Immunogenicity and safety of a booster dose of the 13-valent pneumococcal conjugate vaccine in children primed with the 10-valent or 13-valent pneumococcal conjugate vaccine in the Czech Republic and Slovakia

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ABSTRACT

Background. Although both the 13-valent pneumococcal conjugate vaccine (PCV13) and the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV) are widely used, it is unclear how interchangeable they are in terms of immunogenicity.

Methods. Two phase 3, open-label, multicenter studies were conducted to assess the immunogenicity and safety of a booster dose of PCV13 in children primed with PHiD-CV or PCV13. In the Czech Republic, 12–15-month-old children received a PCV13 booster after 3-dose priming with either PHiD-CV or PCV13. In Slovakia, 11–12-month-old children received PCV13 following 2-dose priming with either PHiD-CV or PCV13. Serum IgG concentrations were assessed by enzyme-linked immunosorbent assay and functional antibodies were assessed by opsonophagocytic assay (OPA) before and 1 and 12 months after the booster dose. The primary objective of these studies was to assess non-inferiority of OPA titers for serotype 19A in PHiD-CV-primed subjects compared to those in PCV13-primed children 1 month post-booster.

Results. A total of 98 subjects in the Czech Republic and 89 subjects in Slovakia were included. One month after the PCV13 booster dose, the IgG and OPA immune responses to serotype 19A in subjects primed with 2 or 3doses of PHiD-CV were not inferior to those in subjects primed with PCV13. Non-inferior and persistent immune responses to most other vaccine serotypes were also observed after the PCV13 booster in PHiD-CV-primed subjects. No safety issues were raised in either study.

Conclusions. Overall, robust IgG and OPA immunological responses were observed after booster vaccination with PCV13 in children primed with 2 or 3 doses of PHiD-CV or PCV13, including for serotypes not included in PHiD-CV. These results suggest that both vaccines are

interchangeable in terms of safety and immunogenicity and that PCV13 can be used as a booster in the context of mixed schedules. (EudraCT numbers: 2012-005366-35 and 2012-005367-27)

Keywords: pneumococcal conjugate vaccine, interchangeability, immunisation schedule, immunogenicity, serotype 19A

1. INTRODUCTION[1]

Since the introduction of the first pneumococcal conjugate vaccine (PCV) in 2000, invasive and non-invasive diseases caused by *Streptococcus pneumoniae* have dramatically decreased worldwide [1, 2]. This 7-valent vaccine (PCV7; Pfizer Inc.) included the capsular polysaccharides of the seven most frequent pneumococcal serotypes in the United States at the time of licensure (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) [3]. However, a small but significant rise in infections caused by non-PCV7 serotypes, notably serotype 19A, has occurred in several countries, thereby eroding the benefits of vaccination [4-6]. The burden of disease caused by serotype 19A is of particular concern because it is highly invasive and frequently multiresistant to antibiotics [7]. To limit the rise of infections by non-vaccine serotypes and increase overall serotype coverage, second-generation PCVs with extended valencies became available in 2009 and have gradually replaced PCV7. The 10-valent

[1] Abbreviations: AE, adverse event; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GMT, geometric mean titer; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine; SAE, serious AE.

pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV; GSK Vaccines) includes the PCV7 serotypes plus serotypes 1, 5, and 7F, whereas the 13-valent PCV (PCV13; Pfizer Inc.) includes the PHiD-CV serotypes plus serotypes 3, 6A, and 19A. Both PHiD-CV and PCV13 have been licensed for protection against invasive pneumococcal disease on the basis of immunological non-inferiority compared to the licensed PCV7 [8].

Both vaccines were originally licensed in a 4-dose schedule consisting of 3 primary doses, plus a booster dose administered at least 6 months after the third dose (3+1 schedule) [9]. The first dose can be administered from 6 weeks of age and the booster dose should be administered between 11 and 15 months of age. Several alternative schedules are also used. One consists of 2 primary doses given 2 months apart, followed by a booster dose at least 6 months after the second dose (2+1 schedule) [9]. This schedule was first evaluated and implemented in the United Kingdom [10] and subsequently in several other countries [6]. In developing countries, a 3-dose primary schedule without a booster dose (3+0 schedule) is often used to match the schedules of the Expanded Programme on Immunisation [11].

Although immunogenicity of both PHiD-CV and PCV13 has been compared to that of PCV7 [12, 13], only a few studies have directly compared PHiD-CV and PCV13 [14-16]. In this study, we directly compared the immunogenicity and safety of PCV13 when administered as a booster dose in children primed with PHiD-CV or PCV13, either as a 2- or 3-dose schedule.

2. MATERIALS AND METHODS

2.1. Study design and subjects

These were two phase 3, multicenter, open-label studies conducted in 10 centers in the Czech Republic (EudraCT, 2012-005366-35) and in 8 centers in Slovakia (EudraCT, 2012-005367-

27) to assess immunogenicity and safety of alternative vaccination schedules with pneumococcal conjugate vaccines. Both study protocols were approved by the relevant independent ethics committees and the studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from parents or legal representatives of all children before enrolment.

In the Czech Republic (3+1 schedule), healthy children were eligible if they were 12–15 months of age and had completed a 3-dose vaccination course before 7 months of age with either the 10-valent PCV (PHiD-CV; *Synflorix*™, GSK Vaccines, Rixensart, Belgium) or the 13-valent PCV (PCV13; *Prevenar/Prevnar 13*™, Pfizer, New York, NY). In Slovakia (2+1 schedule), healthy children were eligible if they were 11–12 months of age and had completed a 2-dose vaccination course before 6 months of age with either PHiD-CV or PCV13.

Children were excluded if they had received a previous booster dose against *S. pneumoniae*, any investigational product within 30 days preceding the study vaccination, immunoglobulins or any blood product within 3 months preceding the study vaccination (or were to receive these during the study period), a vaccine (except licensed influenza or diphtheria-tetanus-acellular pertussis vaccines) within 30 days before or after the study vaccination, immunosuppressants or other immune-modifying drugs for >14 days since birth; had any confirmed or suspected immunodeficient condition or family history of immunodeficiency, serious chronic disease, history of neurological disorders or seizures; had any contraindications to vaccination such as allergies; had acute infection or fever at enrollment.

2.2. Study vaccines

All children received one booster dose of PCV13 by intramuscular injection (0.5 mL) in the anterolateral region of the thigh or the deltoid region. This vaccine contains capsular polysaccharides of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, all

conjugated to the diphtheria CRM_{197} carrier protein. Co-administration of the routine diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-*H. influenzae* type b vaccine (DTaP-HBV-IPV/Hib; *Infanrix hexa*™; GSK Vaccines) was allowed in the Czech Republic and was mandatory in Slovakia.

2.3. Assessment of immunogenicity

Blood samples were taken for immunogenicity analyses before the booster dose (study month 0) and 1 and 12 months after the booster dose. Sera were stored at –20°C until analysis. Analyses were performed at the World Health Organization pneumococcal reference laboratory (University College London, United Kingdom). Immunoglobulin G (IgG) serum concentrations specific for the 13 vaccine serotypes were measured using an enzyme-linked immunosorbent assay (ELISA) after adsorption with cell-wall and 22F polysaccharides to increase the assay specificity [17]. A standardized opsonophagocytic assay (OPA) was used to measure functional antibodies against the same serotypes [18]. The OPA response to vaccine-related serotype 6C [19] was also measured, although not planned in the protocol. The OPA titer was defined as the reciprocal of the lowest serum dilution that induces ≥50% bacterial cell death compared to the assay control.

