Author/s:
Leach, AJ;Mulholland, EK;Santosham, M;Torzillo, PJ;McIntyre, P;Smith-Vaughan, H;Wilson, N;Arrowsmith, B;Beissbarth, J;Chatfield, MD;Oguoma, VM;Licciardi, P;Skull, S;Andrews, R;Carapetis, J;McDonnell, J;Krause, V;Morris, PS

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Interchangeability, immunogenicity and safety of a combined 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (Synflorix) and 13-valent-PCV (Prevenar13) schedule at 1-2-4-6 months: PREVIX_COMBO, a 3-arm randomised controlled trial

Amanda Jane Leach [a,b,*], Edward Kim Mulholland [c,d], Mathuram Santosham [e], Paul John Torzillo [f,g], Peter McIntyre [h], Heidi Smith-Vaughan [a,b], Nicole Wilson [a,b], Beth Arrowsmith [a,b], Jemima Beissbarth [a,b], Mark D. Chatfield [i,a,b], Victor M. Oguoma [a,b,j], Paul Licciardi [k], Sue Skull [l], Ross Andrews [a,b,m], Jonathan Carapetis [n,o], Joseph McDonnell [p], Vicki Krause [q], Peter Stanley Morris [a,b,r]

[a] *Child Health Division, Menzies School of Heath Research, PO Box 41096, Casuarina, Australia*
[b] *Charles Darwin University, Northern Territory, Australia*
[c] *Murdoch Children's Research Institute, Department of Paediatrics, University of Melbourne, Australia*
[d] *London School of Hygiene and Tropical Medicine, UK*
[e] *Johns Hopkins Bloomberg School of Public Health, Baltimore, USA*
[f] *Prince Alfred Hospital, Sydney, Australia*
[g] *University of Sydney, Sydney, Australia*
[h] *National Centre for Immunization Research and Surveillance, Sydney, Australia*
[i] *Centre for Health Services Research, Faculty of Medicine, University of Queensland, Brisbane, Queensland, Australia*
[j] *Health Research Institute, University of Canberra, Canberra, Australia*
[k] *Murdoch Children's Research Institute, Dept of Paediatrics, University of Melbourne, Melbourne, Australia*
[l] *Dept of Child Health Research, Perth Children's Hospital, University of Western Australia, Perth, Australia*
[m] *Australian National University, Canberra, Australia*
[n] *Telethon Kids Institute, University of Western Australia, Australia*
[o] *Perth Children's Hospital, Perth, Australia*
[p] *Consultant Statistician, 3291TR Strijen, Netherlands*
[q] *Centre for Disease Control, Northern Territory Department of Health, Darwin, Australia*
[r] *Department of Paediatrics, Royal Darwin Hospital, Darwin Northern Territory, Australia*

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ABSTRACT

Background: Aboriginal children living in remote communities are at high risk of early and persistent otitis media. *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* (NTHi) are primary pathogens. Vaccines with potential to prevent early OM have not been evaluated in this population. We compared immunogenicity (ELISA and opsonophagocytic activity) of a combination of Synflorix™ (PHiD-CV10, 10 serotypes and protein D of NTHi) and Prevenar13™ (PCV13, 10 serotypes plus 3, 6A, and 19A), with recommended schedules.

Methods: This open-label superiority trial randomised (1:1:1) Aboriginal infants at 28 to 38 days of age, to PCV13 (P) at 2–4-6 months (_PPP), PHiD-CV10 (S) at 2–4-6 months (_SSS), or PHiD-CV10 at 1–2–4 plus PCV13 at −6 months (SSSP). Primary outcomes (blinded) were immunogenicity against PCV13-only serotypes 3, 6A, 19A, and PHiD-CV10-only protein D at 7 months. Secondary outcomes include immunogenicity against all serotypes at 2, 4 and 7 months.

Findings: Between 2011 and 2017, 425 infants were allocated to _PPP(143), _SSS(141) or SSSP(141). An intention to treat approach including all available data was used. The SSSP group had superior immunogenicity against serotypes 3, 6A, and 19A compared to _SSS (OPA GMT ratios 8.1 to 59.5, $p < 0.001$), and

* Corresponding author at: Child Health Division, Menzies School of Heath Research, PO Box 41096, Casuarina, Northern Territory, Australia.
E-mail addresses: amanda.leach@menzies.edu.au (A.J. Leach), Kim.Mulholland@lshtm.ac.uk (E.K. Mulholland), Msantosham@jhu.edu (M. Santosham), paul.torzillo@sydney.edu.au (P.J. Torzillo), peter.mcintyre@health.nsw.gov.au (P. McIntyre), Heidi.Smith-Vaughan@menzies.edu.au (H. Smith-Vaughan), nictwil1136@gmail.com (N. Wilson), Beth.Arrowsmith@telethonkids.org.au (B. Arrowsmith), Jemima.Beissbarth@menzies.edu.au (J. Beissbarth), m.chatfield@uq.edu.au (M.D. Chatfield), victor.oguoma@menzies.edu.au (V.M. Oguoma), paul.licciardi@mcri.edu.au (P. Licciardi), Sue.skull@uwa.edu.au (S. Skull), Ross.andrews@menzies.edu.au (R. Andrews), jonathan.carapetis@telethonkids.org.au (J. Carapetis), joseph.mcdonnell@hotmail.com (J. McDonnell), Vicki.Krause@nt.gov.au (V. Krause), Peter.morris@menzies.edu.au (P.S. Morris).

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against protein D compared to _PPP (GMC ratio 11.9 (95%CI 9.7 to 14.6)). Immune responses to protein D and 3, 6A, and 19A in SSSP were not significantly lower (i.e. no harm) than either _SSS or _PPP. For ten common serotypes responses at 2, 4 and 7 months were superior for SSSP (following 1-, 2-, and 4- doses) than _SSS and _PPP (following 0-, 1-, and 3- doses). At 4 months, _SSS was superior to _PPP. Reactogenicity and hospitalisations were rare and unrelated to the intervention.

Interpretation: From two months, the 1–2–4–6-month combined schedule (SSSP) was safe and significantly more immunogenic than 2–4–6-month schedules. The earlier responses may be beneficial in high-risk populations.

1. Introduction

In remote communities of northern Australia, we previously demonstrated that the onset of otitis media (OM) in Aboriginal infants was preceded by acquisition of bacterial pathogens that colonise the nasopharynx (NP) within weeks of birth [1]. Persistent and ongoing nasopharyngeal acquisition and co-colonisation with multiple strains of *Streptococcus pneumoniae* (Spn) and non-typeable *Haemophilus influenzae* (NTHi) cause OM, chronic hearing loss and associated disadvantage throughout critical early learning years [2–4]. Risk factors include overcrowding, smoke exposure, limited handwashing with soap, and under-resourced primary health care services [2,5]. Prevention strategies that address these risk factors have not been evaluated in high quality studies. Pneumococcal conjugate vaccines (PCVs) prevent OM caused by vaccine serotypes, and at the time of designing this trial (2009) one PCV with protein D of NTHi as conjugate (11Pn-PD) also prevented NTHi-OM and NTHi nasopharyngeal carriage [6,7]. Two PCVs are licenced in Australia as a 2–4-6 month infant series; 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10, Synflorix™, S) and 13-valent PCV (PCV13, Prevenar13™, P). Our hypothesis was that both PCVs could be used to broaden immune responses to OM pathogens. At commencement of our trial, there were few data available on safety or efficacy of neonatal PHiD-CV10 or PCV13 immunisation in high-risk infants. PCV7 trials had demonstrated no difference in immunogenicity of newborn versus standard schedules [8,9]. Immunological data indicated that three doses of PHiD-CV10 provided significantly higher levels of anti-protein D antibodies than two doses [10], and an additional study demonstrated that a single dose of PCV13 at 12 months of age and following a PCV7 infant series was immunogenic against the 6 additional serotypes [11].

Our overall objective was therefore to evaluate safety and immunogenicity of a combination PCV schedule of PHiD-CV10 (S) given at 1–2–4 months plus PCV13 (P) given at 6 months (SSSP) compared to either vaccine alone when given at 2–4-6 months (_SSS or _PPP). The aim being to provide early immune responses against OM pathogens when measured at 2, 4, and 7 months of age. [12] We also aimed to show that the ratio of vaccine doses in the combination did not compromise immunogenicity compared to standard schedules. Head-to-head immunogenicity comparisons for the ten serotypes common to PHiD-CV10 and PCV13 are reported at 2, 4, and 7 months.

2. Methods

2.1. Trial design

The trial protocol has been published, [12] brief methods are provided below. The PREVIX_COMBO trial is an open-label, allocation concealed, primary outcome assessor (immunologist) blinded, randomized controlled trial with three parallel groups (1:1:1). The PREVIX_COMBO trial was approved by the relevant Human Research Ethics Committees.

2.2. Setting and participants

The trial took place in five remote Aboriginal communities in the Northern Territory and Western Australia. [12] *Inclusion criteria*: Aboriginal or Torres Strait Islander male and female infants living in a participating remote community, 28 to 38 days of age, eligible for National Immunisation Program routine vaccines. *Exclusion criteria*: Gestational age < 32 weeks. Not the eldest of multiple births. Research nurses were notified of all pregnancies and obtained written informed consent or assent from parents at infant age 28 to 38 days of age.

2.3. Interventions

Synflorix™ (GSK, Rixensart, Belgium) is a 10-valent PCV in which 1 μg of polysaccharide for each of serotypes 1, 5, 6B, 7F, 9 V, 14, and 23F, and 3 μg of serotype 4 polysaccharide are conjugated to protein D of *Haemophilus influenzae*; 3 μg serotype 18C polysaccharide is conjugated to 8 μg tetanus toxoid, and 3 μg of serotype 19F polysaccharide is conjugated to 5 μg diphtheria toxoid. Prevenar/Prevnar 13™ (Pfizer, New York, NY) is a 13-valent PCV in which each dose contains 2 μg of polysaccharide for 12 serotypes and 4 μg of polysaccharide for serotype 6B, each conjugated to cross-reacting material CRM_{197} of diphtheria. See Table 1 for schedule of procedures.

Nomenclature used in this manuscript are: italics *P* and *S* indicate vaccine (*P*revenar13™ or *S*ynflorix™) received at the time point of interest, and to indicate the comparison of interest. Vaccine schedules studied were _PPP, _SSS, and SSSP at 1,2,4,6 months.

2.4. Relevant concomitant care

Throughout this study, the Australian Indigenous infant vaccination schedule was EngerixB™ at birth, Rotarix® at 2–4 months, Infanrix® Hexa, and (for non-study participants) Prevenar13 at 2–4-6 months. Study staff provided treatment or referral for all concomitant conditions according to local guidelines.

