Immunogenicity of a 2-Dose Priming and Booster Vaccination With the 10-Valent Pneumococcal Nontypeable *Haemophilus influenzae* Protein D Conjugate Vaccine

Sven Arne Silfverdal, MD, Birthe Hogh, MD,† Marianne Riise Bergsaker, MD,‡ Helena Skerlikova, MD,§ Patricia Lommel, BScb,¶ Dorota Borys, MD,¶ and Lode Schuerman, MD¶*

Background: The immunogenicity of the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV) was determined following a simplified 2-dose priming and the more commonly employed 3-dose priming both followed by a booster dose.

Methods: A total of 351 healthy subjects were primed with PHiD-CV at either 3 and 5 or 3, 4 and 5 months of age followed in all subjects by a booster dose at 11 to 12 months of age. Serotype-specific pneumococcal responses were measured by 22F-inhibition ELISA and opsonophagocytic assays 1 month following primary and booster vaccinations.

Results: Depending on the serotype, the percentages of subjects reaching the ELISA antibody threshold of 0.2 μg/mL were 92.8% to 98.0% following 2 primary doses and 96.1% to 100% following 3 primary doses except for serotype 6B (55.7% and 63.1%, respectively) and serotype 23F (69.3% and 77.6%, respectively). Opsonophagocytic activity (OPA) could be measured in 74.4% to 100% and 88.9% to 100% of the subjects after the 2-dose or 3-dose priming, respectively, except for serotype 1 (60.8% and 62.9%, respectively). In both groups, robust increases in ELISA antibodies and OPA titers were observed for all serotypes after the booster dose. Higher postprimary and postbooster ELISA antibody levels and OPA titers were observed for most serotypes following the 3+1 schedule.

Conclusion: PHiD-CV was immunogenic in both schedules, but further effectiveness data are needed to fully understand the public health benefit to be expected from these schedules in terms of prevention against invasive and mucosal infections.

Key Words: pneumococcal conjugate 10-valent vaccine, immunogenicity, 2-dose primary schedule (2+1 schedule)

(*Pediatr Infect Dis J* 2009;28: e276–e282)

Accepted for publication June 24, 2009.

From the *Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden; †Department of Pediatrics, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark; ‡Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway; §GPP Practice, Dolny Kubin, Slovakia; and ¶Global Clinical Development Center, GlaxoSmithKline Biologicals, Rixensart, Belgium.

Supported by GlaxoSmithKline Biologicals, Rixensart, Belgium. Dr. SA Silfverdal received consulting fees in the past three years from the commercial entity that sponsored the study. P. Lommel is employed by the commercial entity that sponsored the study. The authors M.R.B., B.H., and H.S. have no conflict of interest to declare. The authors L.S. and D.B. are employed by the commercial entity that sponsored the study and have stock ownership.

Infanrix hexa and Infanrix IPV Hib are trademarks of the GlaxoSmithKline group of companies.

Address for correspondence: Sven Arne Silfverdal, MD, Department of Clinical Sciences, Pediatrics, Umeå University, SE 901 85 Umeå, Sweden. E-mail: sven.arne.silfverdal@pediatri.umu.se.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

ISSN: 0891-3668/09/2810-0276
DOI: 10.1097/INF.0b013e3181b48ca3

Pneumococcal conjugate vaccines (PCV) have been designed to prevent pneumococcal disease in young children. Several vaccines have been developed containing capsular polysaccharides from 7 up to 13 *Streptococcus pneumoniae* serotypes conjugated individually to different carrier proteins such as CRM197 (a nontoxic cross-reacting mutant of diphtheria toxin), outer membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B, and tetanus or diphtheria toxoids. One 7-valent vaccine (PCV7-CRM containing capsular polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to CRM197) has been licensed in many countries based on its efficacy against invasive pneumococcal disease (IPD) caused by vaccine serotypes.[1,2] Both PCV7-CRM and another vaccine containing the same polysaccharides conjugated to OMPC (PCV7-OMPC) have demonstrated efficacy against acute otitis media (AOM) caused by pneumococcal vaccine serotypes.[3,4]

A 10-valent pneumococcal conjugate vaccine (PHiD-CV) was developed containing 3 more serotypes (1, 5, 7F) than the PCV7-CRM vaccine. It is a mixed carrier vaccine containing 8 capsular polysaccharides (1, 4, 5, 6B, 7F, 9V, 14, 23F) conjugated individually to nontypeable *Haemophilus influenzae* protein D (PD) and the remaining 2 conjugated to tetanus (serotype 18C) or diphtheria (serotype 19F) toxoids. Protein D, which is a cell-surface protein highly conserved among *H. influenzae* strains,[5–8] was selected for its potential to provide protection against *H. influenzae* infections.[9,10] In a double-blind randomized controlled efficacy study, an earlier formulation of an 11-valent PD conjugate vaccine was shown to be safe and immunogenic in children and demonstrated significant protective efficacy against AOM caused by pneumococcal vaccine serotypes and also *H. influenzae.*[11] The safety and immunogenicity of the PHiD-CV formulation have been documented.[12–14]

The vaccination schedule recommended for PCV and used in most clinical studies, including those demonstrating efficacy against IPD[1,2] and AOM,[3,11] consists of 4 vaccine doses, ie, a 3-dose primary series in the first 6 months of life followed by a fourth booster dose in the second year of life. As this 3+1 schedule is in line with most national routine vaccination schedules in developed countries, it allows concomitant administration of PCV with other childhood vaccines. However, some countries in Europe employ a 2+1 schedule with a 2-dose primary series in the first 6 months of life, followed by a booster dose between 11 and 15 months of age. For these countries administration of PCV would be facilitated if a 2+1 schedule could be recommended. In addition, a reduced primary schedule would lower the cost of a vaccination course of PCV. In this study we determined the immunogenicity of PHiD-CV following both 2+1 and 3+1 vaccination schedules.

MATERIALS AND METHODS

Study Design and Participants

This was an open randomized study (105539/NCT00307034) conducted in Denmark, Norway, Slovakia, and Sweden. The pri-

mary objective was to assess the postprimary pneumococcal antibody response of a 2-dose primary vaccination schedule (2+1 group) relative to 3-dose priming (3+1 group). Secondary objectives included the assessment of the pneumococcal antibody persistence and the immunogenicity of a booster dose. Eligible participants were healthy male or female infants aged 8 to 16 weeks at the time of the administration of the first vaccine dose. 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. Written informed consent was obtained from each subjects' parent/guardian prior to the performance of any study-specific procedures.

Vaccines, Vaccinations, and Blood Sampling

The PHiD-CV vaccine (GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium) contained 1 μg of each capsular polysaccharide for pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14, and 23F and 3μg for serotype 4 conjugated to Protein D, 3μg of capsular polysaccharide of serotype 18C conjugated to tetanus toxoid and 3μg of capsular polysaccharide of serotype 19F conjugated to diphtheria toxoid.

Subjects were randomized (1:1) to be vaccinated with PHiD-CV vaccine at approximately 3, 5, and 11 to 12 months of age (2+1 group) or at approximately 3, 4, 5, and 11 to 12 months of age (3+1 group).

In both groups, diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-Hib vaccine (Infanrix Hexa, GSK Biologicals, Rixensart, Belgium) in Slovakia and Sweden or diphtheria-tetanus-acellular pertussis-inactivated polio-Hib vaccine (Infanrix IPV Hib, GSK Biologicals, Rixensart, Belgium) in Denmark and Norway were coadministered at approximately 3, 5, and 11 to 12 months of age according to national recommendations. The PHiD-CV vaccine was administered by intramuscular injection into the thigh, with coadministered vaccines injected into the opposite thigh.

Blood samples were obtained approximately 1 month following primary vaccination, just prior to and approximately 1 month following booster vaccination and sera were stored at −20°C until analysis.

Serological Methods

Serum antipneumococcal IgG antibody concentrations to each of the serotypes included in PHiD-CV were measured at GSK Biologicals Rixensart laboratories by an ELISA, which included adsorption with serotype 22F polysaccharide to increase the assay specificity (22F-ELISA) as previously described.[15] The assay cut-off was 0.05 μg/mL. It has been established[16] that an antibody concentration of 0.2 μg/mL as determined by this 22F-ELISA corresponds to an antibody concentration of 0.35 μg/mL as determined by the WHO reference laboratory ELISA without 22F inhibition and proposed by WHO to be used as reference antibody concentration for demonstration of noninferiority of the immune response of a new PCV compared with the registered vaccine.[17] The percentages of subjects with antibody concentration ≥0.35 μg/mL as measured with GSK's 22F-ELISA are also provided (corresponding to an antibody concentration of ≥0.5 μg/mL when using the WHO reference laboratory ELISA).

Opsonophagocytic activity (OPA) was measured using a modification of the HL-60 cell reference method[18,19] which has been validated using sera collected following primary vaccination with PCV7-CRM.[20] The results are presented as the reverse/reciprocal dilution of serum (opsonic titer) able to sustain 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).

IgG antibodies to the *H. influenzae* protein D were measured by ELISA and were expressed in ELISA units (EL.U) per mL, the cut-off of the assay is 100 EL.U/mL. Antidiphtheria and antitetanus antibodies were determined by ELISA, with an assay cut-off of 0.1 IU/mL, above which there is a good correlation between the ELISA and in vivo neutralization tests.[21,22]

Descriptive Analyses

Immunogenicity was analyzed on the according to protocol (ATP) immunogenicity cohort. The following parameters were calculated with 95% confidence intervals (CI) for each treatment group and for all blood sampling time-points: (i) the percentages of subjects with 22F-ELISA pneumococcal antibody concentration ≥0.2 μg/mL or ≥0.35 μg/mL and geometric mean antibody concentrations (GMCs) for each individual pneumococcal vaccine serotype, (ii) the percentages of subjects with OPA titer ≥8 and geometric mean OPA titers (GMTs) for each individual pneumococcal vaccine serotype, (iii) the percentages of subjects with anti-PD antibody concentration ≥100 EL.U/mL and anti-PD antibody GMCs and (iv) antibody GMCs for antidiphtheria and antitetanus antibodies.

A post hoc analysis of pneumococcal ELISA and OPA immune responses stratified according to age at first dose was also performed.

Inferential Analysis

The standardized asymptotic 95% CIs for the difference between groups (3+1 group minus 2+1 group) in terms of the percentages of subjects with pneumococcal antibody concentration ≥0.2 μg/mL 1 month after primary vaccination, prior to and 1 month after booster vaccination were computed for each serotype. Two-sided *P*-values, to detect statistically significant differences, were computed using Chi-Square Test (for percentages) or Schuirmann Test (for concentrations/titers).

RESULTS

Participants

A total of 351 subjects were enrolled and vaccinated (175 in the 2-dose priming group and 176 in the 3-dose priming group) and 342 subjects completed the study. Figure 1 shows the exclusions from the ATP immunogenicity cohort. The demographic profiles of the ATP immunogenicity cohort were comparable between the 2-dose and 3-dose priming groups, with respect to mean weight at birth (3.6 and 3.5 kg) and age at first vaccination (12.0 and 12.2 weeks), gender repartition (50.6% and 51.9% male) and racial distribution (98.7% and 96.8% white Caucasian). PHiD-CV vaccine doses were administered at 12.0 ± 1.91, 20.8 ± 2.06 weeks and 11.1 ± 0.61 months of age in the 2+1 group, and at 12.2 ± 1.90, 16.7 ± 2.09, 21.4 ± 2.27 weeks and 11.2 ± 0.63 months of age in the 3-dose priming group.

Immune Responses

Impact of Vaccination Schedule on Pneumococcal Responses

Serum antipneumococcal IgG antibody levels are presented in Table 1 and illustrated in Figure (Supplemental Digital Content 1, http://links.lww.com/INF/A168).