2.4. Assessment of safety and reactogenicity

Solicited local reactions (pain, redness, and swelling at the injection site) and solicited systemic reactions (drowsiness, fever, irritability/fussiness, and loss of appetite) were recorded by parents on diary cards for 4 days after vaccination (days 0–3). Unsolicited adverse events (AEs) were recorded for 31 days (days 0–30) after vaccination and serious adverse events (SAEs) were recorded over the entire study period. The intensity of solicited reactions and AEs was scored on a scale from 1 (mild) to 3 (severe). Redness and swelling at the injection site were scored as 1 for >0 to ≤20 mm diameter, 2 for >20 to ≤30 mm, and 3 for

>30 mm. Fever was defined as rectal temperature ≥38.0°C or oral, axillary, or tympanic temperature ≥37.5°C. Grade 3 fever was defined as rectal temperature >40°C or axillary, oral, or tympanic temperature >39.5°C. Pain was scored as 1 for minor reaction to touch, 2 for crying on touch, and 3 for crying when limb is moved or spontaneously painful. The other solicited reactions were scored as 1 if they did not affect normal activity, 2 if they interfered with normal activity, and 3 if they prevented normal daily activity. All solicited local reactions were considered vaccination-related. Causality of solicited systemic reactions, unsolicited AEs, and SAEs was determined by the investigators.

2.5. Statistical analysis

Safety was analyzed in the full analysis set, which included all subjects who received a booster dose of PCV13. Immunogenicity was analyzed in the per protocol set, which included all subjects who received the booster dose of PCV13 according to protocol and who had at least one valid IgG or OPA result.

At each visit and for each serotype, antibody geometric mean concentrations (GMCs) and OPA geometric mean titers (GMTs) were determined with 2-sided 95% confidence intervals (CI). The percentages of participants reaching the predefined immunological thresholds (OPA titer ≥8 and IgG concentration ≥0.35 μg/mL) were also calculated [20, 21]. No formal statistical tests were performed for safety data.

The primary objective of the studies was to assess non-inferiority of the OPA GMT to serotype 19A in subjects primed with PHiD-CV compared to those primed with PCV13, 1 month after the PCV13 booster dose administered at 12–15 months in the Czech Republic (3+1) and at 11–12 months in Slovakia (2+1). The secondary confirmatory objective was to assess non-inferiority of the IgG response to serotype 19A 1 month post-booster. Non-inferiority was demonstrated if the lower limit of the 2-sided 95% CI for the OPA GMT ratio

or the IgG GMC ratio (PHiD-CV group over PCV13 group) was >0.5. Other secondary exploratory objectives included IgG and OPA responses against the other serotypes at month 1 and against all serotypes at month 12, and assessment of safety and reactogenicity of the PCV13 booster dose.

In both studies, a sample size of 92 subjects (46 participants in both vaccine groups) was calculated to provide 90% power for assessing non-inferiority in pneumococcal serotype 19A antibody response rate with a limit of 10% at a 2-sided 5% significance level, assuming a 98% response rate in the PHiD-CV group and a 10% drop-out rate. All significance tests were two-tailed. A *P*-value ≤0.05 was considered to indicate statistical significance. Statistical analyses were performed with SAS™ version 9.1 or higher (SAS Institute, Cary, NC).

3. RESULTS

3.1. Subjects

The study in the Czech Republic was conducted between March 12, 2013 and October 7, 2014 and the study in Slovakia between March 5, 2013 and November 14, 2014. Overall, 98 children were enrolled in the Czech Republic and 89 in Slovakia (Figure 1). All subjects in both studies received the PCV13 booster dose and were included in the full analysis set. Twelve months post-booster, 94.1%–98.0% of subjects per group were included in the per protocol set. In the Czech Republic, DTaP-HBV-IPV/Hib was co-administered in 44 subjects (83.0%) in the PHiD-CV-primed group and 39 (86.7%) in the PCV13-primed group. In Slovakia, one subject in the PCV13 group did not receive DTaP-HBV-IPV/Hib concomitantly with PCV13, although requested by the protocol.

In both studies, most participants were male and Caucasian (Table 1). In the Czech Republic, 73.3% of the subjects in the PCV13 group were male compared to 56.6% in the PHiD-CV

group. Per study design, participants were older in the Czech Republic (mean age, 12.6 months) than in Slovakia (mean age, 11.3 months).

3.2. Immune responses against serotype 19A

Before the booster dose, OPA titers against serotype 19A were higher in children primed with PCV13 than those primed with PHiD-CV in both studies (Supplementary Table 1). One month post-booster, OPA GMTs increased markedly with both schedules and, subsequently, decreased between months 1 and 12. In both studies, post-booster 19A OPA titers were comparable in PHiD-CV and PCV13 groups. At month 1, all subjects had 19A OPA GMTs ≥8 in both groups in both studies, whereas at 12 months, fewer subjects had GMTs ≥8 in the PHiD-CV groups (84.3%–86.8%) than in the PCV13 groups (93.9%–97.8%) (Supplementary Table 2).

One month post-booster, the OPA GMT ratio (PHiD-CV group over PCV13 group) for serotype 19A was 1.24 (95% CI, 0.84–1.82) with the 3+1 schedule and 0.81 (95% CI, 0.54–1.20) with the 2+1 schedule (Figure 2). Because the lower limit values of the 95% CI were higher than the 0.5 arbitrary margin in both studies, the OPA titers in PHiD-CV-primed children were not inferior to those in PCV13-primed children. Thus, the primary confirmatory objective was met in both studies. However, 12 months post-booster, 19A OPA titers in PHID-CV-primed children were inferior to those in PCV13-primed children in both studies.

One month post-booster, 19A IgG GMCs were similar between the two schedules (Supplementary Table 3). Compared to pre-booster concentrations, they increased 15–29 fold with the 3+1 schedule and 12–24 fold with the 2+1 schedule, and decreased between months 1 and 12. All subjects had GMCs ≥0.35 mg/mL at month 1, and between 82.4% and 98.0%. at months 12 (Supplementary Table 4). In both studies, the percentages were comparable between groups. The mean IgG GMC ratio for serotype 19A 1 month post-booster was 1.36

(95% CI, 0.90–2.08) with the 3+1 schedule and 1.25 (95% CI, 0.83–1.87) with the 2+1 schedule (Figure 2). Therefore, in both studies, the antibody concentrations in PHiD-CV-primed children were not inferior to those in PCV13-primed children. Non-inferiority was also shown at 12 months.

3.3. OPA responses to the other vaccine serotypes

One month post-booster, the OPA titers increased substantially for all the other serotypes in both studies (Supplementary Table 1). With both schedules, OPA GMTs were similar between groups for serotypes 1, 3, 5, 6B, 18C, 19F, and 23F but, with the 3+1 schedule, they were higher in the PCV13 group for serotypes 4, 6A, 6C, 7F, 9V, and 14. In both studies, all subjects had OPA titers ≥8 in the two groups for all serotypes, except for serotypes 3 and 6A in the PHiD-CV groups (≥97.4%) (Supplementary Table 2). For the vaccine-related serotype 6C, the proportions were slightly lower in the 2+1 than in the 3+1 schedule (92.1%–93.9% vs. 98%–100%).

With the 3+1 schedule, OPA GMTs in PHiD-CV-primed subjects were not inferior to those in PCV13-primed subjects for six serotypes (1, 3, 5, 6B, 18C, and 19F) and were inferior for seven (4, 6A, 6C, 7F, 9V, 14, 23F) (Figure 2). With the 2+1 schedule, OPA GMTs were not inferior in PHiD-CV-primed subjects for seven serotypes (1, 3, 5, 6A, 6B, 9V, and 19F) and inferior for six (4, 6C, 7F, 14, 18C, and 23F).