2.5. Immunogenicity outcomes

Serotype-specific IgG concentration was measured using a modified 3rd generation ELISA based on WHO recommendations against 13 PCV serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9 V, 14, 18C, 19A, 19F, and 23F) and 11 non-PCV polysaccharide vaccine (PPV) serotypes (2, 8, 9 N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F). Protein D of *H. influenzae* (provided by GSK) IgG was measured and expressed in ELISA units, EL.U/mL. Multiplex opsonophagocytosis activity (MOPA) was measured for all PCV serotypes, expressed as a geometric mean titre (GMT). [13]

Table 1
Schedule of enrolment, interventions, and assessments.

	allocation	Study period post allocation				
Study visit number	**1**	**1**	**2**	**3**	**4**	**5**
Age (months)	**1**	**1**	**2**	**4**	**6**	**7**
Eligibility screen	x					
Informed consent signed	x					
Randomisation	x					
Interventions – Pneumococcal conjugate vaccines						
Prevenar13 (_PPP)		_	P	P	P	
Synflorix (_SSS)		_	S	S	S	
COMBO (SSSP)		S	S	S	P	
Rotarix[R]			x	x		
Infanrix[R] Hexa			x	x	x	
Outcome assessments						
Risk factor data and interviews						
Fixed e.g. sex, birthweight, gestational age, maternal education	x					
Not fixed e.g. household occupancy, smoke exposure, breastfeeding	x				x	
Blood draw (heel, finger prick, or venepuncture)			x*	x*		x
Ear assessment						
Tympanometry		x	x	x	x	x
Video otoscopy				x	x	x
Nasopharyngeal swab		x§	x	x	x	x
General health (skin, chest, nose, temp, weight, length) and medical record review		x	x	x	x	x

* blood draw occurs at either 2 months or 4 months of age (decided by a random process). § NP swab collection at one month of age commenced late 2014 (NT) or 2015 (WA). S is PHiD-CV10 (Synflorix™). P is PCV13 (Prevenar13™)

2.6. Sample size

425 participants were expected to provide 339 evaluable infants and 270 sera at 7 months [12]. This was estimated to provide 99% power to detect at least a 30% (absolute) difference in the proportion of infants with immunogenicity above threshold against 3, 6A, or 19A, and 90% power to detect at least a 21% difference in protein D responses [12]. All available outcome data were used (Fig. 1).

2.7. Randomisation and blinding

Eligible infants were randomly allocated (1:1:1) by the study nurses who called the NHMRC CTC randomisation service, to _PPP, _SSS, or SSSP. Stratification was by community [12]. The immunologist was blinded to the intervention allocation [12]. Research nurses were trained in giving vaccines, paediatric blood collection, and in standardised ear and general health checks. See Table 1 for schedule of procedures.

2.8. Statistical methods

Vaccine group comparisons of IgG (μg/mL) were tested with the Mann-Whitney *U* test, and Fisher's exact test for the proportion of infants above threshold IgG; 95% confidence intervals (95%CI) were calculated. IgG concentrations below threshold for detection were given the lowest detectable value multiplied by 0·5. All tests were 2-sided and a P value < 0·05 was considered statistically significant. All data were analysed using Stata/IC 15.1 [14].

2.9. Data safety monitoring

The study was overseen by an independent Data Safety and Monitoring Board (iDSMB). Adverse events (reactogenicity at intensity level 3) were solicited on days 0 to 3 following vaccination, including pain, fever, irritability, drowsiness, loss of appetite. Level 3 intensity generally prevents normal activity. All admissions to hospital were reported as serious adverse events [12].

2.10. Role of funding source

The funders had no role in design, collection, analysis, interpretation of data, writing the report or decision to submit for publication. As corresponding author, AJL had full access to all the data in the study and had final responsibility for the decision to submit for publication. AJL was not paid by any agency to write this article.

3. Results

3.1. Participant flow, recruitment and baseline data

Five communities[12] commenced between September 2011 and August 2014. Randomisations completed on 21st September 2017. Of 1018 pregnancy notifications, 593 were excluded, 425 infants were randomised to _PPP (143), _SSS (141) or SSSP (141). 213 and 212 infants were randomly allocated to a blood draw at 2 or 4 months, respectively. 396 (93%) infants were randomised within 28 to 38 days of age. Overall, infant birth characteristics were similar between groups (Table 2). At 7 months, there were 403 (95%) sera of adequate volume for testing serotypes 3, 6A, and 19A, and 393 (92%) for protein D (Fig. 1 and Table 3). Exclusion of protocol deviations or violations made no difference to our findings.

3.2. Immunogenicity ***outcomes***

Co-primary outcomes: *Serotypes 3, 6A, 19A, and protein D: superiority of SSSP at 7 months*

Broadened immunogenicity of the combination schedule at 7 months of age was confirmed. The SSSP group had significantly higher protein D IgG than _PPP (GMC ratio ~ 12, 57% difference in proportion of infants with IgG ≥ 100 EL.U/mL, Table 3) and significantly higher serotype 3, 6A, and 19A immunogenicity than _SSS (GMC ratios ~ 3 to ~ 8, 18% to 61% difference in proportions ≥ 0·35 μg/ml) (Table 3, Figs. 2 and 3). Opsonophagocytic activity (OPA) GMT ratios support our primary hypothesis of superior immunogenicity against serotypes 3, 6A, and 19A in

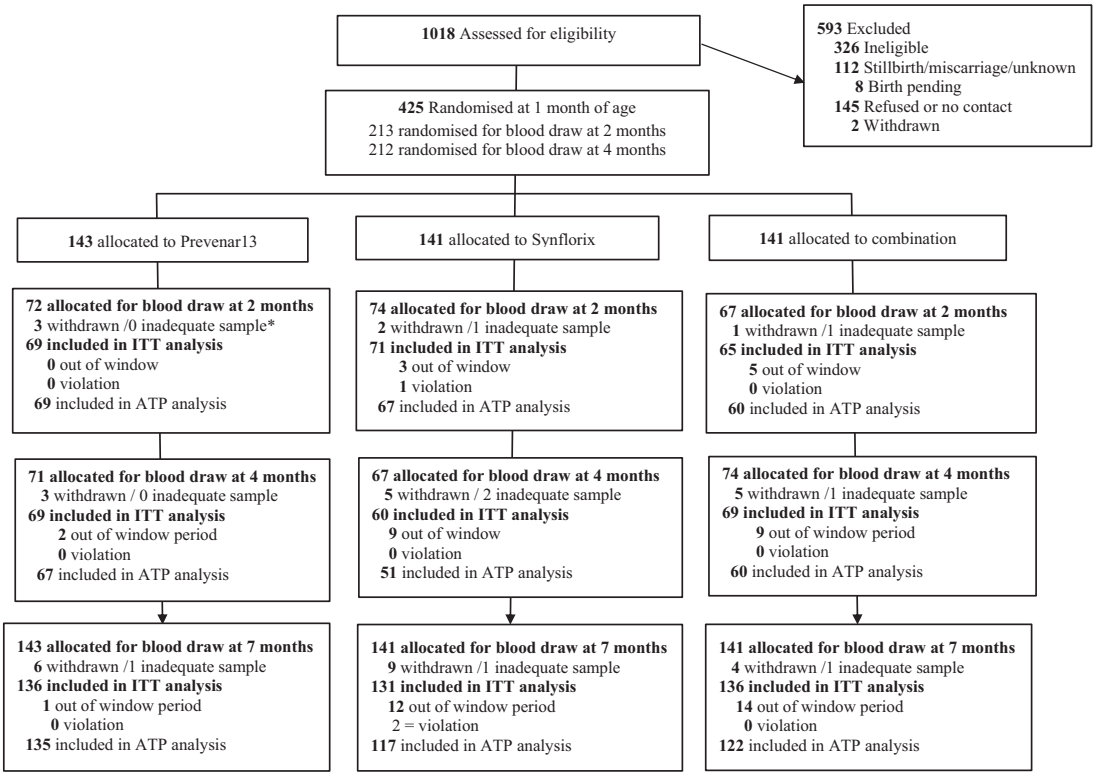

Fig. 1. Trial profile. * This Figure shows number of sera with adequate volumes for testing serotypes 3, 6A, and 19A. See Tables for numbers of sera with adequate volumes for Protein D, ten additional serotypes, and OPA.

Table 2
Baseline Characteristics.

Characteristics		Prevenar13 (_PPP) N = 143	Synflorix (_SSS) N = 141	Combo (SSSP) N = 141
Sex /gender	Male	77/143 (54%)	69/141 (49%)	70/141 (50%)
	Female	66/143 (46%)	72/141 (51%)	71/141 (50%)
Gestational age at birth (Weeks)	Mean (SD)	38·4 (1·42)	38·4 (1·40)	38·1 (1·62)
Birth weight (kg)	Mean (SD)	3·15 (0·47)	3·19 (0·49)	3·07 (0·53)
Weight at randomisation (kg)	Mean (SD)	4·26 (0·54)	4·24 (0·61)	4·04 (0·68)
Age at randomisation (days)	Mean (SD)	33·1 (3·33)	32·5 (3·77)	32·5 (3·94)
Community	1 Wurrumiyanga	29/143 (20%)	32/141 (23%)	30/141 (21%)
	2 Wadeye	53/143 (37%)	50/141 (36%)	50/141 (36%)
	3 Kununurra	26/143 (18%)	25/141 (18%)	27/141 (19%)
	4 Alice Springs	7/143 (5%)	6/141 (4%)	6/141 (4%)
	5 Maningrida	28/143 (20%)	28/141 (20%)	28/141 (20%)
Have any of your other children had runny ears?	Yes	19/98 (19%)	22/97 (23%)	19/93 (20%)
How many children under 5 will live with you and baby?	Median (Q1-Q3)	2·00 (1·00-2·00)	2·00 (1·00-2·50)	2·00 (1·00-2·00)
Are you breast feeding only?	Yes	98/117 (84%)	98/115 (85%)	93/114 (82%)
Are you breastfeeding?	Yes	120/124 (97%)	117/124 (94%)	116/121 (96%)
Are you bottle feeding only?	Yes	4/123 (3%)	6/120 (5%)	4/119 (3%)
Are you bottle feeding?	Yes	24/122 (20%)	26/125 (21%)	25/119 (21%)
Did you smoke when you were pregnant?	Yes	58/119 (49%)	60/124 (48%)	61/120 (51%)
Does anyone smoke at your house?	Yes	27/124 (22%)	31/125 (25%)	22/121 (18%)
Do you cook with or sit near a wood fire?	Yes	23/123 (19%)	22/125 (18%)	28/121 (23%)

the SSSP group compared to _SSS (GMT ratios 8 to 59.5) (Table 6, Fig. 4).

