

# Divergent memory B cell responses in a mixed infant pneumococcal conjugate vaccine schedule

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**Abbreviated Title** B cell memory in a mixed pneumococcal vaccine schedule

**Running Title** B cell memory to pneumococcal vaccines

**Abstract**

**Background:** Vaccine-induced immunity against pneumococcal infection relies on the generation of high concentrations of antibody and B-cell memory. Both the 10- and the 13-valent pneumococcal conjugate vaccines (PCV-10 and PCV-13) effectively reduce disease caused by vaccine serotypes. It is unknown whether the generation of B-cell memory requires several doses of the same vaccine or whether different PCVs are interchangeable.

**Methods:** Children in the UK (n=178) who had previously received PCV-13 at 2 and 4 months were randomized 1:1 to receive a PCV-13 or PCV-10 booster at age 12 months. Peripheral blood memory B cells ( $B_{MEM}$ ) were quantified before and at 1 and 12 months following vaccination using a cultured ELISpot assay for pneumococcal serotypes 1, 3, 4, 9V, 14, 19A and diphtheria and tetanus toxoid. Correlations between  $B_{MEM}$  frequencies and simultaneously measured antibody (IgG and OPA) was also assessed.

**Results:** A significant rise in post-booster  $B_{MEM}$  frequency was seen for 5 out of six serotypes in the PCV-13 group and none in the PCV-10 group. In the PCV-13 group, there was a particularly large increase in serotype 3-specific  $B_{MEM}$  associated with only a small increase in antibody. Post-booster  $B_{MEM}$  responses correlated positively with antibody, but correlations between pre-booster  $B_{MEM}$  and subsequent  $B_{MEM}$  and antibody responses were inconsistent.

**Conclusions:** Following priming with PCV-13 in early infancy, a booster dose of PCV-10 does not induce detectable peripheral blood  $B_{MEM}$  responses but a PCV-13 booster does induce robust responses. Booster responses to pneumococcal conjugate vaccines may be dependent on homologous carrier protein priming.

(250 words)

## Introduction

Vaccination against *S. pneumoniae* with currently used 10- and 13-valent pneumococcal conjugate vaccines (PCV-10 and PCV-13) has been shown to dramatically reduce vaccine-type invasive pneumococcal disease when included in childhood immunization schedules [1–5]. Both vaccines are immunogenic and have been shown to induce immune memory [6,7].

There is limited information on the interchangeability of pneumococcal conjugate vaccines, however there are potential reasons why it may be advantageous to include PCV-10 in the infant vaccination schedule. *Non-typeable Haemophilus influenzae* (NTHi) protein D is not included in any concurrent or previously administered vaccine. Its use may therefore reduce the risk of immune interference and theoretically increase the immune response to the polysaccharides. Immune interference can occur if the same carrier protein is used in concurrent or sequential vaccinations, and can suppress the immune response to the desired antigen [8]. *In addition*, use of NTHi protein D as a carrier protein may confer additional protection against NTHi infection, a common cause of otitis media in children [9], *although effectiveness of PCV-10 against NTHi carriage or disease has not been demonstrated in previous vaccine trials* [10–12].

Ideally, a vaccine should provide long-lasting protection in the form of antibodies, but should also induce immunological memory through the formation of memory B cells ( $B_{MEM}$ ). A number of investigators have studied this outcome by measuring  $B_{MEM}$  frequency in peripheral blood post-vaccination [13–16]. The majority of  $B_{MEM}$  are thought to reside in lymphoid tissue but small numbers of circulating  $B_{MEM}$  can be detected in peripheral blood even years after vaccination. The frequency of these cells in peripheral blood rises shortly after vaccination, particularly after booster doses [17]. Re-encounter with antigen triggers the proliferation of  $B_{MEM}$  and their differentiation into antibody-secreting plasma cells, giving rise to a rapid and effective secondary immune response. The presence of  $B_{MEM}$  may also contribute to the maintenance of circulating antibody by intermittent or continuous differentiation into antibody-secreting plasma cells in response to antigen-dependent or independent stimulation [18].

This study evaluated the potential for the use of PCV-10 as a booster following priming with PCV-13 in infancy by assessing non-inferiority of the proportions of participants with post-booster IgG  $\geq 0.35$   $\mu\text{g/ml}$  for PCV-10 serotypes. The results of antibody measurements in this cohort of children have already been published and showed that in PCV-13 primed infants, a booster dose of PCV-10 induces a strong antibody response, which is generally less pronounced than the response following a PCV-13 booster [19]. In the analysis presented here, we investigated the frequencies of peripheral blood B<sub>MEM</sub> before and at 1 and 12 months following a 12-month booster dose of PCV-10 or PCV-13. Using a cultured enzyme-linked immunospot (ELISpot) assay, we studied the B<sub>MEM</sub> response to two different pneumococcal vaccines, of which only one had previously been given to study participants. By comparing the booster response to the two vaccines, which differ in the concentrations of pneumococcal polysaccharides, the method of conjugation and the type and concentrations of the carrier proteins, we had the unique opportunity to study the effects of a mixed vaccination schedule on B cell biology.

