similarly the MenC rSBA titres in the single infant dose MenC-TT group was also significantly higher than those in the control group (GMR 7.99, 95% CI: 3.63 to 17.60;  $p<0.0001$ ). In contrast no significant difference was noted in the day 28 MenC titres of the two infant dose MenC-CRM compared with the control group (Table 1). A comparison of the MenC rSBA titres between the differently primed groups showed no significant difference between the single infant dose MenC-CRM and MenC-TT groups. However, the MenC rSBA titres were 2.7 times and 5.3 times higher in the single infant dose MenC-CRM and MenC-TT groups, respectively, when

compared to the two infant dose MenC-CRM group (GMR 2.67, 95% CI: 1.31 to 5.44;  $p=0.007$  and 5.28, 95% CI: 2.54 to 10.96;  $p<0.0001$ , respectively, Table 1).

Intriguingly the increase in the number of MenC specific IgG memory B-cells was almost 5 times higher in the participants receiving their first MenCC dose at 12 months compared with those primed with one or two MenC-CRM doses (GMR 4.85, 95%CI: 1.29 to 18.29;  $p=0.02$  and GMR 4.63, 95%CI: 0.10 to 21.48,  $p=0.05$ ) (Table 2). There was no significant difference in the memory B-cell responses between participants primed with MenC-TT in infancy compared with those in the control and the MenC-CRM primed groups (Table 2).

## Discussion

The significantly higher MenC rSBA titres as measured by the GMRs of primed compared with unprimed groups six days after a 12 month Hib-MenC-TT booster dose, shows that the MenC rSBA responses can be used to distinguish the presence or lack of MenCC priming. A significantly higher proportion of participants primed in infancy had MenC rSBA titres  $\geq 8$  six days after Hib-MenC-TT vaccination at 12 months of age compared with those who were never primed. This demonstrates the importance of infant priming to attain high post-boost MenC rSBA titres that exceed the protective threshold of  $\geq 8$  and reflects the significantly higher number of MenC IgG memory B-cells detected 6 days after the 12 month Hib-MenC-TT vaccine in primed compared with unprimed children.

Prior to boosting, around 60% of participants in each of the primed groups had undetectable MenC rSBA titres  $\leq 4$ , although MenC IgG memory B-cells were still

detectable in their bloodstream (Supplementary table 1). A direct comparison of these children with those in the control group revealed significantly higher post-boost MenC rSBA titres, again demonstrating the presence of priming despite the undetectable pre-boost MenC rSBA titres. This observation was not reflected in similar significant differences in the number of MenC memory B-cells, very likely from the small number of participants with MenC rSBA titres  $\leq 4$  who had blood available for B-cell analysis. Interestingly the anamnestic response in children primed with a MenCC vaccine in infancy but who had undetectable pre-boost titres by the time they were 12 months of age was not significantly different from those with detectable titres who were primed with the same MenCC formulation and schedule in infancy. Despite differences in the persistence of MenC antibody in children primed with similar MenCC vaccine schedules in infancy, it appears that immune memory is similar, reflected by a similar rise in antibody titres to a booster dose.

Following the 12 month Hib-MenC-TT vaccine, we observed a tendency towards a decline in the post-boost MenC rSBA titres at 28 days when compared with the titres measured at 6 days. Our observation is similar to the drop in MenC rSBA titres seen from 7-10 days to 21-25 days after a MenC polysaccharide vaccine challenge in toddlers vaccinated with a MenC-CRM vaccine 12 months previously and following a MenC glycoconjugate or pure polysaccharide vaccine in 18-50 year old adults previously vaccinated with a MenC-TT vaccine [13,14]. In contrast, following MenCC vaccination of adolescents and adults who never received a MenC vaccine previously or who were vaccinated with a MenC pure polysaccharide or a MenCC vaccine, the MenC rSBA titres rose rapidly in the first 5-7 days and continued to increase to 28 days later [15-17]. Such discrepancies could be due to variations in the

MenC vaccine formulations used, vaccine scheduling, time elapsed from first immunisation to boosting in the different studies, differences in meningococcal C carriage rates, as well as differing memory B-cell responses in infants and children from that of adults in different populations.

