Immunogenicity of the 10-Valent Pneumococcal Non-typeable *Haemophilus influenzae* Protein D Conjugate Vaccine (PHiD-CV) When Coadministered With Different *Neisseria meningitidis* Serogroup C Conjugate Vaccines

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Background: Immunogenicity of the candidate 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV) was assessed when coadministered with other routine pediatric vaccines including different *Neisseria meningitidis* serogroup C conjugate vaccines.

Methods: One thousand five hundred forty-eight healthy infants received, according to a balanced (1:1:1:1) randomization, either PHiD-CV coadministered with (1) DTPa-HBV-IPV/Hib (*Infanrix hexa*™) and MenC-CRM (*Meningitec*™), (2) DTPa-HBV-IPV/Hib and MenC-TT (*NeisVac-C*™), or (3) DTPa-HBV-IPV (*Infanrix penta*™/*Pediarix*™) and Hib-MenC-TT (*Menitorix*™); or 7vCRM (*Prevenar*™/*Prevnar*™) coadministered with DTPa-HBV-IPV and Hib-MenC-TT at 2-4-6 months of age with a booster dose at 11–18 months. Serotype-specific pneumococcal responses were measured by 22F-inhibition ELISA and opsonophagocytic (OPA) assay.

Results: In all 3 coadministration groups, PHiD-CV was immunogenic for each of the 10 pneumococcal vaccine serotypes as assessed by post-primary and post-booster antibody ELISA and OPA responses. When coadministered with DTPa-HBV-IPV, Hib, and MenC antigens, PHiD-CV responses after the third primary dose were within the same range as 7vCRM responses in terms of the percentage of subjects achieving an ELISA antibody concentration ≥ 0.2 μg/mL for all common vaccine serotypes (over 92% of subjects) except for serotype 6B (at least 87% of subjects). ELISA and OPA immune responses were also evident after the second primary doses of PHiD-CV or 7vCRM vaccine, although antibody levels were below that achieved after 3 primary doses, particularly for serotypes 6B and 23F. The kinetics of the immune responses from after the second dose to after the booster dose were similar for most of the serotypes in both PHiD-CV and 7vCRM groups.

Conclusions: PHiD-CV was immunogenic when coadministered with other routine pediatric vaccines including MenC conjugate vaccines.

Key Words: pneumococcal conjugate vaccine, ELISA, opsonophagocytic activity

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Pneumococcal conjugate vaccines have been designed to prevent invasive pneumococcal disease (IPD) in young children. The manifestations of IPD, defined by isolation of the pneumococcus from a normally sterile site, include meningitis, sepsis, bacteraemic pneumonia, joint or other local infections, as well as occult bacteraemia. In countries where widespread vaccination against *Haemophilus influenzae* type b (Hib) has been introduced, *Streptococcus pneumoniae* along with *Neisseria meningitidis* are usually the major causes of pediatric bacterial meningitis in children aged 1 month or older.[1] Like Hib and *N. meningitidis*, *S. pneumoniae* are encapsulated bacteria for which vaccine-induced protection can be conferred by antibodies directed against the polysaccharide capsule. Pediatric vaccines against all 3 pathogens are based on bacterial capsular polysaccharides conjugated to carrier proteins.

GlaxoSmithKline (GSK) Biologicals has developed a candidate 10-valent pneumococcal conjugate vaccine (PHiD-CV) containing 3 additional serotypes (1, 5, 7F) compared with the 7vCRM vaccine. It contains 8 capsular polysaccharides conjugated individually to non-typeable *H. influenzae* protein D (PD) and the remaining 2 serotypes to tetanus or diphtheria toxoid. PD, which is a highly conserved cell-surface protein among *H. influenzae* strains[2–5] was selected for its potential to provide protection against *H. influenzae* infections.[6,7] Along with *S. pneumoniae*, non-typeable *H. influenzae* is one of the leading bacterial causes of acute otitis media (AOM) in children.[8–14] Clinical evidence of a protective effect of the PD carrier protein was provided by a double-blind randomized controlled AOM efficacy study with an experimental 11-valent vaccine formulation, which demonstrated significant protective efficacy against AOM caused by *H. influenzae* in addition to AOM caused by pneumococcal vaccine serotypes.[15]

To date, 1 pneumococcal conjugate vaccine has been licensed based on its efficacy against vaccine serotype IPD.[16,17] This 7-valent vaccine (7vCRM) contains capsular polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, each conjugated

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to CRM_{197} (a nontoxic cross-reacting mutant of diphtheria toxin).[16,17] It has been proposed by the World Health Organization (WHO) that licensure of any new pneumococcal conjugate vaccine for protection against IPD can be based on the percentage of subjects reaching a reference ELISA antibody concentration after primary vaccination, compared with the licensed 7vCRM vaccine.[18–20] In addition to ELISA antibody concentrations, opsonophagocytic (OPA) activity must also be measured to demonstrate the functionality of induced antibodies. Evidence of booster responses, which confirm priming of the immunologic system and indicate induction of immune memory, is also required.

The vaccination schedules recommended for pneumococcal conjugate vaccination are a 3-dose primary series in the first 6 months of life, followed by a fourth booster dose in the second year or in some countries, a 2-dose primary series in the first 6 months of life, followed by a booster dose at 11–15 months. Because the same schedules are used for other routine pediatric vaccinations, concomitant administration with other vaccines is required. The number of pediatric vaccines to be included in the routine immunization schedules is continuously increasing and it is important to assess new pneumococcal conjugate vaccines in this context. Meningococcal serogroup C (MenC) conjugate vaccines using CRM_{197} (MenC-CRM) or tetanus toxoid (MenC-TT) as carrier proteins are now widely used and a new Hib-MenC conjugate vaccine combining MenC and Hib tetanus toxoid conjugates has been introduced in Europe.[21] In the present report, we assessed the immunogenicity of PHiD-CV when coadministered with combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio vaccine (with or without Hib) together with either MenC-CRM, MenC-TT or with the combined Hib-MenC conjugate vaccine.

MATERIALS AND METHODS

Study Design and Participants

The study was conducted in centers in Germany, Poland, and Spain between June 12, 2006 and January 21, 2008. The protocol and study documents were approved by the appropriate Independent Ethics Committees or Institutional Review Boards and the study was conducted in accordance with the Somerset West 1996 version of the Declaration of Helsinki. Eligible participants were healthy males or females aged 6 to 16 weeks at the time of the first primary vaccination. Written informed consent was obtained from each subjects' parent/guardian before the performance of any study-specific procedures.

Subjects were randomized (1:1:1:1) to 4 study groups as indicated in Table 1. There were 2 study phases; a primary randomized open controlled phase (107005/NCT00334334) and a booster open controlled phase (109507/NCT00463437). The primary objective of both study phases was to evaluate the noninferiority of the safety (in terms of fever) of the PHiD-CV vaccine relative to the 7vCRM vaccine. Assessment of immunogenicity was a secondary objective of both phases.

Vaccines

The PHiD-CV vaccine (GSK Biologicals, Rixensart, Belgium) contained 1 μg of each capsular polysaccharide for pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14, and 23F; and 3 μg for serotype 4 conjugated to PD, 3 μg of capsular polysaccharide of serotype 18C conjugated to tetanus toxoid, and 3 μg of capsular polysaccharide of serotype 19F conjugated to diphtheria toxoid. The 7vCRM vaccine (*Prevenar™/Prevnar™*, Wyeth Lederle Vaccines SA, Pearl River, NY) contained capsular polysaccharide from 7 pneumococcal serotypes conjugated to CRM_{197} (2 μg of each capsular polysaccharide for serotypes 14, 9V, 14, 18C, 19F, and 23F and 4 μg for serotype 6B).

Coadministered vaccines were: (1) diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-Hib vaccine (DTPa-HBV-IPV/Hib, *Infanrix hexa™*); (2) diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-vaccine (DTPa-HBV-IPV, *Infanrix penta™/Pediarix™*); (3) combined Hib-meningococcal C conjugate vaccine (Hib-MenC-TT, *Menitorix™*), all from GSK Biologicals, Rixensart, Belgium; and (4) Meningococcal C conjugate vaccine conjugated to tetanus toxoid (MenC-TT, *NeisVac-C™* from Baxter Healthcare SA, Zurich, Switzerland) or to CRM_{197} (MenC-CRM, *Meningitec™* from Wyeth Lederle Vaccines SA, Pearl River, NY).

In Spain, DTPa-combined coadministration for the booster vaccination was: DTPa-IPV/Hib (*Infanrix™* IPV Hib, GSK Biologicals) in PHiD-CV+MenC-CRM and PHiD-CV+MenC-TT groups or DTPa-IPV (*Infanrix* IPV, GSK Biologicals) in PHiD-CV+Hib-MenC and 7vCRM+Hib-MenC groups.

Safety Evaluation

All safety/reactogenicity data (including primary objective of both primary and booster phases) are reported elsewhere.[22]

Serological Methods

Serological follow-up was performed for a subset of subjects (180 per group) in preselected centers. Blood samples were collected at 2 months after the second and 1 month after the third primary vaccine doses, and before and 1 month after booster vaccination.