## Materials and Methods

### Subjects and Vaccines

Healthy children who had been vaccinated with the 13-valent pneumococcal conjugate vaccine at 2 and 4 months of age were recruited as previously described [19] (Supplementary Figure 1). Ethical approval was obtained from the Oxfordshire Research Ethics Committee (reference number 11/SC/0473) and the study was registered on Clinicaltrials.gov (registration number NCT01443416). Following enrolment into the study, these children were randomized to receive either PCV-10 (Synflorix®, GSK Biologicals, containing capsular polysaccharides of serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F, conjugated to NTHI protein D, tetanus toxoid (18C) or diphtheria toxoid (19F), respectively) or PCV-13 (Prevenar 13®, Pfizer, containing serotypes 3, 6A and 19A in addition to PCV-10 serotypes, conjugated to diphtheria toxin mutant CRM<sub>197</sub>). Blood samples were taken on three visits: at day 0 immediately prior to vaccination (12 months of age) and at 1 and 12 months post-vaccination (13 and 24 months of age).

## PBMC isolation and ELISpot

The cultured ELISpot assay for the detection of antigen-specific B<sub>MEM</sub> was performed as previously described [20]. Firstly, PBMCs were isolated from whole blood by density gradient centrifugation with lymphoprep (Axis-Shield). Cells were then cultured for 6 days at 37 °C in 5% carbon dioxide and 95% humidity with an antigen stimulation mix containing *Staphylococcus aureus* Cowan strain (Calbiochem-Novabiochem) at a 1:5000 dilution, poke weed mitogen (Sigma-Aldrich) at a final concentration of 83 ng/ml and CpG oligonucleotide (InvivoGEN) at a final concentration of 1.7 µg/ml. After harvesting, cells were washed and seeded at  $2 \times 10^5$  viable cells per well onto a 96 well plate with PVDF membranes (Millipore). Membranes were pre-coated with either pneumococcal polysaccharides (1, 3, 4, 9V, 14 and 19A; LCG Promochem) conjugated to methylated human serum albumin (NIBSC UK), diphtheria toxoid (10ug/ml, also used to represent the CRM<sub>197</sub> carrier protein; Statens Serum Institut), or tetanus toxoid (5ug/ml, Statens Serum Institut). Pneumococcal serotypes were chosen in order to reflect i) serotypes contained in both PCV-10 and PCV-13 (serotypes 1, 4, 9V, 14) or unique to PCV-13 (serotypes 3, 19A). Plates included phosphate buffered saline (PBS) wells and polyvalent goat anti-human immunoglobulin (10 µg/mL) wells as negative and positive controls respectively. Following overnight incubation, plates were washed and bound IgG antibody was detected using a goat anti-human IgG alkaline phosphatase conjugate (Calbiochem) and alkaline phosphatase substrate kit (Bio-Rad). Plates were dried overnight before being read using an automated ELISpot reader (AID ELR03, AID Diagnostika). All plate readings were manually checked to exclude artefacts.

## Data analysis

The average number of spots per well was used to calculate the number of antibody-secreting cells (ASCs) per million PBMCs. If any spots were observed in the control PBS wells, the average count across these control wells was subtracted from the average count from each type of antigen-coated well. Wells coated with total immunoglobulin were used as positive controls. Results of zero were assigned the value of 0.625 per million PBMCs (half of the lower limit of detection). Geometric mean frequencies (GMF) and corresponding 95% confidence intervals (CI) were calculated for each group

and study time point. Differences between groups and time points were investigated using two-sample t-tests of logarithmically transformed  $B_{MEM}$  frequencies and Satterthwaite's correction for unequal variances. Correlations between responses at different time points were assessed using the Pearson correlation coefficient.

## Results

### Samples

Out of a total of 434 blood samples across both groups and the 3 study visits from the original serological analysis [19], 377 (87%) had cells available for  $B_{MEM}$  and antibody analysis. The breakdown of samples by visit and vaccine group is shown in Table 1.

### Antigen-specific memory B cell ( $B_{MEM}$ ) geometric mean frequencies (GMF) by antigen and vaccine group

At 12 months of age,  $B_{MEM}$  frequencies were low and no significant differences between the groups were seen in  $B_{MEM}$  GMF for all antigens tested (Figure 1 and Supplementary Table 1). One month following booster vaccination at 13 month of age, significantly higher  $B_{MEM}$  GMF were found for serotypes 1, 4, 9V and 3 in the PCV-13 compared with the PCV-10 group whereas  $B_{MEM}$  responses to tetanus toxoid were statistically higher in PCV-10 than in PCV-13 recipients (Figure 1 and Supplementary Table 1). One year post-booster at 24 months of age, no significant differences were detected between the groups for any of the antigens tested (Figure 1 and Supplementary Table 1).