The lower rise in MenC rSBA titres at 6 days post Hib-MenC-TT vaccination in unprimed than in primed individuals indicates that these antibodies are likely derived from low numbers of B cells that are proliferating during the primary response before sufficient time has passed for germinal centre output. In primed infants a secondary response is observed as strong rapid high avidity response as measured by SBA. The subsequent relatively steeper decline in bactericidal titres at 28 days from Hib-MenC-TT vaccination in MenCC naive participants may suggest that this component of the primary response arises from the early, possibly pre-germinal centre, production of short-lived plasma cells.

Infants primed with the two dose MenC-CRM schedule had a lower number of memory B-cells prior to the booster at 12 months of age than did those who had only one dose suggesting that administering multiple doses of the vaccine during priming may affect the memory pool and perhaps accounting for the differences in functional antibody between the two groups one month after the booster.

No studies have previously investigated the post-boost MenC rSBA or circulating memory B-cell kinetics in children primed with different MenCC vaccines. We have shown that the kinetics of the immune response are affected by the MenCC vaccine formulation and schedule used for priming in infancy. The significantly higher MenC rSBA titres seen at 6 days after the 12 month Hib-MenC-TT boost in participants primed in infancy with one MenC-TT vaccine dose compared with one/two MenC-

CRM dose priming indicate that this vaccine formulation induces more robust immunologic memory than the MenC-CRM vaccine. Furthermore, the decline in MenC rSBA at the 28<sup>th</sup> compared with the 6<sup>th</sup> day after the 12 month Hib-MenC-TT vaccination is significantly less in children primed with MenC-TT compared with the MenC-CRM primed groups, an effect that persists until 24 months of age [10].

We did not observe any significant differences in the 6 day post-boost antibody kinetics in children previously primed with a single or two dose MenC-CRM schedule. However, the MenC rSBA titres at the 28<sup>th</sup> compared with the 6<sup>th</sup> day, after boosting were significantly higher in the single infant dose MenC-CRM group than in the two infant dose MenC-CRM group. In addition, the MenC rSBA response was not significantly different in the two infant dose MenC-CRM group compared with the control group. The differences in the antibody kinetics between children primed with one or two MenC-CRM doses in infancy are similar to that which was observed in children primed with one or two doses of MenC-TT vaccine in infancy and then challenged with a low dose of a MenAC polysaccharide vaccine at 12 months of age in a different study [18]. Thus it appears that a higher quantity of antigen used for priming negatively impacts the booster response at 12 months of age to an extent that the antibody kinetics at the 28<sup>th</sup> compared with the 6<sup>th</sup> day after boosting are not significantly different from those who were not primed in infancy.

The clinical relevance of the MenC antibody kinetics induced by a 12 month MenCC vaccine following MenCC priming, or no priming, in infancy lies in the persistence of

