Immunogenicity of the 10-Valent Pneumococcal Non-typeable *Haemophilus influenzae* Protein D Conjugate Vaccine (PHiD-CV) Compared to the Licensed 7vCRM Vaccine

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Background: The immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV) was assessed and compared with the 7-valent pneumococcal conjugate vaccine (7vCRM).

Methods: Healthy subjects (1650) were randomized to be vaccinated with 3 doses of PHiD-CV or 7vCRM (*Prevenar*™/*Prevnar*™) at 2-3-4 months of age and a fourth booster dose at 12–18 months. Serotype-specific pneumococcal responses (GlaxoSmithKline's ELISA with 22F-inhibition) and opsonophagocytic activity (OPA) were measured 1 month after primary and booster vaccinations.

Results: The primary objective to demonstrate noninferiority of PHiD-CV versus 7vCRM (in terms of percentage of subjects with antibody concentration ≥0.2 μg/mL) for at least 7 of the 10 vaccine serotypes was reached as noninferiority was demonstrated for 8 serotypes. Although, noninferiority could not be demonstrated for ELISA responses against serotypes 6B and 23F, a post-hoc analysis of the percentage of subjects with OPA titers ≥8 suggested noninferiority for the 7 serotypes common to both vaccines including 6B and 23F.

Priming of the immune system against all vaccine serotypes was confirmed by robust increases in ELISA antibody levels (~6.0–17 fold) and OPA titers (~8–93 fold) after a fourth consecutive dose of PHiD-CV.

Conclusions: PHiD-CV induces ELISA and functional OPA antibodies for all vaccine serotypes after primary vaccination and is noninferior to 7vCRM in terms of ELISA and/or OPA threshold responses. Effective priming is further indicated by robust booster responses.

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Streptococcus pneumoniae and nonencapsulated (non-typeable) *Haemophilus influenzae* are 2 major bacterial pathogens of the respiratory tract.[1] *S. pneumoniae* is generally assumed to be the most common cause of community-acquired pneumonia, and also causes bacteremia, septicaemia, and bacterial meningitis.[2] The highest incidence of invasive pneumococcal disease (IPD) is found in young children and the elderly.[3,4] In developing countries, *S. pneumoniae* causes up to 1 million deaths every year among children under 5 years of age,[5,6] most of which are caused by pneumonia and occur in the first year of life. In addition, *S. pneumoniae* and non-typeable *H. influenzae* (NTHi) are the 2 leading causes of acute otitis media (AOM) in children.[7–13]

The polysaccharide capsule is the major immunogen and also principal virulence factor of *S. pneumoniae* by virtue of its antiphagocytic properties. As vaccines containing plain bacterial capsular polysaccharides are not immunogenic or effective in young children,[14] pediatric pneumococcal vaccines based on capsular polysaccharides conjugated to carrier proteins have been developed. To date, one 7-valent pneumococcal conjugate vaccine containing capsular polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, each conjugated to CRM_{197} (a nontoxic cross-reacting mutant of diphtheria toxin) has been licensed for use in several parts of the world. This 7vCRM vaccine has demonstrated efficacy against vaccine serotype pneumococcal disease.[8,15]

The candidate 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV; GlaxoSmithKline (GSK) Biologicals) contains polysaccharides from pneumococcal serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F. Protein D (PD), which is a highly conserved cell-surface protein among *H. influenzae* strains[16–19] was selected for its potential to provide protection against *H. influenzae* infections.[20,21] 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.[22]

S. pneumoniae is an encapsulated bacterium for which vaccine-induced protection can be conferred by antibodies directed against the polysaccharide capsule. These antibodies facilitate phagocytosis via opsonization of the pathogen. It is generally accepted that protection against invasive diseases is achieved when circulating functional antibodies reach a minimal level.[23–25] Since the introduction of the 7vCRM vaccine, a consensus on licensure criteria for new pneumococcal conjugate vaccines in terms of

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protection against IPD was reached at the World Health Organization (WHO) Expert Committee meeting in 2003.[23,24,26] It was recommended that licensure be based on post-primary immunogenicity comparisons with the licensed 7vCRM and the percentage of vaccinated subjects, reaching a reference ELISA IgG antibody concentration. In addition, demonstration of the functionality of the induced antibodies as measured by opsonophagocytosis (OPA) is required, and also demonstration of boostability. The present report evaluates the immunogenicity of the candidate PHiD-CV vaccine compared with the licensed 7vCRM vaccine, based on these WHO licensure criteria.

MATERIALS AND METHODS

Study Design and Participants

This study was conducted in Finland, France, and Poland between 04 November 2005 and 04 June 2007. 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. There were 2 study phases. The primary randomized controlled phase (105553/NCT00307554) was designed to evaluate noninferiority of the immune response and safety (in terms of fever) of the candidate PHiD-CV vaccine relative to the 7vCRM vaccine after a 3-dose primary vaccination course as well as consistency of 3 consecutive production lots of PHiD-CV vaccine. The booster phase (107046/NCT00370396) was partially randomized, controlled and evaluated the immunogenicity, and safety of a booster dose of PHiD-CV vaccine in the second year of life.

Eligible participants were healthy male or female infants aged 6 to12 weeks at the time of the first primary vaccination and 12 to 18 months at the time of the booster dose. Informed consent was obtained from each subjects' parent/guardian, before initiation of the primary and booster vaccination phases.

Vaccines and Vaccinations

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 non-typeable *H. influenzae* (NTHi) Protein D, 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 S.A., 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).

Three different lots of PHiD-CV were used, and in the primary phase subjects were randomized (1:1:1:1) to receive 3 doses of either PHiD-CV (3 groups each with a different vaccine lot) or 7vCRM (1 group) according to a 2, 3, and 4 month of age schedule. In the booster phase at age 12–18 months, approximately two-thirds of the subjects who received PHiD-CV in the primary phase were vaccinated with 1 dose of PHiD-CV (the remaining one-third of PHiD-CV primed subjects were invited to participate in a separate booster vaccination study described elsewhere.[27] All of the subjects who received 7vCRM in the primary phase were subrandomized to receive 1 booster dose of either PHiD-CV or 7vCRM (3:1 ratio).

Diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-Hib vaccine (DTPa-HBV-IPV/Hib, *Infanrix hexa*™; GSK Biologicals, Rixensart, Belgium) was coadministered with all pneumococcal vaccine doses, except for the second dose in France where diphtheria-tetanus-acellular pertussis-inactivated polio-Hib vaccine (DTPa-IPV/Hib, *Infanrix*™ IPV Hib; GSK Biologicals, Rixensart, Belgium) was coadministered.