Serum samples were stored at −20°C until blinded analyses at GSK Biologicals laboratories, Rixensart, Belgium. An ELISA, with 22F polysaccharide adsorption (GSK's 22F-ELISA) in addition to the capsular polysaccharides (CPS) adsorption, was used to measure the pneumococcal serotype specific total IgG antibodies

TABLE 1. Study Groups

Group	Pneumococcal Conjugate Vaccine	Coadministered Vaccines	
PHiD-CV + MenC-CRM	PHiD-CV	DTPa-HBV-IPV/Hib or DTPa-IPV/Hib (booster in Spain)	MenC-CRM (primary doses at 2 and 4 mos only*)
PHiD-CV + MenC-TT	PHiD-CV	DTPa-HBV-IPV/Hib or DTPa-IPV/Hib (booster in Spain)	MenC-TT (primary doses at 2 and 4 mos only*)
PHiD-CV + Hib-MenC	PHiD-CV	DTPa-HBV-IPV or DPTa-IPV (booster in Spain)	Hib-MenC-TT
7vCRM + Hib-MenC	7vCRM	DTPa-HBV-IPV or DPTa-IPV (booster in Spain)	Hib-MenC-TT

Primary phase: vaccines administered at 2, 4, and 6 months. Booster phase: vaccines administered at 11–18 months.

*In Poland, to comply with national recommendations, subjects were offered a third dose of MenC vaccines at ±7 months of age.

7vCRM indicates *Prevenar/Prevnar*; DTPa-HBV-IPV/Hib, *Infanrix hexa*; DTPa-HBV-IPV, *Infanrix penta/Pediarix*; DTPa-IPV/Hib, *Infanrix* IPV Hib; DTPa-IPV, *Infanrix* IPV; MenC-CRM, *Meningitec*; MenC-TT, *NeisVac-C*; Hib-MenC-TT, *Menitorix*.

to increase the specificity of the assay.[23,24] The antibody concentrations were determined by calibration with the standard reference serum 89-SF (courtesy of Dr. Frasch US FDA).[25] The assay cut-off was 0.05 μg/mL. It has been previously established[24] that an antibody concentration of 0.2 μg/mL as determined by GSK's 22F-ELISA is equivalent to an antibody concentration of 0.35 μg/mL as determined by the WHO reference laboratory ELISA without 22F-inhibition, which is proposed by WHO for comparison of post-primary pediatric immune responses between pneumococcal conjugate vaccines.[20] The 0.35 μg/mL threshold with GSK's 22F-ELISA assay (corresponding to a threshold of approximately 0.5 μg/mL when using the WHO reference laboratory ELISA), was also evaluated and given for information.

OPA activity was determined using a modification of the HL-60 cell WHO reference method,[26,27] which has been validated using sera collected after primary vaccination with the 7vCRM vaccine.[28] The results are presented as the dilution of serum (opsonic titer) able to sustain 50% killing of live pneumococci under the assay conditions. The cut-off of the assay was set at an opsonic titer of 8 (serum dilution of 1:8).

IgG antibodies to the *H. influenzae* PD were measured by a classic ELISA with the nonlipidated PD as coating material and were expressed in ELISA units (EL.U) per mL, the cut-off of the assay is 100 EL.U/mL. Serological responses to components of the coadministered vaccines in this study are reported elsewhere.[29]

Descriptive Analyses

The following parameters were calculated with 95% confidence intervals (CI) for each individual pneumococcal vaccine serotype and cross-reactive serotypes 6A and 19A for each treatment group: (1) the percentage of subjects with ELISA pneumococcal antibody concentrations ≥0.2 μg/mL, ≥0.35 μg/mL, and ELISA geometric mean antibody concentrations (GMCs); and (2) the percentage of subjects with OPA titers ≥8 and OPA geometric mean titers (GMTs). In addition, the geometric mean of the individual ratios of post-booster to pre-booster ELISA antibody concentrations and post-booster to pre-booster OPA titers were calculated with the 95% CI.

The percentage of subjects with anti-PD antibody concentrations ≥100 EL.U/mL and antibody GMCs were calculated. GMCs/GMTs were calculated by taking the antilog of the mean of the log concentration/titer transformations. Antibody concentrations and OPA titers below the assay cut-off were given an arbitrary value of one-half the cut-off for the purpose of GMC and GMT calculations.

All comparisons were exploratory and statistical significances were based on the nonoverlapping of the 95% CIs boundaries. These exploratory comparisons therefore only indicate possible differences between groups and should be interpreted with caution.

Immunogenicity analysis was performed on the according to protocol (ATP) immunogenicity cohorts for primary and booster vaccination, respectively. After the second primary dose, anti-pneumococcal immune responses were analyzed for a random subset of sera (about one-third) from the PHiD-CV groups and all available sera from 7vCRM vaccines. To give an overview of the kinetics of the immune responses, anti-pneumococcal immune responses were analyzed on the "Booster ATP immunogenicity cohort" for the post-dose 2 subset.

RESULTS

Participants

Of the 1548 subjects considered in the total vaccinated cohort, 1499 completed the primary phase and for the booster phase 1437 subjects were part of the total vaccinated cohort (1430 subjects completed this phase). Figure 1 shows the numbers of subjects

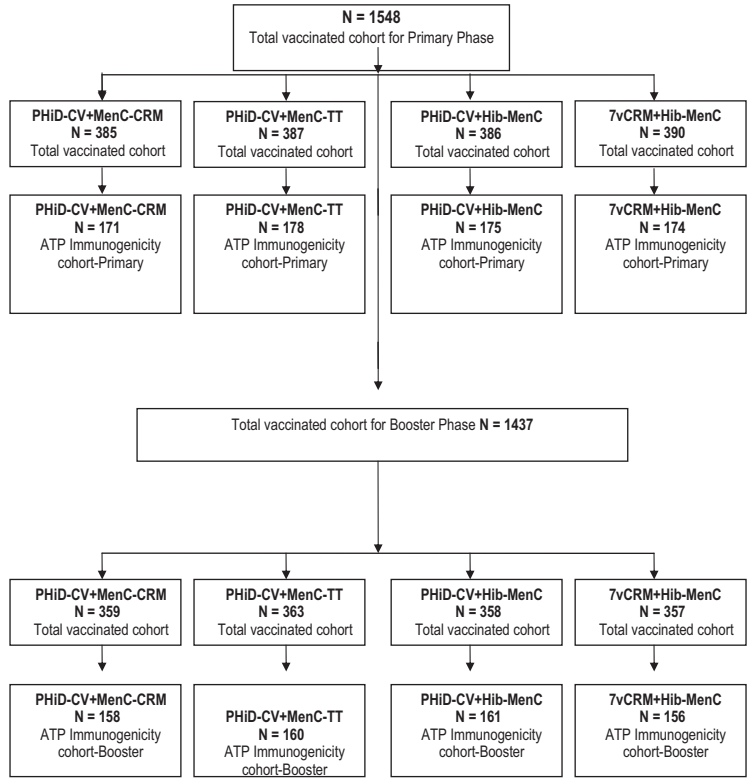

FIGURE 1. Trial profile.

TABLE 2. Demographic Characteristics (ATP Immunogenicity Cohorts)

	PHiD-CV + MenC-CRM	PHiD-CV + MenC-TT	PHiD-CV + Hib-MenC	7vCRM + Hib-MenC
Primary	**N = 171**	**N = 178**	**N = 175**	**N = 174**
Age (in weeks)				
Mean (SD)	8.0 (2.23)	8.1 (2.13)	8.1 (2.19)	8.1 (2.37)
Median (range)	7.0 (6–16)	8.0 (6–16)	8.0 (6–16)	7.0 (6–16)
Gender (%)				
Female	49.7	46.1	58.9	46.6
Male	50.3	53.9	41.1	53.4
Race (%)				
Asian—-Central/South Asian heritage	0.6	0.0	0.0	0.0
Asian—-East Asian heritage	0.0	0.0	0.6	0.0
Asian—-South East Asian heritage	0.0	0.6	0.0	0.0
White—-Arabic/North African heritage	1.8	0.6	0.0	0.6
White—-Caucasian/European heritage	93.0	95.5	93.7	97.7
Other	4.7	3.4	5.7	1.7
Booster	**N = 158**	**N = 160**	**N = 161**	**N = 156**
Age (in months)				
Mean (SD)	14.2 (1.68)	14.2 (1.68)	14.2 (1.68)	14.2 (1.72)
Median (range)	14.0 (11–18)	14.0 (11–18)	14.0 (11–18)	14.0 (11–18)

included in the ATP immunogenicity cohorts. There were no major differences between the 4 study groups in the demographic characteristics for the ATP immunogenicity cohorts although the female/male ratio was slightly higher in the PHiD-CV + Hib-MenC group (Table 2).

Immunogenicity to Pneumococcal Serotypes

Primary Vaccination

ELISA and OPA data after the third primary dose are presented in Table 3. One month after the third dose of PHiD-CV, at least 92.5% of subjects had ELISA antibody concentrations ≥0.2 μg/mL for each of the pneumococcal vaccine serotypes except for serotype 6B (88.6% for PHiD-CV+MenC-TT group and 87.3% for PHiD-CV+Hib-MenC group). Although in the same range for all 3 PHiD-CV groups, there was a tendency towards lower ELISA antibody GMCs in the PHiD-CV+Hib-MenC group. For serotype 18C, the post-primary antibody GMC was markedly higher in the PHiD-CV+MenC-TT group relative to the other 2 PHiD-CV groups.

The percentages of subjects with antibody concentrations ≥0.2 μg/mL against the serotypes common to both PHiD-CV and 7vCRM vaccines were within the same range for all PHiD-CV and 7vCRM groups. The maximum difference was observed for serotype 6B (87.3% [81.4–91.9] in the PHiD-CV+Hib-MenC group vs. 94.1% [89.4–97.1] in the PHiD-CV+MenC-CRM group). Relative to the 7vCRM+Hib-MenC group, post-primary ELISA antibody GMCs in the 3 PHiD-CV groups were lower for serotypes 4, 6B, 9V, 14, and 23F but higher for serotype 19F and the additional serotypes 1, 5, and 7F.

The PHiD-CV vaccine induced post-primary OPA titers ≥8 in at least 90.4% of subjects except for serotypes 1 (ranging between 50.3% and 54.3%), serotype 5 (between 86.5% and 92.8%), and serotype 6B (between 81.8% and 87.8%). Post-primary OPA GMTs were higher for serotype 18C in the PHiD-CV+MenC-TT group and for serotype 19F in the PHiD-CV+MenC-CRM group compared with the other 2 PHiD-CV groups. All other OPA GMTs were within the same range between the 3 PHiD-CV groups although for some serotypes there was a trend toward lower OPA GMTs in the PHiD-CV+Hib-MenC group. The percentage of subjects with OPA titers ≥8 was higher for serotypes 6B in the 7vCRM+Hib-MenC group relative to the 3 PHiD-CV groups. Slightly lower percentages of subjects with OPA titers ≥8 were observed for serotypes 18C and 23F in the PHiD-CV+Hib-MenC group compared with the other study groups. OPA GMTs were higher in all 3 PHiD-CV groups for serotype 19F and the additional serotypes 1, 5, and 7F and lower for serotypes 6B, 14, and 23F relative to those observed in the 7vCRM+Hib-MenC group.

Booster Vaccination

After booster vaccination, over 96% of subjects in all groups had antibody concentrations ≥0.2 μg/mL for the 7 serotypes contained in both PHiD-CV and 7vCRM vaccines (Table 4). At least 98.8% of subjects in the PHiD-CV groups had antibody concentrations ≥0.2 μg/mL against the 3 additional serotypes compared with fewer than 8% in the 7vCRM-group. Booster vaccination induced robust increases in pneumococcal antibody GMCs for all vaccine serotypes in all groups. The fold increase in post-booster antibody GMCs compared with pre-booster levels ranged between 4.4- to 11.6-fold for PHiD-CV vaccinees and 6.1- to 21.8-fold for 7vCRM vaccinees, depending on the serotype. Antibody GMCs were lower for serotypes 4, 6B, 9V, 14, and 23F and higher for 19F and the additional serotypes 1, 5, and 7F in the PHiD-CV groups relative to the 7vCRM group.

Booster vaccination also induced robust increases in OPA GMTs relative to pre-booster levels (Table 4) for all serotypes including serotype 1 for which a low post-primary OPA response was observed. Post-booster OPA GMTs were lower for serotypes 4, 6B, 9V, and 14 and higher for 19F and the additional serotypes 1, 5, and 7F in the PHiD-CV groups relative to the 7vCRM group but the percentages with OPA titers ≥8 were within the same range. Over 94% of both PHiD-CV and 7vCRM vaccinees had OPA titers ≥8 for the shared serotypes and at least 90% of PHiD-CV vaccinees had OPA titers ≥8 for the 3 additional serotypes.

Kinetics of the Immune Response

The kinetics of the ELISA and OPA responses for each vaccine serotype from 2 months after the second primary vaccination dose to 1 month after booster vaccination are illustrated in Figure 2 for both PHiD-CV (pooled data for the 3 groups) and 7vCRM vaccines. In addition, data after the second primary dose are presented in Table 5.

Already after 2 PHiD-CV doses, 87.8% to 99.4% of subjects had ELISA antibody concentrations ≥0.2 μg/mL for serotypes 1, 4, 5, 7F, 9V, 14, 18C, and 19F, at least 64.1% of subjects reached the ELISA threshold for serotype 6B and at least 75.0% for serotype 23F. Results within the same range

TABLE 3. ELISA Antibody Concentrations and Opsonophagocytic Titers Against Individual Pneumococcal Serotypes 1 Month After the Third Primary Vaccination Dose (ATP Immunogenicity Cohort-Primary)

Pneumococcal Serotype	Group*	ELISA PHiD-CV/MenC-CRM (N = 169) PHiD-CV/MenC-TT (N = 175) PHiD-CV/Hib-MenC (N = 173) 7vCRM/Hib-MenC (N = 170)			OPA PHiD-CV/MenC-CRM (N = 162) PHiD-CV/MenC-TT (N = 168) PHiD-CV/Hib-MenC (N = 161) 7vCRM/Hib-MenC (N = 156)	
		% ≥ 0.20 μg/mL (95% CI)	% ≥ 0.35 μg/mL (95% CI)	GMC μg/mL (95% CI)	% ≥ 8 (95% CI)	GMT Dil (95% CI)
Serotype 1	PHiD-CV+MenC-CRM	96.4 (92.4–98.7)	90.5 (85.1–94.5)	1.15 (1.00–1.32)	54.3 (46.3–62.2)	23.9 (17.9–32.0)
	PHiD-CV+ MenC-TT	97.7 (94.2–99.4)	88.5 (82.8–92.8)	1.09 (0.96–1.24)	51.2 (43.4–59.0)	18.8 (14.4–24.4)
	PHiD-CV+ Hib-MenC	93.1 (88.2–96.4)	84.4 (78.1–89.5)	1.00 (0.86–1.15)	50.3 (42.3–58.3)	19.7 (14.9–26.1)
	7vCRM+ Hib-MenC	0.6 (0.0–3.2)	0.6 (0.0–3.2)	0.03 (0.03–0.03)	1.3 (0.2–4.6)	4.2 (3.9–4.5)
Serotype 4	PHiD-CV+MenC-CRM	100 (97.8–100)	100 (97.8–100)	1.88 (1.70–2.09)	100 (97.6–100)	697.4 (617.8–787.3)
	PHiD-CV+ MenC-TT	99.4 (96.8–100)	98.9 (95.9–99.9)	1.96 (1.76–2.19)	100 (97.7–100)	755.6 (660.9–863.7)
	PHiD-CV+ Hib-MenC	98.3 (95.0–99.6)	97.7 (94.2–99.4)	1.70 (1.52–1.92)	97.5 (93.7–99.3)	669.8 (553.8–810.0)
	7vCRM+ Hib-MenC	100 (97.9–100)	100 (97.9–100)	2.78 (2.46–3.14)	100 (97.6–100)	926.2 (779.5–1100.4)
Serotype 5	PHiD-CV+MenC-CRM	100 (97.8–100)	98.8 (95.8–99.9)	1.96 (1.78–2.17)	92.8 (87.5–96.4)	91.7 (72.7–115.7)
	PHiD-CV+ MenC-TT	100 (97.9–100)	98.9 (95.9–99.9)	1.87 (1.69–2.08)	86.5 (80.3–91.3)	71.4 (56.5–90.4)
	PHiD-CV+ Hib-MenC	98.8 (95.9–99.9)	97.1 (93.4–99.1)	1.69 (1.49–1.91)	88.7 (82.7–93.2)	77.4 (61.0–98.2)
	7vCRM+ Hib-MenC	2.4 (0.7–6.0)	0.6 (0.0–3.3)	0.03 (0.03–0.04)	2.0 (0.4–5.6)	4.2 (4.0–4.5)
Serotype 6B	PHiD-CV+MenC-CRM	94.1 (89.4–97.1)	87.0 (81.0–91.7)	0.96 (0.82–1.12)	87.8 (81.5–92.6)	459.1 (334.2–630.8)
	PHiD-CV+ MenC-TT	88.6 (82.9–92.9)	81.1 (74.5–86.6)	0.85 (0.72–1.01)	84.7 (77.9–90.0)	404.6 (287.7–569.1)
	PHiD-CV+ Hib-MenC	87.3 (81.4–91.9)	75.7 (68.6–81.9)	0.71 (0.59–0.86)	81.8 (74.6–87.6)	354.2 (243.4–515.3)
	7vCRM+ Hib-MenC	92.9 (87.9–96.3)	87 (81.0–91.7)	1.32 (1.12–1.57)	97.4 (93.4–99.3)	1575.3 (1230.8–2016.0)
Serotype 7F	PHiD-CV+MenC-CRM	100 (97.8–100)	100 (97.8–100)	2.82 (2.54–3.14)	100 (97.6–100)	2513.3 (2106.1–2999.3)
	PHiD-CV+ MenC-TT	99.4 (96.9–100)	98.9 (95.9–99.9)	2.57 (2.32–2.86)	98.8 (95.7–99.9)	2821.3 (2297.9–3463.9)
	PHiD-CV+ Hib-MenC	98.8 (95.9–99.9)	97.1 (93.4–99.1)	2.25 (1.98–2.55)	96.8 (92.8–99.0)	2290.5 (1802.3–2910.9)
	7vCRM+ Hib-MenC	3.0 (1.0–6.8)	0.6 (0.0–3.3)	0.04 (0.03–0.04)	15.2 (9.7–22.3)	8.7 (6.3–11.9)
Serotype 9V	PHiD-CV+MenC-CRM	98.8 (95.8–99.9)	98.8 (95.8–99.9)	1.77 (1.58–2.00)	99.3 (96.4–100)	1005.6 (825.3–1225.2)
	PHiD-CV+ MenC-TT	97.7 (94.3–99.4)	95.4 (91.2–98.0)	1.72 (1.52–1.95)	98.7 (95.4–99.8)	1108.8 (905.9–1357.1)
	PHiD-CV+ Hib-MenC	98.3 (95.0–99.6)	96.5 (92.6–98.7)	1.58 (1.40–1.77)	100 (97.6–100)	1122.6 (938.9–1342.3)
	7vCRM+ Hib-MenC	98.8 (95.8–99.9)	98.2 (94.9–99.6)	3.17 (2.75–3.64)	99.3 (96.3–100)	1305.0 (1046.3–1627.6)
Serotype 14	PHiD-CV+MenC-CRM	100 (97.8–100)	98.2 (94.9–99.6)	3.75 (3.25–4.31)	98.1 (94.4–99.6)	797.8 (655.3–971.2)
	PHiD-CV+ MenC-TT	100 (97.9–100)	99.4 (96.9–100)	3.79 (3.37–4.26)	97.0 (93.2–99.0)	879 (709.1–1089.5)
	PHiD-CV+ Hib-MenC	100 (97.9–100)	98.3 (95–99.6)	3.36 (2.91–3.88)	96.3 (92.0–98.6)	779.9 (628.1–968.3)
	7vCRM+ Hib-MenC	99.4 (96.7–100)	97.0 (93.2–99.0)	5.97 (5.05–7.07)	98.1 (94.4–99.6)	1539.4 (1230.2–1926.2)
Serotype 18C	PHiD-CV+MenC-CRM	98.8 (95.8–99.9)	97.0 (93.2–99.0)	2.43 (2.07–2.84)	96.8 (92.6–98.9)	174.9 (144.1–212.3)
	PHiD-CV+ MenC-TT	98.9 (95.9–99.9)	98.3 (95.1–99.6)	3.92 (3.38–4.54)	98.2 (94.7–99.6)	282.8 (234.7–340.8)
	PHiD-CV+ Hib-MenC	99.4 (96.8–100)	98.3 (95.0–99.6)	2.34 (2.01–2.71)	91.7 (86.3–95.5)	142.7 (113.8–179.1)
	7vCRM+ Hib-MenC	98.8 (95.8–99.9)	97.0 (93.2–99.0)	3.01 (2.65–3.42)	100 (97.6–100)	212.8 (174.9–258.9)
Serotype 19F	PHiD-CV+MenC-CRM	98.2 (94.9–99.6)	98.2 (94.9–99.6)	4.93 (4.28–5.68)	98.1 (94.5–99.6)	387.5 (305.0–492.2)
	PHiD-CV+ MenC-TT	99.4 (96.9–100)	98.9 (95.9–99.9)	4.71 (4.09–5.42)	93.9 (89.1–97.1)	298.4 (230.3–386.7)
	PHiD-CV+ Hib-MenC	98.8 (95.9–99.9)	97.7 (94.2–99.4)	3.81 (3.32–4.37)	94.3 (89.5–97.4)	261.0 (200.9–339.0)
	7vCRM+ Hib-MenC	100 (97.9–100)	99.4 (96.8–100)	2.56 (2.29–2.86)	90.5 (84.5–94.7)	52.0 (40.8–66.4)
Serotype 23F	PHiD-CV+MenC-CRM	95.9 (91.7–98.3)	94.1 (89.4–97.1)	1.30 (1.13–1.49)	95.2 (90.4–98.1)	1066 (811.9–1399.6)
	PHiD-CV+ MenC-TT	96.0 (91.9–98.4)	88.6 (82.9–92.9)	1.20 (1.02–1.40)	94.9 (90.1–97.8)	1219.6 (930.7–1598.2)
	PHiD-CV+ Hib-MenC	92.5 (87.5–95.9)	83.8 (77.5–89.0)	0.96 (0.82–1.13)	90.4 (84.4–94.7)	880.7 (633.1–1225.1)
	7vCRM+ Hib-MenC	94.1 (89.4–97.1)	91.1 (85.8–94.9)	2.46 (2.04–2.98)	99.3 (96.3–100)	5469.2 (4410.2–6782.6)
Serotype 6A	PHiD-CV+MenC-CRM	52.7 (44.8–60.5)	41.3 (33.8–49.2)	0.24 (0.19–0.29)	81.0 (72.1–88.0)	137.7 (94.2–201.5)
	PHiD-CV+ MenC-TT	44.3 (36.7–52.0)	33.3 (26.4–40.9)	0.18 (0.15–0.22)	85.6 (77.0–91.9)	155.2 (107.6–223.7)
	PHiD-CV+ Hib-MenC	44.2 (36.6–51.9)	28.5 (21.9–35.9)	0.16 (0.13–0.19)	74.0 (64.0–82.4)	100.9 (65.9–154.6)
	7vCRM+ Hib-MenC	56.6 (48.7–64.3)	43.4 (35.7–51.3)	0.24 (0.19–0.29)	87.4 (79.0–93.3)	231.5 (155.1–345.4)
Serotype 19A	PHiD-CV+MenC-CRM	59.3 (51.4–66.8)	43.1 (35.5–51.0)	0.27 (0.22–0.32)	32.4 (23.4–42.3)	11.2 (8.1–15.4)
	PHiD-CV+ MenC-TT	59.5 (51.8–66.9)	37.6 (30.3–45.2)	0.22 (0.19–0.27)	24.5 (16.7–33.8)	8.4 (6.4–11.0)
	PHiD-CV+ Hib-MenC	45.0 (37.4–52.8)	32.2 (25.2–39.7)	0.18 (0.15–0.22)	19.8 (12.5–28.9)	7.1 (5.6–9.0)
	7vCRM+ Hib-MenC	27.7 (21.1–35.2)	12.7 (8.0–18.7)	0.12 (0.1–0.14)	1.0 (0.0–5.6)	4.1 (3.9–4.2)

*Group definitions are given in Table 1.
N indicates number of subjects with available results for at least one serotype (the actual number of subjects tested can slightly vary for the different serotypes depending on the number of sera available); Dil, Dilution of serum able to kill 50% of viable pneumococci.

were obtained in the 7vCRM group for the serotypes common to both PHiD-CV and 7vCRM except that the percentage of subjects with antibody concentrations ≥0.2 μg/mL was lower for serotype 6B and higher for serotype 18C compared with PHiD-CV. Two doses of PHiD-CV induced OPA titers ≥8 for serotypes 4, 7F, 9V, 14, and 23F in at least 96.8% of subjects. The lowest OPA response was observed for serotypes 1 (48.8% of subjects with titers ≥8) and 18C (59.8%). The percentage of subjects with OPA titer ≥8 was lower in the 7vCRM group for serotypes 6B and 19F compared with the PHiD-CV pooled groups and higher for serotype 18C.

The third PHiD-CV dose had an important impact on the percentage of subjects with ELISA antibody concentrations ≥0.2 μg/mL for 6B (increased from 64.1% to 89.9%) and 23F (increased from 75.0% to 93.7%). The same observation was made for the third 7vCRM dose for which the impact on 6B was even more marked (increased from 30.8% to 92.1%). For most serotypes, antibody GMCs were higher after the third dose than after the second dose, with the most marked increase observed for both vaccines for serotypes 6B and 23F. After the third vaccine dose, the percentage of subjects with OPA titers ≥8 increased for serotypes 6B, 18C, and 19F in both groups, for serotype 5 in the PHiD-CV group, and for serotype

TABLE 4. ELISA Antibody Concentrations and Opsonophagocytic Titers Against Individual Pneumococcal Serotypes 1 Month After Booster Vaccination (ATP Immunogenicity Cohort-Booster)

Pneumococcal Serotype	Group*	ELISA: PHiD-CV/MenC-CRM (N = 158), PHiD-CV/MenC-TT (N = 153), PHiD-CV/Hib-MenC (N = 160), 7vCRM/Hib-MenC (N = 153)				OPA: PHiD-CV/MenC-CRM (N = 145), PHiD-CV/MenC-TT (N = 143), PHiD-CV/Hib-MenC (N = 146), 7vCRM/Hib-MenC (N = 143)		
		% ≥ 0.20 μg/mL (95% CI)	% ≥ 0.35 μg/mL (95% CI)	GMC μg/mL (95% CI)	Fold Increase vs. Pre-booster†	% ≥ 8 (95% CI)	GMT Dil (95% CI)	Fold Increase vs. Pre-booster†
Serotype 1	PHiD-CV+MenC-CRM	100 (97.7–100)	97.5 (93.6–99.3)	2.26 (1.95–2.62)	9.8 (8.7–11.1)	94.3 (89.1–97.5)	226.8 (173.1–297)	25.5 (19.2–34.0)
	PHiD-CV+ MenC-TT	100 (97.6–100)	99.3 (96.4–100)	2.14 (1.85–2.47)	10.1 (8.8–11.5)	95.7 (90.8–98.4)	205.8 (159.7–265.2)	26.9 (19.9–36.3)
	PHiD-CV+ Hib-MenC	100 (97.7–100)	98.8 (95.6–99.8)	2.12 (1.83–2.45)	9.5 (8.3–11.0)	90.7 (84.6–95.0)	153.9 (115.6–204.8)	18.1 (13.5–24.2)
	7vCRM+ Hib-MenC	2.6 (0.7–6.6)	2.0 (0.4–5.7)	0.03 (0.03–0.04)	1.1 (1.0–1.2)	8.8 (4.6–14.8)	5.0 (4.4–5.8)	1.1 (0.9–1.3)
Serotype 4	PHiD-CV+MenC-CRM	100 (97.7–100)	100 (97.7–100)	3.94 (3.42–4.54)	8.9 (7.7–10.4)	100 (97.3–100)	1919.6 (1631.0–2259.2)	67.6 (44.6–102.5)
	PHiD-CV+ MenC-TT	100 (97.6–100)	100 (97.6–100)	4.15 (3.65–4.71)	9.1 (7.8–10.7)	100 (97.3–100)	2068.1 (1746.8–2448.5)	87.7 (58.9–130.4)
	PHiD-CV+ Hib-MenC	100 (97.7–100)	100 (97.7–100)	3.53 (3.15–3.95)	8.5 (7.2–9.9)	100 (97.4–100)	1539.9 (1295.5–1830.4)	71.3 (47.8–106.4)
	7vCRM+ Hib-MenC	100 (97.6–100)	100 (97.6–100)	5.26 (4.49–6.17)	10.8 (9.3–12.6)	100 (97.3–100)	3281.0 (2766.1–3891.6)	94.3 (62.5–142.3)
Serotype 5	PHiD-CV+MenC-CRM	100 (97.7–100)	98.7 (95.5–99.8)	2.94 (2.54–3.42)	5.8 (5.1–6.6)	97.6 (93.1–99.5)	192.9 (149.8–248.6)	10.1 (8.0–12.7)
	PHiD-CV+ MenC-TT	100 (97.6–100)	97.4 (93.4–99.3)	2.66 (2.29–3.09)	5.8 (5.0–6.6)	97.7 (93.4–99.5)	170.0 (136.0–212.4)	10.9 (8.3–14.3)
	PHiD-CV+ Hib-MenC	98.8 (95.6–99.8)	97.5 (93.7–99.3)	2.54 (2.18–2.96)	5.6 (4.9–6.4)	96.3 (91.6–98.8)	148.8 (116.4–190.1)	9.8 (7.7–12.3)
	7vCRM+ Hib-MenC	7.3 (3.7–12.7)	2.7 (0.7–6.7)	0.05 (0.04–0.05)	1.3 (1.2–1.4)	3.1 (0.9–7.9)	4.3 (4.0–4.7)	1.0 (0.9–1.1)
Serotype 6B	PHiD-CV+MenC-CRM	98.1 (94.6–99.6)	96.8 (92.8–99.0)	2.48 (2.12–2.9)	5.0 (4.3–5.7)	94.4 (89.2–97.5)	693.5 (513.1–937.2)	22.3 (14.8–33.7)
	PHiD-CV+ MenC-TT	96.7 (92.5–98.9)	96.1 (91.7–98.5)	2.16 (1.83–2.55)	5.0 (4.3–5.8)	94.1 (88.7–97.4)	702.8 (523.2–944)	29.7 (18.9–46.8)
	PHiD-CV+ Hib-MenC	97.5 (93.7–99.3)	97.5 (93.7–99.3)	2.04 (1.75–2.38)	4.4 (3.8–5.0)	95.1 (90.1–98.0)	627.5 (475.8–827.6)	18.8 (12.3–28.7)
	7vCRM+ Hib-MenC	99.3 (96.4–100)	98.0 (94.4–99.6)	4.96 (4.22–5.82)	16.1 (13.3–19.4)	98.6 (94.9–99.8)	4670.0 (3720.5–5861.9)	136.2 (85.1–218.1)
Serotype 7F	PHiD-CV+MenC-CRM	100 (97.7–100)	100 (97.7–100)	4.94 (4.41–5.54)	5.9 (5.3–6.6)	100 (97.4–100)	4539.3 (3970.8–5189.2)	4.4 (3.6–5.4)
	PHiD-CV+ MenC-TT	100 (97.6–100)	100 (97.6–100)	4.87 (4.31–5.5)	6.2 (5.4–7.0)	100 (97.3–100)	5462.6 (4700.0–6348.9)	5.0 (3.9–6.5)
	PHiD-CV+ Hib-MenC	100 (97.7–100)	100 (97.7–100)	4.21 (3.76–4.72)	5.2 (4.6–5.9)	100 (97.4–100)	4950.2 (4186.9–5852.7)	5.7 (4.3–7.6)
	7vCRM+ Hib-MenC	1.3 (0.2–4.7)	1.3 (0.2–4.7)	0.03 (0.03–0.04)	1.1 (1.0–1.2)	47.3 (37.8–57.0)	61.4 (35.2–106.9)	1.3 (0.9–2.0)
Serotype 9V	PHiD-CV+MenC-CRM	99.4 (96.5–100)	99.4 (96.5–100)	4.73 (4.12–5.44)	5.8 (5.1–6.5)	100 (97.5–100)	2317.7 (1938–2771.7)	8.0 (6.2–10.2)
	PHiD-CV+ MenC-TT	99.3 (96.4–100)	99.3 (96.4–100)	4.41 (3.83–5.09)	5.6 (4.9–6.3)	100 (97.4–100)	2666.1 (2266.8–3135.7)	8.8 (7.1–11)
	PHiD-CV+ Hib-MenC	100 (97.7–100)	100 (97.7–100)	4.03 (3.58–4.55)	5.3 (4.6–6.1)	100 (97.5–100)	2334.1 (1951.7–2791.4)	8.4 (6.5–10.8)
	7vCRM+ Hib-MenC	100 (97.6–100)	100 (97.6–100)	8.23 (7.11–9.51)	8.9 (7.8–10.3)	100 (97.3–100)	4800.6 (4032–5715.8)	16.9 (12.9–22.2)
Serotype 14	PHiD-CV+MenC-CRM	99.4 (96.5–100)	98.7 (95.5–99.8)	5.91 (5.08–6.87)	5.9 (5.0–7.0)	100 (97.3–100)	1751.4 (1458.7–2102.9)	6.6 (5.2–8.4)
	PHiD-CV+ MenC-TT	100 (97.6–100)	99.3 (96.4–100)	5.87 (5.08–6.78)	5.1 (4.3–6.1)	100 (97.4–100)	1808.3 (1516.5–2156.2)	8.5 (6.4–11.4)
	PHiD-CV+ Hib-MenC	99.4 (96.6–100)	99.4 (96.6–100)	5.62 (4.85–6.52)	6.9 (5.8–8.3)	100 (97.4–100)	1537.7 (1279.6–1847.8)	7.9 (5.8–10.9)
	7vCRM+ Hib-MenC	100 (97.6–100)	100 (97.6–100)	11.28 (9.61–13.25)	6.1 (5.3–7.1)	100 (97.3–100)	3020.8 (2519.1–3622.4)	7.4 (6.0–9.1)
Serotype 18C	PHiD-CV+MenC-CRM	100 (97.7–100)	100 (97.7–100)	7.19 (6.42–8.05)	11.4 (10–13)	100 (97–100)	813.7 (630.5–1050.1)	103.6 (74.0–145.0)
	PHiD-CV+ MenC-TT	100 (97.6–100)	100 (97.6–100)	9.99 (8.74–11.43)	11.3 (9.7–13.1)	98.4 (94.5–99.8)	1030.5 (814.1–1304.5)	71.3 (47.9–106.3)
	PHiD-CV+ Hib-MenC	100 (97.7–100)	100 (97.7–100)	7.20 (6.36–8.15)	11.6 (10.1–13.3)	100 (97–100)	717.1 (559.1–919.9)	91.5 (63.0–132.9)
	7vCRM+ Hib-MenC	100 (97.6–100)	100 (97.6–100)	7.58 (6.6–8.7)	13 (11.4–14.8)	96.5 (91.3–99)	754.2 (540.6–1052.2)	90.5 (60.2–136.0)
Serotype 19F	PHiD-CV+MenC-CRM	99.4 (96.5–100)	98.7 (95.5–99.8)	7.77 (6.68–9.03)	7.5 (6.4–8.8)	97.1 (92.7–99.2)	875.9 (688.1–1114.8)	33.1 (24.7–44.3)
	PHiD-CV+ MenC-TT	99.3 (96.4–100)	99.3 (96.4–100)	7.38 (6.28–8.69)	6.4 (5.3–7.6)	96.4 (91.7–98.8)	763.6 (577.3–1010)	24.5 (17.9–33.4)
	PHiD-CV+ Hib-MenC	100 (97.7–100)	100 (97.7–100)	6.78 (6.01–7.65)	7.1 (6.0–8.5)	100 (97.4–100)	551.3 (443.2–685.9)	26.3 (20.1–34.5)
	7vCRM+ Hib-MenC	98.7 (95.3–99.8)	98.7 (95.3–99.8)	3.72 (3.23–4.28)	13.7 (11.0–17.1)	98.5 (94.7–99.8)	321.3 (251.2–411.0)	40.2 (27.7–58.3)
Serotype 23F	PHiD-CV+MenC-CRM	98.7 (95.5–99.8)	98.1 (94.6–99.6)	3.49 (2.99–4.07)	6.4 (5.5–7.3)	100 (97.5–100)	3032.2 (2569.3–3578.5)	3.9 (2.8–5.4)
	PHiD-CV+ MenC-TT	99.3 (96.4–100)	98 (94.4–99.6)	3.27 (2.82–3.81)	7.1 (6.1–8.3)	100 (97.5–100)	3378.9 (2877.8–3967.3)	6.5 (4.5–9.5)
	PHiD-CV+ Hib-MenC	98.8 (95.6–99.8)	96.3 (92–98.6)	2.55 (2.17–2.98)	6.9 (6–7.8)	100 (97.5–100)	2836.7 (2430.8–3310.2)	7.2 (4.7–11.1)
	7vCRM+ Hib-MenC	99.3 (96.4–100)	98 (94.4–99.6)	9.42 (7.84–11.33)	21.8 (18.5–25.7)	100 (97.5–100)	25321.1 (20390.5–31443.9)	22.8 (15.7–33.3)
Serotype 6A	PHiD-CV+MenC-CRM	85.9 (79.4–90.9)	72.4 (64.7–79.3)	0.9 (0.72–1.12)	4.7 (4.1–5.5)	91.3 (85–95.6)	315.2 (230.9–430.2)	5.4 (3.5–8.2)
	PHiD-CV+ MenC-TT	79.6 (72.3–85.7)	68.4 (60.4–75.7)	0.74 (0.58–0.94)	5 (4.2–5.9)	93.0 (87.1–96.7)	339 (255.8–449.1)	8.5 (5.9–12.3)
	PHiD-CV+ Hib-MenC	79.9 (72.8–85.8)	69.8 (62.0–76.8)	0.67 (0.53–0.84)	4.4 (3.7–5.1)	91.4 (85.1–95.6)	293.7 (217.9–395.8)	5.3 (3.6–7.9)
	7vCRM+ Hib-MenC	92.8 (87.4–96.3)	88.8 (82.7–93.3)	1.91 (1.5–2.44)	15.1 (12.3–18.6)	97.5 (93.0–99.5)	1166.4 (877.2–1550.9)	33.9 (22.0–52.3)
Serotype 19A	PHiD-CV+MenC-CRM	89.8 (84.0–94.1)	80.9 (73.9–86.7)	1.19 (0.96–1.47)	7.1 (6.1–8.3)	62.1 (52.9–70.7)	48 (32.1–71.9)	10.1 (6.6–15.4)
	PHiD-CV+ MenC-TT	84.3 (77.6–89.7)	76.5 (68.9–82.9)	1.12 (0.89–1.42)	5.8 (4.9–6.9)	56.8 (47.9–65.4)	46.6 (30.8–70.4)	9.2 (5.9–14.3)
	PHiD-CV+ Hib-MenC	82.5 (75.7–88.0)	71.3 (63.6–78.1)	0.75 (0.6–0.94)	5.6 (4.7–6.7)	38.5 (30.1–47.4)	18.9 (13.0–27.4)	4.8 (3.2–7.2)
	7vCRM+ Hib-MenC	68.4 (60.4–75.7)	44.1 (36.0–52.4)	0.35 (0.29–0.42)	5.3 (4.5–6.2)	23.6 (16.4–32.1)	8.7 (6.6–11.4)	2.0 (1.6–2.7)

*Group definitions are given in Table 1.

†Geometric mean of the individual ratios of post-booster to pre-booster ELISA antibody concentrations/OPA titers calculated for all subjects with both pre-booster and post-booster samples.

N indicates number of subjects with available results for at least one serotype (the actual number of subjects tested can slightly vary for the different serotypes depending on the number of sera available).

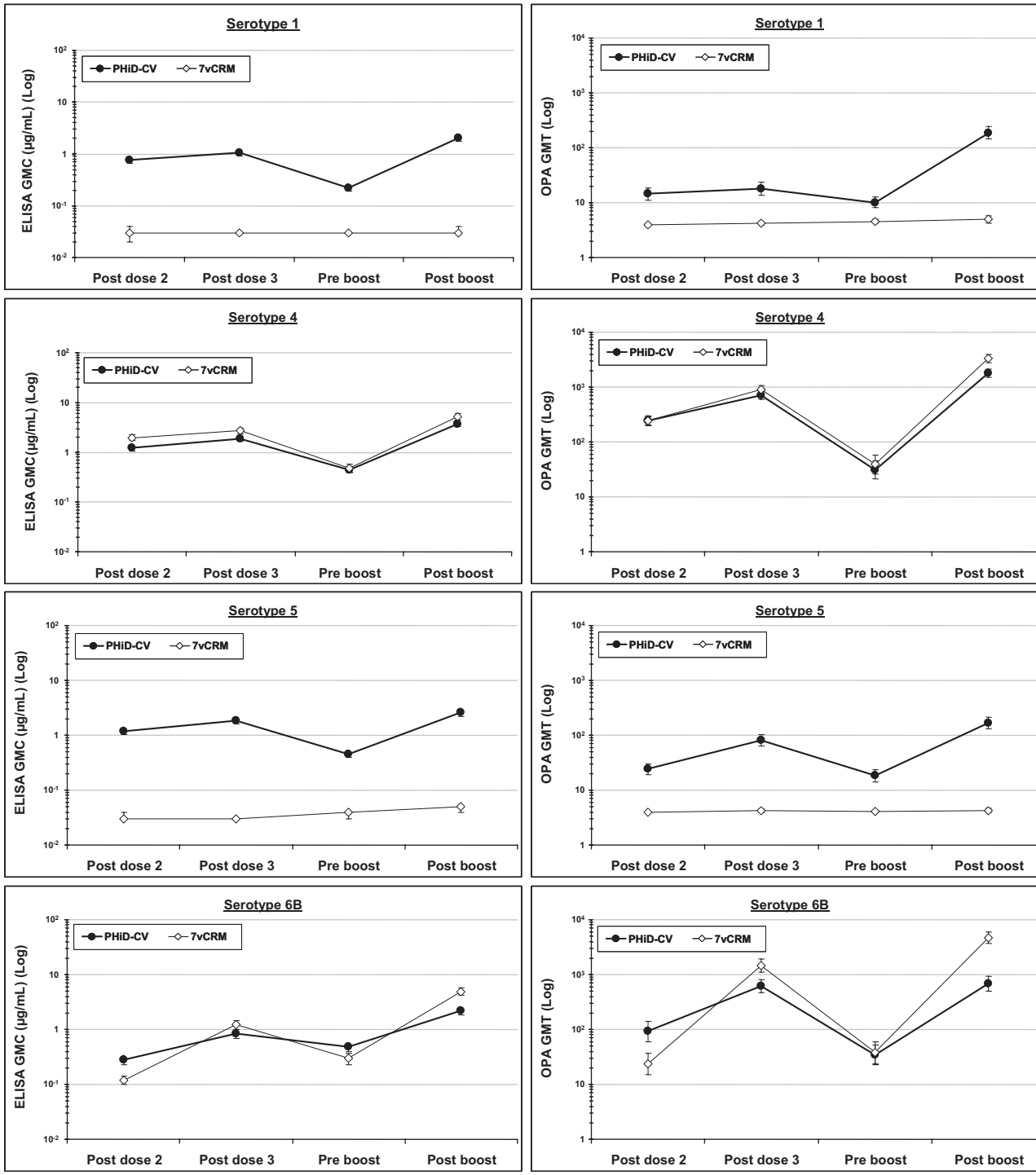

FIGURE 2. Evolution of pneumococcal antibody concentrations (ELISA, GMC) and opsonophagocytic activity (GMT) measured 2 months after the second primary dose (post-dose 2), 1 month after the third primary dose (post-dose 3), just before (pre-boost), and 1 month after the booster dose (post-boost).

23F in the 7vCRM group. In both groups, the third dose increased OPA GMTs for all common serotypes and for serotypes 5 and 7F in the PHiD-CV group.

A marked decline in ELISA antibody GMCs was observed in the time period after the third primary dose and before booster vaccination. Nevertheless, before the booster dose over 80.0% to 94.8% of PHiD-CV primed subjects still had persisting anti-pneumococcal antibody concentrations ≥ 0.2 μg/mL for all vaccine serotypes except serotype 1 (53.9%). In the 7vCRM group, 77.2% to 96.0% of vaccinees still had anti-

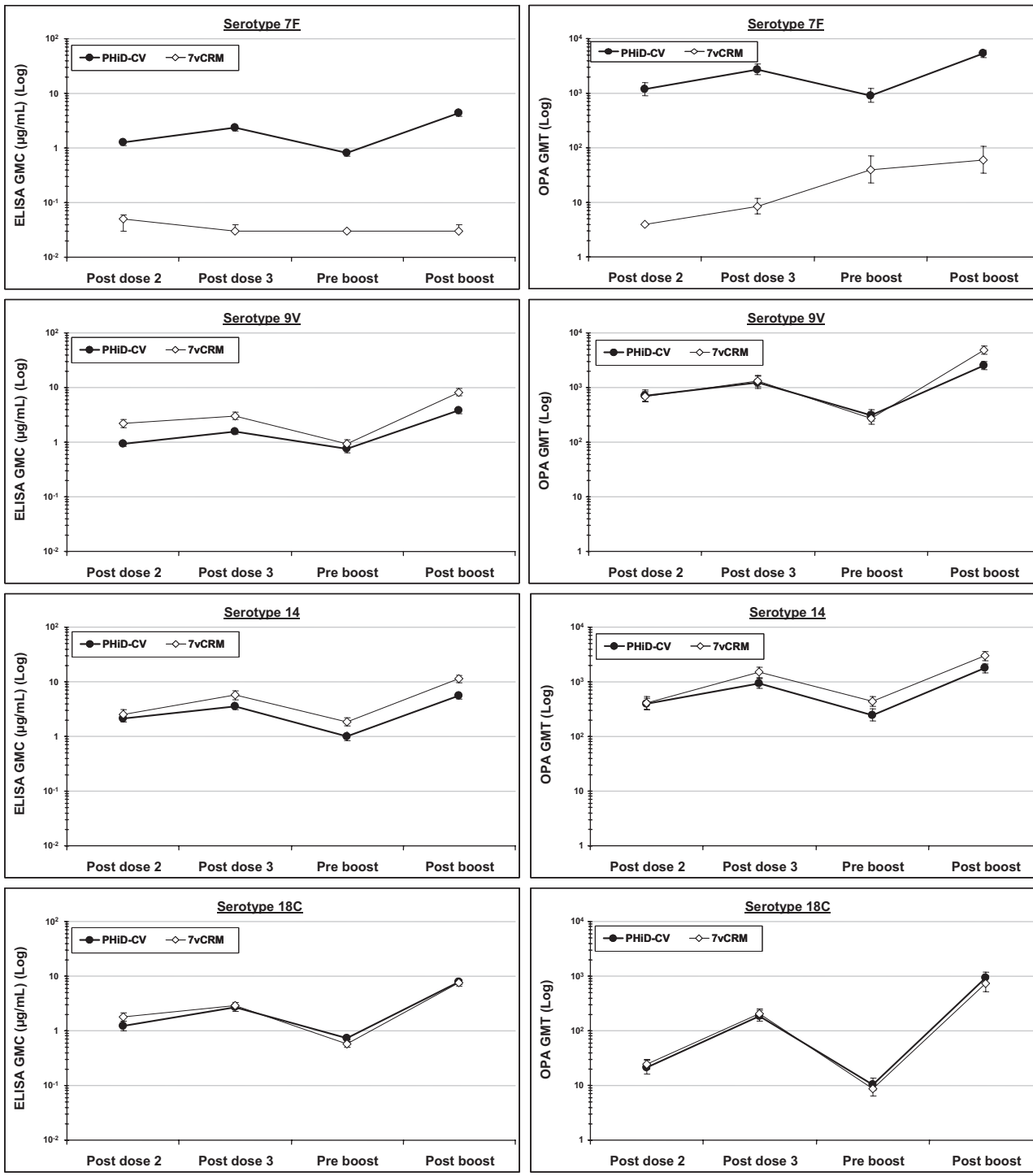

FIGURE 2. (*Continued*)

pneumococcal antibody concentrations ≥0.2 g/mL for each of the shared serotypes but lower persistence rates were observed for serotypes 6B (57.8%) and 19F (46.6%). In PHiD-CV primed subjects, the persistence of antibody concentrations ≥0.2 µg/mL for serotypes 1, 5, and 7F ranged between 53.9% and 94.8%, compared with less than 4.8% in 7vCRM-primed subjects. It is noteworthy that for serotypes 4, 9V, and 23F, despite the lower post-primary antibody GMCs observed in PHiD-CV-primed subjects compared with 7vCRM-primed subjects, GMCs were in the same range before the booster vaccination, indicating a less pronounced decline of antibody concentrations in PHiD-CV-primed subjects for those serotypes. OPA GMTs also declined over the same period, however, a high proportion of subjects still had OPA titers ≥8 before booster for serotypes 7F

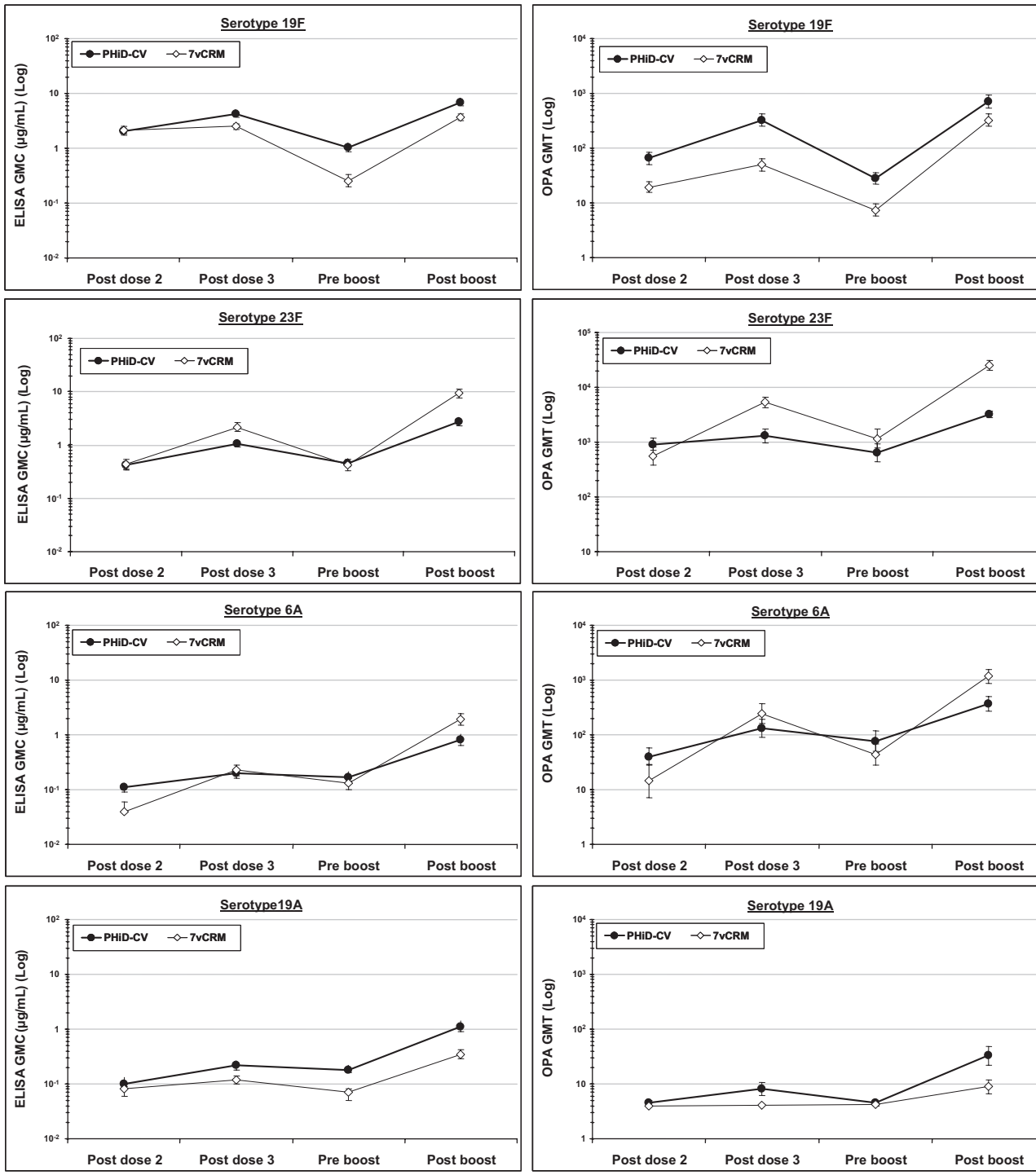

FIGURE 2. (*Continued*)

(95.8%), 9V (96.4%), 14 (93.8%), 19F (76.8%), and 23F (88.7%) in the PHiD-CV groups and serotypes 9V (96.9%), 14 (99.2%), and 23F (90.3%) in 7vCRM primed subjects. Low persistence was observed for serotype 18C in both PHiD-CV (37.9%) and 7vCRM (28.3%) primed subjects and for 19F (20.6%) in 7vCRM primed subjects. After booster vaccination both ELISA GMCs and OPA GMTs were restored to above post-primary level for all vaccine serotypes in both vaccine groups.

Immunogenicity to PD

After primary vaccination, all except 1 PHiD-CV vaccinee had measurable antibodies against PD (≥100 EL.U/mL) with anti-PD antibody GMCs of 1715.5 to 2114.0 EL.U/mL compared with 72.3 EL.U/mL in the 7vCRM group (Table 6). At least 94.0% of PHiD-CV vaccinees still had measurable antibodies before booster vaccination and a booster response within the same range was observed in the 3 PHiD-CV groups.

TABLE 5. ELISA Antibody Concentrations and Opsonophagocytic Titers Against Individual Pneumococcal Serotypes 2 Months After the Second Primary Vaccination Dose (ATP Immunogenicity Cohort-Booster Post-dose 2 subset*)

Pneumococcal Serotype	Group†	ELISA PHiD-CV Groups Pooled (N = 156) 7vCRM/Hib-MenC (N = 146)		OPA PHiD-CV Groups Pooled (N = 140) 7vCRM/Hib-MenC (N = 137)
		% ≥ 0.20 μg/mL (95% CI)	% ≥ 0.35 μg/mL (95% CI)	% ≥ 8 μg/mL (95% CI)
Serotype 1	PHiD-CV groups pooled	95.8 (91.0–98.4)	82.4 (75.1–88.3)	48.8 (39.8–57.9)
	7vCRM + Hib-MenC	4.2 (0.5–14.3)	4.2 (0.5–14.3)	0.0 (0.0–8.0)
Serotype 4	PHiD-CV groups pooled	98.7 (95.4–99.8)	89.7 (83.8–94.0)	97.8 (93.7–99.5)
	7vCRM + Hib-MenC	99.3 (96.2–100)	97.9 (94.1–99.6)	95.6 (90.6–98.4)
Serotype 5	PHiD-CV groups pooled	96.5 (91.9–98.8)	88.7 (82.2–93.4)	74.6 (65.9–82.0)
	7vCRM + Hib-MenC	2.1 (0.1–11.3)	2.1 (0.1–11.3)	0.0 (0.0–8.0)
Serotype 6B	PHiD-CV groups pooled	64.1 (55.9–71.6)	53.6 (45.4–61.7)	63.0 (54.4–71.1)
	7vCRM + Hib-MenC	30.8 (23.5–39.0)	13.7 (8.6–20.4)	35.2 (26.9–44.1)
Serotype 7F	PHiD-CV groups pooled	98.6 (94.9–99.8)	90.0 (83.8–94.4)	96.8 (91.9–99.1)
	7vCRM + Hib-MenC	6.4 (1.3–17.5)	4.3 (0.5–14.5)	0.0 (0.0–8.8)
Serotype 9V	PHiD-CV groups pooled	96.1 (91.7–98.5)	87.6 (81.3–92.4)	98.5 (94.8–99.8)
	7vCRM + Hib-MenC	96.6 (92.2–98.9)	93.8 (88.6–97.1)	99.3 (95.9–100)
Serotype 14	PHiD-CV groups pooled	99.4 (96.5–100)	97.4 (93.6–99.3)	97.1 (92.7–99.2)
	7vCRM + Hib-MenC	97.9 (94.1–99.6)	93.2 (87.8–96.7)	93.3 (87.7–96.9)
Serotype 18C	PHiD-CV groups pooled	87.8 (81.6–92.5)	82.1 (75.1–87.7)	59.8 (51.0–68.3)
	7vCRM + Hib-MenC	97.3 (93.1–99.2)	89.0 (82.8–93.6)	77.0 (69.0–83.8)
Serotype 19F	PHiD-CV groups pooled	96.2 (91.8–98.6)	90.4 (84.6–94.5)	84.3 (77.2–89.9)
	7vCRM + Hib-MenC	99.3 (96.2–100)	95.9 (91.2–98.5)	67.9 (59.4–75.6)
Serotype 23F	PHiD-CV groups pooled	75.0 (67.4–81.6)	60.9 (52.8–68.6)	97.1 (92.8–99.2)
	7vCRM + Hib-MenC	74.7 (66.8–81.5)	56.8 (48.4–65.0)	87.8 (80.9–92.9)
Serotype 6A	PHiD-CV groups pooled	33.1 (25.4–41.6)	20.1 (13.8–27.8)	60.5 (51.3–69.1)
	7vCRM + Hib-MenC	4.3 (0.5–14.5)	0.0 (0.0–7.5)	26.8 (14.2–42.9)
Serotype 19A	PHiD-CV groups pooled	26.6 (19.5–34.8)	15.1 (9.6–22.2)	4.3 (1.4–9.8)
	7vCRM + Hib-MenC	12.8 (4.8–25.7)	4.3 (0.5–14.5)	0.0 (0.0–8.6)

*Post-dose 2 subset indicates subset of subjects randomly selected for post-dose 2 immunogenicity testing.
†Group definitions are given in Table 1.
N indicates number of subjects with available results for at least 1 serotype (the actual number of subjects tested can slightly vary for the different serotypes depending on the number of sera available).