351 MenC rSBA GMTs above the protective threshold of  $\geq 8$  which is important for  
352 sustained protection against invasive MenC disease. This is because the incubation  
353 period of meningococcal disease is short with the majority of secondary cases of IMD  
354 occurring 24 hours to 7 days following exposure to an index case [19]. A rise in  
355 MenC rSBA titres to above the threshold of protection of  $\geq 8$  only occurs around the  
356 9<sup>th</sup> day following acquisition of MenC carriage in individuals with undetectable MenC  
357 bactericidal antibodies [20]. Similarly, the immune memory response in previously  
358 vaccinated adolescents and adults following a MenCC vaccine challenge is delayed by  
359 5-7 days [21,22]. As shown by surveillance data from countries with routine MenCC  
360 vaccination, relying on an anamnestic response to antigenic exposure from  
361 colonisation or revaccination would not be rapid enough to prevent invasive disease in  
362 those with unprotective titres [23,24], since protection is dependent on having MenC  
363 rSBA titres  $\geq 8$  [25]. As we have shown in this study infant priming results in a greater  
364 increase in MenC-rSBA titres following a 12 month MenCC vaccine compared with  
365 no priming, an effect that is more pronounced with a single infant MenC-TT rather  
366 than with MenC-CRM vaccine priming. A single dose of MenCC vaccine at 12  
367 months of age still resulted in MenC rSBA GMTs  $\geq 8$  in 80% of children 28 days after  
368 vaccination (Table 1), a proportion that might be acceptable in countries like the UK  
369 where MenC disease has been controlled following a robust MenC infant prime and  
370 boost vaccination programme and an initial catch up MenCC vaccine campaign [26].  
371 The herd protection that has been established from such a MenCC vaccine programme  
372 is likely to be sustained through adolescent vaccination and may make it possible to  
373 remove the infant priming dose in the future. The 12 month MenCC vaccine might,  
374 however, still be important for a robust rise in MenC rSBA GMTs above the threshold  
375 of  $\geq 8$  following an adolescent boost.

376

377 **Conclusion**

378 MenC glycoconjugate vaccines can be used to demonstrate the induction of immune  
379 memory, a regulatory requirement that is necessary to define a successful MenCC  
380 vaccine in clinical trials. Immune memory may, however, be affected by the MenCC  
381 vaccine formulation and number of doses used for infant vaccination

382

383

384 **Acknowledgements**

385 We would like to thank all children taking part in the study as well as their  
386 parents/guardians, the study staff in the research centres at Bristol, London, Malta,  
387 Oxford and Southampton and the NIHR Oxford Biomedical Research Centre, UK, the  
388 NIHR Medicines for Children Network South West and London (now NIHR Clinical  
389 Research Network: Paediatrics), the Southampton NIHR Wellcome Trust Clinical  
390 Research Facility and NIHR Respiratory Biomedical Research Unit, GlaxoSmithKline  
391 Biologicals, Belgium and European Society of Paediatric Infectious Diseases for  
392 financially supporting this study. The research team acknowledges the support of the  
393 National Institute for Health Research Clinical Research Network. AJP and MDS are  
394 Jenner Institute Investigators.

395

396 **Conflict of interest statement**

397 AJP has previously conducted studies on behalf of Oxford University funded by  
398 vaccine manufacturers, but currently does not undertake industry funded clinical  
399 trials. AJP chairs the UK Department of Health's (DH) Joint Committee on



Vaccination and Immunisation (JCVI); the views expressed in this manuscript do not necessarily reflect the views of JCVI or DH.

MDS, AF, SNF, and PTH act as investigators for clinical trials conducted on behalf of their respective Universities and NHS Hospital Trusts sponsored by vaccine manufacturers and have participated in advisory boards but receive no personal payments from these activities. MDS, DP, AK and SNF have had travel and accommodation expenses paid by vaccine manufacturers to attend international conferences related to paediatric infectious disease. RB performs contract research on behalf of Public Health England for Baxter Bioscience, GlaxoSmithKline, Pfizer, Sanofi Pasteur, Sanofi Pasteur MSD, and Novartis Vaccines. The other authors have no conflicts of interest.

## References

- [1] World Health Organization. Recommendations for the production and control of meningococcal group C conjugate vaccines: WHO Technical Report Series. 2004, 924; Annex 2.
- [2] Richmond P, Borrow R, Miller E, Clark S, Sadler F, Fox A, Begg N, Morris R, Cartwright K. Meningococcal serogroup C conjugate vaccine is immunogenic in infancy and primes for memory. J Infect Dis. 1999 Jun;179(6):1569-72.