### Antigen-specific $B_{MEM}$ frequency fold changes

Significant increases between  $B_{MEM}$  measured at age 12 months and 13 months were seen for most pneumococcal serotypes in the PCV-13 group (with the exception of serotype 14, which showed a 1.6 fold increase with a borderline p-value of 0.05) and none in the PCV-10 group (Table 2). For antigens representing carrier proteins, a significant rise in  $B_{MEM}$  frequencies was seen for both diphtheria and tetanus toxoid in the PCV-10 group but only for diphtheria toxoid in the PCV-13 group (Table 2). When adjusted for baseline  $B_{MEM}$  values, age, sex and ethnicity, changes in  $B_{MEM}$  frequencies between

age 12 and 13 months were significantly greater in the PCV-13 compared with the PCV-10 group for all pneumococcal serotypes and statistically superior in PCV-10 compared with PCV-13 recipients only for tetanus toxoid (Table 2). Frequencies of B<sub>MEM</sub> were not significantly different between age 12 and 24 months for most serotypes in both groups. In the PCV-10 group, significantly higher B<sub>MEM</sub> frequencies were seen for serotypes 14 (GMF of 3.83 vs. 1.91) and 19A (4.76 vs. 2.66) at 24 months compared with 12 months of age whereas in the PCV-13 group a significant fold increase was only seen for serotype 3 (2.88 vs. 1.61) (Supplementary Table 2). However, when comparing these fold changes from age 12 to 24 months between the vaccine groups, no differences were seen when adjusted for baseline values, age, sex and ethnicity (data not shown).

### Correlation between serotype-specific B<sub>MEM</sub> and antibody responses

Pearson correlation was used to investigate correlations between log-transformed B<sub>MEM</sub> frequencies at different time points and between B<sub>MEM</sub> and antibody responses (both IgG concentration and opsonophagocytic assay (OPA) titers). All correlations and their statistical significance are shown in Figure 2. The most striking correlations were seen between B<sub>MEM</sub> and antibody, both at age 13 months. Antibody and B<sub>MEM</sub> were correlated at 12 months and at 24 months of age for some serotypes, however this relationship was less consistent than that seen at age 13 months. B<sub>MEM</sub> at 12 and 13 months of age were also predictive of later antibody responses for some serotypes in each group, but again this relationship was not consistent across serotypes.

A significant increase in B<sub>MEM</sub> frequencies from 12 to 13 months of age against the majority of the tested serotypes was only observed for the PCV-13 group but the extent of the response was serotype-dependent (Table 2). In PCV-13 recipients, the immune response against serotype 3 was associated with a strong increase in peripheral B<sub>MEM</sub> (Figure 2) and associated with a weak antibody responses compared with other serotypes [19]. In contrast, antibody responses against serotype 14 were strong [19] whereas it was associated with only a small increase in post-booster B<sub>MEM</sub> frequency (Figure 2).



## Discussion

This study provides an assessment of the  $B_{MEM}$  responses following booster vaccination with either PCV-13 or PCV-10 in children previously primed with two doses of PCV-13. Our results show that PCV-13 primed children do not generate peripheral  $B_{MEM}$  in response to a PCV-10 booster. This is a surprising finding as 4 of the 6 serotypes that were evaluated are contained in both vaccines. However, the vaccines differ in the concentrations of pneumococcal polysaccharides, the method of conjugation and the type and concentrations of the carrier proteins, which may result in diverging presentation and processing of antigens.

One recent study assessed the short-term (7-9 days post-booster) immunogenicity as well as the plasma and memory B cell response around a booster dose given at 11 months of age following a vaccination series with either PCV-10 or PCV-13 [21,22]. This study showed that the pneumococcal serotype-specific frequencies of peripheral blood  $B_{MEM}$  measured before and shortly after a PCV booster were significantly higher in PCV-13 compared with PCV-10 recipients for 3 out of 4 serotypes common to both vaccines [21]. No such differences were seen for serotype-specific plasma cell responses [21] but the study also showed statistically superior post-booster IgG responses in the PCV-13 compared with the PCV-10 group to the majority of serotypes common to both vaccines [22]. Following 4 doses of PCV-10, a significant  $B_{MEM}$  booster response was observed [21], however even in that study PCV-13 appeared to be a more potent inductor of memory B cells than PCV-10. Interchangeability of pneumococcal conjugate vaccines was not assessed in that study as children received the same booster vaccine as they had received in infancy.

In the present clinical trial, in the PCV-10 group, no change in serotype-specific  $B_{MEM}$  frequencies was seen between pre-booster and 1 month following booster vaccination whereas a significant increase in  $B_{MEM}$  specific for diphtheria and tetanus toxoid was noted between these 2 study time points. Children who were allocated to the PCV-10 group had previously received 2 doses of PCV-13 and were therefore already primed with all pneumococcal serotypes contained in PCV-10. However, their immune system had not been exposed to the same conjugates and the carrier protein D derived from NTHi in the form of a vaccine although some may have encountered it through carriage