Depending on the serotype, 92.8% to 98.0% of the subjects who received a 2-dose priming and 96.1% to 100% of the subjects who received a 3-dose priming had antibody concentrations ≥0.2 μg/mL 1 month postprimary vaccination, except for serotype 6B (55.7% in the 2+1 group and 63.1% in the 3+1 group, $P = 0.1944$), and serotype 23F (69.3% in the 2+1 group and 77.6% in the 3+1 group, $P = 0.0987$). Persistence of the immune response

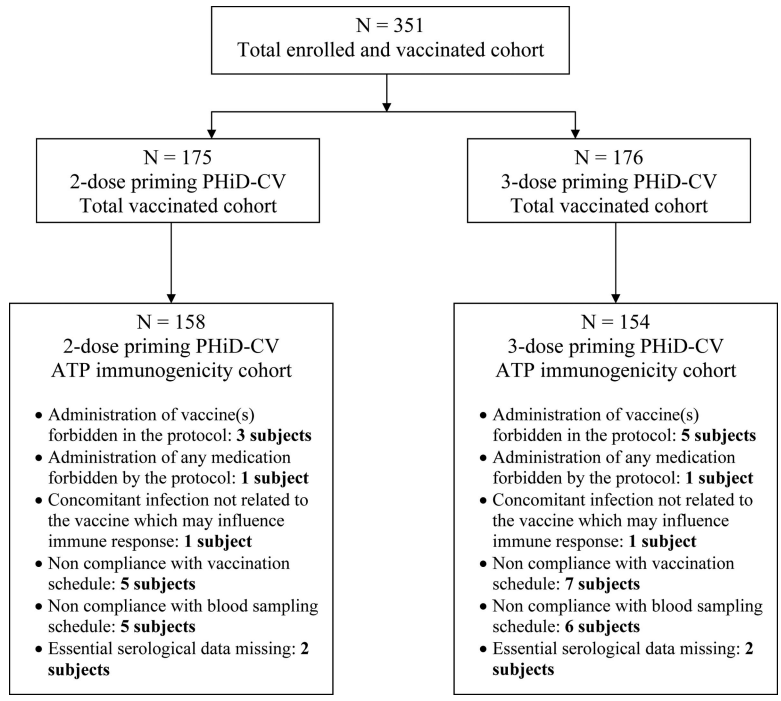

FIGURE 1. Trial profile.

TABLE 1. ELISA Antibody Responses Against Individual Pneumococcal Serotypes 1 Month Following Primary Vaccination and 1 Month Following Booster Vaccination (ATP Cohort for Immunogenicity)

Pneumococcal Serotype	Study Group	Postprimary			Postbooster		
		% IgG Antibody ≥0.2 μg/mL (95% CI)	% IgG Antibody ≥0.35 μg/mL (95% CI)	IgG Antibody GMC (μg/mL) (95% CI)	% IgG Antibody ≥0.2 μg/mL (95% CI)	% IgG Antibody ≥0.35 μg/mL (95% CI)	IgG Antibody GMC (μg/mL) (95% CI)
Serotype 1	2+1	97.4 (93.4–99.3)	86.3 (79.8–91.3)	1.03 (0.90–1.18)	99.4 (96.5–100)	95.5 (91.0–98.2)	1.85 (1.59–2.15)
	3+1	98.7 (95.3–99.8)	90.7 (84.9–94.8)	1.23 (1.07–1.42)	100 (97.5–100)	97.3 (93.2–99.3)	1.88 (1.62–2.17)
Serotype 4	2+1	98.0 (94.4–99.6)	94.8 (90.0–97.7)	1.37 (1.21–1.55)	100 (97.6–100)	100 (97.6–100)	3.06 (2.68–3.49)
	3+1	99.3 (96.4–100)	94.8 (90.0–97.7)	1.71 (1.47–1.99)	100 (97.5–100)	100 (97.5–100)	3.47 (3.03–3.98)
Serotype 5	2+1	96.1 (91.6–98.5)	94.7 (89.9–97.7)	1.32 (1.14–1.52)	100 (97.6–100)	98.7 (95.4–99.8)	2.65 (2.31–3.03)
	3+1	100 (97.6–100)	99.3 (96.3–100)	1.85 (1.63–2.10)	100 (97.5–100)	99.3 (96.3–100)	3.21 (2.81–3.67)
Serotype 6B	2+1	55.7 (47.3–63.8)	45.0 (36.8–53.3)	0.19 (0.15–0.24)	88.5 (82.4–93.0)	87.2 (80.9–92.0)	1.12 (0.88–1.41)
	3+1	63.1 (54.8–70.8)	49.0 (40.7–57.3)	0.31 (0.25–0.38)	96.6 (92.2–98.9)	95.2 (90.4–98.1)	1.85 (1.54–2.22)
Serotype 7F	2+1	96.7 (92.5–98.9)	92.8 (87.5–96.4)	1.28 (1.13–1.46)	100 (97.7–100)	100 (97.7-100)	2.81 (2.51–3.15)
	3+1	99.3 (96.4–100)	99.3 (96.4–100)	2.14 (1.90–2.40)	100 (97.5–100)	100 (97.5–100)	3.88 (3.45–4.37)
Serotype 9V	2+1	93.4 (88.2–96.8)	86.2 (79.7–91.2)	0.92 (0.81–1.05)	99.4 (96.5–100)	99.4 (96.5–100)	2.95 (2.59–3.37)
	3+1	99.3 (96.4–100)	96.1 (91.7–98.5)	1.47 (1.29–1.68)	100 (97.5–100)	100 (97.5–100)	3.97 (3.49–4.50)
Serotype 14	2+1	96.1 (91.6–98.5)	90.8 (85.0–94.9)	1.72 (1.45–2.05)	99.4 (96.5–100)	98.7 (95.4–99.8)	4.19 (3.62–4.85)
	3+1	100 (97.6–100)	98.0 (94.3–99.6)	2.57 (2.22–2.97)	98.6 (95.2–99.8)	98.6 (95.2–99.8)	5.47 (4.68–6.40)
Serotype 18C	2+1	96.1 (91.6–98.5)	87.5 (81.2–92.3)	1.26 (1.06–1.51)	100 (97.7–100)	100 (97.7–100)	6.24 (5.43–7.18)
	3+1	99.3 (96.4–100)	97.4 (93.4–99.3)	3.42 (2.87–4.07)	99.3 (96.3–100)	99.3 (96.3–100)	7.20 (6.08–8.52)
Serotype 19F	2+1	92.8 (87.4–96.3)	91.4 (85.8–95.4)	2.43 (1.97–2.98)	96.2 (91.8–98.6)	95.5 (91.0–98.2)	5.58 (4.65–6.69)
	3+1	96.1 (91.6–98.5)	94.7 (89.9–97.7)	4.43 (3.60–5.45)	98.0 (94.2–99.6)	98.0 (94.2–99.6)	6.95 (5.92–8.17)
Serotype 23F	2+1	69.3 (61.3–76.5)	55.6 (47.3–63.6)	0.38 (0.30–0.47)	96.1 (91.7–98.6)	92.2 (86.8–95.9)	2.41 (1.98–2.94)
	3+1	77.6 (70.2–84.0)	64.5 (56.3–72.1)	0.52 (0.42–0.63)	95.9 (91.3–98.5)	95.9 (91.3–98.5)	2.78 (2.31–3.35)

GMC = geometric mean concentration.
2+1 group: N = 158; 3+1 group: N = 154 (actual number of subjects tested per serotype and time point may slightly vary according to the volume of serum available).

was evaluated prior to the booster dose (data not shown) when at least 78.9% of subjects in the 2+1 group and 89.3% of subjects in the 3+1 group still had antibody concentrations ≥0.2 μg/mL for each vaccine pneumococcal serotype except for serotypes 1, 6B, and 23F. There was a trend towards a lower proportion of subjects in the 2+1 group maintaining antibody concentrations ≥0.2 μg/mL particularly for serotypes 1 (51.7% vs. 68.7%, $P = 0.0028$), 4 (78.9% vs. 91.9%, $P = 0.0014$), and 18C (86.4% vs.

96.6%, $P = 0.0014$). One month postbooster vaccination, the percentages of subjects with antibody concentrations ≥0.2 μg/mL against vaccine pneumococcal serotypes were in the same range in both groups, except for serotype 6B (88.5% in the 2+1 group and 96.6% in the 3+1 group, $P = 0.0075$).

For most serotypes postprimary antibody GMCs were higher (no overlap of CIs) after the 3-dose priming than after the 2-dose priming, particularly for serotype 18C and to a lesser extent 19F. This trend continued for postbooster antibody GMCs for most serotypes, except for serotypes 1 and 4 for which the observed antibody GMCs were in the same range for both groups. In the time period after primary and before booster vaccination, a marked decline in serotype-specific antibody GMCs was observed in both groups for all serotypes except 6B and 23F. In both groups, robust increases in antibody GMCs were observed for all serotypes after the booster dose compared with prebooster values (4–11 fold and 4–6 fold for the 2+1 and 3+1 groups respectively) and postbooster antibody GMCs also exceeded the postprimary values for all serotypes.

Opsonophagocytic activity data are presented in Table 2 and illustrated in Figure (Supplemental Digital Content 2, http://links.lww.com/INF/A169). One month following primary vaccination, serotype specific OPA ≥8 was induced in 74.4% to 100% of the subjects in the 2+1 group and 88.9% to 100% in the 3+1 group, except for serotype 1 (60.8% and 62.9%, respectively). A trend towards lower postprimary percentages of subjects with OPA ≥8 was evident in the 2+1 group compared with the 3+1 group for most serotypes, but particularly for serotypes 6B, 18C, and 23F.

Prior to the booster dose (data not shown), persistence of OPA ≥8 was lowest for serotype 1 (9.6% in the 2+1 group and 15.7% in the 3+1 group) and highest for 9V (95.8% in the 2+1 group and 97.8% in the 3+1 group). There was a trend towards lower persistence of OPA ≥8 in the 2+1 group particularly for serotypes 7F (66.4% vs. 82.5%), 14 (56.9% vs. 78.9%), 18C (24.6% vs. 49.2%), and 19F (63.8% vs. 78.4%).

One month postbooster vaccination, the percentages of subjects with opsonophagocytic activity ≥8 against vaccine pneumococcal serotypes were in the same ranges in both study groups, except for serotypes 5, 6B, and 23F which tended to be lower in the 2+1 group. Higher postprimary and postbooster OPA GMTs were observed following the 3+1 vaccination course relative to the 2+1 vaccination course. This was most evident for serotypes 18C, 19F, and 23F following primary vaccination. The booster dose induced substantial increases in OPA GMTs relative to prebooster levels (4–46-fold and 5–64-fold for the 2+1 and 3+1 groups, respectively) for all serotypes in both groups and postbooster GMTs exceeded the postprimary values for all serotypes.

Overall, the percentages of subjects reaching the 22F-ELISA threshold of 0.2 μg/mL for each serotype correlated well with the percentages reaching the OPA cut-off of 8 with some exceptions. For serotype 1 and serotype 5, a higher percentage of subjects reached the ELISA threshold following both primary and booster vaccination (apart from the 3+1 schedule for serotype 5). Similarly, for serotype 18C, a higher percentage of subjects reached the ELISA threshold following primary vaccination in the 2+1 schedule, and the same trend was observed for serotypes 7F and 19F. For serotypes 6B and 23F on the contrary, a lower percentage of subjects reached the ELISA threshold compared with the OPA cut-off following primary vaccination in both schedules, whereas an opposite trend was observed for serotype 6B following booster vaccination.

Impact of Schedule on Response to Protein D

The GMCs for anti-PD antibodies were lower in the 2+1 group relative to the 3+1 group following both primary (861.8 EL.U/mL vs. 1223.3 EL.U/mL, $P = 0.0008$) and booster vaccinations (1629.8 EL.U/mL vs. 2113.0 EL.U/mL, $P = 0.0392$).

Impact of Schedule on Response to Diphtheria and Tetanus Toxoids

Although only 2 primary doses of concomitant vaccines were administered, all subjects developed antitetanus antibody

TABLE 2. Anti-Pneumococcal Opsonophagocytic Activity Against Individual Pneumococcal Serotypes 1 Month Following Primary Vaccination and 1 Month Following Booster Vaccination (ATP Cohort for Immunogenicity)

Pneumococcal Serotype	Study Group	Post-primary		Post-booster	
		% OPA Titers ≥8 (95% CI)	OPA GMT (95% CI)	% OPA Titers ≥8 (95% CI)	OPA GMT (95% CI)
Serotype 1	2+1	60.8 (51.8–69.2)	21.9 (16.4–29.1)	80.9 (73.1–87.3)	109.9 (76.1–158.7)
	3+1	62.9 (54.0–71.1)	26.5 (19.8–35.4)	77.8 (69.5–84.7)	100.6 (68.9–146.9)
Serotype 4	2+1	100 (97.3–100)	462.6 (410.4–521.4)	96.8 (92.0–99.1)	634.6 (496.3–811.3)
	3+1	99.2 (95.9–100)	758.9 (647.8–888.9)	99.0 (94.6–100)	1204.0 (990.7–1463.2)
Serotype 5	2+1	82.6 (75.0–88.6)	48.3 (37.7–61.8)	87.2 (80.3–92.4)	102.1 (75.8–137.6)
	3+1	90.8 (84.4–95.1)	68.4 (54.0–86.5)	97.5 (92.9–99.5)	157.2 (123.1–200.7)
Serotype 6B	2+1	74.4 (65.8–81.8)	157.8 (104.7–237.8)	81.1 (73.3–87.4)	220.3 (146.9–330.3)
	3+1	88.9 (82.1–93.8)	379.6 (272.4–529.1)	90.3 (82.9–95.2)	468.5 (311.6–704.3)
Serotype 7F	2+1	90.6 (84.1–95.0)	844.8 (591.4–1206.7)	100 (97.2–100)	1843.4 (1494.2–2274.1)
	3+1	98.5 (94.6–99.8)	2176.5 (1759.2–2692.7)	100 (96.7–100)	3290.6 (2709.1–3996.8)
Serotype 9V	2+1	100 (97.3–100)	875.1 (732.0–1046.1)	100 (97.2–100)	1068.1 (874.7–1304.2)
	3+1	100 (97.2–100)	1343.4 (1130.8–1596.0)	100 (96.7–100)	1706.9 (1438.5–2025.3)
Serotype 14	2+1	98.5 (94.6–99.8)	692.6 (559.1–858.0)	100 (96.6–100)	835.5 (672.1–1038.5)
	3+1	100 (97.2–100)	1125.3 (946.2–1338.3)	100 (96.4–100)	1280.7 (1054.5–1555.5)
Serotype 18C	2+1	82.8 (75.4–88.8)	56.2 (42.9–73.7)	97.8 (93.7–99.5)	330.0 (259.1–420.3)
	3+1	96.2 (91.3–98.7)	218.6 (176.1–271.4)	98.5 (94.6–99.8)	490.8 (395.3–609.4)
Serotype 19F	2+1	87.0 (80.0–92.3)	101.0 (74.9–136.0)	96.2 (91.3–98.7)	251.3 (193.4–326.6)
	3+1	93.8 (88.1–97.3)	356.7 (263.2–483.4)	96.1 (91.2–98.7)	734.7 (568.3–949.8)
Serotype 23F	2+1	86.3 (79.2–91.6)	489.7 (342.6–700.0)	92.5 (86.7–96.4)	1047.3 (748.1–1466.3)
	3+1	97.7 (93.4–99.5)	1233.7 (991.7–1534.7)	98.3 (94.2–99.8)	1528.9 (1171.2–1996.0)

GMT = geometric mean titer.
2+1 group: N = 158; 3+1 group: N = 154 (actual number of subjects tested per serotype and time point may slightly vary according to the volume of serum available).

levels >0.1 IU/mL and the percentage of subjects with antidiphtheria antibody levels >0.1 IU/mL was at least 97.4% regardless the group. The postprimary GMCs for antibodies against diphtheria and tetanus were higher in the group that received 3 primary doses of PHiD-CV relative to the group that received 2 primary doses of PHiD-CV (antidiphtheria: 3.123 IU/mL vs. 1.791 IU/mL; antitetanus: 4.602 IU/mL and 2.504 IU/mL, respectively). This trend continued for postbooster antibody GMCs (data not shown).

Impact of Age at First Vaccine Dose on Pneumococcal Response

Pneumococcal ELISA and OPA responses were stratified by age of first vaccination (8–12 or 13–16 weeks) for the 2+1 schedule (Table, Supplemental Digital Content 3, http://links.lww.com/INF/A170 and Table, Supplemental Digital Content 4, http://links.lww.com/INF/A171). When stratified post hoc by age of first vaccine dose administration, 63% of subjects in the 2+1 group were aged 8 to 12 weeks and 37% were aged 13 to 16 weeks. Both ELISA and OPA responses appeared to be similar in the 2 age categories, although there was an overall trend towards slightly higher antibody responses in the older age group for some serotypes (1.3 fold higher postprimary for serotypes 18C and 23F and 1.5 fold higher for serotype 19F). Similar observations were made for the 3+1 schedule (data not shown).

DISCUSSION

In this study we evaluated the immune responses to the PHiD-CV vaccine in infants following a simplified 2-dose priming and the more commonly employed 3-dose priming both followed by a booster dose. Both vaccination schedules elicited serum IgG and functional OPA immune responses against all vaccine pneumococcal serotypes following primary vaccination and strong antibody responses following the booster dose indicating the induction of immunological memory. For most vaccine serotypes a trend towards lower postprimary and postbooster immune responses was observed in children primed with 2 vaccine doses. This trend seemed to be more pronounced for functional OPA responses, a notable finding since this is the first study to monitor the effect of 2-dose PCV priming on OPA responses.

The clinical relevance of these observations is unknown as for all serotypes, with the exception of serotypes 6B and 23F, over 92% of the infants primed with 2 doses achieved the 22F-ELISA threshold of 0.2 μg/mL 1 month following primary vaccination. Further evidence of adequate priming comes from the strong postbooster responses to all serotypes evoked at approximately 12 months of age. This implies that exposure of the vaccinees to *S. pneumoniae* vaccine serotypes subsequent to primary vaccination with 2 dose of PHiD-CV would result in a vigorous anamnestic response, including functional opsonophagocytic antibodies which are considered to reflect the primary mechanism of host defense against pneumococcal disease.[23,24]

The postprimary ELISA antibody levels for serotypes 6B and 23F, following both vaccination schedules, were lower than for other vaccine serotypes and also tended to be lower after the 2-dose primary vaccination than after the 3-dose primary vaccination. Previous studies have indicated that the kinetics of the primary vaccination response to PCV7-CRM, PCV9-CRM, and PCV7-OMPC vaccines is serotype-specific,[25–27] and studies investigating the use of a 2-dose primary schedule with PCV7-CRM[28–30] or PCV9-CRM[31] found that responses to serotypes 6B and 23F were markedly lower relative to a 3-dose primary schedule. Following 2-dose priming with PCV7-CRM the percentages of subjects reaching the putative protective ELISA threshold of 0.35 μg/mL ranged, depending on the serotype, from 76% to 100% except for serotype 6B (40%),[28] from 91.3% to 97.8% except for serotypes 6B and 23F (69.6%)[29] and from 89.2% to 96.8% except for serotypes 6B (61.1%) and 23F (70.1%).[30] These observations are similar to those in the present study where following 2 primary PHiD-CV doses 92.8% to 98.0% of subjects reached the putative protective GSK 22F ELISA threshold of 0.2 μg/mL (which corresponds to an antibody concentration of 0.35 μg/mL, using the WHO reference laboratory ELISA) except for serotype 6B (55.7%) and serotype 23F (69.3%) and 86.2% to 94.8% of subjects developed antibody concentrations ≥0.35 μg/mL (which corresponds to an antibody concentration of ≥0.5 μg/mL, using the WHO reference laboratory ELISA) except for serotypes 6B (45.0%) and 23F (55.6%).

Even following 3 primary doses, the lowest ELISA antibody levels are usually observed for serotypes 6B and 23F. This is not the case for functional OPA responses to these serotypes, especially 23F OPA responses which are usually among the highest. It is also noteworthy that despite the relatively lower ELISA responses to 6B and 23F, serotype-specific measures of PCV7-CRM vaccine effectiveness against IPD show that serotype 6B and 23F are as highly effective as other serotypes.[32] Furthermore significant vaccine efficacy against AOM due to serotypes 6B and 23F has been demonstrated.[3,4,11] This suggests that lower antibody concentrations as measured by ELISA are sufficient to confer protection against diseases caused by serotypes 6B and 23F.

Although for most serotypes postprimary ELISA antibody GMCs and OPA GMTs were higher after the 3-dose priming with PHiD-CV than after the 2-dose priming, this was most evident for serotype 18C (conjugated to tetanus toxoid) and 19F (conjugated to diphtheria toxoid). It has been documented that tetanus and diphtheria carriers employed in conjugates can influence the response of concomitantly administered tetanus and diphtheria containing vaccines.[33–36] Indeed in this study diphtheria and tetanus antibody GMCs were higher in the 3+1 group compared with the 2+1 group even though the concomitantly administered tetanus and diphtheria containing vaccines were administered in both groups according to the 2-dose priming schedule. There is also evidence that carrier priming can enhance immune responses to polysaccharides in subsequent doses of conjugate vaccine.[37–39] Thus, unlike the other serotypes conjugated to PD, it is possible that 18C and 19F responses to the third primary vaccine dose of PHiD-CV may have been enhanced by stronger carrier priming. These data therefore indicate that the immunization schedule of concomitant vaccines needs to be taken into account when evaluating 2-dose priming PCV immunogenicity data.

In line with other studies,[12,13,40] the lowest postprimary OPA response following both schedules was observed for serotype 1. However, there was a substantial OPA booster response for serotype 1. Furthermore, as previously observed in the antibody kinetics study performed with the PCV9-CRM in South Africa,[26] the number of primary vaccine doses did not appear to influence the level of responses against serotype 1. This is of interest for countries where a 2-dose primary schedule is routinely employed for infants and where serotype 1 is responsible for a high proportion of IPD cases.

Overall, the percentages of subjects achieving the OPA cut-off of 8 correlated well with the percentages achieving the 22F-ELISA threshold of 0.2 μg/mL with the notable exceptions of serotypes 1 and 5 where less subjects had OPA ≥8 and serotypes 6B and 23F where more subjects had OPA ≥8. A previous study with PCV7-CRM observed differences between pneumococcal serotypes in antibody concentrations required to induce opsonophagocytosis, with serotype 19F needing the highest antibody concentrations and serotypes 6B and 23F the lowest.[20] Our results

would indicate that serotypes 1 and 5 may also require relatively high antibody concentrations for opsonophagocytic activity.

An impact of immunization schedule was observed on the response to the PD carrier protein, with lower antibody levels induced in the 2+1 group relative to the 3+1 group. The response to protein D is important in the context of protection against nontypeable *H. influenzae* AOM.[11] However, in the absence of a correlate of protection it is not clear to what extent a reduced schedule would influence efficacy against AOM caused by nontypeable *H. influenzae*.

Recently the use of a 2+1 schedule has been approved for PCV7-CRM in Europe, with the acknowledgment that most data indicates that smaller proportions of infants achieve threshold ELISA levels against serotypes 6B and 23F and that GMCs are lower for antibodies against most serotypes relative to a 3-dose infant series.[41] Despite this, for all 7 PCV7-CRM serotypes ELISA antibody responses to booster doses in toddlers following either a 2-dose or 3-dose priming were comparable, indicating that both infant regimens had elicited adequate priming.[41] The impact of a 2+1 schedule on functional OPA responses against PCV7-CRM serotypes has not been documented so far. However, a recent study showed lower ELISA and functional OPA responses against serotype 6B following 2-dose PCV7-CRM priming when compared with post dose 2 PHiD-CV responses.[13]

Although immunogenicity data suggest that a 2+1 schedule may be a practical option for implementation of PCV in routine pediatric immunization programs, it is important to demonstrate its effectiveness in clinical practice. Whitney et al[32] demonstrated that in the US a PCV7-CRM schedule of 2 doses within 7 months of age, plus a booster at 12 to 16 months was highly protective (98%) against IPD compared with no vaccination, although too few children were vaccinated on this schedule to compare its effectiveness directly with other schedules. A key finding of the Whitney et al study was the importance of the booster dose in the second year of life, which gives added benefit over all doses given in the first year of life.[32,42] This implies that a 2+1 schedule may well confer significant benefit over a 3+0 schedule where no booster is given following a 3 dose priming. More recent studies on 2 dose priming PCV7-CRM schedules outside the US have reported effectiveness values against IPD of 72% in England and Wales,[43] 74% in Norway,[44] and 93% in Canada.[41,45] Denmark also reported an estimated decrease in IPD incidence of about 30% after less than a year with a 2+1 schedule and a catch-up program.[46]

Effectiveness of a 2+1 immunization schedule with PCV7-CRM against pneumonia or acute otitis media has not been established. Of interest however are data from Dagan et al indicating that a 2+1 schedule was associated with higher nasopharyngeal carriage of serotypes 6B and 6A relative to a 3+1 schedule.[47]

In summary, the results of our study with PHiD-CV are in line with data generated for PCV7-CRM and other PCV vaccines, demonstrating that a 2+1 schedule results in a smaller proportion of infants achieving threshold ELISA antibody levels against serotypes 6B and 23F compared with the 3+1 schedule and lower antibody GMCs for most serotypes. The application of the functional OPA assay in our study also suggests that a third dose may be important to optimize functional responses. Experience with PCV7-CRM in the 3+1 schedule indicates that the lower ELISA responses observed for 6B and 23F relative to other vaccine serotypes have minimal impact on effectiveness against IPD. The 2+1 schedule seems to be efficacious against IPD as well, but additional effectiveness data for a 2+1 schedule are needed to fully understand the public health benefit to be expected from these schedules especially in terms of prevention of the mucosal infections.

ACKNOWLEDGMENTS

The authors thank the parents and their children who participated in this trial. The authors also thank the study nurses, clinicians, laboratory personnel, and other staff members for their assistance in conducting this study, and in particular all the investigators involved in this study: T. Arvidsson, L. Ekholm, C.-E. Flodmark, L. Gothefors, H. Hauge, M. Horvathova, D. Papuchova, M. Zanova. In addition, they would like to thank the clinical and serological laboratory teams of GlaxoSmithKline Biologicals for their contribution as well as Liliana Manciu (GlaxoSmithKline Biologicals) for clinical report writing, Dr Miriam Hynes (Freelance, UK) for medical writing and Dr Christine Vanderlinden (GlaxoSmithKline Biologicals) for editorial assistance and manuscript coordination.

REFERENCES

1. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J*. 2000;19:187–195.
2. O'Brien KL, Moulton LH, Reid R, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet*. 2003;362:355–361.
3. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med*. 2001;344:403–409.
4. Kilpi T, Ahman H, Jokinen J, et al. Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children. *Clin Infect Dis*. 2003;37:1155–1164.
5. Akkoyunlu M, Ruan M, Forsgren A. Distribution of protein D, an immunoglobulin D-binding protein, in *Haemophilus* strains. *Infect Immun*. 1991; 59:1231–1238.
6. Janson H, Ruan M, Forsgren A. Limited diversity of the protein D gene (hpd) among encapsulated and nonencapsulated *Haemophilus influenzae* strains. *Infect Immun*. 1993;61:4546–4552.
7. Munson RS Jr, Sasaki K. Protein D, a putative immunoglobulin D-binding protein produced by *Haemophilus influenzae*, is glycerophosphodiester phosphodiesterase. *J Bacteriol*. 1993;175:4569–4571.
8. Song XM, Forsgren A, Janson H. The gene encoding protein D (hpd) is highly conserved among *Haemophilus influenzae* type b and nontypeable strains. *Infect Immun*. 1995;63:696–699.
9. Janson H, Melhus A, Hermansson A, et al. Protein D, the glycerophosphodiester phosphodiesterase from *Haemophilus influenzae* with affinity for human immunoglobulin D, influences virulence in a rat otitis model. *Infect Immun*. 1994;62:4848–4854.
10. Janson H, Carlén B, Cervin A, et al. Effects on the ciliated epithelium of protein D-producing and -nonproducing nontypeable *Haemophilus influenzae* in nasopharyngeal tissue cultures. *J Infect Dis*. 1999;180:737–746.
11. Prymula R, Peeters P, Chrobok V, et al. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae*: a randomized double blind efficacy study. *Lancet*. 2006;367:740–748.
12. Vesikari T, Wysocki J, Chevallier B, et al. Immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) compared to the licensed 7vCRM vaccine. *Pediatr Infect Dis J*. 2009;28:S66–S76.
13. Wysocki J, Tejedor JC, Grunert D, et al. 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. *Pediatr Infect Dis J*. 2009;28:S77–S88.
14. Chevallier B, Vesikari T, Brzostek J, et al. Safety and reactogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) when co-administered with routine childhood vaccines. *Pediatr Infect Dis J*. 2009;28:S109–S118.
15. Concepcion N, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol*. 2001;8:266–272.

16. Henckaerts I, Goldblatt D, Ashton L, et al. Critical differences between pneumococcal polysaccharide enzyme-linked immunosorbent assays with and without 22F inhibition at low antibody concentrations in pediatric sera. *Clin Vaccine Immunol*. 2006;13:356–360.

17. World Health Organization. Pneumococcal conjugate vaccines. Recommendations for production and control of pneumococcal conjugate vaccines. *WHO Tech Rep Ser*. 2005;927(Annex 2):64–98.

18. Centers for Disease Control and Prevention, Emory University. *Streptococcus pneumonia*e opsonophagocytosis using differentiated HL-60 cells (promyelocytic leukemia cell line). Laboratory protocol December 20th 1999. Available at: http://www.vaccine.uab.edu. Accessed 10 February, 2009.

19. Romero-Steiner S, Frash C, Concepcion N, et al. Multilaboratory evaluation of a viability assay for measurement of opsonophagocytic antibodies specific to the capsular polysaccharides of *Streptococcus pneumoniae*. *Clin Diagn Lab Immunol*. 2003;10:1019–1024.

20. Henckaerts I, Durant N, De Grave D, et al. Validation of a routine pneumococcal opsonophagocytosis assays to predict invasive pneumococcal disease efficacy of conjugate vaccine in children. *Vaccine*. 2007;25:2518–2527.

21. Melville-Smith ME, Balfour A. Estimation of *Corynebacterium diphtheriae* antitoxin in human sera: a comparison of enzyme-linked immunosorbent assay with the toxin neutralization test. *J Med Microbiol*. 1988;25:279–283.

22. Melville-Smith ME, Seagroatt VA, Watkins JT. A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralization test in mice as a method for the estimation of tetanus antitoxin in human sera. *J Biol Stand*. 1983;11:137–144.