- [3] Gold R, Lepow ML, Goldschneider I, Gotschlich EC. Immune Response of human infants of polysaccharide vaccines of group A and C *Neisseria meningitidis*. J Infect Dis. 1977 Aug;136 Suppl:S31-5.
- [4] MacDonald NE, Halperin SA, Law BJ, Forrest B, Danzig LE, Granoff DM. Induction of immunologic memory by conjugated vs plain meningococcal C polysaccharide vaccine in toddlers: a randomized controlled trial. JAMA. 1998 Nov 18;280(19):1685-9.
- [5] Richmond P, Kaczmarek E, Borrow R, Findlow J, Clark S, McCann R, Hill J, Barker M, Miller E. Meningococcal C polysaccharide vaccine induces immunologic hyporesponsiveness in adults that is overcome by meningococcal C conjugate vaccine. J Infect Dis. 2000 Feb;181(2):761-4.
- [6] Granoff DM, Pollard AJ. Reconsideration of the use of meningococcal polysaccharide vaccine. Pediatr Infect Dis J. 2007 Aug;26(8):716-22.
- [7] Borrow R, Fox AJ, Richmond PC, Clark S, Sadler F, Findlow J, Morris R, Begg NT, Cartwright KA. Induction of immunological memory in UK infants by a meningococcal A/C conjugate vaccine. Epidemiol Infect. 2000 Jun;124(3):427-32.
- [8] World Health Organization (WHO) ECoBS. Proposed replacement of: TRS 926, Annex 3. Part C. Clinical evaluation of group C meningococcal conjugate vaccines. 2007. Available at [http://www.who.int/biologicals/expert\\_](http://www.who.int/biologicals/expert_)

448 committee/BS 2065%20 Mening%20+%20line%20number.pdf. Accessed  
 449 30th November 2015.

450  
 451 [9] Blanchard Rohner G, Snape MD, Kelly DF, John T, Morant A, Vu L et al. The  
 452 magnitude of the antibody and memory B cell responses during priming with a  
 453 protein-polysaccharide conjugate vaccine in human infants is associated with  
 454 the persistence of antibody and the intensity of booster response. J. Immunol.  
 455 2008;180:2165-73.

456  
 457 [10] Pace D, Khatami A, McKenna J, Campbell D, Attard-Montalto S,  
 458 Birks J, Voysey M, White C, Finn A, Macloed E, Faust SN, Kent AL, Heath  
 459 PT, Borrow R, Snape MD, Pollard AJ. Immunogenicity of reduced dose  
 460 priming schedules of serogroup C meningococcal conjugate vaccine followed  
 461 by booster at 12 months in infants: open label randomised controlled trial.  
 462 BMJ. 2015 Apr 1;350:h1554.

463  
 464 [11] Kelly DF, Snape MD, Perrett KP, Clutterbuck EA, Lewis S, et al.  
 465 Plasma and memory B-cell kinetics in infants following a primary schedule of  
 466 CRM 197-conjugated serogroup C meningococcal polysaccharide vaccine.  
 467 Immunol. 2009;127: 134–143.

468  
 469 [12] Khatami A, Clutterbuck EA, Thompson AJ, McKenna JA, Pace D,  
 470 Birks J, Snape MD, Pollard AJ. Evaluation of the induction of immune  
 471 memory following infant immunisation with serogroup C *Neisseria*  
 472 *meningitidis* conjugate vaccines--exploratory analyses within a randomised  
 473 controlled trial. PLoS One. 2014 Jul 14;9(7):e101672.