3.4. Anti-pneumococcal IgG responses to the other serotypes

IgG GMCs increased for all serotypes 1 month post-booster in both studies (Supplementary Table 3). GMCs were comparable between groups for all vaccine serotypes, except for serotypes 6B and 19F, which were higher in the PHiD-CV group than in the PCV13 group in both studies. All subjects had antibody concentrations ≥0.35 mg/mL for serotypes 1, 4, 7F,

14, 18C, and 19F in the two groups in both studies (Supplementary Table 4). For serotypes 3, 5, 6A, 6B, 9V, and 23F, the percentages ranged between 84.2% and 100.0%. When PHiD-CV or PCV13 were administered as 3+1 or 2+1 schedules, non-inferiority of the IgG GMCs after PHiD-CV priming was shown for all the other serotypes, except for serotype 14 in the 2+1 schedule (Figure 3).

3.5. Persistence of the OPA and IgG responses to the other vaccine serotypes

Twelve months after the booster dose, the OPA GMTs and the IgG GMCs decreased approximately to baseline levels for all the other serotypes after both 3+1 and 2+1 schedules (Supplementary Tables 1 and 3). Although the proportions of subjects with OPA titers ≥8 also decreased over time, they remained >90% for serotypes 6B, 7F, and 23F and between 47.4% and 100% for serotypes 4, 5, 6A, 6C, 9V, and 19F (Supplementary Table 2). The lowest proportions were for serotypes 1 and 3 (13%−61%). Overall, no major differences were seen between groups, except for serotype 1 in the 3+1 schedule (43.1% in the PHiD-CV group vs. 13.3% in the PCV13 group) and serotype 4 in the 2+1 schedule (47.4% in the PHiD-CV group vs. 81.6% in the PCV13 group). The proportions of subjects with IgG concentrations above the protective threshold of 0.35 µg/mL also decreased over time and were ≥76.5% for serotypes 6A, 6B, 7F, 14, and 19F and between 15.7% and 65.8% for serotypes 1, 3, 4, 5, 9V, 18C, and 23F. In both studies, no major differences were seen between vaccine groups.

Twelve months post-booster, the OPA GMTs in PHiD-CV-primed subjects were not inferior to those in PCV13-primed subjects for serotypes 1, 3, 5, 6B, 14, 18C, and 19F with the 3+1 schedule and for serotypes 1, 3, 5, and 6A with the 2+1 schedule (Figure 2). The IgG concentrations after PHiD-CV priming were not inferior for all serotypes with the 3+1 schedule and for all serotypes except serotypes 4 and 23F with the 2+1 schedule (Figure 3).

3.6. Safety and reactogenicity of the PCV13 booster dose

Overall, solicited local and systemic reactions were reported in 82.7% of subjects in the 3+1 schedule and 86.5% in the 2+1 schedule (Table 2). In the 3+1 schedule, one subject had a grade 3 local reaction (pain) and two subjects had grade 3 systemic reactions (irritability/fussiness in both). In the 2+1 schedule, three subjects had grade 3 pain at the injection site. One subject had one grade 3 systemic reaction (irritability/fussiness) and one subject had two (irritability/fussiness and loss of appetite).

Overall, approximately 40% of subjects in both studies had unsolicited AEs within 1 month after the PCV13 booster dose (Table 2). These AEs were mainly respiratory conditions and of mild intensity. In the 3+1 schedule, the frequency of subjects reporting AEs and the number of AEs were approximately two fold lower in the PHiD-CV group than in the PCV13 group. Two AEs, erythema and rhinitis, were considered vaccine-related by the investigators, both in the PCV13 group. They began within 3 days of the booster dose, were of mild intensity, and resolved without sequelae. In the 2+1 schedule, one subject in the PHiD-CV group had two AEs (acute gastroenteritis and dehydration) and one subject in the PCV13 group had one AE (sideropenic anemia) considered vaccine-related.

Four subjects in the 3+1 schedule had SAEs (acute laryngitis, head and neck burn, herniotomy in the PCV13 group; falling on head in the PHiD-CV group) and three in the 2+1 schedule (severe dyspepsia in the PCV13 group; respiratory tract inflammation [rhinopharyngitis, obstructive bronchitis, and unilateral pneumonia] and acute gastroenteritis with dehydration in the PHiD-CV group). None of the SAEs were considered related to the study vaccine.

4. DISCUSSION

Our results show that a PCV13 booster dose, either in a 3+1 or a 2+1 schedule, is immunogenic and well tolerated in PCV13- or PHiD-CV-primed children. The primary and secondary confirmatory objectives were met for both studies. One month post-booster, the IgG and OPA immune responses to serotype 19A in PHiD-CV-primed children were not inferior to those in PCV13-primed children, as part of either 3+1 or 2+1 schedules.

An IgG concentration of 0.35 µg/mL correlates with protection at a population level and is therefore used as a protective threshold for evaluating PCVs [8]However, because antibody production determined by ELISA does not necessarily reflect functional potential, in vitro OPA assays are considered the best surrogate to evaluate protection provided by PCVs [22, 23]. Although non-inferiority was shown for more serotypes in terms of IgG than OPA responses, nearly all subjects primed with PHiD-CV or PCV13 had OPA titers above the chosen threshold at month 1. This suggests that functional antibodies are induced even for serotypes for which the immune response was considered inferior in PHiD-CV-primed children. The IgG GMCs and the OPA GMTs decreased over time but the proportions of subjects with immune responses above the thresholds remained high for most serotypes 12 months post-booster. This suggests that, although the immune responses waned after the booster dose, this decline may be of limited clinical relevance for most serotypes.

Although serotypes 3, 6A, and 19A are not included in PHiD-CV, high IgG GMCs and OPA GMTs against these serotypes were found after the PCV13 booster dose in PHiD-CV-primed subjects. Mixed PHiD-CV-PCV13 schedules may therefore offer a reasonable alternative in settings with high occurrence of 19A disease. Indeed, in Quebec, Canada, a mixed schedule consisting of 2 primary PHiD-CV doses followed by a PCV13 booster provided similar

protection against overall and vaccine-type invasive pneumococcal disease, including for serotype 19A, as compared to schedules using only PHiD-CV or PCV13 [24].

One previous study has evaluated the interchangeability of PHiD-CV and PCV13 when given as booster doses in mixed schedules [14]. The study showed that a PHiD-CV booster is generally less immunogenic than a PCV13 booster in PCV13-primed children. Our studies evaluated for the first time a booster dose with PCV13 in children primed with PHiD-CV or PCV13. Our results suggest that these two vaccines are interchangeable as boosters and induce strong immune responses against vaccine serotypes. Using mixed schedules would have several advantages over standard schedules. The nontypeable *H. influenzae* protein D is used as a carrier protein for 8 of the 10 serotypes included in PHiD-CV. Because this protein is not included in any other vaccine, the risk of immune interference is minimal [25].

The reactogenicity and safety profiles of the PCV13 booster dose were comparable both schedules. In both studies, the frequency and severity of the solicited reactions and the unsolicited AEs in both study groups corresponded to those commonly observed after administration of vaccines. These results suggest that using a PCV13 booster after PHiD-CV priming does not raise any safety issues, as expected. Indeed, a switch from PHiD-CV to PCV13 has been introduced in the immunization programs in several countries, and no safety issues were raised during the transition period [26] (ref).

Our studies have some limitations. The sample size of the studies, which was powered based on serotype 19A responses, may not be sufficient for testing non-inferiority of the other serotypes. Furthermore, multiplicity of comparisons was not taken into account, which could influence the results in any direction. The 3+1 and 2+1 schedules were assessed in two different studies conducted in different countries, which may also limit comparisons between the studies. However, the immunological analyses were performed by the same laboratory to limit the risk of bias.