Serological Methods

Serum samples were stored at −20°C until blinded analyses were conducted at GSK laboratories, Rixensart, Belgium. An ELISA (22F-ELISA), including adsorption with serotype 22F polysaccharide to increase assay specificity,[28,29] was used to measure the pneumococcal serotype specific total IgG antibodies. Antibody concentrations were determined by calibration with the standard reference serum 89-SF (courtesy of Dr. Frasch, US FDA).[30] The assay cut-off was 0.05 μg/mL. It has been previously established that an antibody concentration of 0.2 μg/mL as determined by GSK's ELISA with 22F-inhibition is equivalent to 0.35 μg/mL, as determined by the WHO reference laboratory ELISA, without 22F-inhibition[29] and therefore corresponds to the threshold proposed by the WHO for noninferiority comparison between pneumococcal conjugate vaccines.[26] Although protocol planned noninferiority based on the percentage of subjects with antibody concentrations $\geq$0.2 μg/mL, the percentage of subjects with at least an antibody concentration of 0.35 μg/mL as measured with the GSK 22F-ELISA assay (corresponding to a concentration of approximately 0.5 μg/mL when using the WHO reference laboratory ELISA), were also calculated for information. Opsonophagocytic (OPA) activity was determined using a modification of the HL-60 cell WHO reference method[31,32] which has been validated using sera collected after primary vaccination with the 7vCRM vaccine.[33] The results are presented as the dilution of serum (opsonic titer) able to confer 50% killing of live pneumococci under the assay conditions. The cut-off of the assay is an opsonic titer of 8 (serum dilution of 1:8). In the primary phase all eligible subjects were analyzed by ELISA and a random subset of approximately 25% of subjects per group by OPA. In the booster phase, a random subset of approximately 50% of subjects in groups boosted with PHiD-CV and all subjects boosted with 7vCRM were analyzed by both ELISA and OPA.

IgG antibodies to the *H. influenzae* protein D were measured by a classic ELISA, with the nonlipidated protein D as coating material and were expressed in ELISA units (EL.U) per milliliter; 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.[34]

Safety Evaluation

Safety/reactogenicity data are reported elsewhere.[35]

Statistical Analyses

Immunogenicity was analyzed for the according to protocol (ATP) immunogenicity cohorts. In the primary vaccination phase, the first co-primary objective was to demonstrate lot-to-lot consistency of 3 consecutive production lots of PHiD-CV in terms of the immune response induced against each of the 10 pneumococcal serotypes and the carrier protein D. Adjusted geometric mean concentration (GMC) ratios with their 95% confidence interval (CI), 1 month post dose 3, were computed for each pair among the 3 PHiD-CV lots using an ANCOVA model for the pneumococcal serotypes and an ANOVA model for protein D. This objective is reached if the 95% CIs of the GMC ratios between each pair of PHiD-CV lots lie within [0.4 to 2.5] for all vaccine pneumococcal serotypes and protein D.

The second co-primary objective was to demonstrate noninferiority of PHiD-CV (pooled data for the 3 lots) compared with 7vCRM. To reach the noninferiority objective, the protocol required demonstration of noninferiority for at least 7 of the 10 vaccine serotypes contained in PHiD-CV, based on the number of

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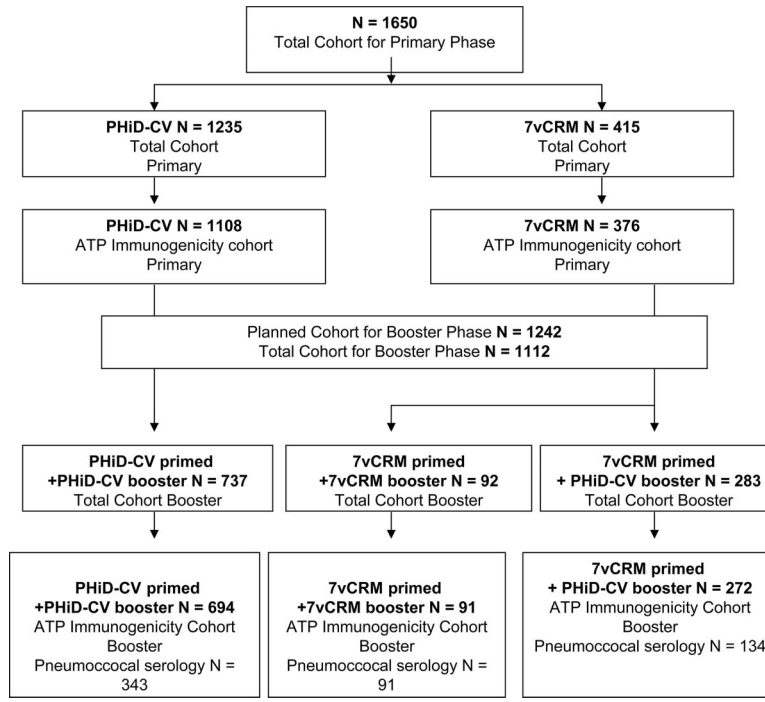

FIGURE 1. Trial profile.

serotypes contained in the registered 7vCRM vaccine and on the WHO indication that noninferiority is not an absolute requirement to be met for each serotype contained in the candidate vaccine. To control the statistical type I error for the demonstration of noninferiority for 7 of the 10 vaccine serotypes, adjustment of the 1-sided alpha ($\alpha = 0.0175 = 0.025$ [7/10]) was applied and thus 96.5% CIs were computed. The noninferiority criterion defined in the protocol for each of the 7 pneumococcal serotypes contained in both vaccines was an upper limit of the 2-sided 96.5% CI (standardized asymptotic CIs) below 10% for the difference between groups (7vCRM minus PHiD-CV) in terms of percentage of subjects with pneumococcal antibody concentrations $\geq$0.2 μg/mL. For each of the 3 additional serotypes not contained in 7vCRM (ie, 1, 5, and 7F), noninferiority was demonstrated if the upper limit of the 96.5% CI (computed using a bootstrap method; Efron Percentile Confidence Interval–2000 iterations[36]) was lower than 10% for the difference between the aggregate response for 7vCRM (aggregate response = percentage of pneumococcal antibody concentrations $\geq$0.2 μg/mL across all 7 7vCRM serotypes) and each of the new serotypes in PHiD-CV, in terms of percentage of subjects with pneumococcal antibody concentrations $\geq$0.2 μg g/mL. A post-hoc inferential analysis was also performed for the percentage of subjects with opsonphagocytic titers $\geq$8.

RESULTS

Participants

A total of 1650 subjects (1235 in the 3 PHiD-CV groups and 415 in the 7vCRM group) were enrolled for the primary vaccination phase and 1112 subjects for the booster phase (737 in the group primed and boosted with PHiD-CV, 92 in the group primed and boosted with 7vCRM, and 283 in the group primed with 7vCRM and boosted with PHiD-CV). Figure 1 shows the numbers of subjects included in the ATP immunogenicity cohorts.

In the primary phase, the demographic characteristics for the ATP immunogenicity cohorts were comparable between the 3 PHiD-CV lot primary vaccination groups (data not shown), as well as between PHiD-CV (pooled data for the 3 lots) and 7vCRM participants (Table 1). Demographic characteristics were also comparable for the 3 booster groups, although the female/male ratio was slightly lower in the 7vCRM booster group (Table 1). Although the mean and median ages are slightly higher in the PHiD-CV groups compared with the 7vCRM group, the observed ranges are within the same range.

Immunogenicity

Primary Vaccination

The lot-to-lot consistency objective was met because the lower and upper limits of the 2-sided 95% CIs of the adjusted GMC ratios for each pair among the 3 PHiD-CV lots were within [0.75 to 1.57], for all vaccine serotypes and protein D. The post-primary immunogenicity data for the 3 PHiD-CV lots could therefore be pooled and are presented with the data for 7vCRM in Table 2.