Secondary outcomes: *Serotypes 3, 6A, 19A, and protein D: no immune compromise of SSSP at 7 months*

We found no overall immune compromise of the single dose P in SSSP versus 3-dose _PPP at 7 months of age, since at least 87% infants had IgG above threshold against serotypes 3, 6A, and 19A following a single dose of PCV13 in SSSP. GMCs in the SSSP group were significantly higher (GMC ratio 1·48) than _PPP against serotype 3 and significantly lower against serotypes 6A and 19A (GMC ratios 0·49 and 0·76) (Table 3, Figs. 2 and 3). In support of our secondary hypothesis regarding no immunological 'harm' of the SSSP schedule compared to _PPP, all serotype 3, 6A, and 19A GMTs were $\geq$ 8 (GMTs 49, 279, and 226, respectively). Higher GMTs in the SSSP group against serotypes 3 and 19A (GMT ratios 1.35 and 1.05) did not reach statistical significance, whereas serotype 6A GMT was significantly lower (GMT ratio 0.37) (Table 6 and Fig. 4).

We found no protein D immune compromise in the early 1–2–4-month SSSP schedule compared to the 2–4-6 month _SSS schedule (GMC ratio 0·93) (Table 3).

Table 3
Vaccine group comparisons of serotype-specific GMCs (μg/mL) (Ratio, 95%CI, p value) and proportion of infants with IgG ≥ 0·35 μg/mL or ≥ 1·0 μg/mL (Difference, 95%CI, p value) against serotypes 3, 6A, 19A, 10 common serotypes, and ≥ 100 EU/mL against protein D, at 7 months.

7 months	_PPP	_SSS	SSSP	SSSP vs _PPP			SSSP vs _SSS			_PPP vs _SSS		
	GMC	GMC	GMC	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	p
n	*136*	*131*	*136*									
3	1·48	0·26	2·19	1·48	(1·23, 1·78)	<0·001	8·27	(6·69, 10·21)	<0·001	5·60	(4·55, 6·90)	<0·001
6A	5·25	0·42	2·59	0·49	(0·35, 0·69)	<0·001	6·14	(4·44, 8·49)	<0·001	12·47	(9·76, 15·94)	<0·001
19A	3·47	0·97	2·63	0·76	(0·58, 0·99)	0·045	2·73	(2·05, 3·63)	<0·001	3·59	(2·78, 4·65)	<0·001
n	*132*	*129*	*132*									
Protein D	88	1121	1047	11·92	(9·72, 14·61)	<0·001	0·93	(0·74, 1·18)	0·73	0·08	(0·06, 0·10)	<0·001
n	*117*	*115*	*120*									
1	3.96	2.18	5.32	1.34	(1.05, 1.71)	0.015	2.44	(1.93, 3.08)	<0.001	1.81	(1.47, 2.24)	<0.001
4	4.27	3.35	3.88	0.91	(0.72, 1.15)	0.15	1.16	(0.94, 1.43)	0.49	1.27	(1.02, 1.59)	0.017
5	2.66	1.07	2.74	1.03	(0.83, 1.28)	0.88	2.57	(2.09, 3.15)	<0.001	2.49	(2.01, 3.08)	<0.001
6B	2.80	2.44	15.05	5.38	(3.82, 7.57)	<0.001	6.17	(4.70, 8.12)	<0.001	1.15	(0.84, 1.57)	0.21
7F	4.02	2.33	3.95	0.98	(0.79, 1.22)	0.48	1.69	(1.37, 2.09)	<0.001	1.72	(1.41, 2.10)	<0.001
9 V	3.12	2.01	4.69	1.50	(1.21, 1.87)	<0.001	2.33	(1.89, 2.88)	<0.001	1.55	(1.26, 1.92)	<0.001
14	8.55	6.23	8.13	0.95	(0.70, 1.29)	0.62	1.30	(0.98, 1.74)	0.035	1.37	(1.02, 1.84)	0.0064
18C	2.92	3.14	2.92	1.00	(0.76, 1.31)	0.98	0.93	(0.71, 1.22)	0.74	0.93	(0.72, 1.20)	0.88
19F	4.77	5.79	12.95	2.72	(2.03, 3.64)	<0.001	2.24	(1.62, 3.09)	<0.001	0.82	(0.62, 1.09)	0.032
23F	3.21	1.89	7.63	2.38	(1.76, 3.21)	<0.001	4.04	(3.11, 5.26)	<0.001	1.70	(1.28, 2.26)	<0.001
	%≥ 0.35	**%≥ 0.35**	**%≥ 0.35**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**
n	*136*	*131*	*136*									
3	96	37	99	3	(-1, 7)	0·28	61	(53, 70)	<0·001	58	(49, 67)	<0·001
6A	98	54	87	−11	(-17, −5)	<0·001	32	(22, 43)	<0·001	44	(35, 52)	<0·001
19A	100	78	96	−4	(-8, −1)	0·03	18	(10, 26)	<0·001	22	(15, 29)	<0·001
	%≥ 100	**%≥ 100**	**%≥ 100**									
n	*132*	*129*	*132*									
Protein D	41	99	98	57	(48, 66)	<0·001	−1	(-4, 1)	0·62	−58	(-67, −50)	<0·001
	%≥ 0.35	**%≥ 0.35**	**%≥ 0.35**									
n	*117*	*115*	*120*									
1	99	100	99	0	(-2, 2)	1.0	−1	(-2, 1)	1.0	−1	(-3, 1)	1.0
4	97	100	100	3	(0, 5)	0.12	0	(0, 0)		−3	(-5, 0)	0.25
5	97	92	99	2	(-2, 5)	0.37	7	(2, 12)	0.0089	5	(0, 11)	0.082
6B	89	97	99	10	(4, 16)	<0.001	2	(-2, 5)	0.36	−9	(-15, −2)	0.017
7F	100	100	99	−1	(-2, 1)	1.0	−1	(-2, 1)	1.0	0	(0, 0)	
9 V	98	97	100	2	(-1, 4)	0.24	3	(0, 6)	0.12	1	(-3, 5)	0.68
14	99	98	99	0	(-2, 2)	1.0	1	(-2, 4)	0.62	1	(-2, 4)	0.62
18C	97	97	97	0	(-5, 5)	1.0	−1	(-5, 4)	1.0	−1	(-5, 4)	1.0
19F	99	97	98	−1	(-4, 2)	1.0	1	(-3, 5)	0.68	2	(-2, 5)	0.37
23F	96	95	99	3	(-1, 7)	0.12	4	(0, 9)	0.061	1	(-5, 6)	0.77
	%>1.0	**%>1.0**	**%>1.0**									
n	*136*	*131*	*136*									
3	71	5	83	13	(3, 22)	0·021	79	(71, 86)	<0·001	66	(58, 74)	<0·001
6A	93	18	69	−24	(−33, −16)	<0·001	51	(41, 62)	<0·001	76	(68, 84)	<0·001
19A	88	47	79	−9	(-18, 0)	0·074	32	(21, 43)	<0·001	41	(31, 51)	<0·001
n	*117*	*115*	*120*									
1	95	83	95	0	(-5, 6)	1.0	12	(4, 19)	0.0053	11	(4, 19)	0.0057
4	96	94	94	−2	(-7, 4)	0.77	0	(-6, 6)	1.0	2	(-4, 8)	0.57
5	86	54	90	4	(-5, 12)	0.43	36	(26, 47)	<0.001	32	(21, 43)	<0.001
6B	78	84	98	20	(12, 28)	<0.001	13	(6, 20)	<0.001	−7	(-17, 3)	0.24
7F	94	86	95	1	(-5, 7)	0.78	9	(1, 16)	0.024	8	(0, 16)	0.05
9 V	92	86	98	5	(0, 11)	0.081	11	(4, 18)	0.0014	6	(-2, 14)	0.14
14	93	95	94	1	(-5, 7)	0.80	−1	(-6, 5)	1.0	−2	(-8, 4)	0.78
18C	84	90	83	0	(-10, 9)	1.0	−6	(-15, 2)	0.19	−6	(-15, 3)	0.25
19F	94	91	98	3	(-2, 9)	0.21	6	(0, 12)	0.047	3	(-4, 9)	0.46
23F	79	78	98	20	(12, 27)	<0.001	20	(12, 28)	<0.001	0	(-10, 11)	1.0

P = PCV13 or Prevenar13; S = PHiD-CV10 or Synflorix. *Italic P or S* represents doses received at each age. GMC, geometric mean concentration. 95%CI, 95% confidence interval.

Secondary outcomes: *Serotypes 3, 6A, 19A, and protein D: early responses to accelerated SSSP schedule*

At 4 months of age post 2-month dose in the _PPP group immunogenicity was superior to post 1–2 month doses in the SSSP group against serotype 3 (GMC ratio 0.35), but not against serotypes 6A or 19A (GMC ratios 1.15 and 1.17, respectively) (Table 4, Figs. 2 and 3). Responses to protein D in the SSSP group were significantly higher than _PPP (GMC ratio 20) and _SSS (GMC ratio 8). Protein D immunogenicity was similar following either two or three doses (Tables 3 and 4).

At 2 months of age there were no group differences in serotype 3, 6A, or 19A immunogenicity (Table 5, Figs. 2 and 3). Responses to protein D following one-month dose in the SSSP group were significantly higher than the non-vaccinated groups (GMC ratio ~ 3, ~40% difference in the proportion of infants with GMCs ≥ 100 EL.U/mL) (Table 5). Protein D immunogenicity was similar following first dose given at either one month or two months of age (Tables 4 and 5).

Secondary outcomes: *Serotypes 3, 6A, 19A, and protein D: Head-to-head comparisons*

At 4 months, following first dose in the standard 2–4-6 schedules, protein D immunogenicity was significantly lower (GMC ratio 0·4) in the _PPP group (Table 4). Responses to serotypes 3, 6A, and 19A were all low (GMCs 0·22 to 0·42 μg/mL). Surprisingly, the _PPP