In conclusion, our results suggest that a PCV13 booster in PHiD-CV primed children could be a promising vaccination strategy to improve clinical protection against three additional serotypes, although the clinical relevance of the observed differences in IgG and OPA responses between groups is currently unknown. When given as simplified 2+1 schedules, mixed schedules could also improve vaccine uptake and reduce the cost of the vaccination course.

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AUTHORS' CONTRIBUTIONS

I.U. and R.P. were the lead investigators and contributed to protocol design, data collection, data analysis, and data interpretation. K.P. and P.K. contributed to protocol design, data analysis, and data interpretation. D.G. and L.R. contributed to protocol design data collection, and interpretation. All authors contributed to the writing of the manuscript, provided critical comments on manuscripts drafts, and approved the final version of the manuscript and the decision to submit for publication.

CONFLICTS OF INTEREST

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TABLES

Table 1. Baseline demographic characteristics of participants

	3+1 schedule (Czech Republic)			2+1 schedule (Slovakia)		
Characteristics, n (%)	**PHiD-CV group N = 53**	**PCV13 group N = 45**	**Total N = 98**	**PHiD-CV group N = 39**	**PCV13 group N = 50**	**Total N = 89**
Age (months)						
Mean (SD)	12.9 (1.1)	12.3 (0.6)	12.6 (1.0)	11.3 (0.6)	11.4 (0.5)	11.3 (0.5)
Range	12.0–16.0	12.0–14.0	12.0–16.0	10.0–13.0	11.0–13.0	10.0–13.0
Sex						
Female	23 (43.4%)	12 (26.7%)	35 (35.7%)	16 (41.0%)	21 (42.0%)	37 (41.6%)
Male	30 (56.6%)	33 (73.3%)	63 (64.3%)	23 (59.0%)	29 (58.0%)	52 (58.4%)
Race						
Caucasian	53 (100.0%)	45 (100.0%)	98 (100.0%)	39 (100.0%)	49 (98.0%)	88 (98.9%)
Other	0	0	0	0	1 (2.0%)	1 (1.1%)

PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-*typeable Haemophilus influenzae* protein D conjugate vaccine; SD, standard deviation.

Table 2. Safety and reactogenicity by subject

	3+1 schedule (Czech Republic)						2+1 schedule (Slovakia)					
	PHiD-CV (N = 53)		PCV13 (N = 45)		Total (N = 98)		PHiD-CV (N = 39)		PCV13 (N = 50)		Total (N = 89)	
Symptom	**n subjects (%)**	**n AEs**	**n subjects (%)**	**n AEs**	**n subjects (%)**	**n AEs**	**n subjects (%)**	**n AEs**	**n subjects (%)**	**n AEs**	**n subjects (%)**	**n AEs**
Reactogenicity												
Local reactions												
Any	45 (84.9%)	-	36 (80.0%)	-	81 (82.7%)	-	32 (82.1%)	-	45 (90.0%)	-	77 (86.5%)	-
Grade 3	1 (1.9%)	1	0	0	1 (1.0%)	1	2 (5.1%)	2	1 (2.0%)	1	3 (3.4%)	3
Systemic reactions												
Any	44 (83.0%)	-	37 (82.2%)	-	81 (82.7%)	-	29 (74.4%)	-	45 (90.0%)	-	74 (83.1%)	-
Grade 3	0	0	2 (4.4%)	2	2 (2.0%)	2	1 (2.6%)	2	1 (2.0%)	1	2 (2.2%)	3
Safety												
Adverse events												
Any	14 (26.4%)	23	26 (57.8%)	60	40 (40.8%)	83	15 (38.5%)	32	21 (42.0%)	31	36 (40.4%)	63
Severe	1 (1.9%)	1	3 (6.7%)	4	4 (4.1%)	5	0	0	1 (2.0%)	1	1 (1.1%)	1
Vaccination -related	0	0	2 (4.4%)	2	2 (2.0%)	2	1 (2.6%)	2	1 (2.0%)	1	2 (2.2%)	3
Serious adverse events												
Any	1 (1.9%)	1	3 (6.7%)	3	4 (4.1%)	4	2 (5.1%)	4	1 (2.0%)	1	3 (3.4%)	5

AEs, adverse events; PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine

LEGENDS TO FIGURES

Figure 1. Flowchart of participants

FAS, full analysis set; DTaP-HBV-IPV/Hib, diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-*Haemophilus influenzae* type b vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine; PPS, per protocol set.

Figure 2. PHiD-CV/PCV13 ratio of anti-pneumococcal OPA titers 1 and 12 months after the PCV13 booster dose

Ratios of OPA geometric mean titer in PHID-CV over PCV13 recipients at 1 month and 12 months after the booster dose. The error bars indicate 95% confidence intervals and the red dotted line the arbitrary margin for non-inferiority.

Figure 3. PHiD-CV/PCV13 ratio of anti-pneumococcal IgG concentrations 1 and 12 months after the PCV13 booster dose

Ratios of IgG geometric mean concentration in PHID-CV over PCV13 recipients at 1 month and 12 months after the booster dose. The error bars indicate 95% confidence intervals and the red dotted line the arbitrary margin for non-inferiority.

Figure 1.

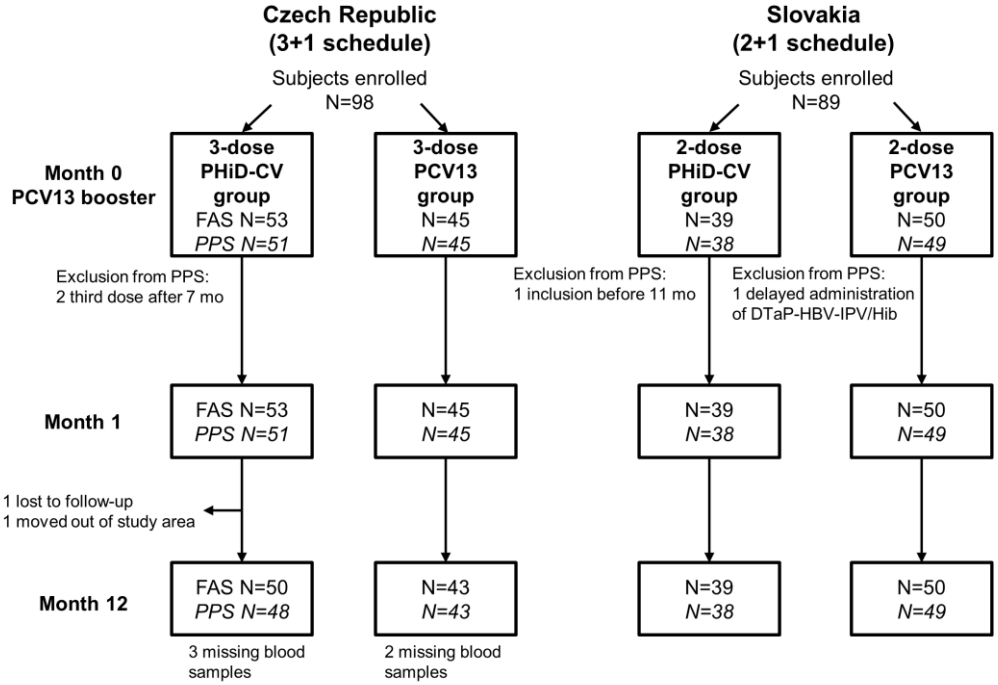

Czech Republic
(3+1 schedule)
Slovakia
(2+1 schedule)
Subjects enrolled
N=98
Subjects enrolled
N=89
Month 0
PCV13 booster
3-dose PHiD-CV group
FAS N=53
PPS N=51
3-dose PCV13 group
N=45
N=45
2-dose PHiD-CV group
N=39
N=38
2-dose PCV13 group
N=50
N=49
Exclusion from PPS:
2 third dose after 7 mo
Exclusion from PPS:
1 inclusion before 11 mo
Exclusion from PPS:
1 delayed administration of DTaP-HBV-IPV/Hib
Month 1
FAS N=53
PPS N=51
N=45
N=45
N=39
N=38
N=50
N=49
1 lost to follow-up
1 moved out of study area
Month 12
FAS N=50
PPS N=48
N=43
N=43
N=39
N=38
N=50
N=49
3 missing blood samples
2 missing blood samples

Figure 2.