1 or disease. Priming and boosting with different carrier proteins has previously been investigated in  
2 children vaccinated against meningitis C [23]. Children primed with a dose of tetanus toxoid–  
3 conjugated polysaccharide (MenC-TT) at 3 months generated better B<sub>MEM</sub> responses to a MenC-TT  
4 booster at 12 months than those primed with either one or two doses of CRM-conjugated  
5 polysaccharide (MenC-CRM). In the present study, B<sub>MEM</sub> booster responses to serotypes 18C and  
6 19F, which are conjugated to tetanus and diphtheria toxoid, respectively, were not assessed.  
7 Investigating 18C and 19F B<sub>MEM</sub> responses to a booster dose of PCV-10 in PCV-13 primed children  
8 may have provided further insight into whether exposure to the carrier protein through routine  
9 vaccination (as these children have already received several doses of diphtheria and tetanus toxoid-  
10 containing vaccines) is enough to generate pneumococcal B cell memory in these children or whether  
11 the carrier protein has to be conjugated to pneumococcal polysaccharides to achieve effective  
12 priming. In the meningitis C study, children primed with MenC-CRM generated inferior booster B  
13 cell responses to a MenC-TT booster despite previous exposure to tetanus toxoid in other routine  
14 vaccinations [23], suggesting that in children of this age group, priming with a similar carrier protein  
15 is essential for B<sub>MEM</sub> generation. Overall the findings of the present study suggest that in children  
16 previously primed with PCV-13, the protein D-conjugated polysaccharides in a booster dose of PCV-  
17 10 appear to evoke a primary rather a secondary immune response. This may involve, in the short  
18 term, processing of antigens similar to plain polysaccharides (which do not generate B<sub>MEM</sub> responses)  
19 rather than recognition as T-dependent antigens. Future studies are needed to explore in detail the  
20 observed lack of B cell responses following PCV-10 vaccination, especially by investigating  
21 responses in children primed with PCV-10 and boosted with PCV-13.

22 In the group of children who were primed and boosted with PCV-13, the B<sub>MEM</sub> peak at age 13 months  
23 was followed by a return to almost baseline by age 24 months for most serotypes, by which time most  
24 cells have probably transited to lymph nodes. In PCV-10 recipients, previously receiving PCV-13  
25 primary vaccination, an increase in B<sub>MEM</sub> frequencies was seen between 12 and 24 months of age for  
26 all serotypes tested, which was significant for serotype 14 and serotype 19A (Supplementary Table 2).  
27 These findings suggest that there was a slower generation of B<sub>MEM</sub> in response to PCV-10 vaccination

or ongoing generation through natural exposure via carriage [24,25] resulting in some increase in B<sub>MEM</sub> frequencies in the peripheral blood 1 year following the booster vaccination.

It is possible that due to differences between polysaccharides in the vaccine and those bound to the ELISpot plates, the assay used in this study was less able to detect B<sub>MEM</sub> induced by the PCV-10 vaccine despite their presence in peripheral blood. However, the fact that a late rise in B<sub>MEM</sub> was detected in the PCV-10 group at age 24 months makes this explanation less likely.

In the PCV-13 group, the most marked B<sub>MEM</sub> response was seen for serotype 3. However, the immune response to serotype 3 polysaccharide has previously been shown to be atypical, and antibody responses to a booster appear to be particularly impaired [26]. Compared with other serotypes, immunogenicity for serotype 3 is attenuated and a serotype 3-containing vaccine has previously failed to show protective efficacy against serotype 3 otitis media [9], although post-implementation surveillance suggests immunization with PCV-13 does have some efficacy against serotype 3 [27]. Despite diminished antibody production, the B<sub>MEM</sub> response to serotype 3 polysaccharide has previously been shown in adults to resemble that of other serotypes [28]. In the present analysis, serotype 3 showed both the greatest increase in B<sub>MEM</sub> frequency and the lowest antibody response of all PCV-13 serotypes (Figure 1) [19], suggesting that one reason for impaired serotype-3 specific antibody concentrations following a booster might be that the immune response is driven towards the generation of B<sub>MEM</sub> rather than antibody-secreting cells but still provides some protection.

A question addressed by a number of studies has been whether baseline B<sub>MEM</sub> positively correlate with later antibody responses [17]. A correlation between B<sub>MEM</sub> frequency following priming and antibody persistence at 1 year was found in children receiving the meningococcal serotype C conjugate vaccine [16], and for some meningococcal serogroups in children receiving the MenACWY vaccine [29], however no relationship between B<sub>MEM</sub> and antibody was found in older children receiving a booster dose of the Hib-MenC conjugate vaccine [30]. Here we show that B<sub>MEM</sub> and antibody responses 1 month post-booster correlate well for most serotypes, particularly in PCV-13 recipients, however baseline B<sub>MEM</sub> are not a consistent predictor of post-vaccination B<sub>MEM</sub> and IgG responses. This may

reflect the unmeasured contribution of other antibody-producing cell types such as long-lived plasma cells.

## Conclusion

Here we present the first study assessing the memory B cell response to a mixed pneumococcal conjugate vaccine schedule. We were unable to detect serotype-specific B<sub>MEM</sub> following a booster dose of PCV-10 given to children who had been primed with PCV-13. In contrast, a strong serotype-specific B<sub>MEM</sub> response was generated in children primed with PCV-13 following receipt of a PCV-13 booster. These findings suggest that immunizing with a pneumococcal conjugate vaccine containing polysaccharides conjugated to a novel carrier protein is not sufficient to generate a rapid and strong B<sub>MEM</sub> response, at least when primary vaccination with PCV-13 is followed by a booster dose of PCV-10. Although the clinical implications of these results are unknown they still indicate that a vaccination series only using PCV-13 is advantageous over a mixed pneumococcal conjugate vaccine schedule consisting of a priming series with PCV-13 and boosting with PCV-10.