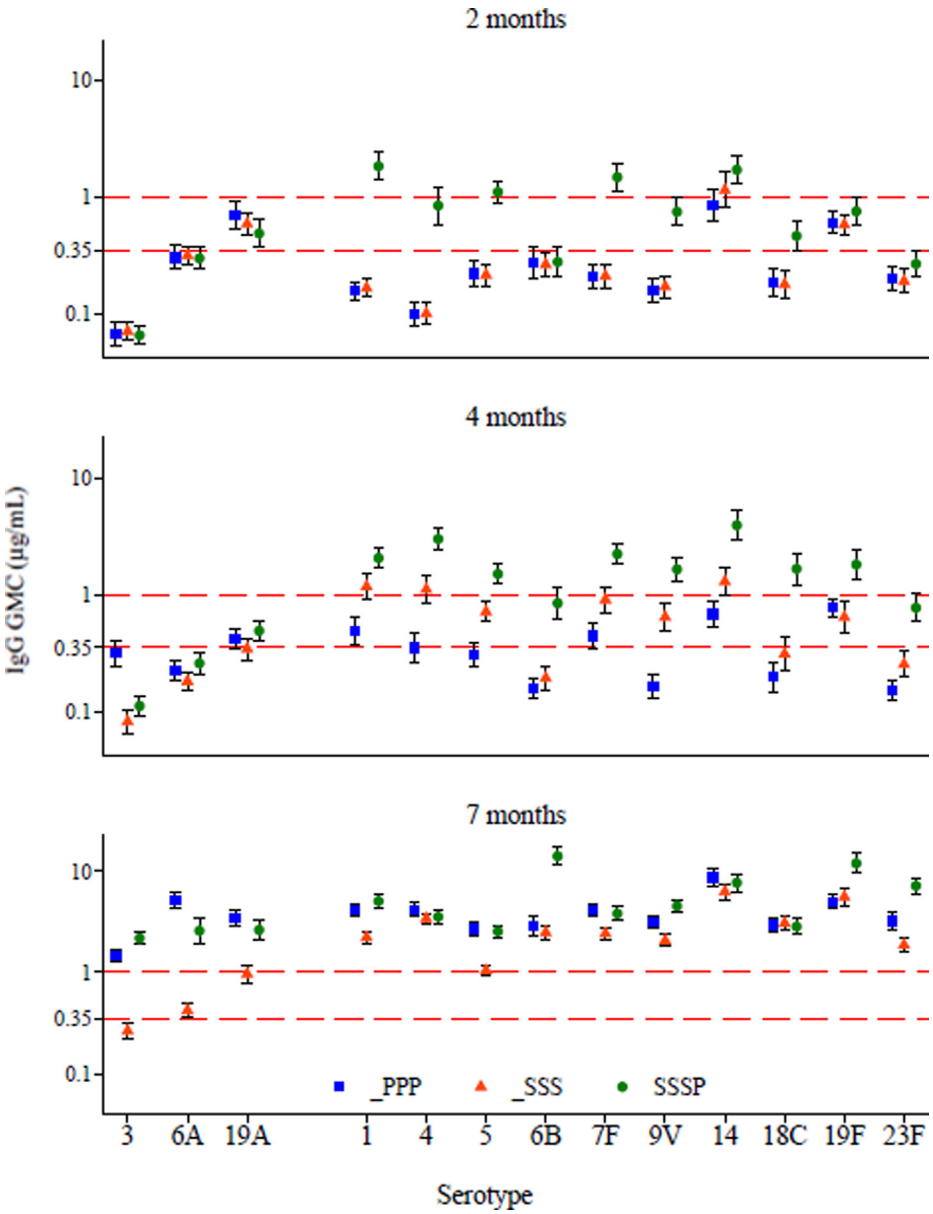

Fig. 2. Vaccine group comparisons of IgG GMCs, µg/mL (95%CI) against serotypes 3, 6A, 19A, and ten common serotypes at 2, 4, and 7 months of age. P = PCV13 or Prevenar13; S = PHiD-CV10 or Synflorix. GMC geometric mean concentration. 95%CI, 95% confidence interval. Seroprotection threshold of 0·35 µg/mL and putative threshold of 1·0 µg/mL for pneumococcal serotypes, are indicated by red dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

response was only significantly higher than *_SSS* against serotype 3 (~35% increase in proportion of infants with IgG ≥ 0·35 µg/mL), but not 6A or 19A (Table 4, Figs. 2 and 3).

At 7 months of age, the *_PPP* protein D immunogenicity was significantly lower (GMC ratio 0·08) than *_SSS* (Table 3) and immunogenicity against serotypes 3, 6A, and 19A was significantly higher (GMC ratios 3·6 to 12·5 and GMT ratios ~ 6 to 163) (Tables 3 and 6, Figs. 2 to 4).

Secondary outcomes: *Ten common serotypes at 7 months of age: 4-dose* versus *3-dose schedules at 7 months*

Compared to the 3-dose *_PPP* group, the 4-dose *SSSP* GMCs were significantly higher against five common serotypes 1, 6B, 9 V, 19F, and 23F (ratios 1·34 to 5·38). The proportion of infants with IgG ≥ 0·35 µg/mL was significantly higher against serotype 6B (89% versus 99%). The proportion of infants with IgG ≥ 1·0 µg/mL was significantly higher against serotypes 6B and 23F (Differences 20% for each) (Table 3, Figs. 2 and 3).

Compared to the 3-dose *_SSS* group, the 4-dose *SSSP* GMCs were significantly higher against eight common serotypes (ratios 1·30 to 6·17), other than serotypes 4 and 18C. The proportion of infants with IgG ≥ 0·35 µg/mL was significantly higher against serotypes 6B (Difference 10%) and 5 (Difference 7%), and at the higher threshold (IgG ≥ 1·0 µg/mL), against seven serotypes (Differences 6% to 36%), other than serotypes 4, 14 and 18C (Table 3, Figs. 2 and 3).

OPA GMTs in all groups were ≥ 8 against all ten common serotypes. OPA confirms significantly higher GMTs of the 4-dose *SSSP* schedule against most serotypes (other than 4, 14 and 18C), and particularly against serotypes 1, 5, 6B, and 19F compared to *_PPP* (GMT ratios ~ 2·3 to 4·2), and also against 7F, 9V, and 23F when compared to *_SSS* (GMT ratios 2·9 to 10·5). The proportion of infants with GMT ≥ 8 was significantly higher against serotype 6B (Difference 10% compared to *_PPP*), 1, and 23F (Differences 19% and 15% compared to *_SSS*) (Table 6, Fig. 4).

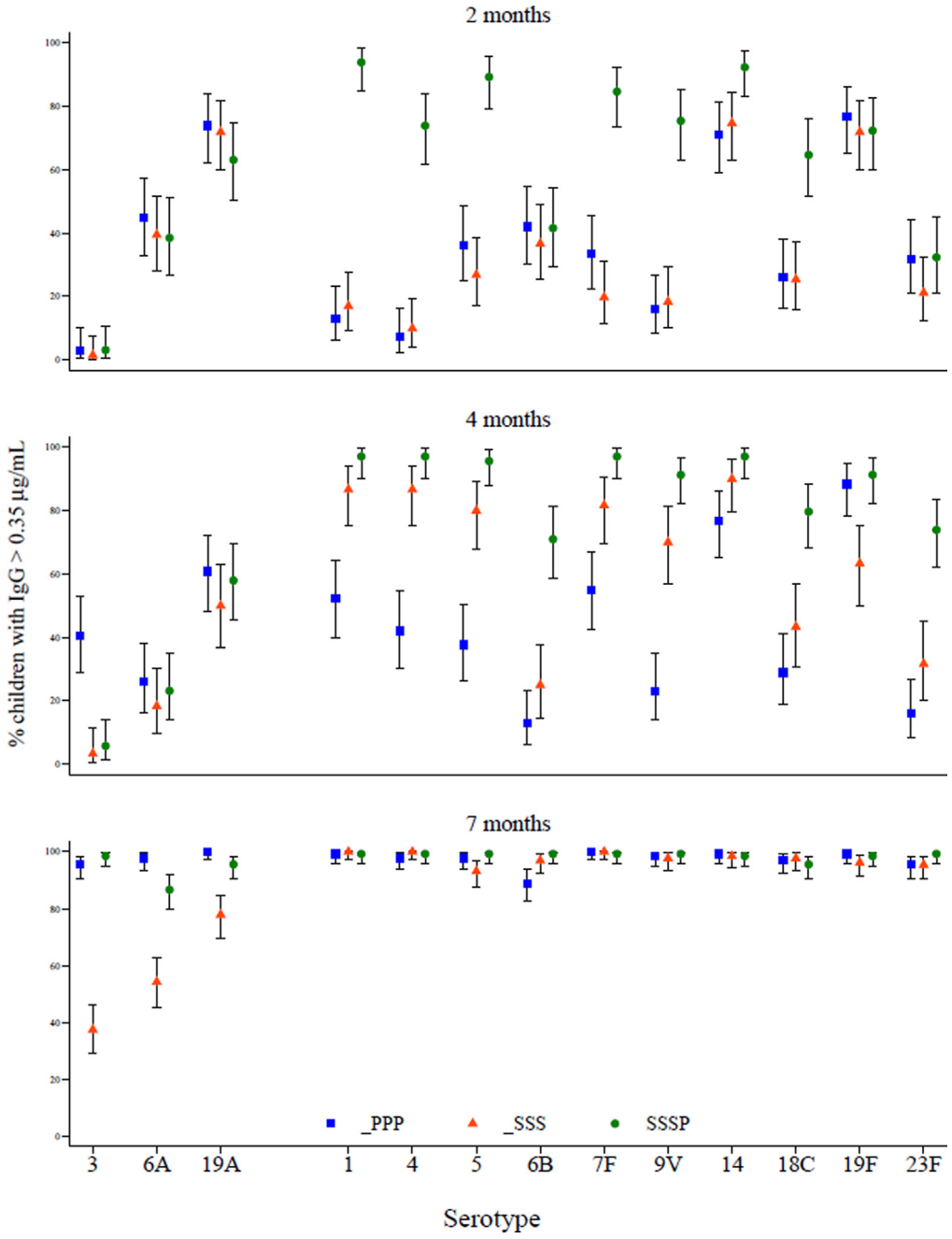

Fig. 3. Vaccine group comparisons of the proportion of infants (%, 95%CI) with IgG ≥ 0·35 µg/mL against serotypes 3, 6A, and 19A, and ten common serotypes, at 2, 4, and 7 months of age. P = PCV13 or Prevenar13; S = PHiD-CV10 or Synflorix. GMC geometric mean concentration. 95%CI, 95% confidence interval.

Secondary outcomes: *Ten common serotypes at 7 months of age: head to head 3-dose comparisons of _PPP and _SSS*

The *_PPP* group GMCs were significantly higher against serotypes 1, 4, 5, 7F, 9 V, 14, and 23F (ratios 1·27 to 2·49) and significantly lower only against serotype 19F (ratio 0·82). Compared to *_SSS*, the *_PPP* proportion of infants with IgG ≥ 0·35 µg/mL was significantly lower against serotype 6B (97% versus 89%, respectively). At the higher threshold *_PPP* was significantly lower against serotypes 1 (95% and 83%, respectively) and 5 (86% and 54%, respectively) (Table 3, Figs. 2 and 3).

The *_PPP* group GMTs were significantly higher against serotypes 4, 6B, 7F and 23F (GMT ratios 1.25 to 6·7), although there were no significant differences in the proportion of infants with GMT ≥ 8. Low OPA titres against serotype 1 in both *_PPP* and *_SSS* (GMTs 20·6 and 13, and proportions with GMT ≥ 8 of 64% and 61%, respectively) may be clinically relevant (Table 6, Fig. 4).

Secondary outcomes: *Ten common serotypes at 4 months of age: early responses to accelerated SSSP schedule*

At 4 months, all ten common vaccine type GMCs (0·82 to 4·00 µg/mL) in the 2-dose *SSSP* group were significantly higher than both the single-dose *_PPP* (ratios 2·3 to 10·4) and *_SSS* (ratios 2·07 to 5·1) groups (Table 4 and Fig. 2).