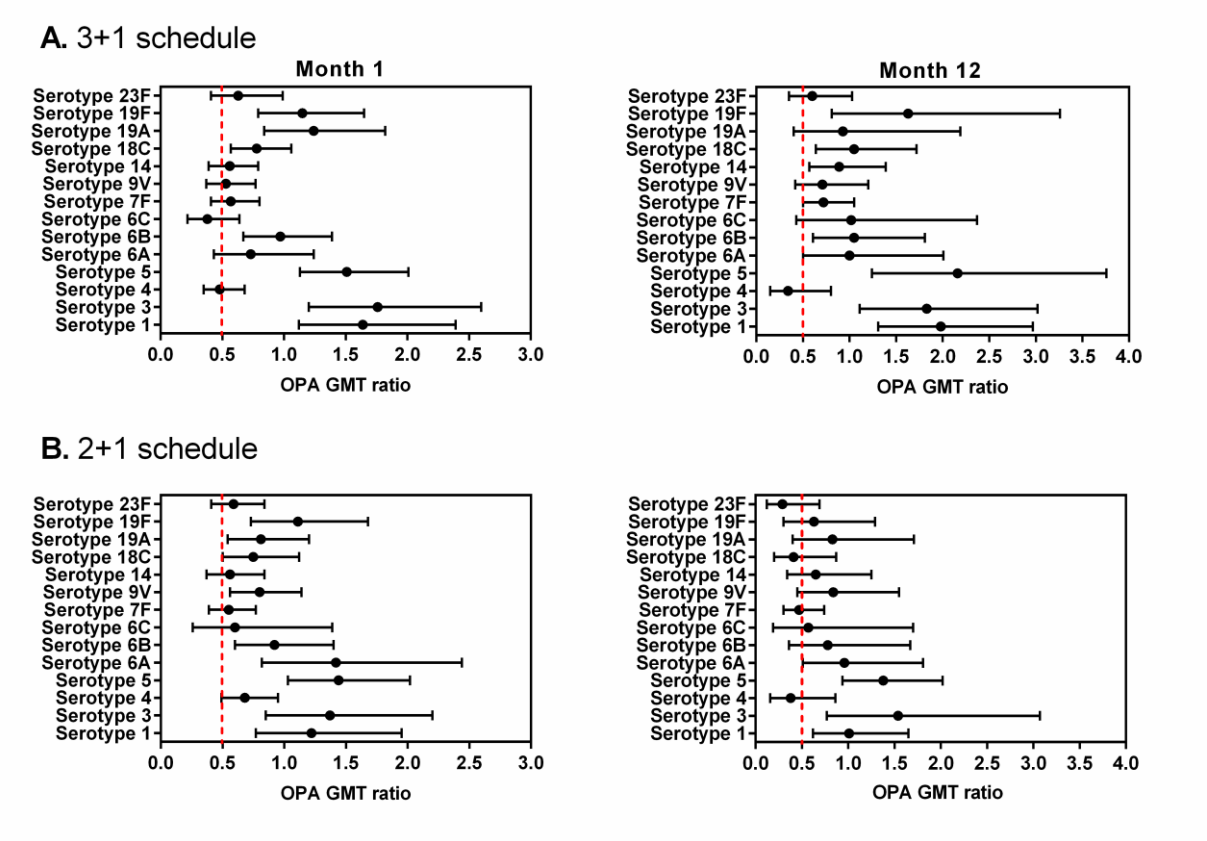
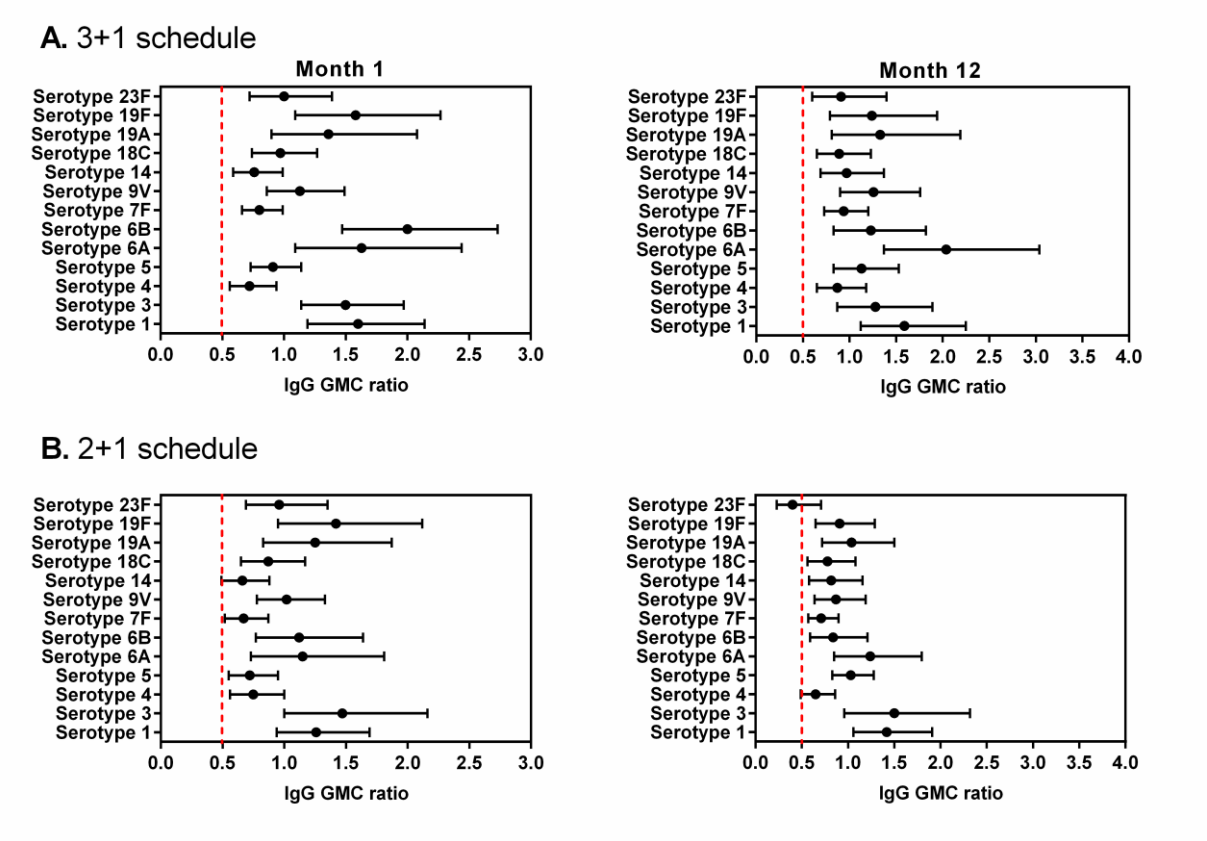

A. 3+1 schedule
Month 1
Month 12
Serotype 23F
Serotype 19F
Serotype 19A
Serotype 18C
Serotype 14
Serotype 9V
Serotype 7F
Serotype 6C
Serotype 6B
Serotype 6A
Serotype 5
Serotype 4
Serotype 3
Serotype 1
0.0 0.5 1.0 1.5 2.0 2.5 3.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
OPA GMT ratio
B. 2+1 schedule

Figure 3.