PHiD-CV induced antibody responses against all pneumococcal serotypes contained in the vaccine including serotypes 1, 5, and 7F. Altogether, in the PHiD-CV group, the percentage of subjects reaching the ELISA threshold of 0.2 μg/mL at 1 month after primary vaccination, was at least 95.4% except for serotypes 6B (65.9%) and 23F (81.4%). The response to 6B was also the lowest in the 7vCRM group with 79.0% reaching the ELISA threshold. Geometric mean ELISA antibody concentrations (GMCs) were lower in the PHiD-CV group for all serotypes common to both vaccines. The noninferiority objective of the study was however met, as the upper limit of the 96.5% CIs for the difference between groups in terms of percentage of subjects reaching the ELISA threshold, was less than 10% for 8 of the

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TABLE 1. Demographic Characteristics (ATP Cohort for Immunogenicity)

	Primary Vaccination Phase		Booster Vaccination Phase		
	PHiD-CV N = 1108	7vCRM N = 376	PHiD-CV/PHiD-CV N = 694	7vCRM/7vCRM N = 91	7vCRM/PHiD-CV N = 272
Age (primary in weeks; booster in months)					
Mean (SD)	9.0 (2.03)	8.8 (2.00)	15.3 (2.08)	14.2 (2.27)	14.2 (2.24)
Median (range)	9.0 (6–12)	9.0 (6–12)	16.0 (12–18)	13.0 (12–18)	13.0 (12–18)
Gender (%)					
Female	50.9	51.6	51.0	45.1	52.9
Male	49.1	48.4	49.0	54.9	47.1
Race (%)					
African heritage/African American	0.5	0.3	0.4	0.0	0.4
Asian-East Asian heritage	0.1	0.0	0.0	0.0	0.0
White-Arabic/North African heritage	1.5	0.5	1.4	0.0	1.1
White-Caucasian/European heritage	97.3	98.7	97.3	98.9	98.2
Other	0.6	0.5	0.9	1.1	0.4

PHiD-CV/PHiD-CV = primary and booster vaccination with PHiD-CV.
7vCRM/7vCRM = primary and booster vaccination with 7vCRM.
7vCRM/PHiD-CV = primary vaccination with 7vCRM and booster vaccination with PHiD-CV.
SD indicates standard deviation.

pneumococcal serotypes contained in the PHiD-CV vaccine (1, 4, 5, 7F, 9V, 14, 18C, and 19F) (Table 3). Noninferiority could not be demonstrated based on the ELISA antibody threshold of 0.2 μg/mL for serotypes 6B and 23F as the observed difference between groups was 13.1% and 12.7%, respectively.

The PHiD-CV vaccine induced a post-primary OPA titer $\geq$8 in at least 90% of the vaccinees tested with the exception of serotype 1 (65.7%) and serotype 19F (87.7%) (Table 2). The OPA geometric mean titers (GMTs) were lower for serotypes 4, 6B, 14, 18C and 23F, and higher for serotype 19F and the additional serotypes 1, 5 and 7F in the PHiD-CV group compared with those in the 7vCRM group. The difference between groups in terms of percentage of subjects with OPA titer $\geq$8 was below 5% for all the pneumococcal serotypes common to both vaccines, including serotypes 6B (3.14%) and 23F (3.83%) (Table 3).

For the cross-reactive serotypes 6A and 19A, the percentages of subjects reaching the ELISA threshold of 0.2 μg/mL were low in both groups ($\sim$22% of PHiD-CV vaccinees and $\sim$30% of 7vCRM vaccinees) (Table 2). The OPA response was stronger than the ELISA response in both groups for serotype 6A (58.0% of PHiD-CV and 68.5% of 7vCRM vaccinees reaching an OPA titer $\geq$8). For serotype 19A, the percentage of vaccinees reaching an OPA titer $\geq$8 was 19.6% in the PHiD-CV group versus 3.4% in the 7vCRM group (Table 2).

Persistence

In the time period after primary and before booster vaccination, a marked decline in ELISA serotype-specific antibody GMCs was observed for most serotypes in both groups (Fig. 2, Table 4). Nevertheless for the 7 common serotypes, 57.3% to 84.6% of PHiD-CV-primed and 30.7% to 93.3% of 7vCRM-primed subjects still had persisting anti-pneumococcal antibody concentrations $\geq$0.2 μg/mL before the booster dose (Table 4). For the 3 additional PHiD-CV serotypes (1, 5, and 7F), the percentage of subjects with persisting antibody concentrations $\geq$0.2 μg/mL was higher in the PHiD-CV group, compared with subjects not primed for these serotypes in the 7vCRM group (Table 4).

A similar decline between the post-primary and the pre-booster time points was observed for OPA GMTs (Fig. 2, Table 5). There was considerable variability between serotypes in the percentage of subjects with persistence of OPA titers $\geq$8 before the booster dose. For the 7 common serotypes, low persistence of OPA titers $\geq$8 was observed for both vaccines for serotype 18C (about 30% for both vaccines). For 19F, the OPA persistence was 45.7% in the PHiD-CV group as compared with less than 20% in the 7vCRM group. Low OPA persistence was also observed for serotypes 1 (16.3%) and 5 (34.4%) in PHiD-CV-primed subjects. However, for both these serotypes and for 7F, the percentages of subjects with OPA titers $\geq$8 remained significantly higher than in the 7vCRM group (Table 5).

Booster Vaccination

One month after the booster dose, robust increases in antibody GMCs were measured in the PHiD-CV and 7vCRM groups which were primed and boosted with the same vaccine (Fig. 2 and Table 4). Compared with pre-booster levels, post-booster antibody GMCs against each vaccine serotype (including 6B and 23F) increased by 6.0- (serotype 9V) to 16.7-fold (serotype 18C) for PHiD-CV vaccinees and by 8.8-(serotype 14) to 27.8-fold (serotype 23F) for 7vCRM vaccinees, depending on the serotype. Post-booster 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 group relative to the 7vCRM group, but over 96% of subjects in both groups had antibody concentrations $\geq$0.2 μg/mL for the 7 serotypes contained in both vaccines. Over 99% of PHiD-CV primed and boosted subjects had antibody concentrations $\geq$0.2 μg/mL against the 3 additional serotypes, compared with fewer than 7.1% in 7vCRM-primed and boosted subjects.

Substantial increases in OPA GMTs were also observed after the booster dose (Fig. 2, Table 5). The fold increase in post-booster OPA GMTs ranged between 7.9- (serotype 9V) to 93.1-fold (serotype 18C) for PHiD-CV primed and boosted subjects, including 31.5-fold for serotype 1, and between 10.6- (serotype 14) to 114.4-fold (serotype 4) for 7vCRM primed and boosted subjects compared with pre-booster levels. In the PHiD-CV group, post-booster GMTs were lower for serotypes 4, 6B, 9V, and 23F relative to the 7vCRM group, in the same range for 14 and 18C, and higher for 19F and the additional serotypes 1, 5, and 7F. Over 94.9% of PHiD-CV vaccinees and over 92.5% of 7vCRM vaccinees had OPA titers $\geq$8 for the shared serotypes.