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## Figure legends

**Figure 1** Geometric mean frequencies (along with 95% CI) of B<sub>MEM</sub> specific for pneumococcal serotypes as well as diphtheria and tetanus toxoid. Groups were compared by independent samples t-tests using log<sub>10</sub>-transformed data with Satterthwaite's correction for unequal variances. Stars indicate the associated p-value (\*\*\* <.001; \*\* <.01; \* <.05).

Dip, diphtheria toxoid; Tet, tetanus toxoid; 1/4/9V/14/3/19A, pneumococcal serotypes 1/4/9V/14/3/19A

**Figure 2** Correlation between B<sub>MEM</sub> frequency at 12, 13 and 24 months of age and B<sub>MEM</sub> frequency at other time points, IgG and OPA. NS = not significant.

Figure 1

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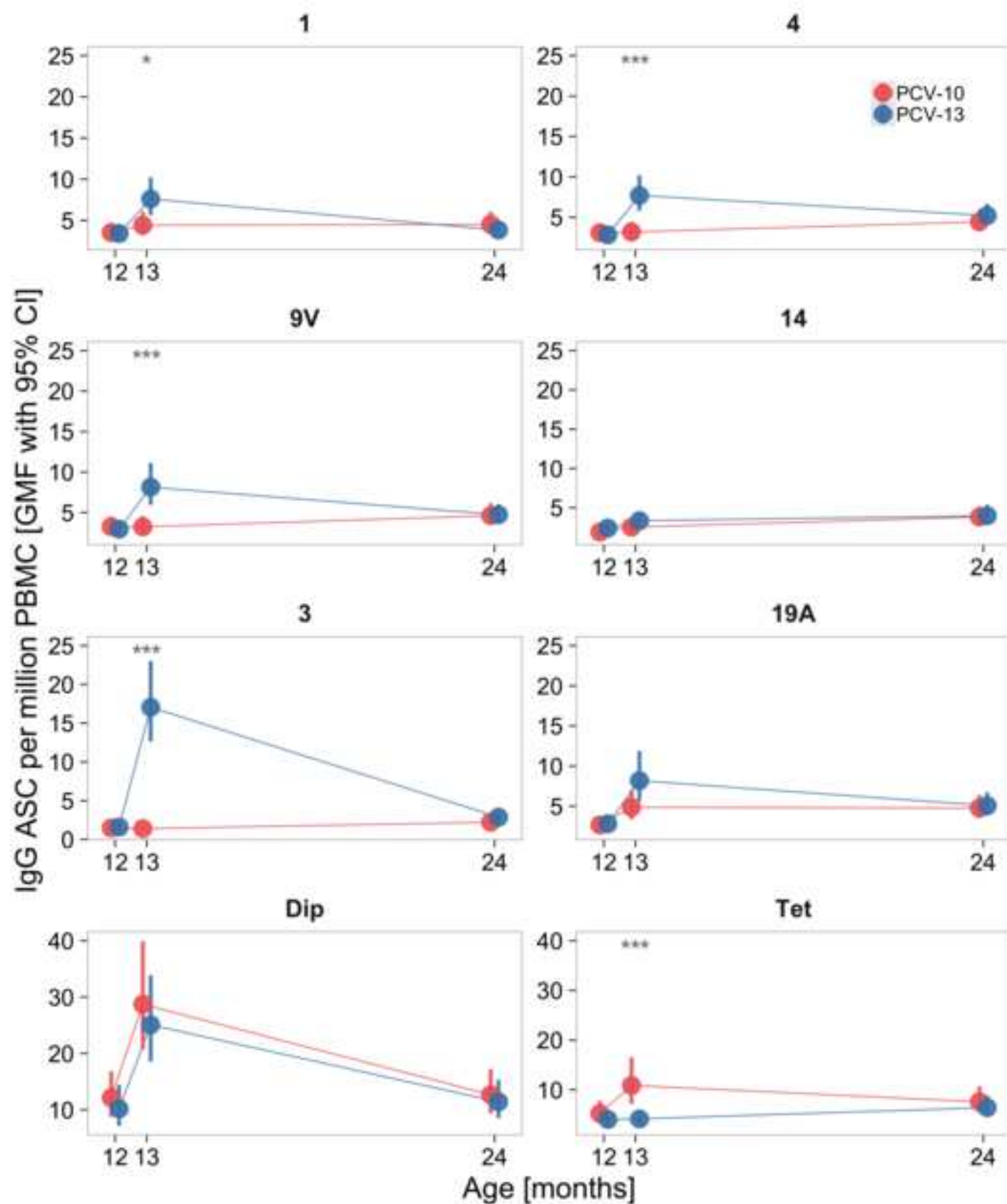
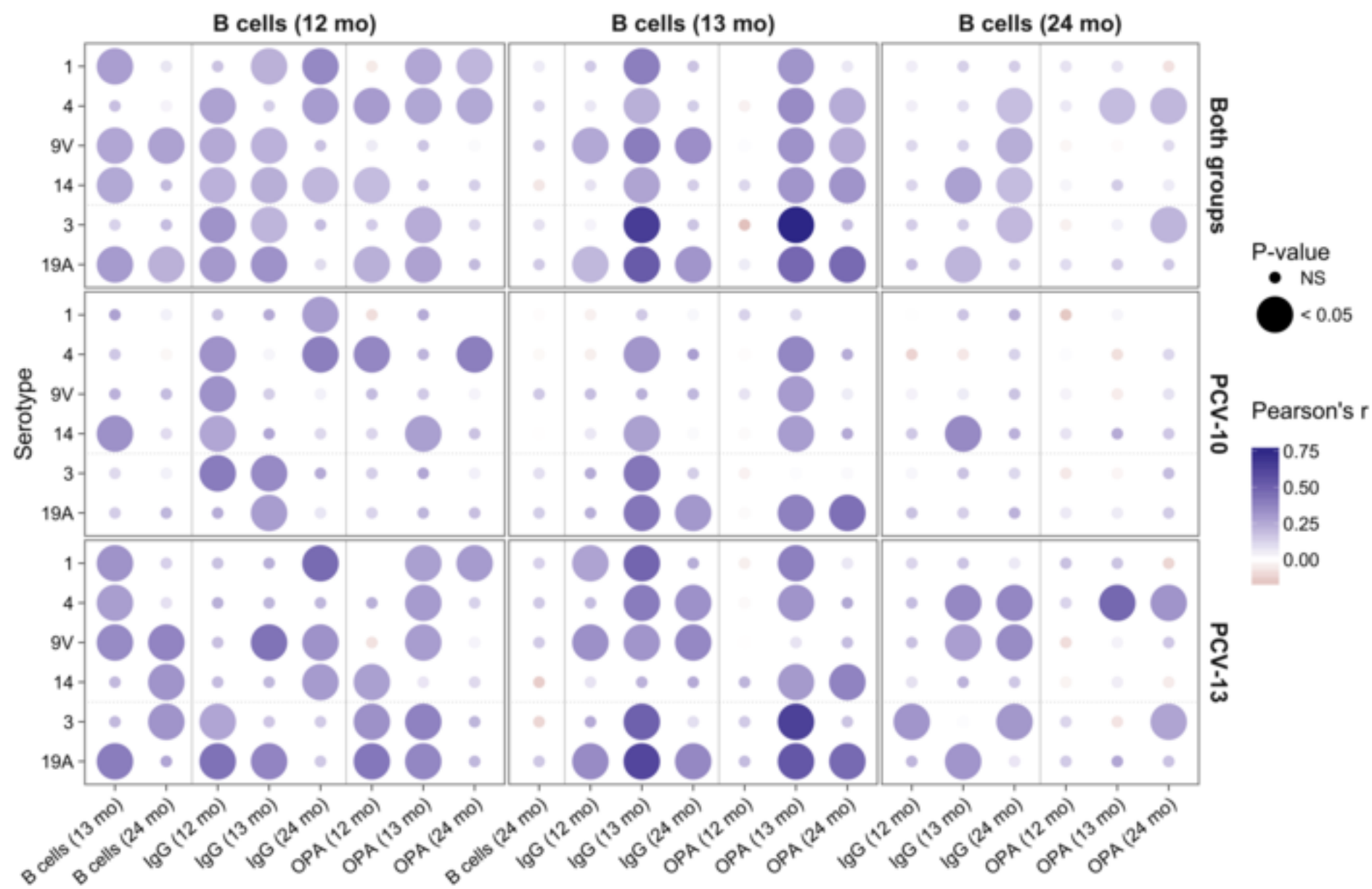


