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Safety, reactogenicity and immunogenicity of a novel pneumococcal protein-based vaccine in adults: A phase I/II randomized clinical study☆

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ABSTRACT

Background: New vaccines containing highly conserved *Streptococcus pneumoniae* proteins such as pneumolysin toxoid (dPly) and histidine-triad protein D (PhtD) are being developed to provide broader protection against pneumococcal disease. This study evaluated the safety, reactogenicity and immunogenicity of different pneumococcal protein-containing formulations in adults.

Methods: In a phase I double-blind study (www.clinicaltrials.gov: NCT00707798), healthy adults (18–40 years) were randomized (1:2:2:2:2:2:2) to receive two doses of one of six investigational vaccine formulations 2 months apart, or a single dose of the control 23-valent pneumococcal polysaccharide vaccine (23PPV; *Pneumovax23*™, Sanofi Pasteur MSD) followed by placebo. The investigational formulations contained dPly alone (10 or 30 μg), or both dPly and PhtD (10 or 30 μg each) alone or combined with the polysaccharide conjugates of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV; *Synflorix*™, GlaxoSmithKline Vaccines). Two groups primed with a formulation containing dPly and PhtD (10 or 30 μg each) continued to the follow-up phase II study (NCT00896064), in which they received a booster dose at 5–9 months after primary vaccination.

Results: Of 156 enrolled and vaccinated adults, 146 completed the primary immunization and 43 adults received a booster dose. During primary and booster vaccination, for any formulation, ≤8.9% of doses were followed by grade 3 solicited local or general adverse events. No fever >39.5 °C (oral temperature) was reported. Unsolicited adverse events considered causally related to vaccination were reported following ≤33.3% of investigational vaccine doses. No serious adverse events were reported for adults receiving investigational vaccine formulations. Formulations containing dPly with or without PhtD were immunogenic for these antigens; polysaccharide conjugate-containing formulations were also immunogenic for those 10 polysaccharides.

Conclusion: Investigational vaccine formulations containing dPly and PhtD were well tolerated and immunogenic when administered to healthy adults as standalone protein vaccine or combined with PHiD-CV conjugates.

Abbreviations: 23PPV, 23-valent pneumococcal polysaccharide vaccine; AE, adverse event; anti-PD, NTHi protein D antibody; ATP, according to protocol; dPly, pneumolysin toxoid; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GMT, geometric mean titer; LU, Luminex units; OPA, opsonophagocytic activity; PCV, pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine; PhtD, histidine-triad protein D; Ply, pneumolysin; PS-conjugates, capsular polysaccharide conjugates; SAE, serious adverse event; TVC, total vaccin.

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1. Introduction

Streptococcus pneumoniae is frequently involved in common mucosal bacterial infections such as pneumonia, and can lead to invasive disease including sepsis, meningitis and invasive pneumonia [1,2]. Worldwide, this pathogen is responsible for approximately 11% of mortality in children under 5 years old [2].

Pneumococcal conjugate vaccines (PCVs) have decreased the burden of pneumococcal disease in children in many countries and provided indirect effect in decreasing vaccine-type disease in non-vaccinated populations [3–5]. However, shifts in serotype epidemiology have occurred and consequently considerable disease burden remains, largely owing to serotypes not included in the currently used PCVs [4–6].

The use of highly conserved pneumococcal proteins as vaccine antigens has the potential to provide broader protection against pneumococcal disease than PCVs. Two candidate antigens for a protein-based pneumococcal vaccine are pneumolysin (Ply) and histidine-triad protein (PhtD). Ply is a thiol-dependent toxin that is present in nearly all pneumococcal serotypes [7]. Its toxoid derivatives (dPly) induce protection against pneumococcal infection in animal models [8–11]. PhtD is exposed on the surface of intact bacteria [12] and may be involved in lung-specific virulence [13]. Immunization with PhtD elicits functional antibodies [14–16] and provides protection against pneumonia in animal models [11,15]. Antibodies against PhtD prevent pneumococcal adherence to human airway epithelial cells [16]. An investigational vaccine containing 10 or 30 μg PhtD was shown to have an acceptable reactogenicity profile in adults, with no safety concerns, and dose-dependent immunogenicity when comparing the 10 and 30 μg formulations [17].

This phase I study provides a safety and reactogenicity assessment of investigational pneumococcal protein-containing formulations in healthy adults before progressing to the target pediatric population. We evaluated six different formulations containing dPly alone or with PhtD, or a combination of dPly and PhtD with the conjugates of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV). After the two-dose primary series, two primed cohorts received a booster dose of a 10 or 30 μg dPly/PhtD formulation in the follow-up phase II study.

2. Methodology

2.1. Study design and objectives

A phase I, randomized, controlled study (primary vaccination study; NCT00707798) was conducted between June 2008 and January 2009. Two groups were further evaluated in a follow-up phase II study (booster vaccination study; NCT00896064) between May and August 2009. Both studies were conducted at a single center in Belgium. The primary vaccination study was open in step 1 (for the group receiving 10 μg dPly). For steps 2 and 3 (encompassing all other groups), data were collected in an observer-blinded manner (vaccine recipients and those responsible for evaluation of any study endpoint were unaware which vaccine was administered) (Fig. 1).

The primary objective of both studies was to assess the safety and reactogenicity of the different investigational pneumococcal vaccine formulations. Secondary objectives included evaluation of the dPly and PhtD protein antibody responses. We also evaluated the non-typeable *Haemophilus influenzae* (NTHi) protein D antibody (anti-PD) response and opsonophagocytic activity (OPA) of vaccine serotypes for the formulations containing capsular polysaccharide conjugates (PS-conjugates).

The study protocols were approved by the Ethics Committee of the Ghent University Hospital. The studies were conducted in line with the Declaration of Helsinki and Good Clinical Practice. Informed consent was obtained from each study participant before enrolment.

These studies have been registered at www.clinicaltrials.gov (NCT00707798; NCT00896064). Protocol summaries are available at http://www.gsk-clinicalstudyregister.com (GSK study IDs: 111651; 112993).

2.2. Participants and vaccines

Eligible participants were healthy adults (18–40 years old), without a history of bacterial pneumonia or invasive pneumococcal disease within 3 years before vaccination. Exclusion criteria included vaccination with diphtheria/tetanus toxoids within 1 month preceding the first study vaccine dose, and chronic administration (>14 days) of immunosuppressants or immune-modifying drugs within 6 months before vaccination. Participants were screened by clinical laboratory analysis (supplementary methods); those with hematological or biochemical abnormalities were not enrolled. Participants were not to use any investigational or non-registered product other than the study vaccine from 30 days before the first vaccine dose until study end. Women of childbearing potential were asked to practice adequate contraception from 30 days pre-vaccination until 2 months after completing the vaccination series.

Participants were enrolled sequentially in three steps preceded by a safety review (Fig. 1). They were randomized (1:2:2:2:2:2:2, block size 4 [step 1], 7 [step 2] and 5 [step 3]) using a central internet randomization system (SBIR) to receive a two-dose primary vaccination series with one of six investigational vaccine formulations (GlaxoSmithKline Vaccines) or a single dose of the 23-valent pneumococcal polysaccharide vaccine (23PPV; *Pneumovax23*™, Sanofi Pasteur MSD) followed by placebo (150 mM NaCl) (Fig. 1; supplementary methods). All vaccines and the placebo were administered intramuscularly into the deltoid region of the non-dominant arm.

Two investigational vaccines contained 10 or 30 μg of dPly alone (dPly-10 and dPly-30, respectively). Two other formulations contained both dPly and PhtD, each at a dose of 10 μg (dPly/PhtD-10) or 30 μg (dPly/PhtD-30). The remaining two formulations contained the 10 PHiD-CV PS-conjugates (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F) [18], in combination with 10 or 30 μg of both dPly and PhtD (PHiD-CV/dPly/PhtD-10 and PHiD-CV/dPly/PhtD-30). Production of PhtD and dPly is described in supplementary methods.

The control group received one dose of 23PPV, containing 25 μg of each capsular polysaccharide for pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F, and placebo (150 mM NaCl) as a second dose.

Participants from the dPly/PhtD-10 and dPly/PhtD-30 groups were invited to participate in the booster vaccination study, to receive a booster dose 5–9 months after completion of the two-dose primary series.

2.3. Safety and reactogenicity assessment

Solicited local and general symptoms were recorded during the 7-day post-vaccination period and unsolicited adverse events (AEs) during the 31-day post-vaccination period. Symptom intensity was graded on a scale of 1 (mild) to 3 (severe). Grade 3 symptoms were defined as follows: for redness or swelling, a diameter >50 mm; for fever, oral temperature >39.5 °C; and for all other events, preventing normal activity.

Serious adverse events (SAEs) were recorded throughout the duration of each study, and were defined as any medical occurrence that resulted in death, disability or incapacity, was life-threatening,

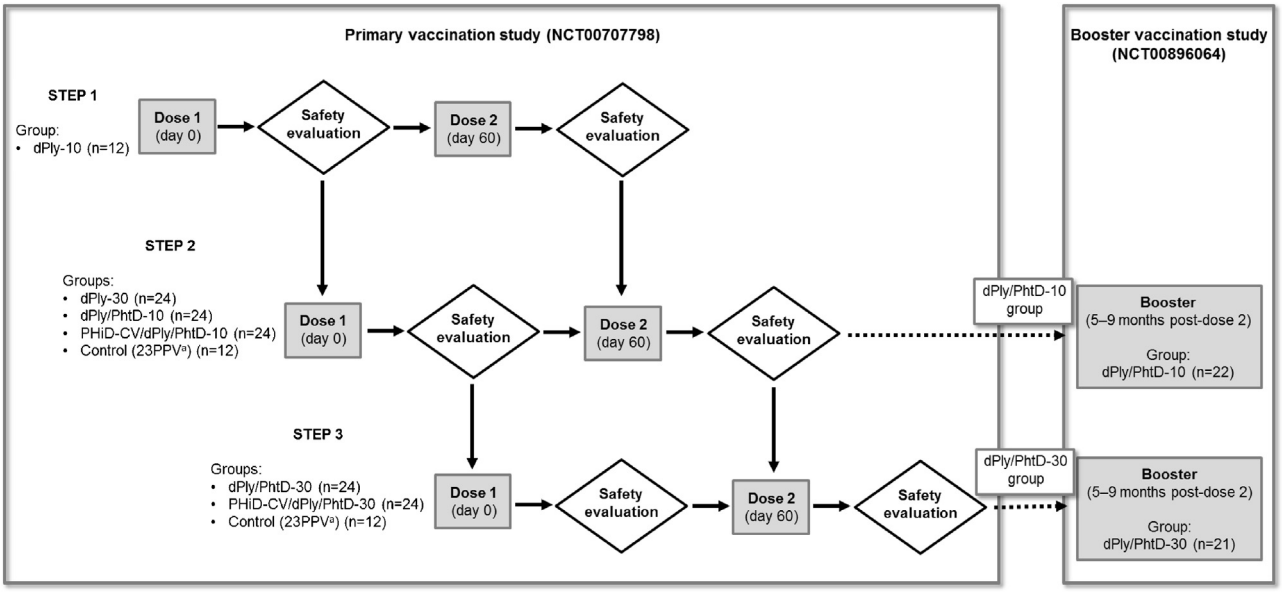

Fig. 1. *Study design* Participants were enrolled in a sequential manner comprising three steps. A safety evaluation was completed prior to proceeding with the dosing or next step of the vaccination schedule. The primary vaccination study encompassed study visits at days 0 (dose 1), 1, 7, 30 (1 month post-dose 1), 60 (dose 2), 61, 67 and 90 (1 month post-dose 2); the booster vaccination study visits were at days 0 (booster dose), 1, 6 and 30 (1 month post-booster). [a]The second dose in the control group was a placebo.

required hospitalization, or any congenital anomaly or birth defect in the descendants of a study participant.

2.4. Immunogenicity assessment

Blood samples for immunogenicity assays were collected before primary and booster vaccination, and 1 month after each dose. Serum samples were stored at −20 °C until analysis at GlaxoSmithKline's laboratory, Rixensart, Belgium and SGS laboratory, Wavre, Belgium.

Antibodies were quantified using an in-house multiplex assay coated with protein D, Ply (non-detoxified) and PhtD (supplementary methods), with assay cut-offs of 112 LU/mL for anti-PD, 599 LU/mL for anti-Ply and 391 LU/mL for anti-PhtD. These cut-offs were based on the lower limit of quantification [19], the global variability of the assay at the highest dilution and the lower limit of linearity. Participants with antibody levels below these technical cut-offs were considered as antibody negative; however, as this is not a clinical cut-off, they were not considered true negatives.

Functional antibodies against the 10 serotype-specific PS-conjugates of PHiD-CV were measured by a pneumococcal killing assay (OPA) with an opsonic titer cut-off of 8, as described previously [20].

2.5. Statistical analysis

Safety analyses were performed on primary and booster total vaccinated cohorts (TVC). Immunogenicity analyses were performed on primary and booster according-to-protocol (ATP) cohorts for immunogenicity, comprising participants who met all eligibility criteria, complied with protocol-defined procedures, and with pre- and post-vaccination results available for at least one assay. All objectives were descriptive. The target sample size of the primary vaccination study was 156 participants: 12 for dPly-10; 24 for the remaining groups. With this sample size, the percentage of participants with grade 3 and related symptoms that would lead to a significant difference between groups with 80% power is 4% in the control group and 39.7% in the investigational formulation groups.

Incidences of solicited and unsolicited AEs were calculated with exact 95% confidence intervals (CIs). Antibody geometric mean concentrations (GMCs), OPA geometric mean titers (GMTs) and seropositivity rates were calculated with their 95% CIs. GMCs and GMTs were calculated by taking the anti-log10 of the mean of the log10 antibody concentration or titer transformations. Antibody concentrations/titers below assay cut-offs were given an arbitrary value of half the cut-off for the purpose of GMC/GMT calculation. Analyses were performed with Statistical Analysis System (SAS® Institute Inc., Cary, NC).

3. Results

3.1. Study participants and demographics

Of 156 vaccinated adults, 146 completed the primary vaccination study. 43 adults who had received two primary doses of dPly/PhtD-10 or dPly/PhtD-30 completed the booster vaccination study (Fig. 2). Demographic characteristics of the groups are shown in Table 1.

3.2. Safety and reactogenicity of two-dose primary vaccination

3.2.1. Solicited local and general symptoms

Pain was the most commonly reported solicited local symptom in all groups, reported by 41.7%–100% of participants post-dose 1 and 71.4%–95.2% post-dose 2 for investigational formulation groups, and 91.7% post-dose 1 and 4.3% (one participant) post-dose 2 for the control group (Fig. 3A–C). Grade 3 local symptoms were reported by up to three participants (0.0%–12.5%) post-dose 1 and up to one participant (0.0%–4.8%) post-dose 2 in groups receiving an investigational formulation, and by one participant (4.2%) post-dose 1 and none of the participants post-dose 2 (placebo) in the control group (Fig. 3A–C).

The most frequently reported solicited general symptoms were fatigue and headache in the investigational groups and fatigue in the control group. Fever was reported by 0.0%–8.3% of participants post-dose 1 and 0.0%–10.0% of participants post-dose 2 in the investigational groups, and by 4.2% post-dose 1 and 0.0% post-dose 2 in

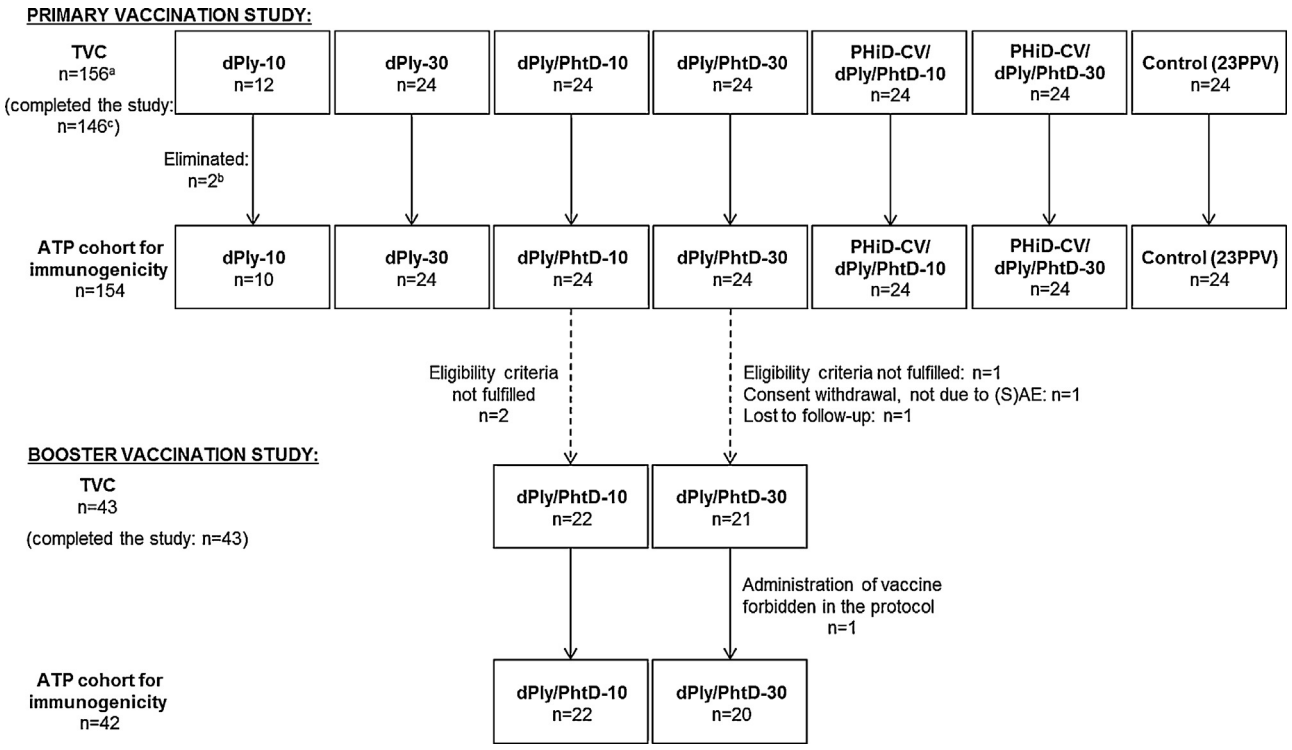

Fig. 2. Participant flow diagram. TVC, total vaccinated cohort; ATP, according to protocol; *n*, number of participants.
[a]In addition to these vaccinated subjects, 39 subjects failed their screening phase. [b]Reasons for exclusion: study vaccine not administered according to protocol ($n = 1$), essential serological data missing ($n = 1$). [c]Two participants in the dPly-30 group were withdrawn due to a non-serious AE, considered vaccine-related. No participants were withdrawn from the study due to a SAE.

the control group. No grade 3 fever was reported in any group. No trend for higher incidence rates of solicited general symptoms after dose 2 compared to dose 1 was observed (Fig. 3D–I).

The combination of pneumococcal proteins with PS-conjugates seemed to be associated with higher incidences of solicited local and general symptoms than the control vaccine (23PPV at dose 1, placebo at dose 2) (Fig. 3). The formulations containing the pneumococcal proteins alone tended to be the least reactogenic.

3.2.2. Unsolicited symptoms and serious adverse events

At least one unsolicited AE was reported after 44.7%–66.7% of primary investigational doses, and 46.8% of control doses. At least one grade 3 unsolicited AE was reported following 4.5%–13.3% of primary investigational doses, and 8.5% of control doses (Table S1). At least one unsolicited AE considered causally related to vaccination was reported following 10.4%–33.3% of investigational vaccine doses and 12.8% of control doses (Table S2).

No SAEs were reported in the investigational groups. One participant in the control group reported two SAEs (myalgia and skeletal injury), which were considered not to be causally related to vaccination.

3.3. Safety and reactogenicity of the booster vaccination

3.3.1. Solicited local and general symptoms

Pain was the most commonly reported solicited local symptom in both groups post-booster (Fig. 3). Redness and swelling tended to be reported more frequently following vaccination with the higher protein-content formulation than the lower protein-content formulation. Grade 3 solicited local symptoms were reported by one participant in each group (Fig. 3).

Headache and fatigue tended to be reported more frequently in the dPly/PhtD-30 group than in the dPly/PhtD-10 group, although one participant in the dPly/PhtD-10 group reported grade 3 fatigue that was considered to be vaccine-related. No other grade 3 solicited general symptoms were reported. Fever was reported by one participant (in the dPly/PhtD-10 group) (Fig. 3).

3.3.2. Unsolicited symptoms and serious adverse events

Unsolicited symptoms post-booster were reported by six participants (27.3%) in the dPly/PhtD-10 group and five participants (23.8%) in the dPly/PhtD-30 group. One participant in each group reported a grade 3 unsolicited AE (pharyngitis [dPly/PhtD-10] and upper respiratory tract infection [dPly/PhtD-30]). One participant in each group reported an unsolicited AE that was considered vaccine-related (aphthous stomatitis [dPly/PhtD-10] and peripheral edema in the right hand of a participant vaccinated in the left arm [dPly/PhtD-30]). No SAEs were reported during the booster study.

No clinically significant changes in the hematology, biochemistry or urinary parameters were observed during the primary and booster study (data not shown).

3.4. Immunogenicity

3.4.1. Immune response after two-dose primary vaccination

Before vaccination, all participants had anti-Ply and anti-PhtD concentrations above the assays cut-offs. All remained seropositive post-dose 1 and post-dose 2. Anti-Ply antibody GMCs increased after each vaccination in all groups except control. For PhtD, antibody GMCs increased following each vaccination in the groups that received a PhtD-containing formulation. A trend toward higher anti-Ply antibody GMCs was observed for dPly/PhtD compared to dPly alone. Antibody GMCs tended to be higher for the 30 μg formulations when compared to the respective 10 μg formulation, although this trend was more pronounced for dPly (1.9- to 2.6-fold higher) than PhtD (1.3- to 1.6-fold higher) (Table 2A and B).

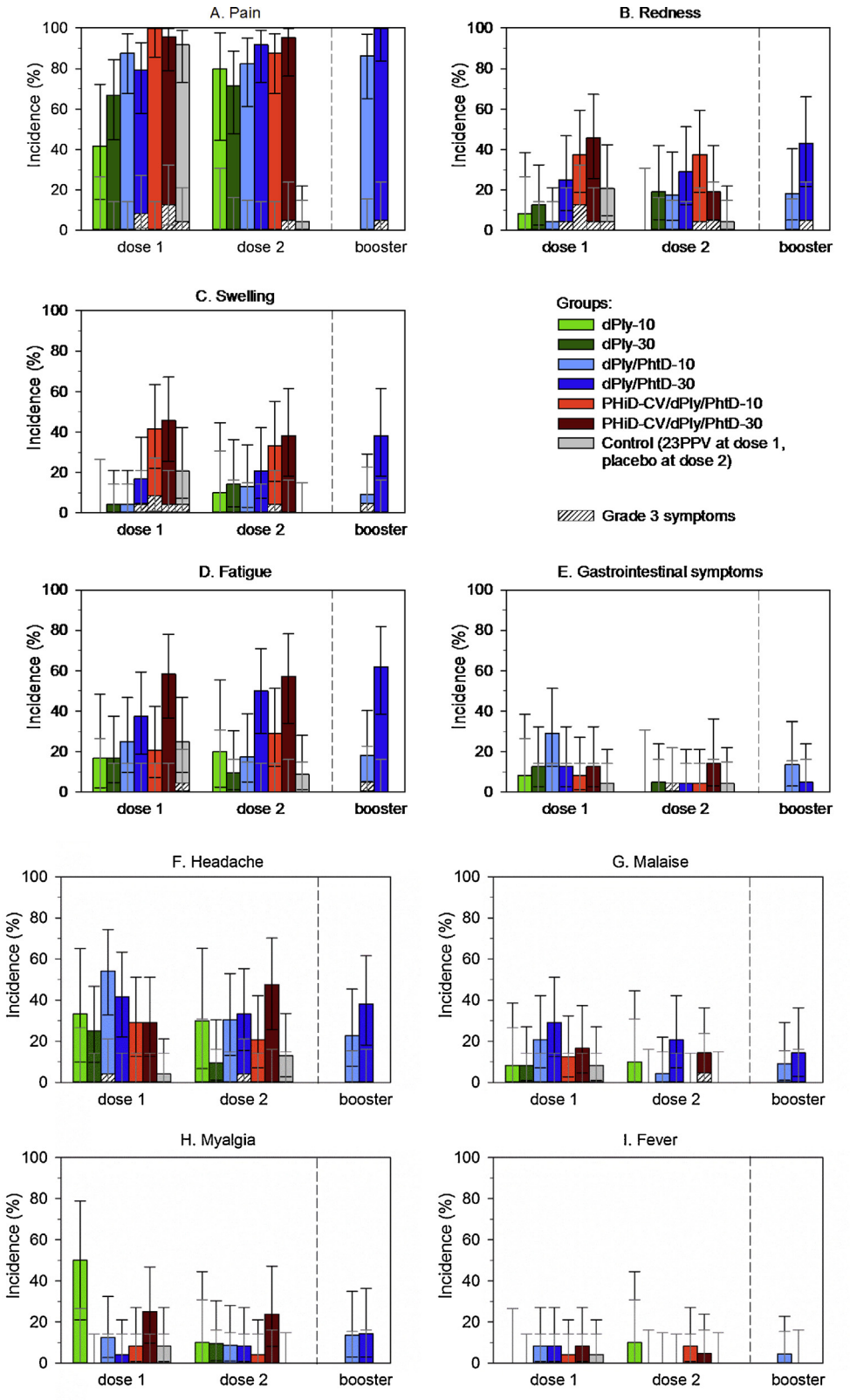

Fig. 3. Incidence of solicited local and general symptoms reported during day 0–6 post-vaccination. The results are shown for the primary total vaccinated cohort (TVC) for dose 1 and 2; and for the booster TVC for the booster dose. Grade 3 symptoms were defined as those that prevented normal activity, redness or swelling with a diameter of >50 mm, or temperature >39.5 °C measured orally. Error bars indicate 95% confidence intervals.

For anti-PD, a marked increase in seropositivity rates and antibody GMC values was observed post-dose 1 compared to pre-vaccination in the groups receiving PD-containing formulations. Antibody GMCs increased from 106.8 LU/mL [95% CI: 73.9–154.4] pre-vaccination to 612.4 LU/mL [95% CI: 409.9–915.1] post-dose 1 for PHiD-CV/dPly/PhtD-10 and from 82.3 LU/mL [95% CI: 62.5–108.4] to 503.9 LU/mL [95% CI: 366.2–693.3] for PHiD-CV/dPly/PhtD-30. One month post-dose 2, anti-PD antibody GMCs

Table 1
Demographic characteristics of participants (total vaccinated cohort).

	dPly-10 (*n* = 12)	dPly-30 (*n* = 24)	dPly/PhtD-10 (*n* = 24)	dPly/PhtD-30 (*n* = 24)	PHiD-CV/dPly/PhtD-10 (*n* = 24)	PHiD-CV/dPly/PhtD-30 (*n* = 24)	Control (23PPV/placebo) (*n* = 24)	Total (*n* = 156)
A. Primary vaccination study								
Mean age at first dose (years ± SD)	26.3 ± 5.74	25.8 ± 5.39	26.3 ± 5.17	23.3 ± 3.87	25.0 ± 6.53	23.0 ± 5.09	26.3 ± 6.90	25.0 ± 5.65
Female, *n* (%)	4 (33.3)	14 (58.3)	16 (66.7)	14 (58.3)	14 (58.3)	15 (62.5)	15 (62.5)	92 (59.0)
Race, *n* (%)								
White–Caucasian/European heritage	11 (91.7)	24 (100)	24 (100)	24 (100)	24 (100)	24 (100)	24 (100)	155 (99.4)
Other	1 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)

	dPly/PhtD-10 (*n* = 22)	dPly/PhtD-30 (*n* = 21)	Total (*N* = 43)
B. Booster vaccination study			
Mean age at first dose (years ± SD)	26.7 ± 4.80	23.9 ± 4.11	25.3 ± 4.64
Female, *n* (%)	15 (68.2)	12 (57.1)	27 (62.8)
Race, *n* (%)			
White–Caucasian/European heritage	22 (100)	21 (100)	43 (100)

n, number of participants; SD, standard deviation

Table 2
Anti-Ply and anti-PhtD antibody geometric mean concentrations (according-to-protocol cohort for immunogenicity).

	dPly-10		dPly-30		dPly/PhtD-10		Ply/PhtD-30		PHiD-CV/dPly/PhtD-10		PHiD-CV/dPly/PhtD-30		Control	
	N	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)
A. Anti-Ply antibody GMC (LU/mL)														
Pre-vac.	10	9198 (5993–14,115)	24	15,333 (10,751–21,867)	24	13,060 (9621–17,729)	23	14,905 (11,128–19,963)	24	12,239 (8648–17,320)	24	19,988 (15,149–26,371)	24	18,661 (12,727–27,363)
Post-dose 1	10	22,982 (15,631–33,790)	24	55,848 (37,181–83,887)	24	43,981 (31,936–60,568)	24	90,445 (62,894–130,065)	24	23,276 (16,926–32,009)	24	61,346 (46,049–81,725)	24	18,685 (12,612–27,683)
Post-dose 2	10	47,010 (30,960–71,379)	21	87,300 (58,202–130,947)	23	63,999 (48,406–84,614)	24	143,923 (106,150–195,138)	24	28,560 (21,331–38,239)	21	73,597 (52,250–103,665)	23	16,573 (11,007–24,954)
Pre-booster		–		–	22	41,823 (30,264–57,797)	20	89,612 (65,851–121,947)		–		–		–
Post-booster		–		–	22	92,943 (65,791–131303)	20	144767 (106912–196026)		–		–		–

	dPly-10		dPly-30		dPly/PhtD-10		dPly/PhtD-30		PHiD-CV/dPly/PhtD-10		PHiD-CV/dPly/PhtD-30		Control	
	N	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)	*N*	GMC (95% CI)
B. Anti-PhtD antibody GMC (LU/mL)														
Pre-vac.	10	9997 (6609–15,121)	24	17,716 (13,161–23,849)	24	16,454 (11,491–23,561)	23	16,801 (13,271–21,271)	24	13,959 (10,650–18,297)	24	16,771 (11,893–23,650)	24	13,120 (10,035–17,153)
Post-dose 1	10	11,351 (6929–18,596)	24	18,095 (13,270–24,673)	24	28,637 (20,778–39,468)	24	37,186 (28,847–47,934)	24	22,446 (17,794–28,314)	24	29,021 (20,873–40,349)	24	12,904 (9568–17,402)
Post-dose 2	10	8054 (5005–12961)	21	14378 (10120–20429)	23	37,671 (29,311–48,414)	24	58,531 (49,364–69,401)	24	24,312 (18,801–31,439)	21	40,142 (29,095–55,382)	23	11,544 (8458–15,756)
Pre-booster		–		–	22	26,672 (19,424–36,625)	20	42,111 (33,408–53,081)		–		–		–
Post-booster		–		–	22	37,851 (29,018–49,371)	20	62,795 (51,696–76,278)		–		–		–

N, number of participants with available results; GMC, geometric mean concentration, 95% CI, 95% confidence interval.

Table 3
Percentage of participants with OPA titers ≥ 8, and OPA geometric mean titers of vaccine pneumococcal serotypes (according-to-protocol cohort for immunogenicity).

Sero-type		PHiD-CV/dPly/PhtD-10			PHiD-CV/dPly/PhtD-30			Control (23PPV)		
		N	% ≥ 8 (95% CI)	GMT (95% CI)	*N*	% ≥ 8 (95% CI)	GMT (95% CI)	*N*	% ≥ 8 (95% CI)	GMT (95% CI)
1	Pre-vac.	21	23.8 (8.2–47.2)	7.4 (4.2–13.0)	22	18.2 (5.2–40.3)	5.7 (4.0–8.0)	21	9.5 (1.2–30.4)	4.6 (3.8–5.7)
	Post-dose 1	24	95.8 (78.9–99.9)	117.1 (67.7–202.5)	23	95.7 (78.1–99.9)	91.9 (56.0–150.9)	24	100 (85.8–100)	391.5 (193.7–791.4)
	Post-dose 2	24	95.8 (78.9–99.9)	139.4 (83.8–231.9)	21	100 (83.9–100)	147.3 (98.7–219.8)	23	95.7 (78.1–99.9)	268.6 (129.0–559.2)
4	Pre-vac.	15	40.0 (16.3–67.7)	21.6 (6.2–75.5)	17	17.6 (3.8–43.4)	9.3 (3.5–24.8)	16	6.3 (0.2–30.2)	5.8 (2.6–12.7)
	Post-dose 1	24	100 (85.8–100)	3468.8 (2091.4–5753.3)	24	100 (85.8–100)	5327.9 (3480.7–8155.3)	24	100 (85.8–100)	3335.4 (1902.8–5846.6)
	Post-dose 2	23	100 (85.2–100)	2548.9 (1735.9–3742.7)	21	100 (83.9–100)	3845.0 (2573.6–5744.6)	23	95.7 (78.1–99.9)	1838.2 (855.4–3950.4)
5	Pre-vac.	24	16.7 (4.7–37.4)	5.7 (4.0–8.1)	23	0.0 (0.0–14.8)	4.0 (4.0–4.0)	23	8.7 (1.1–28.0)	5.5 (3.5–8.7)
	Post-dose 1	23	95.7 (78.1–99.9)	266.9 (125.0–569.8)	24	95.8 (78.9–99.9)	202.5 (116.2–352.8)	24	100 (85.8–100)	416.9 (185.6–936.8)
	Post-dose 2	24	95.8 (78.9–99.9)	212.9 (111.2–407.6)	21	100 (83.9–100)	225.7 (139.7–364.8)	23	91.3 (72.0–98.9)	180.9 (70.3–465.7)
6B	Pre-vac.	13	76.9 (46.2–95.0)	144.9 (37.6–558.8)	12	66.7 (34.9–90.1)	122.9 (23.3–648.5)	10	70.0 (34.8–93.3)	141.7 (22.4–897.5)
	Post-dose 1	24	100 (85.8–100)	991.5 (583.3–1685.2)	24	100 (85.8–100)	1775.1 (961.8–3276.3)	23	95.7 (78.1–99.9)	1724.5 (837.6–3550.7)
	Post-dose 2	24	100 (85.8–100)	1202.7 (792.4–1825.3)	21	100 (83.9–100)	1935.8 (1193.7–3139.2)	23	91.3 (72.0–98.9)	940.3 (399.2–2214.7)
7F	Pre-vac.	11	100 (71.5–100)	2107.1 (964.6–4602.7)	12	91.7 (61.5–99.8)	764.2 (193.3–3022.0)	14	85.7 (57.2–98.2)	586.5 (156.8–2194.4)
	Post-dose 1	24	100 (85.8–100)	3239.0 (2209.1–4749.1)	24	100 (85.8–100)	4668.3 (2811.9–7750.4)	22	100 (84.6–100)	6960.6 (4531.6–10,691.5)
	post-dose 2	24	100 (85.8–100)	4547.2 (3105.4–6658.4)	21	100 (83.9–100)	6056.7 (4211.8–8709.6)	23	100 (85.2–100)	4886.4 (2956.8–8075.1)
9V	Pre-vac.	24	91.7 (73.0–99.0)	216.7 (103.5–453.8)	22	100 (84.6–100)	271.2 (177.8–413.6)	20	95.0 (75.1–99.9)	304.4 (148.5–624.0)
	Post-dose 1	24	100 (85.8–100)	2037.4 (1208.5–3434.7)	24	100 (85.8–100)	2666.3 (1586.8–4480.2)	24	100 (85.8–100)	3945.9 (2390.3–6514.1)
	Post-dose 2	24	100 (85.8–100)	3915.8 (2467.4–6214.3)	21	100 (83.9–100)	5498.0 (3334.3–9065.8)	23	100 (85.2–100)	2659.0 (1463.6–4830.9)
14	Pre-vac.	17	100 (80.5–100)	618.2 (364.9–1047.4)	16	93.8 (69.8–99.8)	245.2 (116.4–516.5)	20	95.0 (75.1–99.9)	308.4 (164.8–576.8)
	Post-dose 1	24	100 (85.8–100)	2968.1 (1713.8–5140.3)	24	100 (85.8–100)	2559.1 (1651.0–3966.7)	24	100 (85.8–100)	3058.8 (1829.4–5114.2)
	Post-dose 2	24	100 (85.8–100)	3045.5 (2007.2–4620.9)	21	100 (83.9–100)	2944.8 (1854.0–4677.5)	22	100 (84.6–100)	2551.9 (1518.7–4287.9)
18C	Pre-vac.	18	33.3 (13.3–59.0)	11.0 (5.2–23.3)	20	20.0 (5.7–43.7)	9.0 (4.1–19.6)	18	22.2 (6.4–47.6)	9.3 (4.1–20.8)
	Post-dose 1	24	95.8 (78.9–99.9)	562.2 (286.0–1105.0)	24	100 (85.8–100)	1132.3 (750.8–1707.6)	24	95.8 (78.9–99.9)	454.3 (217.1–950.7)
	Post-dose 2	24	100 (85.8–100)	928.5 (613.2–1405.8)	21	100 (83.9–100)	1830.0 (1220.6–2743.7)	22	90.9 (70.8–98.9)	311.6 (134.9–719.5)
19F	Pre-vac.	21	47.6 (25.7–70.2)	19.7 (7.8–49.8)	20	55.0 (31.5–76.9)	15.2 (7.7–30.1)	20	35.0 (15.4–59.2)	7.6 (4.9–11.7)
	Post-dose 1	21	100 (83.9–100)	690.6 (374.4–1273.7)	23	100.0 (85.2–100)	2218.9 (1351.2–3643.6)	20	100 (83.2–100)	412.5 (189.4–898.4)
	Post-dose 2	24	100 (85.8–100)	992.1 (532.9–1847.2)	21	100 (83.9–100)	2179.0 (1404.4–3380.9)	23	95.7 (78.1–99.9)	311.7 (141.7–685.6)
23F	Pre-vac.	19	68.4 (43.4–87.4)	240.5 (58.6–987.6)	17	70.6 (44.0–89.7)	235.4 (55.6–996.7)	15	73.3 (44.9–92.2)	236.9 (56.8–988.4)
	Post-dose 1	24	100 (85.8–100)	1968.9 (1256.5–3085.1)	24	100 (85.8–100)	3306.3 (2280.7–4793.1)	24	100 (85.8–100)	2024.2 (1237.8–3310.1)
	Post-dose 2	24	100 (85.8–100)	1882.9 (1176.2–3014.3)	21	100 (83.9–100)	2987.4 (1977.1–4513.9)	23	95.7 (78.1–99.9)	1559.7 (778.7–3123.8)

N, number of participants for which the results were available; pre-vac., pre-vaccination; GMT, geometric mean titers; CI, confidence interval.

remained within the same ranges as post-dose 1 (data not shown).

At both 1 month post-dose 1 and 1 month post-dose 2, for each vaccine pneumococcal serotype, at least 95.7% of participants in the PHiD-CV/dPly/PhtD groups had OPA titers ≥8. In the control group, these percentages were at least 95.7% 1 month post-dose 1 (23PPV) and at least 90.9% 1 month after dose 2 (placebo), compared to at least 6.3% before vaccination (Table 3).

After each primary dose, for 7 of 10 pneumococcal serotypes, observed OPA GMTs seemed to be higher in the PHiD-CV/dPly/PhtD-30 group than in the PHiD-CV/dPly/PhtD-10 group. For several pneumococcal serotypes, increases in OPA GMTs from post-dose 1 to post-dose 2 were observed (Table 3).

3.4.2. Persistence and post-booster immune response to dPly and PhtD

Before and 1 month post-booster, all participants in the dPly/PhtD-10 and dPly-PhtD-30 groups had antibody concentrations ≥599 LU/mL for anti-Ply and ≥391 LU/mL for anti-PhtD antibodies.

Anti-Ply and anti-PhtD antibody GMCs decreased between the post-dose 2 and pre-booster timepoint. For both the 10 and 30 μg formulations, a trend for increased anti-Ply and anti-PhtD antibody GMCs was observed post-booster compared to pre-booster. Post-booster antibody GMCs were in a similar range as those post-dose 2, except for dPly in the dPly/PhtD-10 group (63,999 LU/mL post-dose 2, 92,943 LU/mL post-booster). A trend toward higher anti-Ply and anti-PhtD antibody GMCs was observed pre- and post-booster with the PHiD-CV/dPly/PhtD-30 formulation compared to the PHiD-CV/dPly/PhtD-10 formulation (Table 2A and B).

4. Discussion

We assessed the safety and immunogenicity of six investigational pneumococcal protein-containing vaccine formulations. All had an acceptable safety profile and were well tolerated. No vaccine-related SAEs were reported. Vaccination with subsequent doses did not lead to increased incidence of solicited symptoms or unsolicited AEs.

There was a trend toward higher incidences of solicited symptoms for the combination of pneumococcal proteins with PS-conjugates than for the control vaccine (particularly redness and swelling). This higher reactogenicity in adults could be related to the carrier proteins of the polysaccharides [21,22]; reactogenicity profiles may differ in young children, the main target group.

Reactogenicity of the formulations containing pneumococcal proteins alone (dPly and dPly/PhtD) was low, and generally in a similar range as previously reported for other investigational pneumococcal protein vaccines containing dPly [23], PhtD [24] or a combination of PhtD and pneumococcal choline-binding protein A (PcpA) [25].

Initial immunogenicity assessments in this small group of adults showed an increase in anti-PhtD and/or anti-Ply antibody GMCs following each investigational vaccine dose. Coadministration of dPly with PhtD did not negatively affect anti-Ply antibody responses. There was a trend toward higher anti-Ply antibody GMCs for dPly/PhtD than for dPly alone. Our results thus confirm the immunogenicity of both antigens, in-line with previous studies [26,27], and suggest that PhtD enhances the anti-Ply immune response. One prospective study reported an increase over time in the levels of natural antibodies against five pneumococcal proteins (including PhtD and Ply) in young children with nasopharyngeal colonization and acute otitis media [26]. Adults have been shown to have circulating memory CD4+ T cells that can be stimulated by PhtD, Ply and other protein vaccine candidate antigens [27]. Young children have a more limited response, indicating that their vaccination would likely require several priming doses to stimulate CD4+ T-cell responses [27].

Before vaccination, all participants already had anti-Ply and anti-PhtD antibody concentrations above the assay cut-off. This high pre-vaccination seropositivity rate most likely reflects previous pneumococcal exposure. In infants and toddlers, increases in naturally-acquired antibody levels against several pneumococcal protein surface antigens (including PhtD) and Ply have been reported with increasing age (from 6 months to 2 years) and exposure (nasopharyngeal carriage, acute otitis media) [26,28–30]. Otitis-prone children and children with treatment failure of acute otitis media also mount a lower IgG serum antibody response to pneumococcal proteins [31]. Several studies have indicated a protective role of naturally acquired anti-Ply antibodies [32,7,33], while antibodies against PhtD prevent pneumococcal adherence to human airway epithelial cells [16]. The presence of these antibodies, as seen in our participants, could thus be contributing to the protection of healthy young adults against pneumococcal disease.

Our immunogenicity results must be interpreted with caution due to the small number of participants and the fact that protective levels of antibodies to pneumococcal proteins have not yet been determined. Additionally, our study was performed in adults aged 18–40 years; these results serve as a safety assessment before progressing to a pediatric population but may not reflect the safety, reactogenicity and immunogenicity data from other age groups. Because PHiD-CV is not licensed for use in adults, no PHiD-CV control group was included and we could thus not assess whether addition of the proteins affects PS-conjugate responses.

Our results support continued development of the investigational pneumococcal protein-containing vaccine and further assessment in younger age groups, who carry the main burden of pneumococcal disease. New pneumococcal protein-containing vaccines are promising and have the potential to also target the serotypes that are currently not covered by PCVs.

Synflorix is a trademark of the GlaxoSmithKline group of companies; Pneumovax23 is a trademark of Sanofi Pasteur.

Conflict of interest

The institution of GLR and FDB received grants from GlaxoSmithKline group of companies. GLR declares he received payment for consultancies for GlaxoSmithKline group of companies, Novartis Vaccines and Diagnostics and Immune Targeting Systems. GLR received travel fees from the GlaxoSmithKline group of companies. JUR was and MT and DB are employees of GlaxoSmithKline group of companies; DB and JUR declares stock and share options ownership in GlaxoSmithKline group of companies. CM has no conflict of interest to declare.

Authors' contributions

GLR and FDB coordinated the clinical aspects of the study. GLR, CM and FDB collected data. MT, JUR and DB planned and designed the study and together with GLR interpreted the results. MT did the statistical analyses. All authors critically reviewed the different drafts of the manuscript and approved the final version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.02.052.

References

[1] Lynch III JP, Zhanel GG. *Streptococcus pneumoniae*: epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines. Curr Opin Pulm Med 2010;16(3):217–25.
[2] O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet 2009;374(9693):893–902.
[3] Grijalva CG, Nuorti JP, Arbogast PG, Martin SW, Edwards KM, Griffin MR. Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. Lancet 2007;369(9568):1179–86.
[4] Crisinel PA, Chevalier I, Rallu F, Tapiero B, Lamarre V, Thibault R, et al. Invasive pneumococcal disease after implementation of a reduced three-dose pneumococcal conjugate vaccine program: a pediatric tertiary care center experience. Eur J Pediatr 2010;169(11):1311–5.
[5] Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis 2010;201(1):32–41.
[6] Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. Lancet 2011;378:1962–73, 9807.
[7] Musher DM, Phan HM, Baughn RE. Protection against bacteremic pneumococcal infection by antibody to pneumolysin. J Infect Dis 2001;183(5):827–30.
[8] Alexander JE, Lock RA, Peeters CC, Poolman JT, Andrew PW, Mitchell TJ, et al. Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of *Streptococcus pneumoniae*. Infect Immun 1994;62(12):5683–8.
[9] Ogunniyi AD, Woodrow MC, Poolman JT, Paton JC. Protection against Streptococcus pneumoniae elicited by immunization with pneumolysin and CbpA. Infect Immun 2001;69(10):5997–6003.
[10] Garcia-Suarez MM, Cima-Cabal MD, Florez N, Garcia P, Cernuda-Cernuda R, Astudillo A, et al. Protection against pneumococcal pneumonia in mice by monoclonal antibodies to pneumolysin. Infect Immun 2004;72(8):4534–40.
[11] Denoel P, Philipp MT, Doyle L, Martin D, Carletti G, Poolman JT. A protein-based pneumococcal vaccine protects rhesus macaques from pneumonia after experimental infection with *Streptococcus pneumoniae*. Vaccine 2011;29(33):5495–501.
[12] Hamel J, Charland N, Pineau I, Ouellet C, Rioux S, Martin D, et al. Prevention of pneumococcal disease in mice immunized with conserved surface-accessible proteins. Infect Immun 2004;72(5):2659–70.
[13] Hava DL, Camilli A. Large-scale identification of serotype 4 *Streptococcus pneumoniae* virulence factors. Mol Microbiol 2002;45(5):1389–406.
[14] Adamou JE, Heinrichs JH, Erwin AL, Walsh W, Gayle T, Dormitzer M, et al. Identification and characterization of a novel family of pneumococcal proteins that are protective against sepsis. Infect Immun 2001;69(2):949–58.
[15] Godfroid F, Hermand P, Verlant V, Denoel P, Poolman JT. Preclinical evaluation of the Pht proteins as potential cross-protective pneumococcal vaccine antigens. Infect Immun 2011;79(1):238–45.
[16] Khan MN, Pichichero ME. Vaccine candidates PhtD and PhtE of Streptococcus pneumoniae are adhesins that elicit functional antibodies in humans. Vaccine 2012;30(18):2900–7.
[17] Leroux-Roels I, Devaster JM, Leroux-Roels G, Verlant V, Henckaerts I, Moris P, et al. Adjuvant system AS02 enhances humoral and cellular immune responses to pneumococcal protein PhtD vaccine in healthy young and older adults: randomised, controlled trials. Vaccine 2013, http://dx.doi.org/10.1016/j.vaccine.2013.10.052, pii: S0264-410X(13)01430-8.
[18] Prymula R, Schuerman L. 10-Valent pneumococcal nontypeable *Haemophilus influenzae* PD conjugate vaccine: Synflorix. Expert Rev Vaccines 2009;8(11):1479–500.
[19] Findlay JW, Smith WC, Lee JW, Nordblom GD, Das I, DeSilva BS, et al. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. J Pharm Biomed Anal 2000;21(6):1249–73.
[20] Henckaerts I, Durant N, De GD, Schuerman L, Poolman J. Validation of a routine opsonophagocytosis assay to predict invasive pneumococcal disease efficacy of conjugate vaccine in children. Vaccine 2007;25(13):2518–27.
[21] Kretsinger K, Broder KR, Cortese MM, Joyce MP, Ortega-Sanchez I, Lee GM, et al. Preventing tetanus, diphtheria, and pertussis among adults: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine recommendations of the Advisory Committee on Immunization Practices (ACIP) and recommendation of ACIP, supported by the Healthcare Infection Control Practices Advisory Committee (HICPAC), for use of Tdap among health-care personnel. MMWR Recomm Rep 2006;55(RR-17):1–37.
[22] Galazka AM, Robertson SE. Immunization against diphtheria with special emphasis on immunization of adults. Vaccine 1996;14(9):845–57.
[23] Kamtchoua T, Bologa M, Hopfer R, Neveu D, Hu B, Sheng X, et al. Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults. Vaccine 2013;31(2):327–33.
[24] Seiberling M, Bologa M, Brookes R, Ochs M, Go K, Neveu D, et al. Safety and immunogenicity of a pneumococcal histidine triad protein D vaccine candidate in adults. Vaccine 2012;30(52):7455–60.
[25] Bologa M, Kamtchoua T, Hopfer R, Sheng X, Hicks B, Bixler G, et al. Safety and immunogenicity of pneumococcal protein vaccine candidates: monovalent choline-binding protein A (PcpA) vaccine and bivalent PcpA-pneumococcal histidine triad protein D vaccine. Vaccine 2012;30(52):7461–8.
[26] Pichichero ME, Kaur R, Casey JR, Xu Q, Almudevar A, Ochs M. Antibody response to *Streptococcus pneumoniae* proteins PhtD, LytB, PcpA, PhtE and Ply after nasopharyngeal colonization and acute otitis media in children. Hum Vaccin Immunother 2012;8(6):799–805.
[27] Sharma SK, Roumanes D, Almudevar A, Mosmann TR, Pichichero ME. CD4 T-cell responses among adults and young children in response to *Streptococcus pneumoniae* and *Haemophilus influenzae* vaccine candidate protein antigens. Vaccine 2013;31(30):3090–7.
[28] Rapola S, Jantti V, Haikala R, Syrjanen R, Carlone GM, Sampson JS, et al. Natural development of antibodies to pneumococcal surface protein A, pneumococcal surface adhesin A, and pneumolysin in relation to pneumococcal carriage and acute otitis media. J Infect Dis 2000;182(4):1146–52.
[29] Holmlund E, Quiambao B, Ollgren J, Nohynek H, Kayhty H. Development of natural antibodies to pneumococcal surface protein A, pneumococcal surface adhesin A and pneumolysin in Filipino pregnant women and their infants in relation to pneumococcal carriage. Vaccine 2006;24(1):57–65.
[30] Holmlund E, Quiambao B, Ollgren J, Jaakkola T, Neyt C, Poolman J, et al. Antibodies to pneumococcal proteins PhtD, CbpA, and LytC in Filipino pregnant women and their infants in relation to pneumococcal carriage. Clin Vaccine Immunol 2009;16(6):916–23.
[31] Kaur R, Casey JR, Pichichero ME. Serum antibody response to five *Streptococcus pneumoniae* proteins during acute otitis media in otitis-prone and non-otitis-prone children. Pediatr Infect Dis J 2011;30(8):645–50.
[32] Huo Z, Spencer O, Miles J, Johnson J, Holliman R, Sheldon J, et al. Antibody response to pneumolysin and to pneumococcal capsular polysaccharide in healthy individuals and *Streptococcus pneumoniae* infected patients. Vaccine 2004;22(9-10):1157–61.
[33] Francis JP, Richmond PC, Pomat WS, Michael A, Keno H, Phuanukoonnon S, et al. Maternal antibodies to pneumolysin but not to pneumococcal surface protein A delay early pneumococcal carriage in high-risk Papua New Guinean infants. Clin Vaccine Immunol 2009;16(11):1633–8.
[34] Guidance for Industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials; 2013.