After booster vaccination a high percentage of subjects in both groups had antibody concentrations $\geq$0.2 μg/mL for the cross-reactive serotypes 6A and 19A (Table 4). Although the percentage of subjects with OPA titers $\geq$8 was high for 6A (85%

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TABLE 2. ELISA Antibody Concentrations and OPA Titers Against Individual Pneumococcal Vaccine Serotypes and Cross-Reactive Serotypes 6A and 19A 1 Month After The Third Primary Vaccine Dose (ATP Cohort for Immunogenicity)

	ELISA						OPA			
	PHiD-CV (N = 1107)			7vCRM (N = 375)			PHiD-CV (N = 268)		7vCRM (N = 89)	
	% ≥0.20 μg/mL (95% CI)	% ≥0.35 μg/mL (95% CI)	GMC μg/mL (95% CI)	% ≥0.20 μg/mL (95% CI)	% ≥0.35 μg/mL (95% CI)	GMC μg/mL (95% CI)	% ≥8 (95% CI)	GMT Dil (95% CI)	% ≥8 (95% CI)	GMT Dil (95% CI)
Vaccine serotypes										
1	97.3 (96.1–98.2)	90.2 (88.3–91.9)	1.05 (1.00–1.10)	4.0 (2.3–6.6)	1.6 (0.6–3.5)	0.03 (0.03–0.03)	65.7 (59.7–71.3)	25.3 (21.0–30.6)	4.5 (1.2–11.1)	4.7 (3.9–5.6)
4	97.1 (95.9–98.0)	94.8 (93.4–96.1)	1.45 (1.38–1.53)	100 (99.0–100)	99.2 (97.7–99.8)	2.78 (2.58–3.00)	99.6 (97.9–100)	734.9 (655.6–823.9)	100 (95.9–100)	1010.4 (846.0–1206.7)
5	99.0 (98.2–99.5)	95.5 (94.1–96.6)	1.70 (1.62–1.78)	1.9 (0.8–3.8)	1.3 (0.4–3.1)	0.03 (0.03–0.03)	90.9 (86.7–94.1)	59.9 (51.4–69.7)	3.4 (0.7–9.6)	4.5 (3.9–5.3)
6B	65.9 (63.0–68.7)	54.8 (51.8–57.8)	0.33 (0.30–0.36)	79.0 (74.5–83.1)	70.7 (65.8–75.3)	0.59 (0.51–0.67)	92.4 (88.5–95.3)	457.4 (373.7–559.9)	95.5 (88.9–98.8)	999.4 (716.4–1394.4)
7F	99.5 (98.8–99.8)	97.4 (96.3–98.2)	1.72 (1.64–1.80)	4.5 (2.7–7.2)	2.7 (1.3–4.8)	0.04 (0.04–0.04)	99.6 (97.9–100)	2253.9 (1966.9–2582.8)	18.2 (10.8–27.8)	10.2 (6.5–15.8)
9V	98.1 (97.1–98.8)	94.0 (92.4–95.3)	1.32 (1.25–1.38)	99.5 (98.1–99.9)	98.4 (96.5–99.4)	2.68 (2.47–2.91)	100 (98.6–100)	1399.7 (1214.7–1577.9)	100 (95.9–100)	1233.3 (988.4–1538.8)
14	99.5 (98.9–99.9)	99.0 (98.2–99.5)	2.90 (2.75–3.05)	99.5 (98.1–99.9)	97.9 (95.8–99.1)	4.49 (4.07–4.96)	99.6 (97.9–100)	1061.0 (924.4–1217.7)	98.9 (93.9–100)	1890.6 (1439.8–2482.5)
18C	96.0 (94.7–97.1)	90.7 (88.8–92.3)	1.66 (1.56–1.77)	98.9 (97.3–99.7)	97.6 (95.5–98.9)	2.46 (2.25–2.69)	93.6 (90.0–96.2)	130.1 (111.1–152.4)	95.5 (88.8–98.7)	212.3 (162.1–278.1)
19F	95.4 (94.0–96.5)	89.1 (87.1–90.9)	1.84 (1.71–1.98)	99.2 (97.7–99.8)	98.1 (96.2–99.2)	3.42 (3.16–3.70)	87.7 (83.1–91.4)	148.6 (117.8–187.5)	92.1 (84.5–96.8)	52.0 (38.9–69.4)
23F	81.4 (79.0–83.7)	66.6 (63.7–69.4)	0.53 (0.50–0.57)	94.1 (91.2–96.3)	87.2 (83.3–90.4)	1.34 (1.18–1.52)	93.9 (90.2–96.5)	1010.0 (820.6–1243.1)	97.7 (91.9–99.7)	4412.9 (3313.2–5877.5)
Cross-reactive serotypes	(N = 279)			(N = 94)			(N = 260)		(N = 89)	
6A	22.2 (17.5–27.6)	9.7 (6.5–13.8)	0.07 (0.06–0.08)	31.2 (22.0–41.6)	19.4 (11.9–28.9)	0.10 (0.08–0.13)	58.0 (51.7–64.1)	40.9 (31.4–53.3)	68.5 (57.8–78.0)	83.3 (51.6–134.3)
19A	22.6 (17.8–27.9)	8.2 (5.3–12.1)	0.08 (0.07–0.09)	28.7 (19.9–39.0)	10.6 (5.2–18.7)	0.11 (0.09–0.13)	19.6 (15.0–25.0)	8.6 (7.1–10.5)	3.4 (0.7–9.5)	4.5 (3.9–5.3)

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); Dil, dilution of serum able to kill 50% of viable pneumococci.

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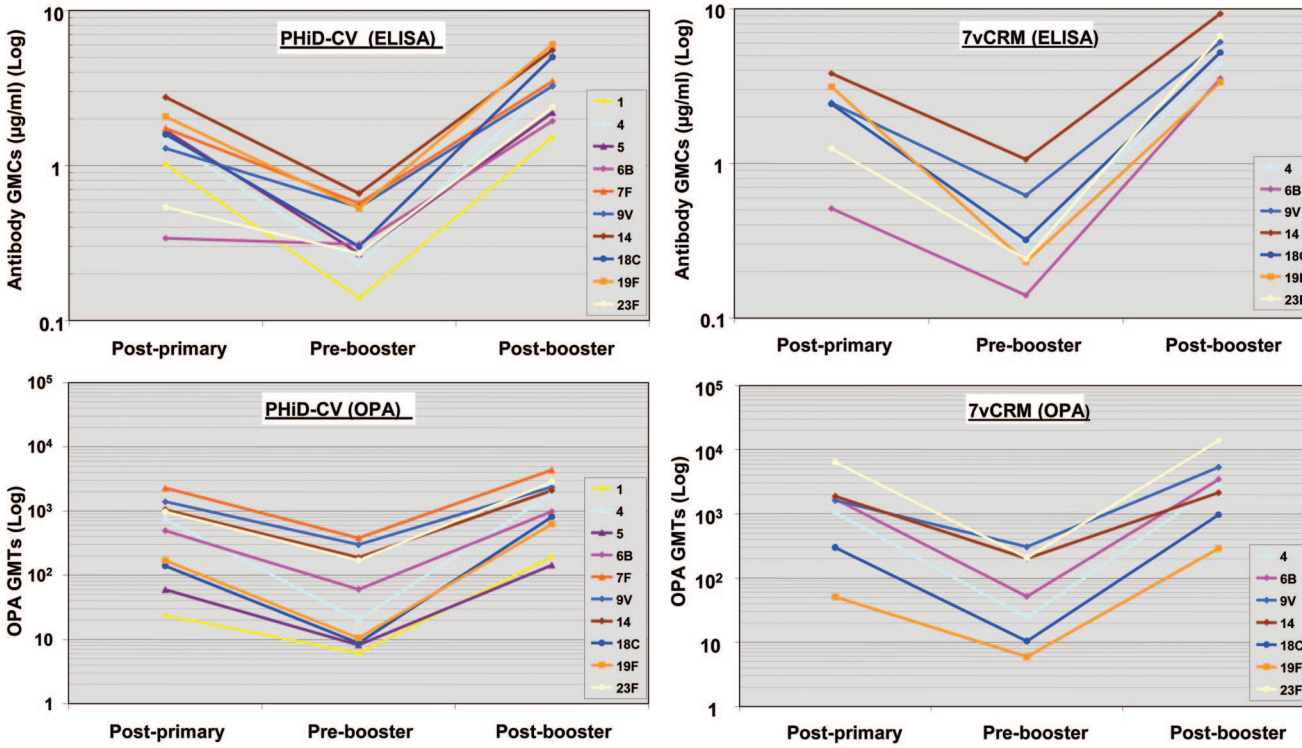
TABLE 3. Comparative Analysis Between the PHiD-CV and 7vCRM Vaccines for ELISA and OPA Responses 1 Month After the Third Primary Vaccine Dose (ATP Cohort for Immunogenicity)