A. 3+1 schedule
Month 1
Serotype 23F
Serotype 19F
Serotype 19A
Serotype 18C
Serotype 14
Serotype 9V
Serotype 7F
Serotype 6B
Serotype 6A
Serotype 5
Serotype 4
Serotype 3
Serotype 1
0.0 0.5 1.0 1.5 2.0 2.5 3.0
IgG GMC ratio
Month 12
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
IgG GMC ratio
B. 2+1 schedule
IgG GMC ratio
IgG GMC ratio

Supplementary Table 1. OPA geometric mean titers before and after the PCV13 booster dose

	3+1 schedule (Czech Republic)				2+1 schedule (Slovakia)			
Serotype	**PHiD-CV group N = 53**		**PCV13 group N = 45**		**PHiD-CV group N = 39**		**PCV13 group N = 49**	
	N	**GMT (95% CI)**	**N**	**GMT (95% CI)**	**N**	**GMT (95% CI)**	**N**	**GMT (95% CI)**
Serotype 1								
Month 0	51	5.6 (4.5; 6.9)	44	5.7 (4.5; 7.1)	37	7.7 (5.4; 10.8)	49	6.8 (5.3; 8.8)
Month 1	51	287.7 (213.7; 387.3)	45	177.6 (135.1; 233.4)	38	291.8 (197.2; 431.8)	49	226.8 (161.7; 318.0)
Month 12	46	11.0 (7.6; 15.8)	44	5.7 (4.3; 7.7)	37	11.4 (7.6; 17.3)	48	11.6 (8.5; 15.7)
Serotype 3								
Month 0	50	4.9 (3.8; 6.2)	44	7.5 (5.2; 10.8)	38	4.4 (3.9. 4.9)	46	11.7 (8.1. 16.8)
Month 1	51	205.5 (164.5; 256.6)	45	128.0 (92.3; 177.6)	38	135.1 (93.5; 195.0)	49	135.4 (105.3; 174.2)
Month 12	40	12.5 (8.6; 18.3)	43	7.9 (5.6; 11.1)	32	14.3 (9.1; 22.5)	40	16.3 (10.6; 25.1)
Serotype 4								
Month 0	49	72.3 (46.1; 113.3)	43	60.0 (33.0; 109.1)	35	43.8 (23.1; 82.9)	47	43.2 (27.2; 68.5)
Month 1	51	1691.3 (1366.1; 2093.9)	45	3455.1 (2672.6; 4466.7)	38	1372.1 (1067.6; 1763.4)	49	1995.2 (1598.0; 2491.2)
Month 12	46	44.5 (25.1; 79.0)	43	112.2 (57.6; 218.6)	36	31.1 (15.1; 64.4)	48	86.8 (52.4; 143.7)
Serotype 5								
Month 0	48	21.2 (13.6; 33.1)	43	22.2 (16.3; 30.4)	36	32.2 (19.6; 53.0)	45	25.4 (18.0; 35.7)
Month 1	51	671.2 (528.4; 852.5)	45	477.1 (376.7; 604.2)	38	733.7 (528.5; 1018.7)	49	470.4 (377.6; 586.1)
Month 12	46	72.8 (50.0; 106.0)	34	38.4 (24.1; 61.0)	35	69.3 (45.4; 105.8)	48	40.1 (28.0; 57.4)
Serotype 6A								
Month 0	49	32.1 (15.3; 67.5)	44	525.5 (349.2; 790.9)	36	34.6 (15.3; 77.9)	47	119.9 (70.5; 203.8)
Month 1	51	4017.0 (2708.5; 5957.8)	45	8807.5 (7174.8; 10811.6)	38	6389.4 (4690.2; 8704.2)	49	5991.7 (3965.5; 9053.2)
Month 12	46	671.0 (414.2; 1087.1)	44	1097.4 (837.5; 1438.1)	38	710.6 (402.0; 1256.2)	49	966.7 (677.0; 1380.4)
Serotype 6B								
Month 0	50	408.6 (307.9; 542.3)	45	146.1 (81.4; 262.2)	36	358.5 (200.4; 641.3)	46	43.8 (21.5; 89.6)
Month 1	51	6289.5 (4807.6; 8228.3)	45	5577.0 (4388.4; 7087.6)	38	6933.8 (5165.6; 9307.2)	49	4332.1 (3203.6; 5858.1)
Month 12	48	657.3 (466.0; 927.0)	43	532.4 (348.2; 814.0)	38	389.2 (224.5; 674.6)	49	236.5 (144.3; 387.7)
Serotype 6C								
Month 0	49	24.3 (11.6; 50.8)	42	189.3 (92.8; 386.3)	36	23.4 (10.3; 53.1)	46	65.0 (31.0; 136.2)
Month 1	51	3207.6 (2164.0; 4754.7)	45	10499.0 (8059.5; 13676.9)	38	1171.8 (602.7; 2278.1)	49	2680.1 (1535.4; 4678.3)
Month 12	41	635.1 (310.3; 1300.1)	42	1031.1 (707.0; 1503.7)	36	189.4 (73.4; 488.9)	45	457.5 (239.0; 875.7)
Serotype 7F								
Month 0	51	585.4 (455.8; 751.9)	45	797.6 (624.4; 1018.8)	37	721.8 (533.7; 976.2)	49	614.3 (486.8; 775.3)
Month 1	51	3130.4 (2605.1; 3761.6)	45	6281.4 (4528.6; 8712.5)	38	2792.3 (2229.7; 3496.8)	49	4801.4 (3698.7; 6232.8)
Month 12	47	901.6 (665.3; 1222.0)	45	1470.7 (1125.1; 1922.5)	38	857.1 (554.2; 1325.7)	49	1781.5 (1405.7; 2257.8)
Serotype 9V								
Month 0	49	212.9 (136.4; 332.2)	41	157.6 (84.4; 294.5)	38	43.6 (22.4; 84.9)	46	44.3 (27.4; 71.8)
Month 1	51	2078.7 (1664.4; 2596.1)	45	4099.2 (3032.7; 5540.8)	38	2305.9 (1797.8; 2957.5)	49	2815.0 (2161.9; 3665.4)
Month 12	44	537.0 (368.8; 781.9)	44	732.9 (525.8; 1021.6)	38	526.4 (336.6; 823.1)	47	679.6 (419.2; 1101.9)
Serotype 14								
Month 0	51	531.8 (373.0; 758.1)	45	612.2 (397.9; 941.8)	36	405.8 (266.1; 618.6)	49	340.7 (226.5; 512.4)
Month 1	51	1780.5 (1444.1; 2195.2)	45	3376.4 (2345.3; 4860.7)	38	1492.7 (1158.4; 1923.4)	49	2598.8 (1932.1; 3495.4)