- [13] Tsai TF, Borrow R, Gnehm HE, Vaudaux B, Heininger U, Desgrandchamps D, Aebi C, Balmer P, Pedersen RD, Fritzell B, Siegrist CA. Early appearance of bactericidal antibodies after polysaccharide challenge of toddlers primed with a group C meningococcal conjugate vaccine: what is its role in the maintenance of protection? *Clin Vaccine Immunol.* 2006 Aug;13(8):854-61.
- [14] de Voer RM, van der Klis FR, Engels CW, Schepp RM, van de Kassteele J, Sanders EA, Rijkers GT, Berbers GA. Kinetics of antibody titres after primary immunization with meningococcal serogroup C conjugate vaccine or secondary immunization with either conjugate or polysaccharide vaccine in adults. *Vaccine.* 2009 Nov 23;27(50):6974-82.
- [15] Snape MD, Kelly DF, Salt P, Green S, Snowden C, Diggle L, Borkowski A, Yu LM, Moxon ER, Pollard AJ. Serogroup C meningococcal glycoconjugate vaccine in adolescents: persistence of bactericidal antibodies and kinetics of the immune response to a booster vaccine more than 3 years after immunization. *Clin Infect Dis.* 2006 Dec 1;43(11):1387-94.
- [16] Borrow R, Southern J, Andrews N, Peake N, Rahim R, Acuna M, Martin S, Miller E, Kaczmarek E. Comparison of antibody kinetics following meningococcal serogroup C conjugate vaccine between healthy adults previously vaccinated with meningococcal A/C polysaccharide vaccine and vaccine-naïve controls. *Vaccine.* 2001 Apr 30;19(23-24):3043-50.

- [17] Jacobson RM, Jackson LA, Reisinger K, Izu A, Odrlic T, Dull PM. Antibody persistence and response to a booster dose of a quadrivalent conjugate vaccine for meningococcal disease in adolescents. *Pediatr Infect Dis J*. 2013 Apr;32(4):e170-7.
- [18] Borrow R, Goldblatt D, Finn A, Southern J, Ashton L, Andrews N, Lal G, Riley C, Rahim R, Cartwright K, Allan G, Miller E. Immunogenicity of, and immunologic memory to, a reduced primary schedule of meningococcal C-tetanus toxoid conjugate vaccine in infants in the United Kingdom. *Infect Immun*. 2003 Oct;71(10):5549-55.
- [19] De Wals P, Hertoghe L, Borlée-Grimée I, De Maeyer-Cleempoel S, Reginster-Haneuse G, Dachy A, Bouckaert A, Lechat MF. Meningococcal disease in Belgium. Secondary attack rate among household, day-care nursery and pre-elementary school contacts. *J Infect*. 1981 Mar;3(1 Suppl):53-61.
- [20] Edwards EA, Devine LF, Sengbusch GH, Ward HW. Immunological investigations of meningococcal disease. III. Brevity of group C acquisition prior to disease occurrence. *Scand J Infect Dis*. 1977;9(2):105-10.
- [21] Findlow H, Borrow R, Hardelid P, Newton E, Frankland S, Naylor S, Miller E, Kaczmarek E, Read RC. Serum antibody kinetics following nasal or parenteral challenge with meningococcal polysaccharide in healthy adults. *Clin Vaccine Immunol*. 2011 Mar;18(3):424-9.

- [22] Snape MD, MacLennan JM, Lockhart S, English M, Yu LM, Moxon RE, Pollard AJ. Demonstration of immunologic memory using serogroup C meningococcal glycoconjugate vaccine. *Pediatr Infect Dis J*. 2009 Feb;28(2):92-7.
- [23] Trotter CL, Andrews NJ, Kaczmarski EB, Miller E, Ramsay ME. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. *Lancet*. 2004 Jul 24-30;364(9431):365-7.
- [24] Cano R, Larrauri A, Mateo S, Alcalá B, Salcedo C, Vázquez JA. Impact of the meningococcal C conjugate vaccine in Spain: an epidemiological and microbiological decision. *Euro Surveill*. 2004 Jul;9(7):11-5.
- [25] Auckland C, Gray S, Borrow R, Andrews N, Goldblatt D, Ramsay M, Miller E. Clinical and immunologic risk factors for meningococcal C conjugate vaccine failure in the United Kingdom. *J Infect Dis*. 2006 Dec 15;194(12):1745-52.
- [26] Miller E, Salisbury D, Ramsay M. Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. *Vaccine*. 2002 Oct 15;20 Suppl 1:S58-67.