Figure 2

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## Supplementary Information

### **Divergent memory B cell responses in a mixed infant pneumococcal conjugate vaccine schedule**

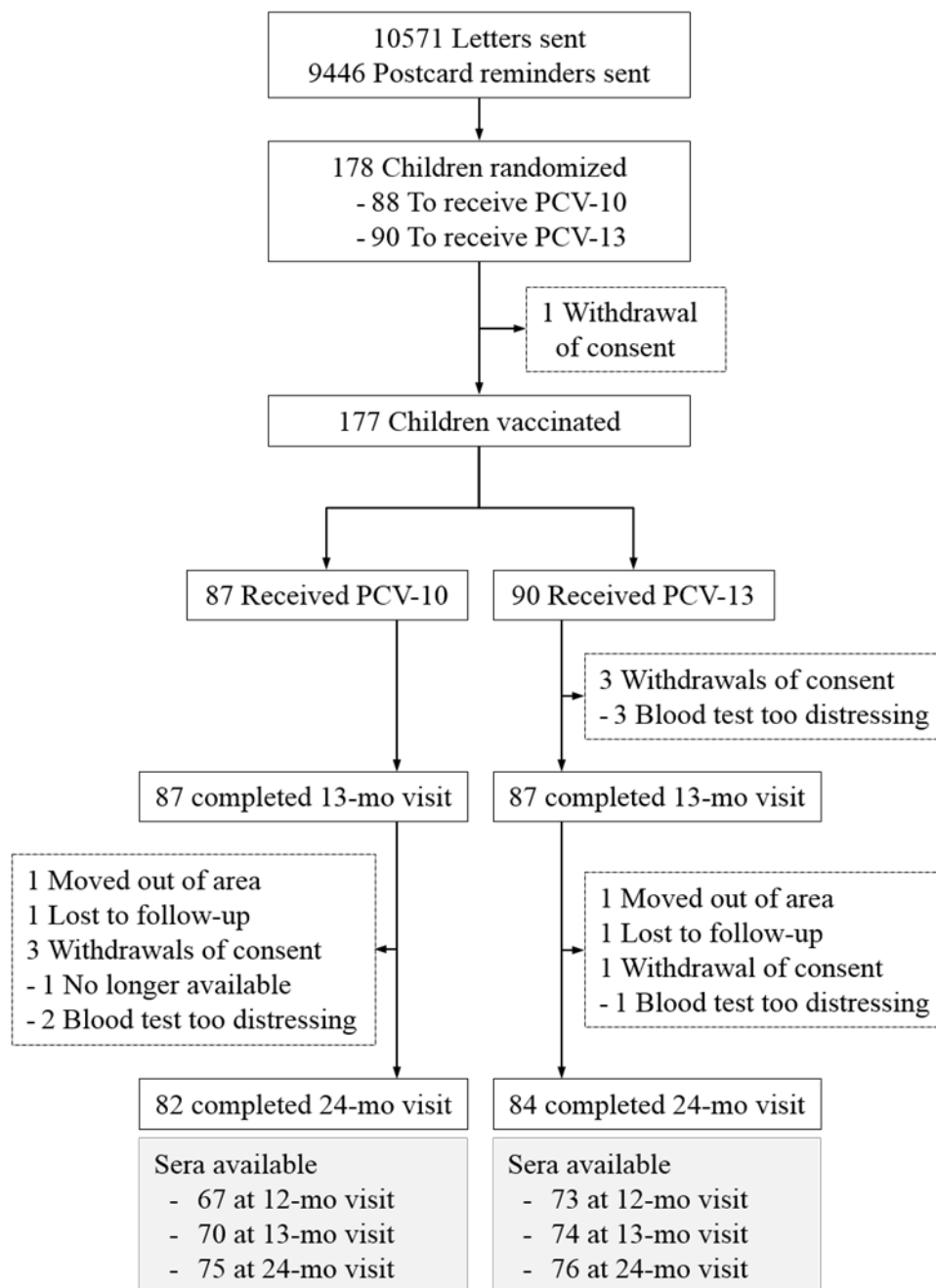
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# Supplementary Figure 1



**Figure S1** CONSORT diagram showing the flow through the study. One study participant receiving PCV-10 was excluded from the analysis of the primary objective because of major protocol violation resulting in n = 69 in the PCV-10 group at the 13-mo visit.

**Supplementary Table 1** Geometric mean B<sub>MEM</sub> frequencies and ratios at age of 12 months (A), 13 months (B) and 24 months (C) by vaccine group. Significant group differences are shown in bold letters and colors indicate group superiority (blue = PCV-13; red = PCV-10)

<b>A</b>									
Serotype	PCV-10 (n = 59)			PCV-13 (n = 63)			PCV-10 / PCV-13		
	N	Geometric Mean	95% CI	N	Geometric Mean	95% CI	Geometric Mean Ratio	95% CI	P-Value
1	59	3.519	2.615, 4.737	63	3.413	2.533, 4.599	1.031	0.680, 1.564	0.885
4	59	3.084	2.324, 4.092	63	2.812	2.088, 3.786	1.097	0.731, 1.647	0.653
9V	59	3.261	2.355, 4.514	63	2.942	2.124, 4.075	1.108	0.703, 1.748	0.656
14	59	1.908	1.432, 2.542	63	2.418	1.832, 3.190	0.789	0.532, 1.171	0.238
3†	59	1.493	1.179, 1.892	63	1.611	1.251, 2.074	0.927	0.658, 1.306	0.662
19A†	59	2.661	1.933, 3.664	62	2.809	2.095, 3.767	0.947	0.616, 1.456	0.803
Dip*	57	12.16	8.765, 16.86	62	10.18	7.166, 14.46	1.194	0.743, 1.920	0.461
Tet**	58	5.233	3.511, 7.798	62	4.041	2.760, 5.917	1.295	0.750, 2.235	0.351