Antibody	ELISA			OPA		
	Difference in % ≥0.2 μg/mL(7vCRM minus PHiD-CV)			Difference in % ≥8 (7vCRM minus PHiD-CV)		
	%	96.5% CI		%	95% CI	
Anti-4	2.89	1.71	4.16	0.37	−3.81	2.09
Anti-6B	13.12	7.53	18.28	3.14	−3.80	8.03
Anti-9V	1.37	−0.28	2.56	0.00	−4.14	1.41
Anti-14	−0.08	−1.66	0.71	−0.75	−5.73	1.14
Anti-18C	2.92	0.88	4.57	1.85	−5.09	6.52
Anti-19F	3.83	1.87	5.50	4.45	−3.72	10.61
Anti-23F	12.72	8.89	16.13	3.83	−2.20	7.96
	Difference in % ≥0.2 μg/mL (Aggregate 7vCRM* minus PHiD-CV)			Difference in % ≥8 (Aggregate 7vCRM† minus PHiD-CV)		
	%	96.5% CI		%	95% CI	
Anti-1	−1.52	−2.50	−0.52	31.42	27.01	36.05
Anti-5	−3.25	−4.02	−2.50	6.22	3.30	9.13
Anti-7F	−3.70	−4.42	−3.01	−2.53	−3.67	−1.32

*Aggregate 7vCRM % ≥0.2μg/mL = 95.8%.
†Aggregate 7vCRM % ≥8 = 97.1%.



N = number of subjects with available results at concerned timepoint (the actual number of subjects tested can slightly vary for the different serotypes, depending on the number of sera available).
Post-primary: PHiD-CV: N= 350 (ELISA) and N= 164 (OPA); 7vCRM: N= 90 (ELISA) and N= 16 (OPA)
Pre-booster: PHiD-CV: N= 347 (ELISA) and N= 327 (OPA); 7vCRM: N= 85 (ELISA) and N= 84 (OPA)
Post-booster: PHiD-CV: N= 343 (ELISA) and N= 340 (OPA); 7vCRM: N= 89 (ELISA) and N= 83 (OPA)

FIGURE 2. Kinetics of PHiD-CV and 7vCRM immune responses at different time points (post-primary, pre-booster and post-booster) for each pneumococcal vaccine serotype (ATP cohort for immunogenicity-Booster).

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TABLE 4. ELISA Antibody Concentrations Against Individual Pneumococcal Vaccine Serotypes and Cross-Reactive Serotypes 6A and 19A, Pre-Booster (PRE) and 1 Month After Booster Vaccination (POST) (ATP Cohort for Immunogenicity-Booster)