Similarly, the proportions of infants with IgG ≥ 0·35 µg/mL in the 2-dose *SSSP* group were between 71% and 97% against the ten common serotypes, which were significantly higher than 1-dose *_PPP* (Differences 20% to 69%) other than against serotype 19F (which was not significantly lower). Proportions were also

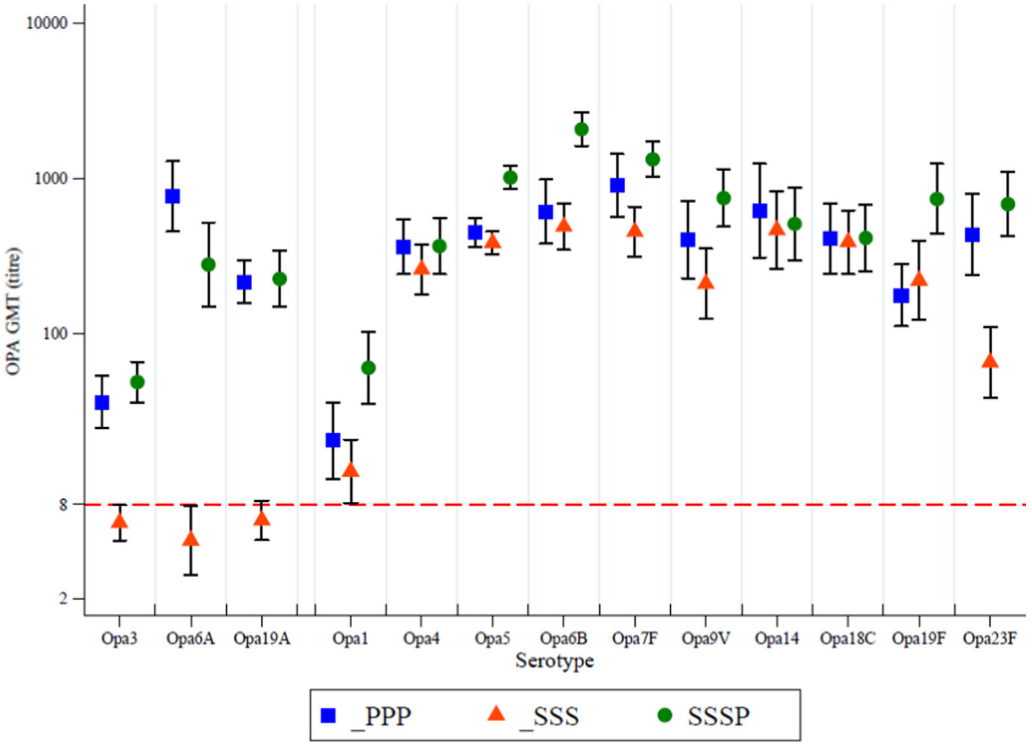

Fig. 4. Vaccine group comparisons of OPA GMTs (95%CI) against serotypes 3, 6A, and 19A, and ten common serotypes, at 7 months of age. P = PCV13 or Prevenar13; S = PHiD-CV10 or Synflorix. OPA opsonophagocytic activity. GMT geometric mean titre. 95%CI, 95% confidence interval. Seroprotection threshold GMT of 8 indicated by red dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly higher than 1-dose _SSS against common serotypes (Differences 14% to 44%) other than serotypes 1, 4 and 14. The 2-dose SSSP group had significantly higher proportions of infants with IgG ≥ 1·0 μg/mL against all common serotypes compared to 1-dose _PPP (Differences 39% to 74%) and 1-dose _SSS (Differences 24% to 51%) (Table 4, Fig. 3).

Secondary outcomes: *Ten common serotypes at 4 months of age – head to head 1-dose comparisons*

Head-to-head comparisons of immunogenicity against the ten common serotypes following a single dose at 2 months showed superiority of _SSS. The _PPP group had lower GMCs (ratios 0·26 to 0·59) against nine serotypes. The proportion of infants with IgG above ≥ 0·35 μg/mL was significantly higher in the _SSS group (Differences 13% to 48%) against serotypes 1, 4, 5, 6B, 7F, and 9 V. Serotype 19F was significantly higher in the _PPP group (Difference 22%). The _SSS group also had significantly higher proportions of infants with IgG ≥ 1·0 μg/mL (Differences 10% to 36%) against serotypes 1, 4, 7F, 9 V, 14, and 23F (Table 4, Figs. 2 and 3).

Secondary outcomes: *Ten common serotypes at 2 months of age – early responses to accelerated SSSP schedule.*

At 2 months, non-vaccinated infant GMCs were < 0·35 μg/mL against serotypes 1, 4, 5, 6B, 7F, 9 V, 18C, and 23F. Less than ~ 40% infants had GMCs ≥ 0·35 μg/mL, and ~ 10% had GMCs ≥ 1·0 μg/mL against these serotypes (Table 5, Figs. 2 and 3).

At 2 months the SSSP group GMCs were higher than the non-vaccinated _PPP and _SSS groups against all vaccine serotypes other than 6B and 23F for which non-significant increases were detected. GMCs differences were highest against serotypes 1, 4 and 7F (GMC ratios 11, 8, 7). The proportion of infants with IgG ≥ 0·35 μg/mL was between ~ 20% (serotype 14) and 81% (serotype 1) higher in the SSSP group. At the higher threshold differences were 20% to 50% higher (Table 5, Fig. 3).

PPV-Non-PCV serotypes

For PPV-non-PCV serotypes, GMCs were generally ≤ 0·35 μg/mL at each timepoint, other than against 9N and 15B, in all vaccine groups. At 7 months of age, the 4-dose *SSSP* group serotype 9N GMC was significantly higher than either *_PPP* or *_SSS* (GMC ratios 1.5 and 2.3, respectively) and the proportions of infants with 9N GMCs above either threshold were also significantly higher (Differences 7% to 27%). There were also some significant vaccine group differences for serotypes 2, 8, 11A, and 33F (data not shown).

Safety

Adverse events were rare. There were two reports of fever, one post dose 2 (38·6°C) and one post dose 3 (38·5°C) in the SSSP group. There were no unsolicited adverse events (day 0 to next dose).

There were 72 serious adverse events (hospital admissions) and one death. There were 23, 21, and 29 SAEs in the _PPP, _SSS, and SSSP groups, respectively. Sixty-two SAEs were unrelated (21, 18, 23, respectively), nine were unlikely to be related including the one death, and two were possibly related. The most common causes of hospitalisation in respective groups were, bronchiolitis (n = 14, 7, 12), other respiratory (n = 3, 3, 3), skin infections (n = 1, 4, 3), and gastroenteritis (n = 4, 1, 2).

4. Discussion

Studies in small populations living in remote areas with high disease burden are difficult to conduct. To our knowledge this is the first RCT to report immunogenicity of a PCV schedule that includes a combination of PCV formulations within the first 6 months of life. The study clearly demonstrates the safety and immunogenicity of the 1–2-4–6-month combination of PHiD-CV10 and PCV13 as a SSSP primary course schedule. We also demonstrate absence of potential deleterious effects due to other

Table 4
Vaccine group comparisons of serotype-specific GMCs (μg/mL) (Ratio, 95%CI, p value) and proportion of infants with IgG ≥ 0·35 μg/mL or ≥ 1·0 μg/mL (Difference, 95%CI, p value) against serotypes 3, 6A, 19A, 10 common serotypes, and ≥ 100 EU/mL against protein D, at 4 months.

4 months	_PPP	_SSS	SSSP	SSSP vs _PPP			SSSP vs _SSS			_PPP vs _SSS		
	GMC	GMC	GMC	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	p
n	*69*	*60*	*69*									
3	0·31	0·08	0·11	0·35	(0·26, 0·48)	<0·001	1·36	(1·01, 1·84)	0·02	3·85	(2·74, 5·42)	<0·001
6A	0·22	0·18	0·26	1·15	(0·86, 1·54)	0·46	1·43	(1·07, 1·90)	0·025	1·24	(0·95, 1·61)	0·16
19A	0·42	0·34	0·49	1·17	(0·89, 1·54)	0·59	1·43	(1·06, 1·93)	0·055	1·22	(0·92, 1·62)	0·15
n	*66*	*54*	*63*									
Protein D	61	150	1231	20·12	(14·21, 28·48)	<0·001	8·21	(5·82, 11·58)	<0·001	0·41	(0·28, 0·59)	<0·001
n	65	59	66									
1	0.49	1.21	2.05	4.16	(2.90, 5.97)	<0.001	1.70	(1.22, 2.36)	0.0036	0.41	(0.28, 0.60)	<0.001
4	0.35	1.13	3.03	8.54	(5.82, 12.54)	<0.001	2.67	(1.85, 3.86)	<0.001	0.31	(0.21, 0.48)	<0.001
5	0.31	0.74	1.54	5.04	(3.73, 6.80)	<0.001	2.07	(1.58, 2.71)	<0.001	0.41	(0.30, 0.56)	<0.001
6B	0.16	0.20	0.86	5.30	(3.68, 7.63)	<0.001	4.22	(2.84, 6.27)	<0.001	0.80	(0.58, 1.09)	0.25
7F	0.45	0.90	2.21	4.87	(3.52, 6.73)	<0.001	2.46	(1.82, 3.32)	<0.001	0.50	(0.35, 0.72)	<0.001
9 V	0.17	0.64	1.72	10.40	(7.38, 14.67)	<0.001	2.68	(1.87, 3.85)	<0.001	0.26	(0.18, 0.37)	<0.001
14	0.69	1.34	4.00	5.84	(3.90, 8.74)	<0.001	2.99	(1.98, 4.51)	<0.001	0.51	(0.36, 0.74)	<0.001
18C	0.20	0.34	1.74	8.61	(5.60, 13.26)	<0.001	5.10	(3.27, 7.96)	<0.001	0.59	(0.38, 0.91)	0.017
19F	0.77	0.68	1.78	2.31	(1.63, 3.27)	<0.001	2.62	(1.71, 4.01)	<0.001	1.13	(0.80, 1.61)	0.22
23F	0.15	0.27	0.82	5.32	(3.80, 7.45)	<0.001	3.05	(2.08, 4.47)	<0.001	0.57	(0.41, 0.79)	0.0025
	%≥ 0.35	**%≥ 0.35**	**%≥ 0.35**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**
n	*69*	*60*	*69*									
3	41	3	6	**−35**	(-48, −22)	<0·001	2	(-5, 10)	0·68	**37**	(25, 50)	<0·001
6A	26	18	23	−3	(-17, 11)	0·84	5	(-9, 19)	0·52	8	(-7, 22)	0·40
19A	61	50	58	−3	(-19, 13)	0·86	8	(-9, 25)	0·38	11	(-6, 28)	0·29
	%≥ 100	**%≥ 100**	**%≥ 100**									
n	*66*	*54*	*63*									
Protein D	27	67	98	**71**	(60, 82)	<0·001	**32**	(19, 45)	<0·001	**−39**	(-56, −23)	<0·001
	%≥ 0.35	**%≥ 0.35**	**%≥ 0.35**									
n	*65*	*59*	*66*									
1	54	88	97	43	(30, 56)	<0.001	9	(0, 18)	0.083	−34	(-49, −20)	<0.001
4	43	88	97	54	(41, 67)	<0.001	9	(0, 18)	0.083	−45	(-60, −30)	<0.001
5	38	83	97	59	(46, 71)	<0.001	14	(3, 24)	0.013	−45	(-60, −29)	<0.001
6B	14	27	71	57	(44, 71)	<0.001	44	(28, 60)	<0.001	−13	(-27, 1)	0.076
7F	55	83	97	42	(29, 54)	<0.001	14	(3, 24)	0.013	−28	(-43, −12)	0.001
9 V	23	71	92	69	(57, 81)	<0.001	21	(8, 34)	0.0022	−48	(-64, −33)	<0.001
14	77	92	97	20	(9, 31)	<0.001	5	(-3, 14)	0.25	−15	(-27, −2)	0.03
18C	29	46	82	53	(38, 67)	<0.001	36	(20, 52)	<0.001	−17	(−33, 0)	0.065
19F	88	66	91	3	(-7, 14)	0.58	25	(11, 39)	<0.001	22	(7, 36)	0.0051
23F	17	32	76	59	(45, 73)	<0.001	44	(28, 59)	<0.001	−15	(-30, 0)	0.059
	%≥ 1.0	**%≥ 1.0**	**%≥ 1.0**									
n	*69*	*60*	*69*									
3	13	2	3	−10	(-19, −1)	0·055	1	(-4, 6)	1·0	11	(3, 20)	0·02
6A	7	0	6	−1	(-10, 7)	1·0	6	(0, 11)	0·12	7	(1, 13)	0·061
19A	13	12	20	7	(-5, 20)	0·36	9	(-4, 21)	0·23	1	(-10, 13)	1·0
n	65	59	66									
1	28	56	80	53	(38, 67)	<0.001	24	(8, 40)	0.0039	−28	(-45, −12)	0.0019
4	18	54	91	72	(61, 84)	<0.001	37	(22, 51)	<0.001	−36	(-52, −20)	<0.001
5	14	24	74	60	(47, 74)	<0.001	51	(35, 66)	<0.001	−10	(-24, 4)	0.17
6B	3	7	47	44	(31, 57)	<0.001	40	(27, 54)	<0.001	−4	(-11, 4)	0.42
7F	23	44	85	62	(48, 75)	<0.001	41	(25, 56)	<0.001	−21	(-37, −5)	0.021
9 V	2	36	76	74	(63, 85)	<0.001	40	(24, 56)	<0.001	−34	(-47, −21)	<0.001
14	34	59	88	54	(40, 68)	<0.001	29	(14, 43)	<0.001	−25	(-42, −8)	0.0066
18C	8	19	68	60	(48, 73)	<0.001	50	(35, 65)	<0.001	−11	(−23, 1)	0.11
19F	34	27	73	39	(23, 55)	<0.001	46	(30, 61)	<0.001	7	(-9, 23)	0.44
23F	0	10	52	52	(39, 64)	<0.001	41	(27, 56)	<0.001	−10	(-18, −2)	0.01

P = PCV13 or Prevenar13; S = PHiD-CV10 or Synflorix. *Italic P or S* represents doses received at each age. GMC, geometric mean concentration. 95%CI, 95% confidence interval.

differences in the combination schedule compared to standard schedules.

Key findings of our study are that at 7 months of age following a single dose of PCV13 in the SSS*P* group, immunogenicity against serotypes 3, 6A, and 19A was not significantly lower than the 3-dose *_PPP*, other than some measures of the 6A response. The opsonophagocytic activity supports these findings. We also found that the 4-dose schedule provided significantly higher immunogenicity including OPA against most common serotypes (1, 5, 6B, 7F 9 V, 19F, and 23F) compared to 3-dose schedules, particularly compared to _SSS. Additional key findings include the substantial response to the first dose of PHiD-CV10 given at one month of age, and superiority of the 2-month first dose of PHiD-CV10 over PCV13 against eight of ten common serotypes. The poor response to the 2-month dose of PCV13 against all 13 serotypes is a new finding that also warrants further investigation. Key findings in relation to protein D were that the first dose of PHiD-CV10 at one-month was immunogenic, and by 4 months of age, following two doses, levels were as high as those achieved following three doses. If immunogenicity also correlates with early impacts on NP carriage and otitis media (which we will report in separate publications), an early 2-dose PHiD-CV10 schedule, followed by a single dose of PCV13 should be evaluated for use in high-risk populations.

To our knowledge this is the first report indicating the potential for mixed vaccine primary course schedules to have benefits

Table 5
Vaccine group comparisons of serotype-specific GMCs (μg/mL) (Ratio, 95%CI, p value) and proportion of infants with IgG ≥ 0·35 μg/mL or ≥ 1·0 μg/mL (Difference, 95%CI, p value) against serotypes 3, 6A, 19A, 10 common serotypes, and ≥ 100 EU/mL against protein D, at 2 months.

2 months	_PPP	_SSS	SSSP	SSSP vs _PPP			SSSP vs _SSS			_PPP vs _SSS		
	GMC	GMC	GMC	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	p
n	*69*	*71*	*65*									
3	0·07	0·07	0·07	0·99	(0·74, 1·32)	0·95	0·93	(0·72, 1·20)	0·79	0·94	(0·71, 1·26)	0·78
6A	0·31	0·32	0·30	0·98	(0·72, 1·35)	0·91	0·95	(0·71, 1·26)	0·87	0·96	(0·72, 1·29)	0·99
19A	0·70	0·59	0·49	0·70	(0·47, 1·04)	0·075	0·83	(0·58, 1·18)	0·22	1·18	(0·82, 1·69)	0·51
n	*69*	*60*	*60*									
Protein D	45	42	133	2·95	(1·99, 4·37)	<0·001	3·20	(2·25, 4·56)	<0·001	1·08	(0·74, 1·60)	0·76
n	*69*	*72*	*68*									
1	0.17	0.17	1.90	11.37	(8.09, 15.99)	<0.001	11.07	(8.00, 15.32)	<0.001	0.97	(0.74, 1.28)	0.89
4	0.11	0.11	0.88	7.89	(5.10, 12.21)	<0.001	8.37	(5.54, 12.63)	<0.001	1.06	(0.76, 1.49)	0.49
5	0.24	0.22	1.12	4.72	(3.37, 6.61)	<0.001	5.12	(3.82, 6.87)	<0.001	1.09	(0.78, 1.51)	0.54
6B	0.30	0.27	0.32	1.07	(0.69, 1.65)	0.59	1.17	(0.80, 1.70)	0.44	1.10	(0.75, 1.60)	0.94
7F	0.22	0.22	1.56	6.99	(4.79, 10.18)	<0.001	7.18	(5.01, 10.29)	<0.001	1.03	(0.72, 1.46)	0.44
9 V	0.17	0.18	0.77	4.57	(3.13, 6.67)	<0.001	4.35	(3.08, 6.14)	<0.001	0.95	(0.68, 1.34)	0.93
14	0.94	1.19	1.79	1.91	(1.26, 2.89)	0.0042	1.50	(0.96, 2.34)	0.078	0.79	(0.49, 1.25)	0.39
18C	0.19	0.19	0.49	2.60	(1.77, 3.84)	<0.001	2.66	(1.79, 3.93)	<0.001	1.02	(0.69, 1.50)	0.89
19F	0.63	0.60	0.85	1.34	(0.93, 1.94)	0.13	1.41	(1.00, 1.99)	0.066	1.05	(0.78, 1.42)	0.66
23F	0.22	0.19	0.29	1.36	(0.94, 1.96)	0.13	1.50	(1.07, 2.12)	0.022	1.11	(0.77, 1.59)	0.51
	%≥ 0.35	**%≥ 0.35**	**%≥ 0.35**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**
n	*69*	*71*	*65*									
3	3	1	3	0	(-6, 6)	1·0	2	(-3, 7)	0·61	1	(-3, 6)	0·62
6A	45	39	38	−6	(−23, 10)	0·49	−1	(-17, 15)	1·0	5	(-11, 22)	0·61
19A	74	72	63	−11	(-26, 5)	0·20	−9	(-24, 7)	0·36	2	(-13, 17)	0·85
	%≥ 100	**%≥ 100**	**%≥ 100**									
n	*69*	*60*	*60*									
Protein D	22	15	58	37	(21, 52)	<0·001	43	(28, 59)	<0·001	7	(-7, 20)	0·37
	%≥ 0.35	**%≥ 0.35**	**%≥ 0.35**									
n	*69*	*72*	*68*									
1	13	18	94	81	(71, 91)	<0.001	76	(66, 87)	<0.001	−5	(-17, 7)	0.49
4	9	11	75	66	(54, 79)	<0.001	64	(51, 76)	<0.001	−2	(-12, 7)	0.78
5	38	28	90	52	(39, 66)	<0.001	62	(49, 75)	<0.001	10	(-6, 25)	0.28
6B	43	38	43	−1	(-17, 16)	1.0	5	(-11, 21)	0.61	6	(-10, 22)	0.50
7F	33	21	85	52	(38, 66)	<0.001	64	(52, 77)	<0.001	13	(-2, 27)	0.13
9 V	17	19	75	58	(44, 71)	<0.001	56	(42, 69)	<0.001	−2	(-15, 11)	0.83
14	72	75	93	20	(8, 32)	0.0029	18	(6, 29)	0.0058	−3	(-17, 12)	0.85
18C	25	26	65	40	(25, 55)	<0.001	38	(23, 54)	<0.001	−2	(-16, 13)	0.85
19F	77	72	74	−3	(-18, 11)	0.70	1	(-13, 16)	1.0	5	(-10, 19)	0.57
23F	33	22	34	0	(-15, 16)	1.0	12	(-3, 26)	0.14	11	(-4, 26)	0.19
	%≥ 1.0	**%≥ 1.0**	**%≥ 1.0**									
n	*69*	*71*	*65*									
3	0	0	0	0	(0, 0)		0	(0, 0)		0	(0, 0)	
6A	7	10	6	−1	(-10, 7)	1.0	−4	(-13, 5)	0.54	−3	(-12, 7)	0.76
19A	30	35	23	−7	(−22, 8)	0.44	−12	(-27, 3)	0.14	−5	(-20, 11)	0.59
n	*69*	*72*	*68*									
1	1	3	68	66	(55, 78)	<0.001	65	(53, 77)	<0.001	−1	(-6, 3)	1.0
4	1	1	43	41	(29, 53)	<0.001	41	(29, 53)	<0.001	0	(-4, 4)	1.0
5	6	4	53	47	(34, 60)	<0.001	49	(36, 62)	<0.001	2	(-6, 9)	0.71
6B	19	8	16	−3	(-15, 10)	0.82	8	(-3, 19)	0.20	11	(-1, 22)	0.086
7F	10	11	68	58	(44, 71)	<0.001	57	(43, 70)	<0.001	−1	(-11, 9)	1.0
9 V	7	6	38	31	(18, 44)	<0.001	33	(20, 45)	<0.001	2	(-6, 10)	0.74
14	51	56	74	23	(7, 39)	0.0081	18	(2, 34)	0.034	−5	(-21, 12)	0.61
18C	6	11	31	25	(13, 37)	<0.001	20	(7, 33)	0.0061	−5	(-14, 4)	0.37
19F	29	25	43	14	(-2, 30)	0.11	18	(2, 33)	0.032	4	(-11, 19)	0.70
23F	4	8	13	9	(0, 18)	0.077	5	(-5, 15)	0.42	−4	(-12, 4)	0.49

P = PCV13 or Prevenar13; S = PHiD-CV10 or Synflorix. *Italic P or S* represents doses received at each age. GMC, geometric mean concentration. 95%CI, 95% confidence interval.

greater than predicted from studies of single formulations. Further research is needed to determine whether the substantial 3, 6A, and 19A immunogenicity following first dose of PCV13 at 6 months in the SSS*P* schedule was primed by the preceding PHiD-CV10 doses. Certainly, the GMCs achieved following first dose of PCV13 in the _*P*PP group were very low compared to those after the first dose of PCV13 in the SSS*P* group. For a 6A and 19A response it is plausible that there has been cross-reaction with 6B and 19F in the preceding PHiD-CV10 doses. However, the lack of 6A and 19A responses in the _SSS group suggests that cross-reaction was not involved.

The proportion of infants having above the aggregate correlate of protection against invasive pneumococcal disease (IPD) of ≥ 0·35 μg/mL was at least 89% against all common serotypes and all groups at 7 months of age. Whilst an aggregate correlate of protection of ≥ 0·35 μg/mL is required to demonstrate protection from IPD for licensing, serotype-specific correlates vary substantially. For serotypes 3, 6A, and 19A, correlates estimated from 706 cases of IPD in the UK and Northern Ireland were 2·83, 0·16 and 1·00 μg/mL, respectively [15]. Our corresponding GMCs at 7 months were 2·19, 2·59, and 2·63 μg/mL in the SSS*P* group and 1·48, 5·25, and 3·47 μg/mL in the _*PPP* group. Both groups, and particularly the _*PPP* group have inadequate serotype 3 responses according to this UK correlate of protection. Our serotype 3 OPA GMT was also lower in the _*PPP* group (GMT 36) compared to SSS*P* (GMT 49), but this difference did not reach statistical

Table 6
Vaccine group comparisons of serotype-specific OPA GMTs (Ratio, 95%CI, p value) and proportion of infants with IgG above threshold (≥8) (Difference, 95%CI, p value), against serotypes 3, 6A, and 19A, and 10 common serotypes at 7 months.

7 mo	*_PPP*		*_SSS*		*SSSP*		*SSSP* vs *_PPP*			*SSSP* vs *_SSS*			*_PPP* vs *_SSS*		
	n	GMT	*n*	GMT	*n*	GMT	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	**p**
3	*47*	36·38	*53*	6·10	*65*	49·05	1·35	(0·84, 2·18)	0·26	8·05	(5·43, 11·93)	<0·001	5·97	(3·77, 9·45)	<0·001
6A	*43*	764·82	*43*	4·69	*57*	279·25	0·37	(0·16, 0·82)	0·026	59·48	(26·76, 132·20)	<0·001	162·91	(79·10, 335·56)	<0·001
19A	*104*	215·99	*98*	6·32	*109*	226·09	1·05	(0·63, 1·75)	0·32	35·80	(21·70, 59·05)	<0·001	34·20	(22·36, 52·30)	<0·001
1	*39*	20.59	*49*	13.04	*62*	60.44	2.94	(1.37, 6.31)	0.0071	4.63	(2.30, 9.32)	<0.001	1.58	(0.77, 3.26)	0.21
4	*102*	363.02	*107*	259.38	*107*	366.77	1.01	(0.57, 1.80)	0.87	1.41	(0.81, 2.46)	0.013	1.40	(0.81, 2.42)	0.019
5	*106*	450.19	*108*	384.34	*108*	1013.68	2.25	(1.72, 2.95)	<0.001	2.64	(2.08, 3.34)	<0.001	1.17	(0.90, 1.53)	0.18
6B	*91*	611.28	*101*	488.89	*71*	2072.72	3.39	(1.98, 5.79)	<0.001	4.24	(2.78, 6.48)	<0.001	1.25	(0.70, 2.24)	0.027
7F	*41*	899.69	*46*	453.20	*61*	1325.47	1.47	(0.87, 2.50)	0.27	2.92	(1.86, 4.60)	<0.001	1.99	(1.10, 3.58)	0.0032
9 V	*46*	404.30	*50*	210.24	*63*	748.53	1.85	(0.91, 3.75)	0.049	3.56	(1.83, 6.91)	<0.001	1.92	(0.90, 4.11)	0.031
14	*43*	621.52	*54*	465.16	*59*	510.10	0.82	(0.34, 1.96)	0.21	1.10	(0.50, 2.40)	0.92	1.34	(0.55, 3.25)	0.19
18C	*47*	411.92	*55*	388.63	*58*	413.56	1.00	(0.49, 2.04)	0.70	1.06	(0.54, 2.10)	0.85	1.06	(0.53, 2.12)	0.87
19F	*46*	177.33	*53*	220.53	*60*	738.14	4.16	(2.10, 8.26)	<0.001	3.35	(1.55, 7.24)	<0.001	0.80	(0.39, 1.68)	0.14
23F	*46*	436.28	*54*	65.51	*59*	684.86	1.57	(0.74, 3.35)	0.3	10.45	(5.21, 20.99)	<0.001	6.66	(3.02, 14.68)	<0.001
	n	**%>8**	***n***	**%>8**	***n***	**%>8**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**	**Diff**	**95%CI**	**p**
3	*47*	83%	*53*	23%	*65*	91%	8%	(-5, 21)	0·26	68%	(55, 81)	<0·001	60%	(45, 76)	<0·001
6A	*43*	95%	*43*	23%	*57*	88%	−8%	(-18, 3)	0·29	64%	(49, 80)	<0·001	72%	(58, 86)	<0·001
19A	*104*	94%	*98*	37%	*109*	91%	−3%	(-10, 4)	0·44	54%	(43, 65)	<0·001	57%	(47, 68)	<0·001
1	*39*	64	*49*	61	*62*	81	17	(-1, 35)	0.10	19	(3, 36)	0.033	3	(-17, 23)	0.83
4	*102*	91	*107*	91	*107*	91	−1	(-8, 7)	1.0	0	(-8, 8)	1.0	1	(-7, 8)	1.0
5	*106*	100	*108*	100	*108*	100	0	(0, 0)		0	(0, 0)		0	(0, 0)	
6B	*91*	90	*101*	96	*71*	100	10	(4, 16)	0.0051	4	(0, 8)	0.14	−6	(-13, 1)	0.15
7F	*41*	98	*46*	100	*61*	100	2	(-2, 7)	0.40	0	(0, 0)		−2	(-7, 2)	0.47
9 V	*46*	93	*50*	96	*63*	98	5	(-3, 13)	0.31	2	(-4, 9)	0.58	−3	(-11, 6)	0.67
14	*43*	91	*54*	91	*59*	90	−1	(-12, 11)	1.0	−1	(-12, 10)	1.0	0	(-12, 12)	1.0
18C	*47*	96	*55*	96	*58*	97	1	(-7, 8)	1.0	0	(-7, 7)	1.0	−1	(-8, 7)	1.0
19F	*46*	98	*53*	89	*60*	95	−3	(-10, 4)	0.63	6	(-4, 16)	0.3	9	(0, 19)	0.12
23F	*46*	93	*54*	81	*59*	97	3	(-5, 12)	0.65	15	(4, 26)	0.013	12	(-1, 25)	0.13

significance. The UK study GMT correlate was 39, suggesting that only the *SSSP* group could deliver serotype 3 immunogenicity. Both groups had adequate serotype 19A responses. The serotype 6A GMC in the *SSSP* group (2·59 μg/mL), although significantly lower than the GMC in the *_PPP* group (5·25 μg/mL), is well above the 0.16 μg/mL correlate for protection [15].

Our data clearly demonstrate superior immunogenicity of the early 1–2-month SSSP schedule. At 2 months, SSSP was superior to non-vaccinated groups for almost all shared serotypes, and all GMCs were above UK-proposed serotype-specific correlates, other than 19F. This response to first dose of PHiD-CV10 at one month of age is a significant finding for populations at high-risk of early onset infections. A vaccine response at this age cannot be assumed, given potential for maternal antibody masking, or failure to respond. Nasopharyngeal carriage occurs within weeks of life in this population [1]. Whilst NP carriage has been observed to prime PCV responses, others have reported that prior carriage compromises immune responses to the colonising serotype [13,16]. The hierarchy of carriage serotypes in this population prior to commencement of this trial was (descending) 16F, 15A, 23F, 11A, 35B, 19F, and 15B [2]. It is plausible that NP carriage has had a role in immune responses. The interactions between carriage, immunogenicity, and otitis media will be reported in subsequent papers.

As mentioned above, in the head-to-head comparisons of *_PPP* and *_SSS*, we found poor or no responses to first dose of PCV13 given at 2 months of age. The PCV13 responses to the 2-month dose were below UK serotype-specific correlates of IPD protection for all nine common serotypes, other than serotypes 14 and 18C. Interestingly, and consistent with the strong responses to the one-month dose of PHiD-CV10, the first dose of PHiD-CV10 given at 2 months was superior to PCV13 for all common serotypes other than serotype 19F, eight of which were above UK serotype-specific correlates of protection. This also suggests that our choice to use PHiD-CV10 for first dose in our early combination schedule was possibly the right choice, and that subsequent combination trials could take this into account. We note that serotype 19F is one of two serotypes in PHiD-CV10 that is not protein D-conjugated which may in part explain lack of PHiD-CV10 superiority for this serotype.

tIn the absence of immune correlates of protection against NP carriage, we have reported the proportion of infants having an IgG concentration ≥ 1·0 μg/mL. For serotypes 3, 6A, and 19A this was at least 71% in the *_PPP* group and at least 69% in the *SSSP* group. For the ten common serotypes, at least 78% achieved this level of immunogenicity in the _PPP and _SSS groups, respectively (other than against serotype 5 in the _SSS group which was 54%), and 83% in the SSSP group. Seroincidence has been proposed as a proxy for NP carriage (i.e. a rise in antibody above that at post vaccination being indicative of NP carriage) [17]. We compared the serotype-specific GMCs (95%CIs) with published correlates for 9 of 10 common serotypes (serotype 1 absent) [17]. Above protective levels (non-overlapping 95%CIs) were achieved at 7 months in the *SSSP* group against serotypes 4, 5, 6B, 7F, 18C and 19F. Point estimate GMCs were above estimated thresholds against serotypes 9V and 23F, but with overlapping 95%CIs. Serotype 14 was lower but not significantly lower. GMCs above proposed NP carriage correlates were achieved at younger ages in the SSSP group for serotypes 4 (at 4 months of age) and serotype 5 (at 2 months of age). Nasopharyngeal carriage data from this trial will help determine vaccine impact on early acquisition and immune responses.

We also found small but significant protein D responses to the one-month dose of PHiD-CV10 in our combination SSSP group. Following the second dose at 2 months of age, levels of protein D IgG were almost 10-fold higher and were similar to levels after three doses in either the *SSSP* or *_SSS* groups at 7 months of age. This suggests that for protein D, a third dose within the primary course offers no further immune benefit. We also show that the shift from standard 2–4-6-months to 1–2–4-months did not compromise protein D responses achieved at 7 months, inter alia demonstrating the persistence of protein D IgG to 3 months after the third dose of S in the *SSSP* group.

There is no immune correlate of protection against protein D although studies commonly report the proportion of participants with IgG $\geq$ 100 EU/mL. Evidence of a clinical or microbiological impact of anti-protein D antibodies on NTHi infection or carriage is variable [6,7,18]. Our study found levels of protein D antibodies of around 1200 EU/mL at 7 months of age, similar to levels reported from other post-primary studies [7,10]. Naturally derived protein D antibody has been associated with reduced NTHi-AOM [19]. In our study we detected very small increases in protein D GMCs in the _PPP group (i.e. naturally derived) at 2, 4, and 7 months. Our systematic review showed no evidence of reduced NP carriage of NTHi following early primary course doses [20]. Small studies have found protein D vaccine responses to be protective of respiratory symptoms[21] and to reduced NTHi lower airway infection [22]. Our cross-sectional studies during PCV7, PHID-CV10, and PCV13 eras found a significant reduction in NTHi-culture-positive middle ear infections in PHiD-CV10-vaccinees and a small reduction in AOM [23,24]. A consistent trend in these studies is that there is potentially a compartmental effect of PHiD-CV10; with reduced NTHi infections and clinical improvements in the ear and lung, not paralleled by reduced NP carriage. Our data on NP carriage and otitis media from this study will add further to the slowly emerging evidence on vaccine-induced NTHi protection.

There are few published trials comparing mixed PHiD-CV10 and PCV13 primary course schedules or head-to-head trials with which to compare our findings. Two head-to-head trials have recently been reported [25,26]. The Papua New Guinean trial [26] compared PHiD-CV10 with PCV13 given at 1–2-3 months of age; at 4 months, serotype 3, 6A, 19A as well as 7F, 19F and 23F GMCs were higher in the PCV13 group. The Vietnamese trial reported immunogenicity against the ten common serotypes 4 weeks after the 2-dose primary course of PCV13 versus PHiD-CV10; [25] the 2-dose PCV13 had significantly higher proportion of infants with IgG concentration $\geq$ 0·35 μg/mL against serotypes 6B and 23F, and higher OPA GMTs against serotypes 1, 9 V, and 23F. Neither trial evaluated mixed schedules nor reported responses after the first dose. A recent systematic review of the interchangeability of mixed schedules opined that the limited data available, primarily from boosting with an alternative vaccine to primary course, were reassuring, but gaps in evidence limited application to policy decisions, particularly for primary course interchangeability [27]. They included data from one 3-arm RCT (published as conference abstract in 2017) that compared 2 + 1 SS-S, PP-S and PS-S; the PS-S arm having mixed vaccines within the infant series (PCV13 at 2 months and PHiD-CV10 at 4 months). Immunogenicity was measured post-primary only; serotype 6A, 19A, and 3 responses were lower in the PS-S group compared to PP-S, which is consistent with our findings of low responses to first PCV13 in infant series.

In 2018 Australia's national Immunisation Program made the lowest recommended age for the first dose of PCV13 6 weeks, but did not require a repeated first dose unless given before 28 days of age. The results of our study confirm the safety and immunogenicity of a first dose of PHiD-CV10 at one month. Although most Australian children are now recommended to have a 2 + 1 PCV schedule, for Aboriginal children living in regions with high incidence of pneumococcal disease a 3 + 1 schedule continues to be recommended. Our data suggest that the persistent problem of severe early-onset otitis media in these children may benefit from scheduling the first dose at 4 weeks of age and that clinical outcomes should be further evaluated. Our data also support flexibility in timing of first dose and opportunistic vaccine recommendations.

Importantly we have demonstrated the safety and superior immunogenicity of combining PCV13 and PHiD-CV10 in a primary course schedule that commences at one month of age, with no immunological compromise or safety concerns of the ratio or timing of vaccine doses (SSSP).

Panel 1. Research in context.

Evidence before this study.

In 2009 there was uncertainty regarding the superiority of pneumococcal conjugate vaccines in preventing otitis media caused by pneumococcus and non-typeable *Haemophilus influenzae*. Whilst there were emerging data on interchangeability of PCVs between primary and booster doses, no trial evaluated safety or immunogenicity of combined PCVs within the primary course. No data from head-to-head trials were available to determine which PCV provided superior immunogenicity during the first months of life. No study had evaluated safety or immunogenicity of a 4-dose primary course schedule.

Added value of this study.

This study confirms broader immunogenicity, without compromise (immunological or adverse events) of the combination 4-dose schedule of PHiD-CV10 at 1–2-4 months plus PCV13 at 6 months of age. Outcomes at 2, 4, and 7 months of age are valuable for high-risk populations. For the first time, we have shown that responses following single dose PCV13 given at 6 months (and following the 1–2-4 PHiD-CV10), are comparable to the 3-dose PCV13 response. We confirmed superior immunogenicity of the early one- and two- month PHiD-CV10 doses in the *SS*SP group against most common serotypes at 2 and 4 months, and additional benefit of the fourth dose against most of the common serotypes, particularly 6B, 19F, and 23F. Head-to-head comparisons revealed superiority of the 2-month dose in the _SSS over _*P*PP for most common serotypes and poor responses to all 13 serotypes following the 2-month dose of PCV13. The clinical relevance of protein D antibody concentration is poorly understood, our study indicated that substantial concentrations can be achieved by 4 months of age following the early 1–2 *SS*SP schedule.

Implications of all the available evidence.

This study provides evidence that PHiD-CV10 can elicit protective immune responses when given as early as one month of age. PHiD-CV10 and PCV13 can be combined safely in a 3:1 primary course ratio to provide broader coverage and higher antibody levels. We document poor immunogenicity of first dose PCV13 given at 2 months of age. Further research is needed to better understand the potential beneficial interactions between these PCVs and how their differences can be used to tailor schedules to meet the needs of different populations. Interchangeability of PCVs in the primary course will simplify vaccine use in countries that already use these vaccines alternatively for primary and booster doses.

5. Contributors

AJL (Principal Investigator, PI) conceived the study, led funding applications, obtained ethical approvals and other regulatory approvals, undertook consultations, reporting and has overseen day-to-day management and implementation of the trial, managed, analysed and interpreted the data, created figures (with MC and VO) and wrote the manuscript. NW managed the trial, staffing, participant recruitment and retention, specimen collection, reported to Ethics committees and data safety monitoring board, managed quality of data and read the final version of the manuscript. BA assisted participant recruitment and retention, specimen collection, managed quality of data and read the final version of the manuscript. JB managed microbiology and serology collections, database and data quality, and read the final version of the manu-

script. MC wrote the data analysis plan in the protocol, analysed data, generated tables and figures and read the final version of the manuscript. VO analysed data, generated tables and figures and read the final version of the manuscript. EKM advised on study design, assisted with funding application, participated in investigator meetings, advised on risk management and read the final version of the manuscript. MS advised on study design, assisted with funding application, participated in investigator meetings, advised on risk management and read the final version of the manuscript. PJT advised on study design, assisted with funding application, participated in investigator meetings, advised on risk management and read the final version of the manuscript. NJB advised on study design, assisted with funding application, participated in investigator meetings and advised on risk management, particularly in relation to engagement of Aboriginal Medical Services and cultural matters. PM advised on study design, assisted with funding application, participated in investigator meetings, advised on risk management and read the final version of the manuscript. HS-V advised on study design, assisted with funding application, participated in investigator meetings, advised on laboratory protocols, particularly microbiology, and reviewed the final version of the manuscript. SS advised on study design, assisted with funding application and read the final version of the manuscript. AB advised on study design, assisted with funding application, participated in investigator meetings and advised on laboratory protocols, particularly immunogenicity. PL advised on laboratory protocols, particularly immunogenicity, and read the final version of the manuscript. RA advised on study design, assisted with funding application. JC advised on study design, assisted with funding application and read the final version of the manuscript. JM advised on study design, assisted with funding application and advised with statistical matters. VK advised on study design, assisted with funding application and advised on immunisation policy implications. PSM advised on study design, assisted with funding application, participated in investigator meetings, advised on risk management and provided day-to-day supervision of clinical training, and read the final version of the manuscript.

Additional investigators: *PREVIX staff (current or at least one year)*: PREVIX research managers and research nurses: Nicole Wilson (Manager), Beth Arrowsmith, Tracy Grierson, Carolyn Gage-Pearson (admin), Nicole Weinert, Natalie Bert, Zeina Hayes, Melanie Schwarz, Julie Wheeler, Bronwyn Nankervis, Laura Bell, Jessica Young, Kelly Whykes, Sabine Sprenger, Melisa Downie, Valerie Coomber, Kate Ranford, Rachel Sharp, Elissa Rowe, Jodie Howes, Chantelle Dowling, Claire Haynes, Christine Byrne, Niki Emmett, Sarah Carlisle, Fiona Hildebrand, Kate Dohle, Cathy O'Driscoll.

PREVIX laboratory team: Jemima Beissbarth (Manager), Vanya Hampton, Nerida Liddle, Christopher Wevill, Yuki Ruzsicska, Donna Woltring, Rebecca Cass, Cain Hendy, Shennelle Waters, Shae Tozer, Erin Gargan, Nicole Smitran, Amy Llewellyn, Katrina Lawrence, Jessie Spargo, Kim Hare. PREVIX data manager: Jemima Beissbarth

PREVIX statistician: Mark Chatfield (to Dec 2017), Victor Oguoma (from Jan 2018), Zhiqiang Wang (2020).

Community Workers: Jeanette Warnir and Georgina Parmbuk (Wadeye), Amanda Turner (Alice Springs), Kaylene Puruntatameri (Wurrumiyanga)

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iDSMB members: David Isaacs (Chair), Hasantha Gunasekera, Terry Nolan, Peta Forder, Heather D'Antoine, Nicholas Wood

6. Trial registration

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Protocol deviations and protocol violations can be provided on written request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2021.100086.

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