23. Tuomanen EI, Austrian R, Masure HR. Pathogenesis of pneumococcal infection. *N Engl J Med*. 1995;332:1280–1284.

24. Poolman J. Pneumococcal vaccine development. *Expert Rev Vaccines*. 2004;3:597–604.

25. Rennels MB, Edwards KM, Keyserling HL, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. *Pediatrics*. 1998;101:604–611.

26. Huebner RE, Mbelle N, Forrest B, et al. Immunogenicity after one, two or three doses and impact on the antibody response to coadministered antigens of a nonavalent pneumococcal conjugate vaccine in infants of Soweto, South Africa. *Pediatr Infect Dis J*. 2002;21:1004–1007.

27. Ekström N, Ahman H, Verho J, et al. Kinetics and avidity of antibodies evoked by heptavalent pneumococcal conjugate vaccines PncCRM and PncOMPC in the Finnish Otitis Media Vaccine Trial. *Infect Immun*. 2005;73:369–377.

28. Käyhty H, Åhman H, Eriksonn K, et al. Immunogenicity and tolerability of a heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 12 months of age. *Pediatr Infect Dis J*. 2005;24:108–114.

29. Esposito S, Pugni L, Bosis S, et al. Immunogenicity, safety and tolerability of heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 11 months postnatally to pre- and full-term infants. *Vaccine*. 2005;23:1703–1708.

30. Givon-Lavi N, Greenberg D, Dagan R. Immunogenicity of CRM-conjugated 7-valent pneumococcal conjugate vaccine (PCV7) administered at 2-dose (4 and 6m) or 3-dose primary regimen (2, 4 and 6m) with a booster at 12m. [Abstract P3–049]. In: 6th International Symposium on Pneumococci and Pneumococcal Diseases; June 8–12, 2008; Reykjavik, Iceland. Abstract 308.

31. Sigurdardottir ST, Davidsdottir K, Arason VA, et al. Safety and immunogenicity of CRM197-conjugated pneumococcal-meningococcal C combination vaccine (9cPnC-MnCC) whether given in two or three primary doses. *Vaccine*. 2008;26:4178–4186.

32. Whitney CG, Pilishvili T, Farley MM, et al. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. *Lancet*. 2006;368:1495–1502.

33. Schmitt HJ, Faber J, Lorenz I, et al. The safety, reactogenicity and immunogenicity of a 7-valent pneumococcal conjugate vaccine (7VPnC) concurrently administered with a combination DTaP-IPV-Hib vaccine. *Vaccin*e. 2003;21:3653–3662.

34. Tichmann-Schumann I, Soemantri P, Behre U, et al. Immunogenicity and reactogenicity of four doses of diphtheria-tetanus-three-component acellular pertussis-hepatitis B-inactivated polio virus-*Haemophilus influenzae* type b vaccine coadministered with 7-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis J*. 2005;24:70–77.

35. Knuf M, Habermehl P, Cimino C, et al. Immunogenicity, reactogenicity and safety of a 7-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a DTPa-HBV-IPV/Hib combination vaccine in healthy infants. *Vaccine*. 2006;24:4727–4736.

36. Tejedor JC, Moro M, Ruiz-Contreras J, et al. Immunogenicity and reactogenicity of primary immunization with a hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-*Haemophilus influenzae* type B vaccine coadministered with two doses of a meningococcal C-tetanus toxoid conjugate vaccine. *Pediatr Infect Dis J*. 2006;25:713–720.

37. Peeters C, Tenenbergen-Meekes AM, Poolman JT, et al. Effect of carrier priming on immunogenicity of saccharide-protein conjugate vaccines. *Infect Immun*. 1991;59:3504–3510.

38. Granoff D, Holmes S, Belshe R, et al. Effect of carrier protein priming on antibody responses to *Haemophilus influenzae* type b conjugate vaccines in infants. *JAMA*. 1994;272:1116–1121.

39. Kurikka S, Käyhty H, Saarinen L, et al. Immunologic priming by one dose of *Haemophilus influenzae* type b conjugate vaccine in infancy. *J Infect Dis*. 1995;172:1268–1272.

40. Schuerman L, Prymula R, Henckaerts I, et al. ELISA IgG concentrations and opsonophagocytic activity following pneumococcal protein D conjugate vaccination and relationship to efficacy against acute otitis media. *Vaccine*. 2007;25:1962–1968.

41. Prevenar summary of product characteristics 6th January 2009. Available at: http://www.emea.europa.eu/humandocs/Humans/EPAR/prevenar/prevenar.htm.

42. O'Brien KL, Levine OS. Effectiveness of pneumococcal conjugate vaccine. *Lancet*. 2006;368:1469–1470.

43. Kaye P, Andrews N, Slack M, et al. Vaccine effectiveness and indirect protection from pneumococcal conjugate vaccine used in a 2 dose infant priming plus booster schedule in England and Wales [Abstract P3–118]. In: 6th International Symposium on Pneumococci and Pneumococcal Diseases; June 8–12, 2008; Reykjavik, Iceland. Abstract 355.

44. Vestrheim DF, Løvoll O, Aaberge IS, et al. Effectiveness of a 2+1 dose schedule pneumococcal conjugate vaccination programme on invasive pneumococcal disease among children in Norway. *Vaccine*. 2008;26:3277–3281.

45. De Wals P, Bolduc D, Boulianne N, et al. Impact of a Three-Dose Pneumococcal Conjugate Vaccine Program on the Epidemiology of Pneumococcal-Related Outcomes in the Province of Quebec, Canada. [Abstract G-1003]. In: 47th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17–20, 2007; Chicago, IL. Abstract 277.

46. Andersen PH. Epi-Nyt no 38, 17 Sept 2008. Available at: www.ssi.dk.epinyt@ssi.dkandhttp://www.ssi.dk/graphics/dk/nyheder/epinyt/2008/PDF/epinyt_38–2008.pdf.

47. Dagan R, Givon-Lavi N, Janco J, et al. Nasopharyngeal carriage of *S. pneumoniae* vaccine serotypes (VT-Sp) in first 2 years of life following 4 different 7-valent CRM-conjugate vaccine (PCV7) schedules. [Abstract P3–046]. In: 6th International Symposium on Pneumococci and Pneumococcal Diseases; June 8–12, 2008; Reykjavik, Iceland. Abstract 306.