Serotype	PHiD-CV/PHiD-CV (N = 347)			7vCRM/7vCRM (N = 89)			7vCRM/PHiD-CV (N = 134)		
	% ≥0.20 μg/mL (95% CI)	% ≥0.35 μg/mL (95% CI)	GMC μg/mL (95% CI)	% ≥0.20 μg/mL (95% CI)	% ≥0.35 μg/mL (95% CI)	GMC μg/mL (95% CI)	% ≥0.20 μg/mL (95% CI)	% ≥0.35 μg/mL (95% CI)	GMC μg/mL (95% CI)
1									
Pre	36.4 (31.3–41.8)	14.8 (11.2–19.0)	0.14 (0.13–0.16)	3.7 (0.8–10.3)	1.2 (0.0–6.6)	0.03 (0.03–0.04)	2.3 (0.5–6.5)	1.5 (0.2–5.3)	0.03 (0.03–0.04)
Post	99.4 (97.9–99.9)	96.2 (93.6–98.0)	1.53 (1.40–1.68)	4.9 (1.4–12.2)	3.7 (0.8–10.4)	0.04 (0.03–0.05)	85.0 (77.7–90.6)	76.7 (68.6–83.6)	0.67 (0.56–0.80)
4									
Pre	57.3 (51.9–62.6)	36.3 (31.2–41.6)	0.23 (0.21–0.26)	67.9 (56.4–78.1)	42.3 (31.2–54.0)	0.30 (0.25–0.37)	75.6 (67.3–82.7)	50.4 (41.5–59.2)	0.35 (0.30–0.41)
Post	99.7 (98.4–100)	99.4 (97.9–99.9)	3.35 (3.06–3.67)	100 (95.9–100)	100 (95.9–100)	4.40 (3.75–5.15)	100 (97.3–100)	100 (97.3–100)	4.47 (3.85–5.19)
5									
Pre	67.2 (61.9–72.1)	40.4 (35.2–45.8)	0.27 (0.25–0.30)	6.0 (2.0–13.3)	2.4 (0.3–8.3)	0.04 (0.04–0.05)	10.4 (5.8–16.9)	3.7 (1.2–8.5)	0.05 (0.04–0.05)
Post	99.4 (97.9–99.9)	97.4 (95.1–98.8)	2.20 (2.00–2.42)	6.1 (2.0–13.7)	6.1 (2.0–13.7)	0.05 (0.04–0.07)	85.7 (78.6–91.2)	75.2 (67.0–82.3)	0.74 (0.60–0.90)
6B									
Pre	67.0 (61.6–72.0)	44.4 (39.0–50.0)	0.31 (0.27–0.35)	30.7 (20.5–42.4)	20.0 (11.6–30.8)	0.14 (0.11–0.19)	52.7 (43.8–61.5)	36.6 (28.4–45.5)	0.26 (0.20–0.33)
Post	96.5 (93.9–98.2)	95.6 (92.8–97.5)	1.94 (1.74–2.17)	97.7 (91.9–99.7)	97.7 (91.9–99.7)	3.53 (2.83–4.41)	98.5 (94.7–99.8)	96.2 (91.4–98.8)	1.74 (1.48–2.05)
7F									
Pre	90.6 (87.0–93.5)	76.2 (71.3–80.6)	0.57 (0.52–0.62)	4.7 (1.3–11.6)	3.5 (0.7–10.0)	0.03 (0.03–0.04)	2.3 (0.5–6.5)	1.5 (0.2–5.3)	0.03 (0.03–0.03)
Post	100 (98.9–100)	99.7 (98.4–100)	3.50 (3.25–3.76)	7.1 (2.6–14.7)	4.7 (1.3–11.6)	0.04 (0.03–0.05)	95.5 (90.4–98.3)	92.5 (86.6–96.3)	1.83 (1.49–2.24)
9V									
Pre	84.6 (80.3–88.2)	69.8 (64.6–74.6)	0.54 (0.48–0.60)	90.9 (82.2–96.3)	80.5 (69.9–88.7)	0.62 (0.51–0.76)	94.6 (89.2–97.8)	84.6 (77.2–90.3)	0.78 (0.68–0.89)
Post	100 (98.9–100)	99.7 (98.4–100)	3.25 (2.99–3.53)	100 (95.9–100)	100 (95.9–100)	6.09 (5.19–7.15)	100 (97.3–100)	100 (97.3–100)	1.94 (1.73–2.19)
14									
Pre	79.8 (75.1–83.9)	68.2 (62.9–73.1)	0.66 (0.56–0.76)	93.3 (85.1–97.8)	84.0 (73.7–91.4)	1.06 (0.82–1.38)	96.2 (91.3–98.7)	92.3 (86.3–96.2)	1.69 (1.40–2.03)
Post	99.1 (97.4–99.8)	98.5 (96.6–99.5)	5.56 (5.01–6.18)	100 (95.8–100)	100 (95.8–100)	9.29 (7.85–10.99)	100 (97.3–100)	99.2 (95.9–100)	4.76 (4.12–5.49)
18C									
Pre	70.4 (65.2–75.2)	45.7 (40.4–51.2)	0.30 (0.28–0.34)	72.3 (61.4–81.6)	51.8 (40.6–62.9)	0.32 (0.26–0.39)	81.7 (74.0–87.9)	51.9 (43.0–60.7)	0.37 (0.32–0.43)
Post	100 (98.9–100)	100 (98.9–100)	5.01 (4.60–5.46)	100 (95.8–100)	100 (95.8–100)	5.21 (4.44–6.11)	99.3 (95.9–100)	98.5 (94.7–99.8)	4.98 (4.17–5.93)
19F									
Pre	78.4 (73.7–82.6)	61.4 (56.0–66.5)	0.53 (0.46–0.61)	44.7 (33.9–55.9)	31.8 (22.1–42.8)	0.23 (0.17–0.31)	56.7 (47.9–65.2)	35.1 (27.0–43.8)	0.31 (0.24–0.41)
Post	99.4 (97.9–99.9)	98.8 (97.0–99.7)	6.05 (5.46–6.71)	100 (95.8–100)	98.9 (93.8–100)	3.35 (2.83–3.97)	97.8 (93.6–99.5)	97.8 (93.6–99.5)	5.06 (4.24–6.04)
23F									
Pre	60.9 (55.5–66.2)	42.0 (36.7–47.5)	0.27 (0.23–0.31)	55.8 (44.1–67.2)	36.4 (25.7–48.1)	0.24 (0.19–0.31)	75.4 (67.1–82.5)	50.8 (41.9–59.6)	0.40 (0.32–0.50)
Post	97.4 (95.0–98.8)	96.2 (93.6–98.0)	2.38 (2.13–2.66)	98.9 (93.8–100)	98.9 (93.8–100)	6.67 (5.38–8.26)	97.0 (92.4–99.2)	96.2 (91.4–98.8)	2.42 (1.99–2.95)
6A									
Pre	32.5 (27.6–37.8)	20.1 (16.0–24.8)	0.13 (0.11–0.15)	23.3 (14.8–33.6)	11.6 (5.7–20.3)	0.09 (0.07–0.12)	29.3 (21.8–37.8)	15.8 (10.0–23.1)	0.12 (0.09–0.15)
Post	84.4 (80.1–88.1)	70.6 (65.4–75.4)	0.71 (0.61–0.83)	89.5 (81.1–95.1)	84.9 (75.5–91.7)	1.53 (1.12–2.08)	77.6 (69.6–84.4)	64.9 (56.2–73.0)	0.59 (0.46–0.76)
19A									
Pre	35.0 (29.9–40.3)	15.6 (11.9–19.9)	0.14 (0.12–0.16)	15.2 (8.1–25.0)	10.1 (4.5–19.0)	0.08 (0.06–0.11)	24.8 (17.7–33.0)	14.3 (8.8–21.4)	0.10 (0.08–0.13)
Post	83.8 (79.4–87.6)	71.8 (66.6–76.5)	0.85 (0.73–1.00)	76.5 (65.8–85.2)	55.6 (44.1–66.6)	0.47 (0.37–0.62)	79.1 (71.2–85.6)	56.7 (47.9–65.2)	0.55 (0.44–0.69)

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 and timepoints depending on the number of sera available).

in the PHiD-CV group and 94.9% in the 7vCRM group), for 19A it was 48.8% in the PHiD-CV group and 27.6% in the 7vCRM group (Table 5).

In 7vCRM primed subjects who received a booster dose of PHiD-CV, post-booster antibody GMCs and OPA GMTs were generally lower compared with 4 consecutive doses of the same

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TABLE 5. OPA Titers Against Individual Pneumococcal Vaccine Serotypes and Cross Reactive Serotypes 6A and 19A Pre-Booster (Pre) and 1 Month After Booster Vaccination (POST) (ATP Cohort for Immunogenicity-Booster)

Serotype	PHiD-CV/PHiD-CV (N = 327)		7vCRM/7vCRM (N = 84)		7vCRM/PHiD-CV (N = 126)	
	% ≥8 (95% CI)	GMT Dil (95% CI)	% ≥8 (95% CI)	GMT Dil (95% CI)	% ≥8 (95% CI)	GMT Dil (95% CI)
1						
Pre	16.3 (12.4–20.7)	6.1 (5.4–6.9)	6.0 (2.0–13.5)	5.0 (4.1–6.0)	5.6 (2.3–11.1)	4.9 (4.2–5.8)
Post	91.0 (87.2–94.0)	192.2 (157.5–234.6)	3.6 (0.8–10.2)	4.3 (3.9–4.7)	31.4 (23.3–40.5)	8.3 (6.7–10.4)
4						
Pre	47.8 (42.0–53.7)	20.3 (16.2–25.5)	51.9 (40.4–63.3)	24.5 (15.4–38.8)	60.7 (51.0–69.8)	37.8 (24.8–57.7)
Post	100 (98.8–100)	1856.3 (1666.1–2068.0)	100 (95.5–100)	2812.6 (2282.5–3465.9)	100 (97.0–100)	1528.9 (1286.5–1817.0)
5						
Pre	34.4 (29.1–40.1)	8.2 (7.2–9.4)	3.8 (0.8–10.7)	4.4 (3.9–4.9)	1.6 (0.2–5.7)	4.1 (3.9–4.3)
Post	96.3 (93.5–98.1)	144.1 (122.1–170.0)	1.2 (0.0–6.5)	4.1 (3.9–4.4)	36.9 (28.3–46.1)	9.5 (7.5–11.9)
6B						
Pre	58.8 (52.8–64.6)	60.3 (44.6–81.7)	53.3 (41.4–64.9)	51.4 (27.1–97.5)	50.0 (40.7–59.3)	36.7 (23.3–57.7)
Post	96.6 (93.9–98.4)	981.2 (830.7–1159.1)	98.7 (93.1–100)	3459.6 (2535.7–4720.3)	94.9 (89.3–98.1)	640.2 (480.7–852.7)
7F						
Pre	78.9 (73.8–83.4)	377.7 (279.8–509.9)	36.8 (26.1–48.7)	34.8 (17.5–69.3)	32.5 (24.1–41.8)	25.3 (15.4–41.6)
Post	99.7 (98.1–100)	330.3 (3836.0–4888.3)	31.1 (20.8–42.9)	25.2 (13.1–48.7)	98.3 (94.0–99.8)	2397.2 (1929.2–2978.7)
9V						
Pre	97.1 (94.5–98.7)	296.9 (259.3–339.9)	96.3 (89.6–99.2)	305.5 (227.1–411.0)	99.2 (95.5–100)	305.1 (248.7–374.4)
Post	100 (98.8–100)	2343.5 (2097.1–2618.7)	100 (95.4–100)	5357.4 (4212.5–6813.6)	100 (97.0–100)	886.8 (747.7–1051.8)
14						
Pre	82.9 (78.2–87.0)	188.1 (149.9–235.9)	83.5 (73.5–90.9)	201.6 (129.4–314.2)	94.9 (89.3–98.1)	391.1 (303.1–504.6)
Post	100 (98.8–100)	2085.9 (1868.0–2329.1)	100 (95.6–100)	2134.2 (1689.1–2696.6)	100 (97.0–100)	977.8 (828.9–1153.5)
18C						
Pre	29.4 (24.4–34.9)	8.7 (7.4–10.3)	34.1 (24.0–45.4)	10.4 (7.4–14.7)	28.9 (21.0–37.9)	8.5 (6.6–10.9)
Post	99.7 (98.2–100)	810.3 (712.4–921.7)	100 (95.3–100)	968.7 (724.1–1295.8)	98.3 (94.2–99.8)	610.7 (480.3–776.4)
19F						
Pre	45.7 (40.2–51.4)	10.5 (8.9–12.3)	14.8 (7.9–24.4)	5.9 (4.5–7.8)	17.9 (11.6–25.8)	7.3 (5.5–9.6)
Post	94.9 (91.7–97.1)	624.3 (509.7–764.7)	92.5 (84.4–97.2)	287.8 (190.8–434.3)	98.3 (94.1–99.8)	530.1 (393.0–715.2)
23F						
Pre	70.8 (65.4–75.9)	171.5 (126.3–232.8)	74.0 (62.8–83.4)	205.8 (110.1–384.6)	85.0 (77.3–90.9)	532.9 (344.4–824.7)
Post	99.7 (98.2–100)	2830.1 (2487.2–3220.3)	98.8 (93.2–100)	13900.7 (10177.4–18986.1)	98.4 (94.2–99.8)	2828.8 (2234.3–3581.6)
6A						
Pre	55.4 (49.5–61.3)	38.0 (29.6–48.8)	50.6 (39.0–62.2)	26.1 (16.5–41.4)	48.1 (38.2–58.1)	24.6 (16.5–36.6)
Post	85.0 (80.4–88.9)	254.8 (202.8–320.1)	94.9 (87.5–98.6)	872.7 (597.1–1275.7)	73.5 (64.3–81.3)	100.8 (68.3–148.8)
19A						
Pre	4.9 (2.8–7.8)	4.8 (4.4–5.3)	2.4 (0.3–8.3)	4.6 (3.8–5.5)	4.8 (1.8–10.1)	5.0 (4.1–6.0)
Post	48.8 (42.9–54.7)	29.2 (22.3–38.3)	27.6 (18.0–39.1)	11.1 (7.3–17.0)	23.1 (16.0–31.7)	9.7 (7.0–13.3)

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 and timepoints depending on the number of sera available).

pneumococcal conjugate (7vCRM) vaccine. Still, at least 97.0% and 94.9% of subjects had 22F-ELISA antibody concentrations ≥0.2 μg/mL and opsonophagocytic antibody titers ≥8 respectively, for the serotypes common to both vaccines (Tables 4, 5). The fold increase in post-booster antibody GMCs for these 7 serotypes ranged from 2.5 (serotype 9V) to 16.3 (serotype 19F) and the fold increase in OPA GMTs from 2.5 (serotype 14) to 72.6 (serotype 19F), compared with pre-booster level. At least 85% of subjects had antibody concentrations ≥0.2 μg/mL for serotypes 1 and 5 and 95.5% for serotype 7F, but the percentage of subjects

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with OPA titer ≥8 remained low for serotypes 1 and 5 (31.4% and 36.9%, respectively), although higher than subjects primed and boosted with the 7vCRM vaccine. For serotype 7F, 98.3% of the subjects who were not primed with this type and only received a booster dose of PHiD-CV, had an OPA titer ≥8. Compared with pre-booster levels, the post-booster antibody GMCs increased by 22.3-fold (serotype 1), 14.8-fold (serotype 5), and 61.0-fold (serotype 7F), while OPA GMTs increased by 1.7-fold (serotype 1), 2.3-fold (serotype 5), and 94.8-fold (serotype 7F).

Response to Protein D

After PHiD-CV primary vaccination, all except 2 subjects had measurable antibodies against protein D (≥100 EL.U/mL) with an anti-PD antibody GMC of 1529.9 EL.U/mL, compared with 66.1 EL.U/mL in the 7vCRM group. A booster response was also observed in PHiD-CV primed subjects with antibody GMCs of 2887.6 EL.U/mL compared with 75.3 EL.U/mL in the group primed and boosted with 7vCRM. In 7vCRM primed subjects who received a booster dose of PHiD-CV, the post-booster anti-PD antibody GMC was 125.5 EL.U/mL, with 50% of subjects with measurable protein D antibodies compared with 18.6% in the 7vCRM booster group.

DISCUSSION

This article presents an evaluation of the immunogenicity in terms of antibody concentrations and opsonophagocytic activity after primary vaccination with the novel PHiD-CV vaccine compared with the licensed 7-valent pneumococcal conjugate vaccine (7vCRM), as well as subsequent antibody persistence and booster responses. In the absence of a definitive serological correlate of protection against IPD, WHO has recommended to use the percentage of vaccinees with ELISA antibody concentration ≥0.35 μg/mL (equivalent to 0.2 μg/mL with GSK's ELISA with 22F inhibition) for comparison of candidate and licensed pneumococcal conjugate vaccines,[26] on the basis of a pooled analysis of data from 3 vaccine efficacy trials with invasive disease endpoints.[15,37,38] In addition, the functionality of the elicited antibodies is also required to be demonstrated using an opsonophagocytic activity (OPA) assay, because it is considered to be the primary mechanism of host defense against pneumococcal disease[39,40] and was observed to correlate better with protection against invasive disease and AOM than antibody levels.[33,41]

The study was a direct immunologic comparison of the registered 7vCRM vaccine with the candidate PHiD-CV, using the percentage of subjects with a postimmunization antibody concentration of at least 0.2 μg/mL as the criterion to determine noninferiority. For the 3 pneumococcal serotypes not contained in the 7vCRM vaccine it was not possible to make a direct comparison, so noninferiority was evaluated compared with the aggregate response of 7vCRM vaccine (ie, percentage of pneumococcal antibody concentrations ≥0.2 μg/mL across all 7 7vCRM serotypes) as recommended by the WHO.[26]

Primary vaccination with PHiD-CV induced ELISA antibody responses against all pneumococcal serotypes contained in the vaccine including serotypes 1, 5, and 7F. For the 7 shared serotypes, postvaccination antibody GMCs in the PHiD-CV group were lower than those observed in the 7vCRM group, but the post-primary ranking of the ELISA antibody GMCs was similar for both vaccines with highest levels for serotypes 14 and 19F and lowest for 6B and 23F. Based on the ELISA threshold however, noninferiority of the PHiD-CV versus 7vCRM vaccine was demonstrated for 8 (1, 4, 5, 7F, 9V, 14, 18C and 19F) of the 10 vaccine serotypes, thus meeting the primary objective of the study to show noninferiority for at least 7 serotypes. Although noninferiority could not be demonstrated for serotypes 6B and 23F based on the 0.2 μg/mL ELISA antibody threshold, primary vaccination with PHiD-CV-induced functional OPA antibodies against all vaccine serotypes, including high response rates (>92%) for serotypes 6B and 23F. A post-hoc analysis based on the percentage of subjects with measurable opsonophagocytic activity suggested noninferiority of the PHiD-CV versus the 7vCRM vaccine, with observed differences below 5% for all the pneumococcal serotypes common to both vaccines, including serotypes 6B and 23F.

It is noteworthy that in the AOM efficacy trial with the 11-valent PD-conjugate vaccine formulation, high and statistically significant efficacy was demonstrated against AOM due to serotypes 6B (87.6%) and 23F (72.3%), despite lower ELISA antibody levels compared with other vaccine serotypes[22] therefore testifying for the excellent biologic functionality of these antibodies.

For new pneumococcal conjugate vaccines, which contain more serotypes than the licensed vaccine, it is important to put head-to-head immunologic comparisons into context by considering the overall potential vaccine effectiveness against IPD. As this will be an aggregate value covering the contributions of all vaccine serotypes, it is likely that minor differences in noninferiority of immunogenicity for 1 or 2 serotypes may well be overshadowed by the additional protection provided by the extra serotypes. The 10-valent PHiD-CV vaccine contains the extra serotypes 1, 5, and 7F which together account for approximately 15% of global pneumococcal morbidity and mortality.[42] A 13-valent vaccine is in development,[43,44] which also contains serotypes 3, 19A, and 6A adding a further 7% to global serotype coverage.[42] The relative epidemiological importance of each vaccine serotype varies however in the different countries. A method to translate comparative immunogenicity data into estimated overall IPD impact taking this geographical variation into account as well as differences in individual serotype-specific effectiveness values was recently proposed and may well prove useful in helping to understand the potential public health benefit provided by new multivalent pneumococcal conjugate vaccines on IPD.[45,46]

As previously reported,[47–49] a decrease in serotype specific antibody levels was observed in the 8–12 months after primary vaccination. Although post-primary antibody levels were higher in the 7vCRM group, the subsequent decline of antibody levels was such that before the booster dose, antibody GMCs and OPA GMTs were similar for PHiD-CV and 7vCRM primed groups. For most serotypes, the majority of vaccinees in both groups still had persisting anti-pneumococcal antibody concentrations ≥0.2 μg/mL before the booster dose, but the opsonophagocytic antibody levels declined below 50% for some serotypes. For serotype 19F, the opsonophagocytic antibodies declined significantly in the recipients of both vaccines, but significantly more in the 7vCRM group.

Robust increases in ELISA and OPA responses compared with pre-booster levels were observed after a fourth consecutive dose of the PHiD-CV vaccine, and confirm priming of the immune system against all vaccine serotypes and induction of immunologic memory. With the exception of serotype 19F, the magnitude of the ELISA and OPA responses were lower in PHiD-CV-primed subjects for the common serotypes. It is noteworthy that despite a relatively low post-primary OPA response in PHiD-CV vaccinees against serotype 1, a substantial booster response was observed for this serotype, indicating adequate priming of the immune system. It is reasonable to assume good IPD protection given the similarities in terms of percentages of subjects reaching the post-primary and post-booster thresholds for OPA and ELISA. However, clinical data will be needed to better understand the impact of PHiD-CV on nasopharyngeal carriage, herd immunity, and pneumonia.

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Booster interchangeability was also explored. A booster dose of PHiD-CV in 7vCRM primed children induced increases in both ELISA and OPA antibody levels compared with pre-booster levels for all serotypes common to both vaccines. Although post-booster antibody levels and OPA GMTs were lower for most serotypes compared with 4 consecutive doses of the same pneumococcal conjugate vaccine, at least 97.0% of subjects reached the ELISA threshold of ≥0.2 μg/mL and 95% reached the OPA seropositivity for the common serotypes. For serotype 9V, ELISA GMCs, and OPA GMTs were lower in the interchangeability group compared with the 2 other study groups. There is currently no explanation for this observation, but 100% of subjects reached the ELISA and OPA thresholds post-booster in all 3 study groups. These results therefore support the use of PHiD-CV for booster vaccination after 7vCRM priming. In addition, after the PHiD-CV booster, at least 85% of the 7vCRM primed subjects also reached the ELISA threshold for serotypes 1 and 5 and 95.0% for serotype 7F. Although OPA responses remained low, they clearly exceeded the OPA responses against serotypes 1, 5, and 7F in subjects primed and boosted with 7vCRM. Whether these responses will be sufficient to provide protection against these additional serotypes after a single booster dose of PHiD-CV, is currently not known.

Finally, it is of interest to monitor responses to the cross-reactive serotypes 6A and 19A, especially in the light of recent emergence of 19A as a cause of IPD in the US and other countries.[50–53] Low ELISA responses against both 6A and 19A were detected after primary vaccination with either PHiD-CV or 7vCRM. Stronger post-primary OPA responses were measured for serotype 6A, but not for 19A for which the OPA response remained weak particularly in 7vCRM primed subjects. Booster vaccination substantially increased the percentage of subjects reaching the ELISA threshold for both 6A and 19A and the OPA threshold for 6A. Post-booster OPA responses remained low particularly in the 7vCRM primed subjects.

In conclusion, the results of this study show that PHiD-CV induces ELISA and functional OPA antibodies for all vaccine serotypes after primary vaccination and overall is noninferior to 7vCRM in terms of ELISA and/or OPA threshold responses. Effective priming is further indicated by robust booster responses. It is therefore reasonable to assume that PHiD-CV will provide at least similar protection against IPD than the licensed 7vCRM vaccine.

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