TABLE 6. ELISA Antibody Concentrations Against Protein D 1 Month After the Third Primary Vaccine Dose, Before and 1 Month After Booster Vaccination (ATP Cohort for Immunogenicity)

Group*	Post-primary Response PHiD-CV/MenC-CRM N = 168 PHiD-CV/MenC-TT N = 174 PHiD-CV/Hib-MenC N = 173 7vCRM/Hib-MenC N = 163		Persistence (Pre-booster) PHiD-CV/MenC-CRM N = 151 PHiD-CV/MenC-TT N = 159 PHiD-CV/Hib-MenC N = 151 7vCRM/Hib-MenC N = 149		Booster Response PHiD-CV/MenC-CRM N = 158 PHiD-CV/MenC-TT N = 152 PHiD-CV/Hib-MenC N = 160 7vCRM/Hib-MenC N = 148	
	% ≥ 100 EL.U/mL (95% CI)	GMC (EL.U/mL) (95% CI)	% ≥ 100 EL.U/mL (95% CI)	GMC (EL.U/mL) (95% CI)	% ≥ 100 EL.U/mL (95% CI)	GMC (EL.U/mL) (95% CI)
PHiD-CV + MenC-CRM	100 (97.8–100)	2114.0 (1847.6–2418.8)	98.0 (94.3–99.6)	778.5 (666.9–908.7)	100 (97.7–100)	3106.0 (2693.8–3581.3)
PHiD-CV + MenC-TT	100 (97.9–100)	1715.5 (1494.9–1968.7)	96.2 (92.0–98.6)	666.4 (564.7–786.5)	99.3 (96.4–100)	2598.4 (2206.9–3059.4)
PHiD-CV + Hib-MenC	99.4 (96.8–100)	1726.7 (1493.3–1996.7)	94.0 (89.0–97.2)	636.8 (531.9–762.3)	100 (97.7–100)	2679.3 (2305.5–3113.6)
7vCRM + Hib-MenC	23.3 (17.1–30.6)	72.3 (64.5–81.1)	39.6 (31.7–47.9)	94.7 (82.0–109.4)	44.6 (36.4–53.0)	96.4 (84.1–110.5)

*Group definitions are given in Table 1.
EL.U indicates ELISA Units.

DISCUSSION

In recent years, the availability of new vaccines has led to an increase in the number of injections required to complete recommended pediatric vaccine schedules. The use of DTP-based combination vaccines, which include hepatitis B, polio, and Hib antigens, such as employed in this study, facilitate the inclusion of new vaccines into pediatric immunization programs. *N. meningitidis* serogroup C (MenC) conjugate vaccines are now also increasingly being incorporated into infant schedules. In this study, we have sought to establish whether the candidate *S. pneumoniae* conjugate PHiD-CV vaccine is compatible with these other frequently used pediatric vaccines, in particular different MenC-conjugated vaccines.

Considering the 3 PHiD-CV groups with different MenC conjugate vaccines, ELISA IgG and functional OPA immune responses were elicited against all vaccines serotypes after primary vaccination, followed by strong booster responses indicating the induction of immunologic memory. In all 3 PHiD-CV groups, a high percentage of subjects achieved an antibody concentration of ≥0.2 μg/mL, with antibody GMCs that were within the same range for most serotypes. Post-primary OPA GMTs were within the same range for most serotypes in all 3 PHiD-CV groups. The higher response in the PHiD-CV+MenC-TT group for serotype 18C, which is conjugated to tetanus toxoid, may be related to the coadministered MenC-TT vaccine. The reason why this was not observed in the

PHiD-CV+Hib-MenC-TT group despite the use of TT as carrier protein for both components of the Hib-MenC vaccine is not fully understood, but could be related to the total amount of TT-carrier protein that is different between the PHiD-CV+MenC-TT [10 μg of meningococcal serogroup C capsular polysaccharide (PSC)] conjugated to 10–20 μg of TT and 10 μg of PRP conjugated to 20–40 μg of TT) and PHiD-CV+Hib-MenC-TT (5 μg of PRP conjugated to 10–20 μg of TT and 5 μg of PSC conjugated to 3.5–12.5 μg of TT) groups. As reported elsewhere,[29] the antitetanus response was the highest in the MenC-TT group and there is evidence that carrier priming can enhance immune responses to polysaccharides in subsequent doses of conjugate.[30–32] Overall, the different coadministered vaccines did not result in differences in PHiD-CV pneumococccal responses that would be considered as clinically relevant. As reported by Knuf et al,[29] immunogenicity was demonstrated in the 3 groups for all the coadministered vaccines including the meningococcal C conjugates. These findings indicate that the PHiD-CV candidate vaccine is compatible with other pediatric vaccines including MenC and Hib-MenC vaccines.

Although not designed for statistical comparison, the study also included a 7vCRM+Hib-MenC group. It was observed that after primary vaccination the percentages of subjects with antibody concentrations ≥0.2 μg/mL against the serotypes common to both PHiD-CV and 7vCRM vaccines were within the same range. Although post-primary GMCs were higher for some serotypes in the 7vCRM group, the decline in serotype specific antibody levels observed in the 8–12 months after primary vaccination resulted in antibody GMCs within the same range for PHiD-CV and 7vCRM primed groups before booster vaccination for serotypes 4, 9V, and 23F. Furthermore, the robust increases in ELISA and OPA responses after the PHiD-CV booster, which were in most cases of the same order of magnitude as those obtained after the 7vCRM booster, would indicate adequate priming of the immune system against all PHiD-CV vaccine serotypes.

Despite lower post-primary immunologic responses observed for some serotypes in the PHiD-CV+Hib-MenC group compared to the other PHiD-CV groups, robust booster responses were observed in all PHiD-CV groups; indicating adequate priming of the immune system.

Comparison of post-primary immunogenicity does not by itself, however, provide an understanding of the relative overall public health impact of new vaccines with a different serotype composition to the existing licensed 7-valent vaccine. Although the 7vCRM vaccine has been estimated to cover 80% to 90% of serotypes responsible for IPD in young children in North America and Australia, the coverage is lower in other parts of the world especially Africa, Latin America, and Asia.[33] Higher valent vaccine formulations such as the 10-valent PHiD-CV and 13-valent CRM-conjugated vaccine in development[34,35] will increase vaccine coverage of IPD-causing serotypes in these regions.[36] Although serotype coverage can give a rough estimate of the public health impact of a new multivalent pneumococcal conjugate vaccine, this does not take into account that vaccine efficacy varies by serotype and is usually less than 100%.[37] A recent approach to providing an estimate of the overall vaccine impact on IPD in different countries uses the results of immunogenicity comparisons, along with published serotype-specific vaccine effectiveness values for 7vCRM[37] and country IPD serotype distribution. A preliminary report of the application of this IPD-Impact-Estimate to immunogenicity data from the present study suggested that the overall impact of PHiD-CV on IPD to be at least as high as that of 7vCRM and potentially higher in countries where the additional 3 serotypes 1, 5, and 7F cause significant disease.[38]

This report also followed the kinetics of the immune response from the second primary vaccination dose up to one month after booster vaccination. Overall, the kinetics for the common serotypes appear to be in the same range for the PHiD-CV and 7vCRM vaccines. It is noteworthy that, apart for serotypes 6B and 23F, at least 87.8% of subjects reached an antibody concentration of at least 0.2 μg/mL after just 2 primary doses of PHiD-CV or 7vCRM vaccine although antibody GMCs were below those achieved after 3 primary doses. For serotype 23F, 97.1% of PHiD-CV vaccinees achieved OPA titers ≥8 and for serotype 6B lower percentages of subjects reaching the ELISA and OPA thresholds were observed in 7vCRM vaccines compared with PHiD-CV vaccinees. A third dose was also required to achieve high percentages of subjects with OPA activity for serotypes 6B, 18C, and 19F (for both PHiD-CV and 7vCRM vaccinees) and for serotype 23F in 7vCRM vaccinees. Recently the use of a 2 primary dose plus booster schedule has been approved for 7vCRM in Europe with the acknowledgment that smaller proportions of infants achieve threshold ELISA antibody levels against serotypes 6B and 23F and that GMCs are lower for antibodies against most serotypes compared with those after a 3-dose infant series.[39] However good booster responses were taken as an indication that 2 doses of 7vCRM would elicit adequate priming.[39]

In summary, assessment of immunogenicity in this study indicated that the PHiD-CV vaccine is compatible with other pediatric vaccines including MenC and Hib-MenC vaccines. PHiD-CV was immunogenic already 2 months after the second dose (with similar responses compared with the post-dose 2 responses in the 7vCRM group), as well as 1 month after the 3-dose primary course and 1 month after the booster dose.

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