	3+1 schedule (Czech Republic)				2+1 schedule (Slovakia)			
Serotype	**PHiD-CV group N = 53**		**PCV13 group N = 45**		**PHiD-CV group N = 39**		**PCV13 group N = 49**	
	N	**GMT (95% CI)**	**N**	**GMT (95% CI)**	**N**	**GMT (95% CI)**	**N**	**GMT (95% CI)**
Month 12	48	576.3 (436.0; 761.6)	44	706.9 (453.2; 1102.6)	31	259.3 (138.0; 487.2)	47	361.4 (256.1; 510.1)
Serotype 18C								
Month 0	51	185.4 (110.0; 312.5)	44	104.6 (59.9; 182.5)	37	102.3 (60.2; 173.8)	48	33.6 (21.3; 52.8)
Month 1	51	2072.9 (1680.9; 2556.4)	45	2552.4 (2002.4; 3253.5)	38	2122.1 (1577.2; 2855.2)	49	2270.4 (1741.1; 2960.5)
Month 12	47	836.6 (595.6; 1175.3)	43	772.7 (536.6; 1112.9)	37	490.0 (276.4; 868.8)	49	714.6 (443.1; 1152.4)
Serotype 19A								
Month 0	50	13.4 (8.6; 20.7)	45	65.1 (39.6; 107.1)	38	13.8 (7.6; 25.0)	46	41.5 (28.0; 61.6)
Month 1	51	2778.6 (2102.1; 3672.9)	45	3004.9 (2391.4; 3775.7)	38	1861.4 (1361.6; 2544.5)	49	2337.1 (1865.1; 2928.7)
Month 12	47	266.9 (150.0; 475.2)	44	355.8 (219.9; 575.6)	36	204.8 (117.1; 358.2)	48	292.0 (183.0; 465.9)
Serotype 19F								
Month 0	51	192.7 (122.4; 303.5)	43	47.5 (28.8; 78.3)	38	157.5 (100.6; 246.7)	48	41.1 (27.3; 61.9)
Month 1	51	3868.0 (3045.0; 4913.3)	45	2769.6 (2152.4; 3563.7)	38	2720.6 (2054.4; 3603.0)	49	2137.0 (1655.0; 2759.4)
Month 12	48	586.7 (427.2; 805.8)	39	267.5 (143.2; 499.5)	38	210.2 (118.7; 372.4)	49	180.5 (115.7; 281.8)
Serotype 23F								
Month 0	49	68.1 (36.3; 127.8)	41	159.0 (79.5; 317.7)	38	83.7 (41.1; 170.6)	46	44.5 (23.0; 85.9)
Month 1	51	3470.9 (2774.5; 4342.0)	45	6561.6 (4277.3; 10065.7)	38	3391.4 (2610.1; 4406.6)	49	5238.2 (3985.7; 6884.4)
Month 12	47	709.3 (464.7; 1082.9)	44	1338.3 (888.9; 2015.0)	36	283.3 (130.1; 616.6)	48	788.1 (454.1; 1367.6)

PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine

Supplementary Table 2. Percentage of subjects with OPA geometric mean titers ≥8 1 and 12 months after the PCV13 booster dose

Serotype	Timing	3+1 schedule (Czech Republic)		2+1 schedule (Slovakia)	
		PHiD-CV group N = 51	PCV13 group N = 45	PHiD-CV group N = 38	PCV13 group N = 49
Serotype 1	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	22 (43.1%)	6 (13.3%)	19 (50.0%)	30 (61.2%)
Serotype 3	Month 1	51 (100.0%)	45 (100.0%)	37 (97.4%)	49 (100.0%)
	Month 12	24 (47.1%)	15 (33.3%)	20 (52.6%)	30 (61.2%)
Serotype 4	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	31 (60.8%)	32 (71.1%)	18 (47.4%)	40 (81.6%)
Serotype 5	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	43 (84.3%)	30 (66.7%)	33 (86.8%)	43 (87.8%)
Serotype 6A	Month 1	50 (98.0%)	45 (100.0%)	38 (100.0%)	48 (98.0%)
	Month 12	44 (86.3%)	44 (97.8%)	35 (92.1%)	49 (100.0%)
Serotype 6B	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	48 (94.1%)	41 (91.1%)	36 (94.7%)	46 (93.9%)
Serotype 6C	Month 1	50 (98.0%)	45 (100.0%)	35 (92.1%)	46 (93.9%)
	Month 12	36 (70.6%)	42 (93.3%)	25 (65.8%)	39 (79.6%)
Serotype 7F	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	47 (92.2%)	45 (100.0%)	37 (97.4%)	49 (100.0%)
Serotype 9V	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	44 (86.3%)	44 (97.8%)	38 (100.0%)	47 (95.9%)
Serotype 14	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	48 (94.1%)	42 (93.3%)	29 (76.3%)	46 (93.9%)
Serotype 18C	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	47 (92.2%)	43 (95.6%)	34 (89.5%)	47 (95.9%)
Serotype 19A	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	43 (84.3%)	44 (97.8%)	33 (86.8%)	46 (93.9%)
Serotype 19F	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	48 (94.1%)	36 (80.0%)	34 (89.5%)	47 (95.9%)
Serotype 23F	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	47 (92.2%)	44 (97.8%)	36 (94.7%)	48 (98.0%)

PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine

Supplementary Table 3. Anti-pneumococcal antibody concentrations (95% CI) before and after the booster dose