<b>B</b>									
Serotype	PCV-10 (n = 56)			PCV-13 (n = 69)			PCV-10 / PCV-13		
	N	Geometric Mean	95% CI	N	Geometric Mean	95% CI	Geometric Mean Ratio	95% CI	P-Value
<b>1</b>	<b>56</b>	<b>4.411</b>	<b>3.199, 6.083</b>	<b>69</b>	<b>7.609</b>	<b>5.653, 10.24</b>	<b>0.580</b>	<b>0.376, 0.894</b>	<b>0.014</b>
<b>4</b>	<b>56</b>	<b>3.174</b>	<b>2.273, 4.432</b>	<b>69</b>	<b>7.700</b>	<b>5.812, 10.20</b>	<b>0.412</b>	<b>0.268, 0.635</b>	<b>&lt;0.001</b>
<b>9V</b>	<b>55</b>	<b>3.229</b>	<b>2.262, 4.611</b>	<b>69</b>	<b>8.139</b>	<b>5.945, 11.14</b>	<b>0.397</b>	<b>0.248, 0.635</b>	<b>&lt;0.001</b>
14	55	2.531	1.775, 3.609	69	3.350	2.516, 4.462	0.756	0.481, 1.187	0.221
<b>3†</b>	<b>56</b>	<b>1.406</b>	<b>1.121, 1.764</b>	<b>69</b>	<b>17.06</b>	<b>12.64, 23.03</b>	<b>0.082</b>	<b>0.057, 0.120</b>	<b>&lt;0.001</b>
19A†	55	4.861	3.347, 7.059	68	8.184	5.633, 11.89	0.594	0.352, 1.002	0.051
Dip*	51	28.72	20.68, 39.91	64	25.08	18.58, 33.86	1.145	0.737, 1.779	0.543
<b>Tet**</b>	<b>53</b>	<b>10.89</b>	<b>7.156, 16.56</b>	<b>67</b>	<b>4.163</b>	<b>2.945, 5.886</b>	<b>2.615</b>	<b>1.526, 4.480</b>	<b>0.001</b>

<b>C</b>									
Serotype	PCV-10 (n = 66)			PCV-13 (n = 64)			PCV-10 / PCV-13		
	N	Geometric Mean	95% CI	N	Geometric Mean	95% CI	Geometric Mean Ratio	95% CI	P-Value
1	66	4.528	3.381, 6.063	63	3.827	2.925, 5.006	1.183	0.799, 1.753	0.398
4	66	4.434	3.277, 6.000	64	5.210	4.060, 6.686	0.851	0.577, 1.255	0.413
9V	65	4.608	3.440, 6.170	64	4.730	3.695, 6.054	0.974	0.667, 1.423	0.892
14	65	3.825	2.809, 5.208	64	3.990	2.955, 5.387	0.959	0.626, 1.468	0.845
3†	66	2.227	1.705, 2.910	64	2.882	2.209, 3.761	0.773	0.532, 1.123	0.174
19A†	63	4.764	3.533, 6.424	63	5.079	3.835, 6.727	0.938	0.625, 1.408	0.755
Dip*	62	12.68	9.327, 17.25	60	11.43	8.517, 15.33	1.110	0.729, 1.691	0.624
Tet**	61	7.601	5.378, 10.74	61	6.431	4.650, 8.893	1.182	0.739, 1.890	0.482

† Serotypes contained in PCV-13 only

\*represents carrier protein CRM<sub>197</sub> (PCV-13) or diphtheria toxoid (PCV-10)

\*\*represents carrier protein tetanus toxoid (contained in PCV-10 only)

Dip, diphtheria toxoid; Tet, tetanus toxoid; 1/4/9V/14/3/19A, pneumococcal serotypes 1/4/9V/14/3/19A



**Supplementary Table 2** Antigen-specific B<sub>MEM</sub> frequency geometric mean fold change from age 12 months to 24 months by vaccine group

PCV-10					PCV-13			
Antigen	N	Geometric Mean Fold	95% CI	P-Value	N	Geometric Mean Fold	95% CI	P-Value
		Rise <sup>‡</sup>				Rise <sup>‡</sup>		
1	44	1.374	0.823,2.292	0.221	45	0.830	0.512,1.344	0.445
4	44	1.494	0.934,2.390	0.093	46	1.518	0.961,2.397	0.073
9V	43	1.326	0.765,2.299	0.310	46	1.353	0.841,2.177	0.209
<b>14</b>	<b>43</b>	<b>2.109</b>	<b>1.263,3.521</b>	<b>0.005</b>	46	1.324	0.815,2.150	0.253
3 <sup>†</sup>	44	1.501	0.978,2.306	0.063	<b>46</b>	<b>1.614</b>	<b>1.055,2.468</b>	<b>0.028</b>
<b>19A<sup>†</sup></b>	<b>42</b>	<b>1.803</b>	<b>1.054,3.087</b>	<b>0.032</b>	44	1.314	0.821,2.103	0.252
Dip*	40	1.046	0.609,1.796	0.869	42	0.926	0.532,1.614	0.785
Tet**	39	1.273	0.683,2.374	0.443	43	1.140	0.608,2.138	0.679

<sup>‡</sup> Geometric mean change from age 12 months to 24 months

<sup>†</sup> Serotypes contained in PCV-13 only

\* represents carrier protein CRM<sub>197</sub> (PCV-13) or diphtheria toxoid (PCV-10)

\*\* represents carrier protein tetanus toxoid (contained in PCV-10 only)

Dip, diphtheria toxoid; Tet, tetanus toxoid; 1/4/9V/14/3/19A, pneumococcal serotypes 1/4/9V/14/3/19A