	3+1 schedule (Czech Republic)				2+1 schedule (Slovakia)			
	PHiD-CV group N = 53		PCV13 group N = 45		PHiD-CV group N = 39		PCV13 group N = 49	
Serotype	**N**	**GMC (95% CI)**	**N**	**GMC (95% CI)**	**N**	**GMC (95% CI)**	**N**	**GMC (95% CI)**
Serotype 1								
Month 0	51	0.22 (0.17; 0.27)	45	0.34 (0.28; 0.42)	38	0.27 (0.21; 0.34)	49	0.53 (0.45; 0.63)
Month 1	51	3.42 (2.72; 4.30)	45	2.54 (2.10; 3.09)	38	3.56 (2.75; 4.61)	49	4.31 (3.57; 5.20)
Month 12	48	0.40 (0.31; 0.51)	45	0.28 (0.23; 0.35)	38	0.41 (0.32; 0.54)	49	0.44 (0.37; 0.52)
Serotype 3								
Month 0	50	0.17 (0.12; 0.23)	45	0.24 (0.17; 0.33)	36	0.16 (0.12; 0.21)	49	0.35 (0.26; 0.47)
Month 1	48	1.12 (0.93; 1.35)	45	0.83 (0.64; 1.08)	34	1.10 (0.79; 1.54)	47	1.17 (0.90; 1.51)
Month 12	47	0.23 (0.17; 0.31)	44	0.20 (0.15; 0.28)	33	0.27 (0.18; 0.40)	47	0.25 (0.19; 0.33)
Serotype 4								
Month 0	51	0.31 (0.25; 0.39)	45	0.3 (0.24; 0.37)	38	0.37 (0.29; 0.49)	49	0.36 (0.30; 0.43)
Month 1	51	2.03 (1.72; 2.40)	45	2.77 (2.23; 3.45)	38	1.97 (1.53; 2.53)	49	2.58 (2.10; 3.17)
Month 12	48	0.21 (0.17; 0.25)	45	0.23 (0.18; 0.30)	38	0.17 (0.13; 0.21)	49	0.25 (0.20; 0.31)
Serotype 5								
Month 0	51	0.3 (0.25; 0.36)	45	0.31 (0.26; 0.38)	37	0.37 (0.30; 0.45)	49	0.34 (0.29; 0.40)
Month 1	51	1.24 (1.04; 1.48)	45	1.39 (1.15; 1.67)	38	1.28 (1.04; 1.59)	49	1.65 (1.34; 2.03)
Month 12	48	0.32 (0.25; 0.40)	45	0.29 (0.23; 0.37)	38	0.35 (0.29; 0.43)	49	0.33 (0.28; 0.40)
Serotype 6A								
Month 0	51	0.27 (0.20; 0.35)	45	0.54 (0.45; 0.65)	38	0.24 (0.17; 0.34)	49	0.68 (0.54; 0.86)
Month 1	51	6.29 (4.38; 9.04)	45	5.82 (4.83; 7.02)	37	4.60 (2.87; 7.37)	49	7.75 (6.27; 9.58)
Month 12	48	0.90 (0.64; 1.26)	45	0.63 (0.50; 0.80)	38	0.87 (0.64; 1.18)	49	1.15 (0.91; 1.45)
Serotype 6B								
Month 0	51	0.71 (0.58; 0.88)	45	0.37 (0.29; 0.47)	38	0.53 (0.40; 0.71)	49	0.24 (0.19; 0.31)
Month 1	51	13.07 (10.15; 16.83)	45	5.17 (4.39; 6.09)	38	10.25 (7.28; 14.42)	48	5.17 (4.02; 6.66)
Month 12	48	1.18 (0.87; 1.60)	45	0.71 (0.56; 0.90)	38	1.06 (0.80; 1.41)	49	0.85 (0.67; 1.10)
Serotype 7F								
Month 0	51	0.68 (0.54; 0.85)	45	0.77 (0.64; 0.93)	38	0.64 (0.51; 0.80)	49	0.72 (0.58; 0.88)
Month 1	51	3.34 (2.83; 3.93)	45	4.34 (3.71; 5.08)	38	2.78 (2.26; 3.42)	49	4.36 (3.55; 5.36)
Month 12	48	0.60 (0.50; 0.73)	45	0.69 (0.56; 0.85)	38	0.48 (0.40; 0.57)	49	0.71 (0.58; 0.87)
Serotype 9V								
Month 0	51	0.31 (0.24; 0.39)	45	0.26 (0.21; 0.31)	38	0.26 (0.21; 0.33)	49	0.29 (0.23; 0.38)
Month 1	51	2.08 (1.75; 2.48)	45	1.72 (1.34; 2.20)	38	2.11 (1.69; 2.63)	49	2.18 (1.76; 2.71)
Month 12	47	0.35 (0.27; 0.47)	45	0.25 (0.20; 0.33)	38	0.31 (0.25; 0.39)	49	0.37 (0.29; 0.48)
Serotype 14								
Month 0	51	1.84 (1.38; 2.45)	45	1.91 (1.46; 2.49)	38	1.83 (1.28; 2.61)	49	1.92 (1.39; 2.65)
Month 1	51	5.69 (4.64; 6.97)	45	7.60 (5.93; 9.75)	38	5.94 (4.75; 7.45)	49	9.16 (7.40; 11.34)
Month 12	48	0.87 (0.66; 1.13)	45	0.93 (0.67; 1.29)	38	0.94 (0.69; 1.28)	49	1.17 (0.91; 1.49)
Serotype 18C								
Month 0	50	0.46 (0.34; 0.62)	44	0.23 (0.18; 0.30)	38	0.43 (0.32; 0.58)	49	0.28 (0.22; 0.35)
Month 1	51	2.67 (2.13; 3.34)	45	2.00 (1.62; 2.47)	38	2.23 (1.73; 2.88)	49	2.22 (1.83; 2.70)
Month 12	48	0.28 (0.22; 0.35)	45	0.21 (0.16; 0.29)	38	0.30 (0.22; 0.39)	49	0.32 (0.26; 0.40)
Serotype 19A								
Month 0	51	0.33 (0.25; 0.42)	45	0.62 (0.45; 0.86)	38	0.38 (0.25; 0.57)	49	0.67 (0.55; 0.83)
Month 1	51	9.66 (6.70; 13.94)	45	9.72 (7.67; 12.32)	38	9.45 (6.80; 13.15)	49	8.22 (6.47; 10.45)
Month 12	48	1.30 (0.92; 1.82)	45	1.11 (0.78; 1.58)	38	1.11 (0.86; 1.44)	49	1.31 (1.00; 1.72)
Serotype 19F								
Month 0	51	1.83 (1.37; 2.44)	45	0.73 (0.59; 0.90)	38	2.03 (1.50; 2.74)	49	0.95 (0.80; 1.13)
Month 1	51	18.35 (13.70; 24.57)	45	7.65 (6.22; 9.40)	38	16.49 (11.94; 22.77)	49	9.36 (7.56; 11.60)
Month 12	48	1.90 (1.34; 2.70)	45	0.91 (0.68; 1.21)	38	1.72 (1.28; 2.30)	49	1.23 (0.98; 1.54)

	3+1 schedule (Czech Republic)				2+1 schedule (Slovakia)			
	PHiD-CV group		PCV13 group		PHiD-CV group		PCV13 group	
Serotype	N = 53		N = 45		N = 39		N = 49	
	N	**GMC (95% CI)**	**N**	**GMC (95% CI)**	**N**	**GMC (95% CI)**	**N**	**GMC (95% CI)**
Serotype 23F								
Month 0	51	0.41 (0.31; 0.55)	45	0.28 (0.22; 0.36)	38	0.34 (0.25; 0.46)	49	0.20 (0.15; 0.26)
Month 1	51	5.56 (4.18; 7.39)	45	4.35 (3.25; 5.82)	38	5.01 (3.56; 7.04)	49	3.62 (2.76; 4.76)
Month 12	48	0.47 (0.32; 0.70)	45	0.37 (0.27; 0.53)	38	0.37 (0.27; 0.53)	49	0.66 (0.41; 1.06)

PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine

Supplementary Table 4. Percentage of subjects with IgG geometric mean concentrations ≥0.35 µg/mL 1 and 12 months after the PCV13 booster dose

Serotype	Timing	3+1 schedule (Czech Republic)		2+1 schedule (Slovakia)	
		PHiD-CV N = 51	**PCV13 N = 45**	**PHiD-CV N = 38**	**PCV13 N = 49**
Serotype 1	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	28 (54.9%)	17 (37.8%)	25 (65.8%)	30 (61.2%)
Serotype 3	Month 1	46 (90.2%)	39 (86.7%)	32 (84.2%)	45 (91.8%)
	Month 12	17 (33.3%)	9 (20.0%)	14 (36.8%)	14 (28.6%)
Serotype 4	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	8 (15.7%)	11 (24.4%)	7 (18.4%)	15 (30.6%)
Serotype 5	Month 1	51 (100.0%)	44 (97.8%)	38 (100.0%)	49 (100.0%)
	Month 12	25 (49.0%)	17 (37.8%)	21 (55.3%)	26 (53.1%)
Serotype 6A	Month 1	49 (96.1%)	45 (100.0%)	35 (92.1%)	49 (100.0%)
	Month 12	39 (76.5%)	38 (84.4%)	32 (84.2%)	47 (95.9%)
Serotype 6B	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	47 (95.9%)
	Month 12	42 (82.4%)	36 (80.0%)	34 (89.5%)	43 (87.8%)
Serotype 7F	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	41 (80.4%)	38 (84.4%)	30 (78.9%)	44 (89.8%)
Serotype 9V	Month 1	51 (100.0%)	43 (95.6%)	38 (100.0%)	48 (98.0%)
	Month 12	24 (47.1%)	14 (31.1%)	13 (34.2%)	25 (51.0%)
Serotype 14	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	42 (82.4%)	37 (82.2%)	33 (86.8%)	46 (93.9%)
Serotype 18C	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	17 (33.3%)	10 (22.2%)	14 (36.8%)	21 (42.9%)
Serotype 19A	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	42 (82.4%)	39 (86.7%)	36 (94.7%)	48 (98.0%)
Serotype 19F	Month 1	51 (100.0%)	45 (100.0%)	38 (100.0%)	49 (100.0%)
	Month 12	46 (90.2%)	40 (88.9%)	37 (97.4%)	47 (95.9%)
Serotype 23F	Month 1	50 (98.0%)	44 (97.8%)	38 (100.0%)	49 (100.0%)
	Month 12	32 (62.7%)	21 (46.7%)	18 (47.4%)	30 (61.2%)

PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine